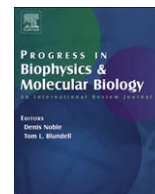




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## Review

# The rules of disorder or why disorder rules

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### ABSTRACT

The finding that a large fraction of proteins (over 30%) in eukaryotic cells lack a unique three-dimensional structure but are functional has forced the scientific community to review its understanding of the structure–function paradigm. The involvement of many of these intrinsically unstructured proteins (IUPs) in intracellular signalling and regulatory processes as well as their central positioning (as interaction hubs) in recently mapped protein interaction networks is particularly intriguing. Here, we review the functional and structural properties of IUPs such as (i) their facilitated regulation via diverse post-translational modifications of specific amino acids (ii) scaffolding and recruitment of different binding partners in space and time via the “fly-casting” mechanism, through peptide motifs and by coupling folding with binding and (iii) conformational variability and adaptability. All of these properties allow these proteins to hold key positions in cellular organisation and regulation which in turn make them tractable as drug targets. In addition, we discuss how such properties, individually and in combination, facilitate combinatorial regulation and re-use of the same component in multiple biological processes.

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## 1. Introduction

As protein–protein interactions are central to all biological processes, a comprehensive determination of all interactions between proteins that take place in an organism provides a fundamental framework for understanding biology as an integrated system. In recent years it has become possible to discern the topology of cellular protein–protein networks on a genomic-scale, which are conventionally represented as “interactome” maps (Barabasi and Oltvai, 2004; Breitkreutz et al., 2008; Li et al., 2004; Oltvai and Barabasi, 2002; Orchard et al., 2007; Russell and Aloy, 2008; Schwikowski et al., 2000; Vidal, 2001; Yu et al., 2008). However, in order to understand (a) cellular processes such as signal transduction, (b) the emergence of phenotype from cellular complexity, and (c) to decipher the complex molecular mechanisms that underlie pathologies like cancer or neurodegeneration, we have to not only map the interactome but also elucidate how structure, dynamics, and the spatio-temporal distributions of the constituent proteins affect protein interaction and the interaction network (Blundell and Srinivasan, 1996; Chothia and Janin, 1975; Jones and Thornton, 1996; Levy and Pereira-Leal, 2008; Levy et al., 2006; Schmid and McMahon, 2007). Although nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography, electron microscopy and other methods (Cowieson et al., 2008; Laskowski and Thornton, 2008) have proven very successful in the determination of protein structures, the exhaustive characterization of binary and higher-order protein complexes present in a cell is an extremely challenging task for several reasons (Laskowski and Thornton, 2008; Robinson et al., 2007): (i) many protein interactions in a cell are weak (Vaynberg and Qin, 2006), transient (Nooren and Thornton, 2003; van der Merwe and Barclay, 1994) and may be promiscuous (Kim et al., 2008; Nobeli et al., 2009); therefore, true transient interactions may not be stable enough to withstand the rigours of purification and hence may not be reliably identified (ii) data from high-throughput proteomics approaches, including pull-down and yeast-2-hybrid experiments, indicate that the total number of protein interactions in a eukaryotic cell significantly exceeds the total number of proteins (Gavin et al., 2006; Krogan et al., 2006; Rual et al., 2005; Stelzl et al., 2005; Tyers and Mann, 2003), (iii) because the number of actual interaction is still much lower than the number of possible interactions, even the most reliable detection method will produce false positives (Levy et al., 2009) and (iv) a large fraction of eukaryotic proteins may lack a tertiary structure when alone in solution – so called intrinsically unstructured proteins (IUPs) – and adopt different folds when interacting with different partners (Dunker et al., 2005, 2001, 2008; Dyson and Wright, 2005; Tompa, 2002; Wright and Dyson, 1999).

Tackling the last point is of growing importance as it has been demonstrated that IUPs are at the heart of many signalling and regulatory cascades in the interaction networks of eukaryotic cells (Chen et al., 2006; Dunker et al., 2008; Iakoucheva et al., 2002; Liu et al., 2006; Lobley et al., 2007; Uversky et al., 2005; Ward et al., 2004; Xie et al., 2007a,b). Joint efforts by structural, molecular and systems biologists have shed new lights on the special properties of IUPs in the last few years. Here, we review these new insights and describe why the unique properties of IUPs predestine them to play a key role in signalling and recognition in cellular systems (Fig. 1).

## 2. Intrinsically unstructured proteins

### 2.1. Definition

Most generally, IUPs can be defined as proteins that lack a unique fold, either entirely or in parts, when alone in solution (Dunker et al., 2001; Wright and Dyson, 1999). Solution-state NMR, circular

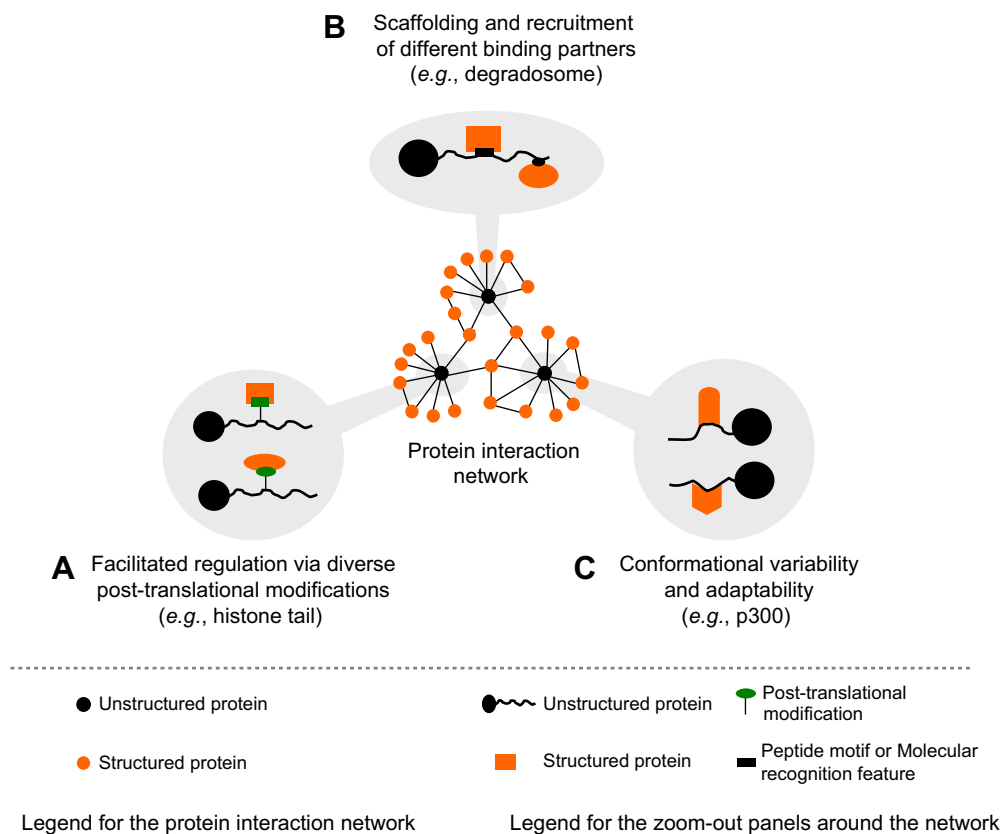
dichroism (CD), fluorescence spectroscopy and small-angle X-ray scattering measurements have provided clear *in vitro* evidence for the existence of IUPs (Daughdrill et al., 1997; Dyson and Wright, 2004; Hecht et al., 2008; Iakoucheva et al., 2002; Kriwacki et al., 1996, 1997; Li and Song, 2007; Mittag and Forman-Kay, 2007; Wells et al., 2008; Zeev-Ben-Mordehai et al., 2003). While some IUPs exhibit partial secondary structure when alone in solution, many lack any detectable secondary or tertiary structure. *In vivo* NMR measurements further indicate that even in the crowded environment of the cell, certain IUPs remain partially or fully unstructured (McNulty et al., 2006). The lack of structure is due to a strong amino acid compositional bias in IUPs. They are rich in hydrophilic and charged residues but often lack bulky hydrophobic residues (Dosztanyi et al., 2005; Garner et al., 1998; Romero et al., 2001; Weathers et al., 2004; Williams et al., 2001). The paucity of hydrophobic residues prevents the formation of a hydrophobic core necessary for a stable 3-dimensional fold. Moreover, the large number of side chain charges present under physiological conditions contributes to destabilize any compact state.

### 2.2. Function

It is important to note that although the biophysical properties of the unstructured chain mediate the function of some IUPs (e.g. titin) (Tompa, 2003), many IUPs adopt a defined 3-dimensional structure upon binding to their cognate partners (Demarest et al., 2002; Dyson and Wright, 2005; Wright and Dyson, 1999). The need for interaction in order to fold was reported by Frankel and Kim (1991) in the early nineties. They showed that certain transcription factor domains are not highly ordered on their own and become structured only upon interaction with other molecules. The process of folding upon binding has since been characterised in great detail, especially for the DNA-binding domains (DBDs) of many transcription factors (Dunker et al., 2008; Dyson and Wright, 2002; Fuxreiter et al., 2004; Levy et al., 2004; Mohan et al., 2006; Sugase et al., 2007; Turjanski et al., 2008; Wright and Dyson, 2009). Well-described examples in the literature include the basic DNA-binding region of the leucine zipper protein GCN4 (Love et al., 1995, 2004; Weiss et al., 1990), the DBD of the retinoid X receptor (Holmbeck et al., 1998; van Tilborg et al., 1999) and the high mobility group (HMG) domain of the lymphoid enhancer-binding factor LEF-1 (Love et al., 1995, 2004). While these DBDs exhibit more localized folding transitions upon binding to DNA, many transcriptional activation domains like the kinase-inducible activation domain of the cAMP regulated transcription factor (CREB) and the acidic activation domain of p53 are completely unstructured when free in solution and fold upon binding to their targets (Kussie et al., 1996; Radhakrishnan et al., 1997). IUPs that fold upon binding are also known to be found among RNA binding proteins, where they play a key role in RNA recognition (Battiste et al., 1996; Mogridge et al., 1998), in vesicle trafficking and membrane fusion (Fiebig et al., 1999; Olesen et al., 2008; Owen et al., 2004; Praefcke et al., 2004; Schmid et al., 2006; Schmid and McMahon, 2007), cell cycle regulation (Kriwacki et al., 1996), and as an integrative part of many different signalling pathways (Bhattacharyya et al., 2006; Chu et al., 2007; Iakoucheva et al., 2002; Mathes et al., 2008; Seldeen et al., 2008). So far, experimental evidence for intrinsic lack of structure could be gathered for more than 500 proteins that are involved in very diverse cellular processes. The database DisProt (Sickmeier et al., 2007) provides a repository for such proteins.

### 2.3. Prediction

The increased interest in IUPs by structural and molecular biologists has triggered the development of a variety of computer



**Fig. 1.** Properties of IUPs that are pivotal to their central role in protein–protein interaction (PPI) networks (shown in the center with nodes representing proteins and links representing physical interactions). Hub proteins in PPI networks are enriched in intrinsically unstructured segments. The specific properties of these unstructured segments facilitate interactions with many diverse partners: (A) conformational flexibility facilitates access to enzymes and effectors that introduce or remove post-translational modifications and read the post-translational code, respectively. Specific post-translational modifications permit combinatorial regulation and the re-use of the same components in multiple biological processes. (B) The availability of molecular recognition features and short linear peptide motifs within the unstructured segments enables the fishing for (“fly-casting”) and gathering of different partners. The interaction specificity provided by these motifs is essential for the precise assembly of macromolecular machines in space and time. (C) Conformational variability enables a nearly perfect moulding to fit the binding surfaces of very diverse partners. Context-dependent folding of an intrinsically unstructured segment can be used to activate or inhibit signalling processes that can have completely orthogonal outcomes.

programs that predict unstructured regions from amino acid sequences. Over 15 different predictor programs have been developed in recent years (Bordoli et al., 2007; Ferron et al., 2006; Oldfield et al., 2005a; Sickmeier et al., 2007). For an overview of protein disorder prediction methods and a list of IUP predictors, please refer to Ferron et al. (2006) and to the table in the Wikipedia article on unstructured proteins at [http://en.wikipedia.org/wiki/Intrinsically\\_unstructured\\_proteins](http://en.wikipedia.org/wiki/Intrinsically_unstructured_proteins). The available approaches to predict unstructured segments can be classified into two categories: (a) methods that rely on sequence composition and their variants and (b) methods that are based on machine learning approaches (e.g., support vector machines or neural networks) that are trained on available experimental data which indicates the absence of structure in proteins (for instance, the lack of electron density in crystallographic data, Ward et al., 2004). It has been estimated that the per residue prediction accuracy of the most elaborate programs reaches 85%, which is comparable to the accuracy of the currently available sequence-based secondary structure prediction methods (Bordoli et al., 2007; Ferron et al., 2006; Oldfield et al., 2005a). The development of computational approaches to predict unstructured segments from protein sequence and the availability of genomic data for various prokaryotic and eukaryotic species has led to an extensive use of these programs in order to quantify the prevalence of IUPs in the different kingdoms of life. One of the key findings that emerged from the independent application of several of these programs to the completely sequenced genomes is that eukaryotes have

a significantly larger fraction (up to 30%) of their proteome that is intrinsically unstructured than prokaryotes (Dunker et al., 2000, 2008; Ward et al., 2004).

### 3. IUPs and their pivotal role in cellular interaction networks

Considering the involvement of IUPs in key cellular processes and that up to 30% of eukaryotic proteins are IUPs, it is not surprising that these proteins were shown to hold key positions (i.e., as hub proteins, which interact with an unusually large number of other proteins; Fig. 1, central toy interaction network) in the recently mapped protein–protein interaction networks of several different eukaryotes (Barrios-Rodiles et al., 2005; Gavin et al., 2006; Giot et al., 2003; Ito et al., 2001; Krogan et al., 2006; Li et al., 2004; Rual et al., 2005; Stelzl et al., 2005; Uetz et al., 2000). It was shown that intrinsic lack of structure is a common feature of hub proteins in interactome networks (Dosztanyi et al., 2006; Dunker et al., 2005; Haynes et al., 2006; Kim et al., 2008; Schlessinger et al., 2007; Singh et al., 2007). Hubs are a characteristic of scale-free networks (Albert, 2005; Barabasi and Oltvai, 2004). Such networks are generally resistant to random removal of any part of the network but are extremely sensitive to hub removal (Albert, 2005; Albert et al., 2000). Indeed, altered expression or mutations of well-known IUP-hubs, such as p53, Mdm2, p300, BRCA1 or XPA, have been associated with severe pathological conditions such as cancer in humans (de Boer et al., 2002; Levine et al., 1991; Mayo and Donner, 2002; Van Heyningen and Yeyati, 2004; Venkitaraman, 2002; Yao et al., 1998).

While generally enriched for unstructured regions, most hubs in protein interaction networks contain a mixture of globular domains and long unstructured segments and interact with their partners with both the structured and unstructured parts (Dyson and Wright, 2005; Kim et al., 2006, 2008; Schlessinger et al., 2007). For instance, p300 is a typical case of an IUP-hub acting as a general transcriptional regulator (Goodman and Smolik, 2000). It has been estimated that p300, which is involved in chromatin remodelling and transcription regulation, binds up to 400 different partners. Experimental evidence and computational predictions suggest that 50% of the 2442 amino acids of this protein are intrinsically unstructured (Dyson and Wright, 2005). The structured parts of p300 such as the TAZ1, TAZ2 and KIX domains are the sites of interaction with unstructured segments of other proteins such as the hypoxia-inducible factor-1 $\alpha$  (HIF1 $\alpha$ ) (Freedman et al., 2002), the tumour suppressor p53 (Avantaggiati et al., 1997; Gu et al., 1997) or the transcriptional activation domain of c-Myb (Zor et al., 2002), respectively. It was shown that the initially unstructured segments of the interaction partners of p300 fold into a well-defined structure during most of these binding events (Dames et al., 2002; Teufel et al., 2007; Zor et al., 2002). The intrinsically unstructured segments in p300 were shown to contain conserved interaction and regulatory motifs (i.e., short exposed peptide sequences) such as the nuclear-receptor interaction domain (Heery et al., 1997) or the transcriptional-repression domain (Snowden et al., 2000) that mediate binding to different structured or unstructured proteins. Besides p300, many other IUPs are highly modular in their architecture and interact with multiple partners via their structured and unstructured segments (Dunker et al., 2008; Tompa and Fuxreiter, 2008).

Many of the IUP-hubs interact with each other and thus form a highly interwoven sub-network bridging and regulating different cellular pathways and processes. While IUP-hubs are known to interact with multiple partners, many of the interactions formed may be short-lived, mutually exclusive or only possible in certain sub-cellular compartments and specific moments of the cell cycle or during development. Nevertheless, the central position of IUPs in the protein-protein interaction networks and their involvement in key regulatory processes raises a fundamental question: which properties of unstructured polypeptide segments are pivotal to earn this status? (Fig. 1)

#### 4. Special properties of intrinsically unstructured polypeptides

##### 4.1. Writing, reading and erasing of diverse post-translational modifications (PTMs)

Post-translational modifications (PTMs) are essential for biological complexity and diversity. PTMs can affect protein stability, turnover, sub-cellular localisation or interaction properties and thereby have a significant impact on protein function. All these aspects of PTMs become particularly relevant for proteins which participate in regulatory and signalling functions (Pawson and Nash, 2003; Seet et al., 2006). In this regard, the conformational flexibility of unstructured regions in IUPs provides two obvious advantages: (i) it allows for the exposure of single or multiple short linear peptide motifs, whose side chains can be accessed by the catalytic sites of modifying enzymes to introduce or remove a modification and (ii) the modified or unmodified peptide motif can be easily accessed by effector proteins (that recognise a specific peptide motif or the modified peptide motif) to mediate distinct outcomes (Davey et al., 2006; Diella et al., 2008; Galea et al., 2008a,b; Hansen et al., 2006; Honnappa et al., 2006; Khan and Lewis, 2005; Seet et al., 2006; Taverna et al., 2007; Yang, 2005) (Fig. 1A).

##### 4.1.1. PTM sites are often found in unstructured regions

Phosphorylation is the most extensively studied PTM until now and it is known to play an important role in most, if not all, cellular processes (Cohen, 1982; Pawson and Scott, 1997; Pawson and Scott, 2005). Computational studies using phosphorylation site prediction methods have suggested that unstructured regions are enriched for sites that can be phosphorylated (Dunker et al., 2008; Iakoucheva et al., 2004). In agreement with this, recent studies have shown that (i) the majority of phosphorylation sites found in mouse forebrain proteins is located in unstructured regions (Collins et al., 2008) and (ii) unstructured proteins in yeast are likely substrates of a large number of kinases (on average, IUPs are substrates of twice as many kinases as structured proteins) (Gsponer et al., 2008). In addition to phosphorylation, acetylation and methylation sites have been found in the intrinsically unstructured parts of certain IUPs (e.g. p53 and histone termini). Although no reliable large-scale studies have been reported so far, it is likely that methylation, acetylation, ADP-ribosylation, ubiquitination, sumoylation and neddylation sites will be more often found in unstructured segments of proteins.

##### 4.1.2. Regulation of diverse processes is facilitated by combinatorial PTMs

Histones, p53 and the cyclin-dependent kinase regulator p27 are likely to be the best characterised examples of IUPs where PTMs are key to their function and regulation (Bode and Dong, 2004; Brooks and Gu, 2003; Galea et al., 2008b; Kouzarides, 2007). In the case of histones, the N-terminal tail domain (NTD) of core histones and the C-terminal tail domain of linker histones are intrinsically unstructured and are involved in the binding of many different partners (Hansen et al., 2006, 1998). Specific patterns of acetylation, ubiquitination, methylation and phosphorylation of lysine, arginine and serine/threonine residues in the core histone NTD influence the binding to diverse partners and are thereby involved in the regulation of transcription, replication, repair and other nuclear processes (Fischle et al., 2003; Kouzarides, 2007; Kurdistani and Grunstein, 2003; Ruthenburg et al., 2007; Taverna et al., 2007). It is believed that the combination of diverse PTMs creates a pattern of 'marks' or a PTM 'code' that is recognised by different binding effectors which then specifically mediate the downstream outcome (Kouzarides, 2007; Ruthenburg et al., 2007; Taverna et al., 2007; Turner, 2002, 2007). Moreover, it has been noted that PTMs modify the local charge density and hydrophobicity, thereby affecting the structural and folding properties of the polypeptide chain and its accessible surface (Wright and Dyson, 2009).

p27 regulates (together with p21) the cell division cycle of mammalian cells by inhibiting cyclin-dependent kinases (CdKs) (Sherr and Roberts, 1999, 2004). Analysis using proteolysis, CD and NMR spectroscopy has shown that large fragments of this protein are intrinsically unstructured and that they fold upon binding to partner proteins (Bienkiewicz et al., 2002; Kriwacki et al., 1996). It was also shown that distinct PTMs regulate the localisation, turnover and activity of p27 (Galea et al., 2008b). Importantly, the inherent flexibility of unstructured segments was shown to allow for sequential phosphorylation events that ultimately led to ubiquitination and proteolytic degradation of p27, which is necessary for progression through the cell division cycle.

Overall, the inherent flexibility of unstructured segments in proteins facilitates binding of different enzymes such as kinases, phosphatases, acetyltransferases, deacetylases, methylases, ubiquitin ligases and others to specific post-translational modification sites that reside in these unstructured protein segments. As it is highly likely that many of the PTMs are used in a combinatorial manner, a plethora of effectors may be necessary to read, write or

erase this PTM 'code' and mediate the specific biological responses. Thus writing, reading and erasing the PTM 'code' is achieved by the binding of many different partners to IUPs (Fig. 1A).

#### 4.2. Scaffolding and recruitment of different binding partners

Cells respond to intra- and extra-cellular stimuli with specific responses that are mediated by complex signalling networks. These signalling networks consist of a multitude of distinct as well as overlapping signalling pathways (Pawson, 1995). Despite the shared use of signalling proteins, there is very little or no cross-talk between different signalling pathways (Pawson, 2004; Schwartz and Madhani, 2004). In recent years, it has become evident that besides the precise spatio-temporal regulation of expression of signalling molecules (Kholodenko, 2006; Schwartz and Madhani, 2004), scaffold proteins provide an additional molecular mechanism for context-specific signalling. They serve as a backbone for the regulated assembly of different signalling partners and anchor the assembly to specific sub-cellular localisations (Bhattacharyya et al., 2006; Morrison and Davis, 2003; Schwartz and Madhani, 2004; Shaw and Filbert, 2009; Vondriska et al., 2004). A variety of structural and biophysical properties such as (i) a high conformational flexibility (ii) the presence of easily accessible molecular recognition features (MoRFs) and short peptide motifs and (iii) a much larger interaction surface, all make IUPs ideal candidates for performing the scaffolding and complex assembly function. Indeed, a large number of IUPs have been found to assist in the assembly, stabilisation and regulation of large multimeric complexes. For instance, ALL-1 is an IUP that assembles a multimeric complex involved in transcriptional regulation (Nakamura et al., 2002). Similarly, BRCA1 regulates the formation of a large complex involved in DNA repair (Wang et al., 2000). An excellent review article in this journal recently provided an exhaustive inventory of the IUP properties that are important for the scaffolding function (Cortese et al., 2008). In the following section, we focus on three specific aspects: (i) the availability of molecular recognition features (MoRFs), (ii) interactions mediated via the fly-casting mechanism and (iii) the presence of a large interaction surface area in IUPs (Fig. 1B).

##### 4.2.1. Molecular recognition features

Molecular recognition features (MoRFs) are short motifs (10–70 amino acids) within large intrinsically unstructured segments that promote specific protein–protein interactions (Mohan et al., 2006; Oldfield et al., 2005b; Vacic et al., 2007). Importantly, upon binding to their partner, MoRFs undergo disorder-to-order transitions, i.e., they fold upon binding (Dunker et al., 2008; Dyson and Wright, 2002; Fuxreiter et al., 2004; Mohan et al., 2006; Sugase et al., 2007; Wright and Dyson, 2009). Dunker et al. analysed MoRFs occurring in the Protein Data Bank and classified them into three subtypes according to their structures in the bound state: alpha-MoRFs that form alpha-helices, beta-MoRFs that form beta-strands and iota-MoRFs that form structures without a regular pattern of backbone hydrogen bonds (Mohan et al., 2006; Oldfield et al., 2005b; Vacic et al., 2007). A combination of MoRFs, separated by intrinsically unstructured segments, enables the concomitant binding and assembly of various partners with high specificity and is, therefore, highly valuable to scaffold proteins. A classical example for a scaffold protein that uses MoRFs in the assembly process is RNase E of the RNA degradosome (Carpousis, 2007; Marcaida et al., 2006). This endonuclease subunit assembles the RNA degradosome, a machinery essential for RNA processing and degradation in prokaryotes. The C-terminal part of RNase E is intrinsically unstructured, as has been shown by far-UV CD and small-angle X-ray scattering, while the N-terminal ribonucleolytic domain is

structured and catalytically active (Callaghan et al., 2004, 2003). Importantly, within the highly flexible C-terminal region lie four MoRFs that are central to the scaffolding function of this protein (Marcaida et al., 2006; Worrall et al., 2007). The MoRFs serve as binding sites for other members of the degradosome, namely an enolase, a polynucleotide phosphorylase and the helicase RhIB, while the intervening unstructured residues provide the flexibility to accommodate these large binding partners.

##### 4.2.2. Fly-casting

The fly-casting model postulates that IUPs have a greater “capture radius” and increased intermolecular association rates because they are less compact than structured proteins (Levy et al., 2004; Pontius, 1993; Shoemaker et al., 2000). The flexible nature of IUPs is supposed to facilitate the binding of targets to peptide motifs located in the unstructured segments. A binding site attached to a flexible chain could be compared to a hook on a fishing rod, which allows for the sampling of large solution volumes and the “fishing” of targets therein. A good example of this involves the membrane-embedded, voltage-activated potassium channel ( $K_v$ ), which mediates the generation and shaping of action potentials in neuronal cells.  $K_v$  is known to interact with PSD-95, a scaffold protein localized in the post-synaptic density of excitatory synapses, which organises the assembly and localisation of many signalling proteins, thereby shaping the synaptic architecture. As  $K_v$  channels have to be precisely localized and clustered in order to ensure an effective transmission of action potentials to post-synaptic cells across the synaptic cleft, it has been suggested that  $K_v$  uses the “fly-casting” mechanism to ensure interaction with the intracellular scaffolding protein, the post-synaptic density 95 (PSD-95) (Magidovich et al., 2006, 2007). In particular, it was shown that the highly flexible, unstructured C-terminal segment of certain  $K_v$  channels mediates the clustering of the channel at the neuro–muscular junction. Specifically, a PDZ-binding peptide motif at the end of this C-terminal segment mediates the interaction of  $K_v$  with PSD-95 (Kim and Sheng, 2004; Magidovich et al., 2006). Hence, in this example, the binding “hook” that is tethered to the rest of the protein by a flexible chain is found in a binding partner ( $K_v$ ) of a scaffold protein (PSD-95).

An important aspect implicit in the fly-casting mechanism is the concept that flexibility of the intrinsically unstructured chain enables an initially weak and non-specific binding, followed by the folding and specific binding to the cognate target. Wright et al. recently provided experimental proof for this mechanism by studying the binding of the phosphorylated KID (pKID) domain of the transcription factor CREB to the KIX domain of CREB binding protein (CBP) (Sugase et al., 2007). Using NMR titrations and  $N^{15}$  relaxation dispersion, they showed that the intrinsically unstructured pKID domain initially forms transient encounter complexes with KIX that are stabilized by non-specific hydrophobic interactions. The subsequent search of the KIX surface for the specific binding site is followed by folding and the formation of a high-affinity complex. Although non-specific encounter complexes have also been observed during the assembly process of complexes that involve solely structured proteins (Iwahara and Clore, 2006; Tang et al., 2006), they are likely to have a more important role for the search of specific and high-affinity interactions when unstructured protein segments are involved in the assembly process (see below).

##### 4.2.3. Large interaction surfaces

It has also been proposed that IUPs provide larger surface areas for intermolecular interactions than do globular proteins of similar size (Gunasekaran et al., 2003). Such extensive surfaces are an advantage for the simultaneous binding to several protein, DNA, or RNA molecules into multimeric assemblies. Chothia et al. have shown that large interaction interfaces in globular proteins require large protein sizes, as measured by the amino acid sequence length

(Janin et al., 1988; Miller et al., 1987). Hence, larger proteins will be needed to enable extensive interaction surfaces to be created using structured domains. Thus, large multi-domain complexes would reach immense sizes if all the constitutive domains were stable, globular proteins (Gunasekaran et al., 2003). IUPs, by contrast, provide a larger available interaction surface area per amino acid due to their unstructured character and they have a larger surface-to-volume ratio than globular proteins (Gunasekaran et al., 2003, 2004). Such large and highly flexible interaction surfaces can be a significant advantage and assist the assembly of intertwined multimeric complexes (Meszaros et al., 2007). In agreement with this, a significant correlation was found recently between the average predicted disorder per complex and the number of complex components (Hegyi et al., 2007). It was also suggested that larger complexes are likely to be assembled from proteins that tend to be more disordered (Hegyi et al., 2007). Flexibility also permits intrinsically unstructured protein segments to wrap around their binding partners, thereby creating an extensive interaction surface. For instance, the C-terminal transactivation domain of HIF1 $\alpha$  is unstructured in solution and folds upon binding to the TAZ1 domain of p300. The structure of the complex reveals that HIF1 $\alpha$  is almost entirely wrapped around TAZ1 (Dames et al., 2002). As a consequence, a high-affinity complex is formed ( $K_D = \sim 7$  nM) that could never be formed by two stable, folded proteins of a comparable size (Dames et al., 2002).

In summary, the assembly of large molecular machines is essential to the functioning of eukaryotic cells. IUPs appear to play an important role in this process by not only providing the conformation flexibility required to find binding partners but also by exposing specific molecular recognition elements that fold upon binding and short peptide interaction motifs for partners to bind within the unstructured segment (Fig. 1B).

#### 4.3. Conformational variability and adaptability

The long-standing dogma of the structure–function paradigm states that proteins need to adopt a well-defined 3D structure in order to be functional. Structure determination methods such as X-ray crystallography and NMR spectroscopy have proven to be very successful in elucidating the atomic coordinates of the native state of globular proteins and certain protein complexes (Cowieson et al., 2008; Laskowski and Thornton, 2008). However, proteins are dynamic, i.e., they sample ensembles of conformations that are in dynamic equilibrium (Akasaka, 2006; Li and Akasaka, 2006), and classical structure determination methods normally identify only the dominant conformation under given environmental conditions. For certain proteins, there is no single predominant conformation under physiological conditions. For instance, it was recently shown that the globular protein lymphotactin can adopt two distinct folds at equilibrium and that shifting between the conformations involves major structural changes (Murzin, 2008; Tuinstra et al., 2008). Importantly, the two conformers have mutually exclusive activities and are essential to the full functioning of the protein *in vivo*. In this regard, IUPs can be seen as highly dynamic proteins that often lack a dominant conformation – though they may have a conformational preference – and exist as a dynamic ensemble of inter-converting conformers. As with lymphotactin, it is likely that some of the sampled conformers are of functional importance, i.e., the conformers are involved in the binding of different targets that exhibit diverse biological functions. A good example of such a case is the N-terminus of p53, which consists of an intrinsically unstructured transactivation domain (TAD). This domain is a promiscuous binding site for p300, components of the transcription machinery and the negative regulators MDM2/MDM4 (Joerger and Fersht, 2008). In complex with MDM2, the promiscuous binding site adopts

an  $\alpha$ -helical conformation (Kussie et al., 1996). NMR studies showed that, when free in solution, the TAD lacks a predominant conformer but does transiently form a helix that is similar to the one found in the p53–MDM2 complex (Lee et al., 2000) (Fig. 1C).

##### 4.3.1. Conformational variability

Although a conformational selection mechanism may be relevant for the binding of some IUPs (Fuxreiter et al., 2004; Tsai et al., 2001), it has more often been observed that partners induce folding in the unstructured segment upon binding (Wright and Dyson, 2009). Due to the intrinsic plasticity of the unstructured segments, diverse conformations can be adopted and induced upon binding to different partners. This mechanism is exemplified by the binding of HIF $\alpha$  to the TAZ1 domain of p300 (Dames et al., 2002) and the asparagine hydroxylase enzyme FIH (Lee et al., 2003). While HIF $\alpha$  adopts a regular helical backbone conformation in the complex with the former, it has an extended backbone conformation when bound to the latter. The auto-inhibition and activation mechanism of the Wiskott–Aldrich syndrome protein (WASP) illustrates how such a context-dependent folding of an intrinsically unstructured segment can affect a signalling process (Kim et al., 2000). The Rho-family GTPase Cdc42 can regulate the actin cytoskeleton through activation of WASP family members (Machesky and Insall, 1999). This is achieved by the binding of Cdc42 to the GTPase-binding domain (GBD) of WASP, which enables interaction of WASP with the actin regulatory machinery. The intrinsically unstructured GDB can also fold back on other parts of WASP and form intramolecular contacts that inhibit actin regulation. Importantly, the structure adopted by GDB in the auto-inhibitory state is different from (and incompatible with) the activated conformation when in complex with Cdc42 (Kim et al., 2000).

##### 4.3.2. Conformational adaptability

The intrinsic conformational plasticity of unstructured segments also enables a nearly perfect moulding to fit the binding surfaces of partners (Tompa et al., 2005). An excellent example for this extraordinary property of IUPs comes from the work of Kleantous et al. (Bonsor et al., 2007; Loftus et al., 2006). They determined and compared the structures of the *Escherichia coli* protein TolB in complex with Pal, its physiological partner, and with the bacterial toxin colicin E9. Colicin competitively recruits TolB with its intrinsically unstructured N-terminal region with an affinity that can be similar to that of Pal ( $K_D = \sim 90$  nM). When comparing the interaction surfaces of the two complexes, they found that large parts of the buried surface coincide but that the intrinsically unstructured segment of colicin E9 makes additional contact by burying side chains more efficiently, i.e., by increasing the complementarity to the TolB interaction surface. Hence, the intrinsically unstructured region of colicin E9 can mimic the surface properties of its binding partner TolB much better than the globular, cognate partner Pal. Achieving such a high degree of complementarity has major consequences for the thermodynamics of binding. The binding of an unstructured segment to its target is associated with a significant entropic cost, in particular if this segment folds upon binding (Brady and Sharp, 1997). Therefore, the thermodynamic driving force for the binding reaction must be a favourable enthalpic contribution (Brady and Sharp, 1997; Wright and Dyson, 1999). If the complementarity is good and the intermolecular interaction surface is big (as seen for the complexes TolB–colicin 9 and TAZ1–HIF $\alpha$ ), the entropic penalty is well compensated and a nanomolar affinity can be achieved. This is, however, not always the case. For many IUPs, coupled folding upon binding gives rise to complexes with relatively low affinities, because the entropic penalty is not fully compensated. Importantly though, these complexes are in most cases highly specific, as their formation is mediated by specific recognition motifs and modulated via characteristic PTMs. It has been

suggested that the phenomenon of specific but low-affinity complexes could be advantageous for signalling proteins that should not only be able to associate specifically to relay a signal but also dissociate easily when conditions change (Dyson and Wright, 2005; Wright and Dyson, 1999).

Thus, the thermodynamic properties of binding, conformational flexibility and adaptability give IUPs a significant advantage over structured proteins with respect to their potential to interact with diverse partners (Fig. 1C). Due to the same reasons, interaction interfaces and polypeptide segments involving intrinsically unstructured regions might make certain IUPs suitable as drug targets (Blundell et al., 2006; Fernandez et al., 2009; Gagna and Lambert, 2006; Wells and McClendon, 2007); and as hubs they are likely to be phenotypically relevant. For example, it was recently shown that certain chemical compounds could specifically target the monomeric unstructured state of the target protein c-Myc rather than the fully folded heterodimeric unit involving the Max protein (Wang et al., 2007). Recent studies that suggest targeting intrinsically unstructured proteins as drug targets have put forward two strategies. These are to develop small molecule drugs that may (i) improve the packing quality of the target protein upon binding thereby steering induced folding (Fernandez et al., 2009) and (ii) enhance binding affinity by inducing conformational disorder in the target protein (Crespo and Fernandez, 2008). Therefore, identification of small molecules or peptide aptamers (Baines and Colas, 2006) that specifically targets interaction interfaces and structural transitions involving unstructured segments may represent a novel drug discovery strategy that could be exploited in the coming years.

## 5. Conclusion

Complex biological processes, such as information processing and decision making, require an elaborate interplay between different layers of biomolecular organisation in a cell. In response to external stimuli, these regulatory layers can change the transcription of specific genes, modulate translation or activate post-translational modifications of particular proteins. In order to be able to respond to different stimuli and external conditions, the cell needs an arsenal of response states, which is defined not only by the characteristic gene expression profiles but also by the regulatory and signalling proteins present in specific functional states and locations. The spectrum of response states available to the cell will depend on the combinatorial use of the functional states of different regulatory proteins. We suggest that IUPs confer greater response variability to the cell because IUPs can sample a broader repertoire of structural and functional states than do structured proteins (Fig. 1). Importantly, transitions between these functional states are fast due to the unique properties of these proteins, which ultimately allow a cell to react in an optimal way to intra- and extra-cellular perturbations.

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## References

- Akasaka, K., 2006. Probing conformational fluctuation of proteins by pressure perturbation. *Chem. Rev.* 106, 1814–1835.
- Albert, R., 2005. Scale-free networks in cell biology. *J. Cell Sci.* 118, 4947–4957.
- Albert, R., Jeong, H., Barabasi, A.L., 2000. Error and attack tolerance of complex networks. *Nature* 406, 378–382.
- Avantaggiati, M.L., Ogryzko, V., Gardner, K., Giordano, A., Levine, A.S., Kelly, K., 1997. Recruitment of p300/CBP in p53-dependent signal pathways. *Cell* 89, 1175–1184.
- Baines, I.C., Colas, P., 2006. Peptide aptamers as guides for small-molecule drug discovery. *Drug Discov. Today* 11, 334–341.
- Barabasi, A.L., Oltvai, Z.N., 2004. Network biology: understanding the cell's functional organization. *Nat. Rev. Genet.* 5, 101–113.
- Barrios-Rodiles, M., Brown, K.R., Ozdamar, B., Bose, R., Liu, Z., Donovan, R.S., Shinjo, F., Liu, Y., Dembowy, J., Taylor, I.W., Luga, V., Przulj, N., Robinson, M., Suzuki, H., Hayashizaki, Y., Jurisica, I., Wrana, J.L., 2005. High-throughput mapping of a dynamic signaling network in mammalian cells. *Science* 307, 1621–1625.
- Battisti, J.L., Mao, H., Rao, N.S., Tan, R., Muhandiram, D.R., Kay, L.E., Frankel, A.D., Williamson, J.R., 1996. Alpha helix-RNA major groove recognition in an HIV-1 rev peptide-RRE RNA complex. *Science* 273, 1547–1551.
- Bhattacharyya, R.P., Remenyi, A., Yeh, B.J., Lim, W.A., 2006. Domains, motifs, and scaffolds: the role of modular interactions in the evolution and wiring of cell signaling circuits. *Annu. Rev. Biochem.* 75, 655–680.
- Bienkiewicz, E.A., Adkins, J.N., Lumb, K.J., 2002. Functional consequences of preorganized helical structure in the intrinsically disordered cell-cycle inhibitor p27(Kip1). *Biochemistry* 41, 752–759.
- Blundell, T.L., Sibanda, B.L., Montalvo, R.W., Brewerton, S., Chelliah, V., Worth, C.L., Harmer, N.J., Davies, O., Burke, D., 1967. 2006 Structural biology and bioinformatics in drug design: opportunities and challenges for target identification and lead discovery. *Philos Trans R Soc Lond B Biol Sci.* 361, 413–423.
- Blundell, T.L., Srinivasan, N., 1996. Symmetry, stability, and dynamics of multidomain and multicomponent protein systems. *Proc. Natl. Acad. Sci. U.S.A.* 93, 14243–14248.
- de Boer, J., Andressoo, J.O., de Wit, J., Huijman, J., Beems, R.B., van Steeg, H., Weeda, G., van der Horst, G.T., van Leeuwen, W., Themmen, A.P., Meradji, M., Hoeijmakers, J.H., 2002. Premature aging in mice deficient in DNA repair and transcription. *Science* 296, 1276–1279.
- Bode, A.M., Dong, Z., 2004. Post-translational modification of p53 in tumorigenesis. *Nat. Rev. Cancer* 4, 793–805.
- Bonsor, D.A., Grishkovskaya, I., Dodson, E.J., Kleantous, C., 2007. Molecular mimicry enables competitive recruitment by a natively disordered protein. *J. Am. Chem. Soc.* 129, 4800–4807.
- Bordoli, L., Kiefer, F., Schwede, T., 2007. Assessment of disorder predictions in CASP7. *Proteins* 69 (Suppl. 8), 129–136.
- Brady, G.P., Sharp, K.A., 1997. Entropy in protein folding and in protein-protein interactions. *Curr. Opin. Struct. Biol.* 7, 215–221.
- Breitkreutz, B.J., Stark, C., Reguly, T., Boucher, L., Breitkreutz, A., Livstone, M., Oughtred, R., Lackner, D.H., Bahler, J., Wood, V., Dolinski, K., Tyers, M., 2008. The BioGRID interaction database: 2008 update. *Nucleic Acids Res.* 36, D637–D640.
- Brooks, C.L., Gu, W., 2003. Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. *Curr. Opin. Cell Biol.* 15, 164–171.
- Callaghan, A.J., Aurikko, J.P., Ilag, L.L., Gunter Grossmann, J., Chandran, V., Kuhnel, K., Poljak, L., Carpousis, A.J., Robinson, C.V., Symmons, M.F., Luisi, B.F., 2004. Studies of the RNA degradosome-organizing domain of the *Escherichia coli* ribonuclease RNase E. *J. Mol. Biol.* 340, 965–979.
- Callaghan, A.J., Grossmann, J.G., Redko, Y.U., Ilag, L.L., Moncrieffe, M.C., Symmons, M.F., Robinson, C.V., McDowall, K.J., Luisi, B.F., 2003. Quaternary structure and catalytic activity of the *Escherichia coli* ribonuclease E amino-terminal catalytic domain. *Biochemistry* 42, 13848–13855.
- Carpousis, A.J., 2007. The RNA degradosome of *Escherichia coli*: an mRNA-degrading machine assembled on RNase E. *Annu. Rev. Microbiol.* 61, 71–87.
- Chen, J.W., Romero, P., Uversky, V.N., Dunker, A.K., 2006. Conservation of intrinsic disorder in protein domains and families: II. Functions of conserved disorder. *J. Proteome Res.* 5, 888–898.
- Chothia, C., Janin, J., 1975. Principles of protein-protein recognition. *Nature* 256, 705–708.
- Chu, I., Sun, J., Arnaout, A., Kahn, H., Hanna, W., Narod, S., Sun, P., Tan, C.K., Hengst, L., Slingerland, J., 2007. p27 phosphorylation by Src regulates inhibition of cyclin E-Cdk2. *Cell* 128, 281–294.
- Cohen, P., 1982. The role of protein phosphorylation in neural and hormonal control of cellular activity. *Nature* 296, 613–620.
- Collins, M.O., Yu, L., Campuzano, I., Grant, S.G., Choudhary, J.S., 2008. Phosphoproteomic analysis of the mouse brain cytosol reveals a predominance of protein phosphorylation in regions of intrinsic sequence disorder. *Mol. Cell. Proteomics* 7, 1331–1348.
- Cortese, M.S., Uversky, V.N., Dunker, A.K., 2008. Intrinsic disorder in scaffold proteins: getting more from less. *Prog. Biophys. Mol. Biol.* 98, 85–106.
- Cowieson, N.P., Kobe, B., Martin, J.L., 2008. United we stand: combining structural methods. *Curr. Opin. Struct. Biol.* 18, 617–622.
- Crespo, A., Fernandez, A., 2008. Induced disorder in protein-ligand complexes as a drug-design strategy. *Mol. Pharmacol.* 5, 430–437.
- Dames, S.A., Martinez-Yamout, M., De Guzman, R.N., Dyson, H.J., Wright, P.E., 2002. Structural basis for Hif-1 alpha/CBP recognition in the cellular hypoxic response. *Proc. Natl. Acad. Sci. U.S.A.* 99, 5271–5276.
- Daughdrill, G.V., Chadsey, M.S., Karlinsky, J.E., Hughes, K.T., Dahlquist, F.W., 1997. The C-terminal half of the anti-sigma factor, FlgM, becomes structured when bound to its target, sigma 28. *Nat. Struct. Biol.* 4, 285–291.

- Davey, N.E., Shields, D.C., Edwards, R.J., 2006. SLiMDisc: short, linear motif discovery, correcting for common evolutionary descent. *Nucleic Acids Res.* 34, 3546–3554.
- Demarest, S.J., Martinez-Yamout, M., Chung, J., Chen, H., Xu, W., Dyson, H.J., Evans, R.M., Wright, P.E., 2002. Mutual synergistic folding in recruitment of CBP/p300 by p160 nuclear receptor coactivators. *Nature* 415, 549–553.
- Diella, F., Haslam, N., Chica, C., Budd, A., Michael, S., Brown, N.P., Trave, G., Gibson, T.J., 2008. Understanding eukaryotic linear motifs and their role in cell signaling and regulation. *Front. Biosci.* 13, 6580–6603.
- Dosztanyi, Z., Chen, J., Dunker, A.K., Simon, I., Tompa, P., 2006. Disorder and sequence repeats in hub proteins and their implications for network evolution. *J. Proteome Res.* 5, 2985–2995.
- Dosztanyi, Z., Csizmok, V., Tompa, P., Simon, I., 2005. The pairwise energy content estimated from amino acid composition discriminates between folded and intrinsically unstructured proteins. *J. Mol. Biol.* 347, 827–839.
- Dunker, A.K., Cortese, M.S., Romero, P., Iakoucheva, L.M., Uversky, V.N., 2005. Flexible nets. The roles of intrinsic disorder in protein interaction networks. *FEBS J.* 272, 5129–5148.
- Dunker, A.K., Lawson, J.D., Brown, C.J., Williams, R.M., Romero, P., Oh, J.S., Oldfield, C.J., Campen, A.M., Ratliff, C.M., Hipps, K.W., Ausio, J., Nissen, M.S., Reeves, R., Kang, C., Kissinger, C.R., Bailey, R.W., Griswold, M.D., Chiu, W., Garner, E.C., Obradovic, Z., 2001. Intrinsically disordered protein. *J. Mol. Graph. Model.* 19, 26–59.
- Dunker, A.K., Obradovic, Z., Romero, P., Garner, E.C., Brown, C.J., 2000. Intrinsic protein disorder in complete genomes. *Genome Inf. Ser. Workshop Genome Inf.* 11, 161–171.
- Dunker, A.K., Oldfield, C.J., Meng, J., Romero, P., Yang, J.Y., Chen, J.W., Vacic, V., Obradovic, Z., Uversky, V.N., 2008. The unfoldomics decade: an update on intrinsically disordered proteins. *BMC Genomics* 9 (Suppl. 2), S1.
- Dyson, H.J., Wright, P.E., 2002. Coupling of folding and binding for unstructured proteins. *Curr. Opin. Struct. Biol.* 12, 54–60.
- Dyson, H.J., Wright, P.E., 2004. Unfolded proteins and protein folding studied by NMR. *Chem. Rev.* 104, 3607–3622.
- Dyson, H.J., Wright, P.E., 2005. Intrinsically unstructured proteins and their functions. *Nat. Rev. Mol. Cell Biol.* 6, 197–208.
- Fernandez, A., Bazan, S., Chen, J., 2009. Taming the induced folding of drug-targeted kinases. *Trends Pharmacol. Sci.* 30, 66–71.
- Ferron, F., Longhi, S., Canard, B., Karlin, D., 2006. A practical overview of protein disorder prediction methods. *Proteins* 65, 1–14.
- Fiebig, K.M., Rice, L.M., Pollock, E., Brunger, A.T., 1999. Folding intermediates of SNARE complex assembly. *Nat. Struct. Biol.* 6, 117–123.
- Fischle, W., Wang, Y., Allis, C.D., 2003. Histone and chromatin cross-talk. *Curr. Opin. Cell Biol.* 15, 172–183.
- Frankel, A.D., Kim, P.S., 1991. Modular structure of transcription factors: implications for gene regulation. *Cell* 65, 717–719.
- Freedman, S.J., Sun, Z.Y., Poy, F., Kung, A.L., Livingston, D.M., Wagner, G., Eck, M.J., 2002. Structural basis for recruitment of CBP/p300 by hypoxia-inducible factor-1 alpha. *Proc. Natl. Acad. Sci. U.S.A.* 99, 5367–5372.
- Fuxreiter, M., Simon, I., Friedrich, P., Tompa, P., 2004. Preformed structural elements feature in partner recognition by intrinsically unstructured proteins. *J. Mol. Biol.* 338, 1015–1026.
- Gagna, C.E., Lambert, W.C., 2006. Novel drug discovery and molecular biological methods, via DNA, RNA and protein changes using structure–function transitions: transitional structural chemogenomics, transitional structural chemoproteomics and novel multi-stranded nucleic acid microarray. *Med. Hypotheses* 67, 1099–1114.
- Galea, C.A., Nourse, A., Wang, Y., Sivakolundu, S.G., Heller, W.T., Kriwacki, R.W., 2008a. Role of intrinsic flexibility in signal transduction mediated by the cell cycle regulator, p27 Kip1. *J. Mol. Biol.* 376, 827–838.
- Galea, C.A., Wang, Y., Sivakolundu, S.G., Kriwacki, R.W., 2008b. Regulation of cell division by intrinsically unstructured proteins: intrinsic flexibility, modularity, and signaling conduits. *Biochemistry* 47, 7598–7609.
- Garner, E., Cannon, P., Romero, P., Obradovic, Z., Dunker, A.K., 1998. Predicting disordered regions from amino acid sequence: common themes despite differing structural characterization. *Genome Inf. Ser. Workshop Genome Inf.* 9, 201–213.
- Gavin, A.C., Aloy, P., Grandi, P., Krause, R., Boesche, M., Marzioch, M., Rau, C., Jensen, L.J., Bastuck, S., Dumpelfeld, B., Edelmann, A., Heurtier, M.A., Hoffman, V., Hoefert, C., Klein, K., Hudak, M., Michon, A.M., Schelder, M., Schirle, M., Remor, M., Rudi, T., Hooper, S., Bauer, A., Bouwmeester, T., Casari, G., Drewes, G., Neubauer, G., Rick, J.M., Kuster, B., Bork, P., Russell, R.B., Superti-Furga, G., 2006. Proteome survey reveals modularity of the yeast cell machinery. *Nature* 440, 631–636.
- Giot, L., Bader, J.S., Brouwer, C., Chaudhuri, A., Kuang, B., Li, Y., Hao, Y.L., Ooi, C.E., Godwin, B., Vitols, E., Vijayadmodar, G., Pochart, P., Machineni, H., Welsh, M., Kong, Y., Zerhusen, B., Malcolm, R., Varrone, Z., Collis, A., Minto, M., Burgess, S., McDaniel, L., Stimpson, E., Spriggs, F., Williams, J., Neurath, K., Ioime, N., Agee, M., Voss, E., Furtak, K., Renzulli, R., Aanensen, N., Carroll, S., Bickelhaupt, E., Lazovatsky, Y., DaSilva, A., Zhong, J., Stanyon, C.A., Finley Jr., R.L., White, K.P., Braverman, M., Jarvie, T., Gold, S., Leach, M., Knight, J., Shimkets, R.A., McKenna, M.P., Chant, J., Rothberg, J.M., 2003. A protein interaction map of *Drosophila melanogaster*. *Science* 302, 1727–1736.
- Goodman, R.H., Smolik, S., 2000. CBP/p300 in cell growth, transformation, and development. *Genes Dev* 14, 1553–1577.
- Gsponer, J., Futschik, M.E., Teichmann, S.A., Babu, M.M., 2008. Tight regulation of unstructured proteins: from transcript synthesis to protein degradation. *Science* 322, 1365–1368.
- Gu, W., Shi, X.L., Roeder, R.G., 1997. Synergistic activation of transcription by CBP and p53. *Nature* 387, 819–823.
- Gunasekaran, K., Tsai, C.J., Kumar, S., Zanuy, D., Nussinov, R., 2003. Extended disordered proteins: targeting function with less scaffold. *Trends Biochem. Sci.* 28, 81–85.
- Gunasekaran, K., Tsai, C.J., Nussinov, R., 2004. Analysis of ordered and disordered protein complexes reveals structural features discriminating between stable and unstable monomers. *J. Mol. Biol.* 341, 1327–1341.
- Hansen, J.C., Lu, X., Ross, E.D., Woody, R.W., 2006. Intrinsic protein disorder, amino acid composition, and histone terminal domains. *J. Biol. Chem.* 281, 1853–1856.
- Hansen, J.C., Tse, C., Wolffe, A.P., 1998. Structure and function of the core histone N-termini: more than meets the eye. *Biochemistry* 37, 17637–17641.
- Haynes, C., Oldfield, C.J., Ji, F., Klitgord, N., Cusick, M.E., Radivojac, P., Uversky, V.N., Vidal, M., Iakoucheva, L.M., 2006. Intrinsic disorder is a common feature of hub proteins from four eukaryotic interactomes. *PLoS Comput. Biol.* 2, e100.
- Hecht, O., Ridley, H., Boetzel, R., Lewin, A., Cull, N., Chalton, D.A., Lakey, J.H., Moore, G.R., 2008. Self-recognition by an intrinsically disordered protein. *FEBS Lett.* 582, 2673–2677.
- Heery, D.M., Kalkhoven, E., Hoare, S., Parker, M.G., 1997. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* 387, 733–736.
- Hegyi, H., Schad, E., Tompa, P., 2007. Structural disorder promotes assembly of protein complexes. *BMC Struct. Biol.* 7, 65.
- Holmbeck, S.M., Dyson, H.J., Wright, P.E., 1998. DNA-induced conformational changes are the basis for cooperative dimerization by the DNA binding domain of the retinoid X receptor. *J. Mol. Biol.* 284, 533–539.
- Honnappa, S., Jahnke, W., Seelig, J., Steinmetz, M.O., 2006. Control of intrinsically disordered stathmin by multisite phosphorylation. *J. Biol. Chem.* 281, 16078–16083.
- Iakoucheva, L.M., Brown, C.J., Lawson, J.D., Obradovic, Z., Dunker, A.K., 2002. Intrinsic disorder in cell-signaling and cancer-associated proteins. *J. Mol. Biol.* 323, 573–584.
- Iakoucheva, L.M., Radivojac, P., Brown, C.J., O'Connor, T.R., Sikes, J.G., Obradovic, Z., Dunker, A.K., 2004. The importance of intrinsic disorder for protein phosphorylation. *Nucleic Acids Res.* 32, 1037–1049.
- Ito, T., Chiba, T., Ozawa, R., Yoshida, M., Hattori, M., Sakaki, Y., 2001. A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc. Natl. Acad. Sci. U.S.A.* 98, 4569–4574.
- Iwahara, J., Clore, G.M., 2006. Detecting transient intermediates in macromolecular binding by paramagnetic NMR. *Nature* 440, 1227–1230.
- Janin, J., Miller, S., Chothia, C., 1988. Surface, subunit interfaces and interior of oligomeric proteins. *J. Mol. Biol.* 204, 155–164.
- Joerger, A.C., Fersht, A.R., 2008. Structural biology of the tumor suppressor p53. *Annu. Rev. Biochem.* 77, 557–582.
- Jones, S., Thornton, J.M., 1996. Principles of protein–protein interactions. *Proc. Natl. Acad. Sci. U.S.A.* 93, 13–20.
- Khan, A.N., Lewis, P.N., 2005. Unstructured conformations are a substrate requirement for the Sir2 family of NAD-dependent protein deacetylases. *J. Biol. Chem.* 280, 36073–36078.
- Kholodenko, B.N., 2006. Cell-signalling dynamics in time and space. *Nat. Rev. Mol. Cell Biol.* 7, 165–176.
- Kim, A.S., Kakalis, L.T., Abdul-Manan, N., Liu, G.A., Rosen, M.K., 2000. Autoinhibition and activation mechanisms of the Wiskott–Aldrich syndrome protein. *Nature* 404, 151–158.
- Kim, E., Sheng, M., 2004. PDZ domain proteins of synapses. *Nat. Rev. Neurosci.* 5, 771–781.
- Kim, P.M., Lu, L.J., Xia, Y., Gerstein, M.B., 2006. Relating three-dimensional structures to protein networks provides evolutionary insights. *Science* 314, 1938–1941.
- Kim, P.M., Sboner, A., Xia, Y., Gerstein, M., 2008. The role of disorder in interaction networks: a structural analysis. *Mol. Syst. Biol.* 4, 179.
- Kouzarides, T., 2007. Chromatin modifications and their function. *Cell* 128, 693–705.
- Kriwacki, R.W., Hengst, L., Tennant, L., Reed, S.L., Wright, P.E., 1996. Structural studies of p21Waf1/Cip1/Sdi1 in the free and Cdk2-bound state: conformational disorder mediates binding diversity. *Proc. Natl. Acad. Sci. U.S.A.* 93, 11504–11509.
- Kriwacki, R.W., Wu, J., Tennant, L., Wright, P.E., Siuzdak, G., 1997. Probing protein structure using biochemical and biophysical methods. Proteolysis, matrix-assisted laser desorption/ionization mass spectrometry, high-performance liquid chromatography and size-exclusion chromatography of p21Waf1/Cip1/Sdi1. *J. Chromatogr. A* 777, 23–30.
- Krogan, N.J., Cagney, G., Yu, H., Zhong, G., Guo, X., Ignatchenko, A., Li, J., Pu, S., Datta, N., Tikuisis, A.P., Punna, T., Peregrin-Alvarez, J.M., Shales, M., Zhang, X., Davey, M., Robinson, M.D., Paccanaro, A., Bray, J.E., Sheung, A., Beattie, B., Richards, D.P., Canadien, V., Lalev, A., Mena, F., Wong, P., Starostine, A., Canete, M.M., Vlasblom, J., Wu, S., Orsi, C., Collins, S.R., Chandran, S., Haw, R., Rilstone, J.J., Gandi, K., Thompson, N.J., Musso, G., St Onge, P., Ghanny, S., Lam, M.H., Butland, G., Altaf-Ul, A.M., Kanaya, S., Shilatifard, A., O'Shea, E., Weissman, J.S., Ingles, C.J., Hughes, T.R., Parkinson, J., Gerstein, M., Wodak, S.J., Emili, A., Greenblatt, J.F., 2006. Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. *Nature* 440, 637–643.
- Kurdistani, S.K., Grunstein, M., 2003. Histone acetylation and deacetylation in yeast. *Nat. Rev. Mol. Cell Biol.* 4, 276–284.

- Kussie, P.H., Gorina, S., Marechal, V., Elenbaas, B., Moreau, J., Levine, A.J., Pavletich, N.P., 1996. Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science* 274, 948–953.
- Laskowski, R.A., Thornton, J.M., 2008. Understanding the molecular machinery of genetics through 3D structures. *Nat. Rev. Genet.* 9, 141–151.
- Lee, C., Kim, S.J., Jeong, D.G., Lee, S.M., Ryu, S.E., 2003. Structure of human FIH-1 reveals a unique active site pocket and interaction sites for HIF-1 and von Hippel-Lindau. *J. Biol. Chem.* 278, 7558–7563.
- Lee, H., Mok, K.H., Muhandiram, R., Park, K.H., Suk, J.E., Kim, D.H., Chang, J., Sung, Y.C., Choi, K.Y., Han, K.H., 2000. Local structural elements in the mostly unstructured transcriptional activation domain of human p53. *J. Biol. Chem.* 275, 29426–29432.
- Levine, A.J., Momand, J., Finlay, C.A., 1991. The p53 tumour suppressor gene. *Nature* 351, 453–456.
- Levy, E.D., Landry, C.R., Michnick, S.W., 2009. How perfect can protein interactomes be? *Sci. Signal.* 2, pe11.
- Levy, E.D., Pereira-Leal, J.B., 2008. Evolution and dynamics of protein interactions and networks. *Curr. Opin. Struct. Biol.* 18, 349–357.
- Levy, E.D., Pereira-Leal, J.B., Chothia, C., Teichmann, S.A., 2006. 3D complex: a structural classification of protein complexes. *PLoS Comput. Biol.* 2, e155.
- Levy, Y., Wolynes, P.G., Onuchic, J.N., 2004. Protein topology determines binding mechanism. *Proc. Natl. Acad. Sci. U.S.A.* 101, 511–516.
- Li, H., Akasaka, K., 2006. Conformational fluctuations of proteins revealed by variable pressure NMR. *Biochim. Biophys. Acta.* 1764, 331–345.
- Li, M., Song, J., 2007. The N- and C-termini of the human Nogo molecules are intrinsically unstructured: bioinformatics, CD, NMR characterization, and functional implications. *Proteins* 68, 100–108.
- Li, S., Armstrong, C.M., Bertin, N., Ge, H., Milstein, S., Boxem, M., Vidalain, P.O., Han, J.D., Chesneau, A., Hao, T., Goldberg, D.S., Li, N., Martinez, M., Rual, J.F., Lamesch, P., Xu, L., Tewari, M., Wong, S.L., Zhang, L.V., Berriz, G.F., Jacotot, L., Vaglio, P., Reboul, J., Hirozane-Kishikawa, T., Li, Q., Gabel, H.W., Elew, A., Baumgartner, B., Rose, D.J., Yu, H., Bosak, S., Sequerra, R., Fraser, A., Mango, S.E., Saxton, W.M., Strome, S., Van Den Heuvel, S., Piano, F., Vandenhaute, J., Sardet, C., Gerstein, M., Doucette-Stamm, L., Gunsalus, K.C., Harper, J.W., Cusick, M.E., Roth, F.P., Hill, D.E., Vidal, M., 2004. A map of the interactome network of the metazoan *C. elegans*. *Science* 303, 540–543.
- Liu, J., Perumal, N.B., Oldfield, C.J., Su, E.W., Uversky, V.N., Dunker, A.K., 2006. Intrinsic disorder in transcription factors. *Biochemistry* 45, 6873–6888.
- Lobley, A., Swindells, M.B., Orengo, C.A., Jones, D.T., 2007. Inferring function using patterns of native disorder in proteins. *PLoS Comput. Biol.* 3, e162.
- Loftus, S.R., Walker, D., Mate, M.J., Bonsor, D.A., James, R., Moore, G.R., Kleanthous, C., 2006. Competitive recruitment of the periplasmic translocation portal TolB by a natively disordered domain of colicin E9. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12353–12358.
- Love, J.J., Li, X., Case, D.A., Giese, K., Grosschedl, R., Wright, P.E., 1995. Structural basis for DNA bending by the architectural transcription factor LEF-1. *Nature* 376, 791–795.
- Love, J.J., Li, X., Chung, J., Dyson, H.J., Wright, P.E., 2004. The LEF-1 high-mobility group domain undergoes a disorder-to-order transition upon formation of a complex with cognate DNA. *Biochemistry* 43, 8725–8734.
- Machesky, L.M., Insall, R.H., 1999. Signaling to actin dynamics. *J. Cell. Biol.* 146, 267–272.
- Magidovich, E., Fleishman, S.J., Yifrach, O., 2006. Intrinsically disordered C-terminal segments of voltage-activated potassium channels: a possible fishing rod-like mechanism for channel binding to scaffold proteins. *Bioinformatics* 22, 1546–1550.
- Magidovich, E., Orr, I., Fass, D., Abdu, U., Yifrach, O., 2007. Intrinsic disorder in the C-terminal domain of the Shaker voltage-activated K<sup>+</sup> channel modulates its interaction with scaffold proteins. *Proc. Natl. Acad. Sci. U.S.A.* 104, 13022–13027.
- Marcaida, M.J., DePristo, M.A., Chandran, V., Carpousis, A.J., Luisi, B.F., 2006. The RNA degradosome: life in the fast lane of adaptive molecular evolution. *Trends Biochem. Sci.* 31, 359–365.
- Mathes, E., O’Dea, E.L., Hoffmann, A., Ghosh, G., 2008. NF-kappaB dictates the degradation pathway of IkappaBalpha. *EMBO J.* 27, 1421.
- Mayo, L.D., Donner, D.B., 2002. The PTEN, Mdm2, p53 tumor suppressor-oncoprotein network. *Trends Biochem. Sci.* 27, 462–467.
- McNulty, B.C., Young, G.B., Pielak, G.J., 2006. Macromolecular crowding in the *Escherichia coli* periplasm maintains alpha-synuclein disorder. *J. Mol. Biol.* 355, 893–897.
- van der Merwe, P.A., Barclay, A.N., 1994. Transient intercellular adhesion: the importance of weak protein–protein interactions. *Trends Biochem. Sci.* 19, 354–358.
- Meszaros, B., Tompa, P., Simon, I., Dosztanyi, Z., 2007. Molecular principles of the interactions of disordered proteins. *J. Mol. Biol.* 372, 549–561.
- Miller, S., Lesk, A.M., Janin, J., Chothia, C., 1987. The accessible surface area and stability of oligomeric proteins. *Nature* 328, 834–836.
- Mittag, T., Forman-Kay, J.D., 2007. Atomic-level characterization of disordered protein ensembles. *Curr. Opin. Struct. Biol.* 17, 3–14.
- Mogridge, J., Legault, P., Li, J., Van Oene, M.D., Kay, L.E., Greenblatt, J., 1998. Independent ligand-induced folding of the RNA-binding domain and two functionally distinct antitermination regions in the phage lambda N protein. *Mol. Cell* 1, 265–275.
- Mohan, A., Oldfield, C.J., Radivojac, P., Vacic, V., Cortese, M.S., Dunker, A.K., Uversky, V.N., 2006. Analysis of molecular recognition features (MoRFs). *J. Mol. Biol.* 362, 1043–1059.
- Morrison, D.K., Davis, R.J., 2003. Regulation of MAP kinase signaling modules by scaffold proteins in mammals. *Annu. Rev. Cell. Dev. Biol.* 19, 91–118.
- Murzin, A.G., 2008. Biochemistry. Metamorphic proteins. *Science* 320, 1725–1726.
- Nakamura, T., Mori, T., Tada, S., Krajewski, W., Rozovskaia, T., Wassell, R., Dubois, G., Mazo, A., Croce, C.M., Canaani, E., 2002. ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. *Mol. Cell* 10, 1119–1128.
- Nobeli, I., Favia, A.D., Thornton, J.M., 2009. Protein promiscuity and its implications for biotechnology. *Nat. Biotechnol.* 27, 157–167.
- Nooren, I.M., Thornton, J.M., 2003. Diversity of protein–protein interactions. *EMBO J.* 22, 3486–3492.
- Oldfield, C.J., Cheng, Y., Cortese, M.S., Brown, C.J., Uversky, V.N., Dunker, A.K., 2005a. Comparing and combining predictors of mostly disordered proteins. *Biochemistry* 44, 1989–2000.
- Oldfield, C.J., Cheng, Y., Cortese, M.S., Romero, P., Uversky, V.N., Dunker, A.K., 2005b. Coupled folding and binding with alpha-helix-forming molecular recognition elements. *Biochemistry* 44, 12454–12470.
- Olesen, L.E., Ford, M.G., Schmid, E.M., Vallis, Y., Babu, M.M., Li, P.H., Mills, I.G., McMahon, H.T., Praefcke, G.J., 2008. Solitary and repetitive binding motifs for the AP2 complex alpha-appendage in amphiphysin and other accessory proteins. *J. Biol. Chem.* 283, 5099–5109.
- Oltvai, Z.N., Barabasi, A.L., 2002. Systems biology: Life’s complexity pyramid. *Science* 298, 763–764.
- Orchard, S., Salwinski, L., Kerrier, S., Montecchi-Palazzi, L., Oesterheld, M., Stumpflen, V., Ceol, A., Chatr-aryamontri, A., Armstrong, J., Woollard, P., Salama, J.J., Moore, S., Wojcik, J., Bader, G.D., Vidal, M., Cusick, M.E., Gerstein, M., Gavin, A.C., Superti-Furga, G., Greenblatt, J., Bader, J., Uetz, P., Tyers, M., Legrain, P., Fields, S., Mulder, N., Gilson, M., Niepmann, M., Burgoon, L., De Las Rivas, J., Prieto, C., Perreau, V.M., Hogue, C., Mewes, H.W., Apweiler, R., Xenarios, I., Eisenberg, D., Cesareni, G., Hermjakob, H., 2007. The minimum information required for reporting a molecular interaction experiment (MIMIx). *Nat. Biotechnol.* 25, 894–898.
- Owen, D.J., Collins, B.M., Evans, P.R., 2004. Adaptors for clathrin coats: structure and function. *Annu. Rev. Cell Dev. Biol.* 20, 153–191.
- Pawson, T., 1995. Protein modules and signalling networks. *Nature* 373, 573–580.
- Pawson, T., 2004. Specificity in signal transduction: from phosphotyrosine-SH2 domain interactions to complex cellular systems. *Cell* 116, 191–203.
- Pawson, T., Nash, P., 2003. Assembly of cell regulatory systems through protein interaction domains. *Science* 300, 445–452.
- Pawson, T., Scott, J.D., 1997. Signaling through scaffold, anchoring, and adaptor proteins. *Science* 278, 2075–2080.
- Pawson, T., Scott, J.D., 2005. Protein phosphorylation in signaling – 50 years and counting. *Trends Biochem. Sci.* 30, 286–290.
- Pontius, B.W., 1993. Close encounters: why unstructured, polymeric domains can increase rates of specific macromolecular association. *Trends Biochem. Sci.* 18, 181–186.
- Praefcke, G.J., Ford, M.G., Schmid, E.M., Olesen, L.E., Gallop, J.L., Peak-Chew, S.Y., Vallis, Y., Babu, M.M., Mills, I.G., McMahon, H.T., 2004. Evolving nature of the AP2 alpha-appendage hub during clathrin-coated vesicle endocytosis. *EMBO J.* 23, 4371–4383.
- Radhakrishnan, I., Perez-Alvarado, G.C., Parker, D., Dyson, H.J., Montminy, M.R., Wright, P.E., 1997. Solution structure of the KIX domain of CBP bound to the transactivation domain of CREB: a model for activator:coactivator interactions. *Cell* 91, 741–752.
- Robinson, C.V., Sali, A., Baumeister, W., 2007. The molecular sociology of the cell. *Nature* 450, 973–982.
- Romero, P., Obradovic, Z., Li, X., Garner, E.C., Brown, C.J., Dunker, A.K., 2001. Sequence complexity of disordered protein. *Proteins* 42, 38–48.
- Rual, J.F., Venkatesan, K., Hao, T., Hirozane-Kishikawa, T., Dricot, A., Li, N., Berriz, G.F., Gibbons, F.D., Dreze, M., Ayivi-Guedehoussou, N., Klitgord, N., Simon, C., Boxem, M., Milstein, S., Rosenberg, J., Goldberg, D.S., Zhang, L.V., Wong, S.L., Franklin, G., Li, S., Albalá, J.S., Lim, J., Fraughton, C., Lamosas, E., Cevik, S., Bex, C., Lamesch, P., Sikorski, R.S., Vandenhaute, J., Zoghbi, H.Y., Smolyar, A., Bosak, S., Sequerra, R., Doucette-Stamm, L., Cusick, M.E., Hill, D.E., Roth, F.P., Vidal, M., 2005. Towards a proteome-scale map of the human protein–protein interaction network. *Nature* 437, 1173–1178.
- Russell, R.B., Aloy, P., 2008. Targeting and tinkering with interaction networks. *Nat. Chem. Biol.* 4, 666–673.
- Ruthenburg, A.J., Li, H., Patel, D.J., Allis, C.D., 2007. Multivalent engagement of chromatin modifications by linked binding modules. *Nat. Rev. Mol. Cell Biol.* 8, 983–994.
- Schlessinger, A., Liu, J., Rost, B., 2007. Natively unstructured loops differ from other loops. *PLoS Comput. Biol.* 3, e140.
- Schmid, E.M., Ford, M.G., Burty, A., Praefcke, G.J., Peak-Chew, S.Y., Mills, I.G., Benmerah, A., McMahon, H.T., 2006. Role of the AP2 beta-appendage hub in recruiting partners for clathrin-coated vesicle assembly. *PLoS Biol.* 4, e262.
- Schmid, E.M., McMahon, H.T., 2007. Integrating molecular and network biology to decode endocytosis. *Nature* 448, 883–888.
- Schwartz, M.A., Madhani, H.D., 2004. Principles of MAP kinase signaling specificity in *Saccharomyces cerevisiae*. *Annu. Rev. Genet.* 38, 725–748.
- Schwikowski, B., Uetz, P., Fields, S., 2000. A network of protein–protein interactions in yeast. *Nat. Biotechnol.* 18, 1257–1261.
- Seet, B.T., Dikic, I., Zhou, M.M., Pawson, T., 2006. Reading protein modifications with interaction domains. *Nat. Rev. Mol. Cell Biol.* 7, 473–483.
- Seldeen, K.L., McDonald, C.B., Deegan, B.J., Farooq, A., 2008. Coupling of folding and DNA-binding in the bZIP domains of Jun–Fos heterodimeric transcription factor. *Arch. Biochem. Biophys.* 473, 48–60.

- Shaw, A.S., Filbert, E.L., 2009. Scaffold proteins and immune-cell signalling. *Nat. Rev. Immunol.* 9, 47–56.
- Sherr, C.J., Roberts, J.M., 1999. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev.* 13, 1501–1512.
- Sherr, C.J., Roberts, J.M., 2004. Living with or without cyclins and cyclin-dependent kinases. *Genes Dev.* 18, 2699–2711.
- Shoemaker, B.A., Portman, J.J., Wolynes, P.G., 2000. Speeding molecular recognition by using the folding funnel: the fly-casting mechanism. *Proc. Natl. Acad. Sci. U.S.A.* 97, 8868–8873.
- Sickmeier, M., Hamilton, J.A., LeGall, T., Vacic, V., Cortese, M.S., Tantos, A., Szabo, B., Tompa, P., Chen, J., Uversky, V.N., Obradovic, Z., Dunker, A.K., 2007. DisProt: the database of disordered proteins. *Nucleic Acids Res.* 35, D786–D793.
- Singh, G.P., Ganapathi, M., Dash, D., 2007. Role of intrinsic disorder in transient interactions of hub proteins. *Proteins* 66, 761–765.
- Snowden, A.W., Anderson, L.A., Webster, G.A., Perkins, N.D., 2000. A novel transcriptional repression domain mediates p21 (WAF1/CIP1) induction of p300 translocation. *Mol. Cell Biol.* 20, 2676–2686.
- Stelzl, U., Worm, U., Lalowski, M., Haenig, C., Brembeck, F.H., Goehler, H., Stroedicke, M., Zenkner, M., Schoenherr, A., Koeppe, S., Timm, J., Mintzlaff, S., Abraham, C., Bock, N., Kietzmann, S., Goedde, A., Toksoz, E., Droegge, A., Krobitsch, S., Korn, B., Birchmeier, W., Lehrach, H., Wanker, E.E., 2005. A human protein–protein interaction database: a resource for annotating the proteome. *Cell* 122, 957–968.
- Sugase, K., Dyson, H.J., Wright, P.E., 2007. Mechanism of coupled folding and binding of an intrinsically disordered protein. *Nature* 447, 1021–1025.
- Tang, C., Iwahara, J., Clore, G.M., 2006. Visualization of transient encounter complexes in protein–protein association. *Nature* 444, 383–386.
- Taverna, S.D., Li, H., Ruthenburg, A.J., Allis, C.D., Patel, D.J., 2007. How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nat. Struct. Mol. Biol.* 14, 1025–1040.
- Teufel, D.P., Freund, S.M., Bycroft, M., Fersht, A.R., 2007. Four domains of p300 each bind tightly to a sequence spanning both transactivation subdomains of p53. *Proc. Natl. Acad. Sci. U.S.A.* 104, 7009–7014.
- van Tilborg, P.J., Mulder, F.A., de Backer, M.M., Nair, M., van Heerde, E.C., Folkers, G., van der Saag, P.T., Karimi-Nejad, Y., Boelens, R., Kaptein, R., 1999. Millisecond to microsecond time scale dynamics of the retinoid X and retinoic acid receptor DNA-binding domains and dimeric complex formation. *Biochemistry* 38, 1951–1956.
- Tompa, P., 2002. Intrinsically unstructured proteins. *Trends Biochem. Sci.* 27, 527–533.
- Tompa, P., 2003. Intrinsically unstructured proteins evolve by repeat expansion. *BioEssays* 25, 847–855.
- Tompa, P., Fuxreiter, M., 2008. Fuzzy complexes: polymorphism and structural disorder in protein–protein interactions. *Trends Biochem. Sci.* 33, 2–8.
- Tompa, P., Szasz, C., Buday, L., 2005. Structural disorder throws new light on moonlighting. *Trends Biochem. Sci.* 30, 484–489.
- Tsai, C.J., Ma, B., Sham, Y.Y., Kumar, S., Nussinov, R., 2001. Structured disorder and conformational selection. *Proteins* 44, 418–427.
- Tuinstra, R.L., Peterson, F.C., Kutlesa, S., Elgin, E.S., Kron, M.A., Volkman, B.F., 2008. Interconversion between two unrelated protein folds in the lymphotactin native state. *Proc. Natl. Acad. Sci. U.S.A.* 105, 5057–5062.
- Turjanski, A.G., Gutkind, J.S., Best, R.B., Hummer, G., 2008. Binding-induced folding of a natively unstructured transcription factor. *PLoS Comput. Biol.* 4, e1000060.
- Turner, B.M., 2002. Cellular memory and the histone code. *Cell* 111, 285–291.
- Turner, B.M., 2007. Defining an epigenetic code. *Nat. Cell Biol.* 9, 2–6.
- Tyers, M., Mann, M., 2003. From genomics to proteomics. *Nature* 422, 193–197.
- Uetz, P., Giot, L., Cagney, G., Mansfield, T.A., Judson, R.S., Knight, J.R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleisch, T., Vijayadamar, G., Yang, M., Johnston, M., Fields, S., Rothberg, J.M., 2000. A comprehensive analysis of protein–protein interactions in *Saccharomyces cerevisiae*. *Nature* 403, 623–627.
- Uversky, V.N., Oldfield, C.J., Dunker, A.K., 2005. Showing your ID: intrinsic disorder as an ID for recognition, regulation and cell signaling. *J. Mol. Recognit.* 18, 343–384.
- Vacic, V., Oldfield, C.J., Mohan, A., Radivojac, P., Cortese, M.S., Uversky, V.N., Dunker, A.K., 2007. Characterization of molecular recognition features, MoRFs, and their binding partners. *J. Proteome Res.* 6, 2351–2366.
- Van Heyningen, V., Yeyati, P.L., 2004. Mechanisms of non-Mendelian inheritance in genetic disease. *Hum. Mol. Genet.* 13 (Spec. No. 2), R225–R233.
- Vaynberg, J., Qin, J., 2006. Weak protein–protein interactions as probed by NMR spectroscopy. *Trends Biotechnol.* 24, 22–27.
- Venkitaraman, A.R., 2002. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* 108, 171–182.
- Vidal, M., 2001. A biological atlas of functional maps. *Cell* 104, 333–339.
- Vondriska, T.M., Pass, J.M., Ping, P., 2004. Scaffold proteins and assembly of multi-protein signaling complexes. *J. Mol. Cell. Cardiol.* 37, 391–397.
- Wang, H., Hammoudeh, D.I., Follis, A.V., Reese, B.E., Lazo, J.S., Metallo, S.J., Prochownik, E.V., 2007. Improved low molecular weight Myc–Max inhibitors. *Mol. Cancer Ther.* 6, 2399–2408.
- Wang, Y., Cortez, D., Yazdi, P., Neff, N., Elledge, S.J., Qin, J., 2000. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev.* 14, 927–939.
- Ward, J.J., Sodhi, J.S., McGuffin, L.J., Buxton, B.F., Jones, D.T., 2004. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. *J. Mol. Biol.* 337, 635–645.
- Weathers, M.A., Paulaitis, M.E., Woolf, T.B., Hoh, J.H., 2004. Reduced amino acid alphabet is sufficient to accurately recognize intrinsically disordered protein. *FEBS Lett.* 576, 348–352.
- Weiss, M.A., Ellenberger, T., Wobbe, C.R., Lee, J.P., Harrison, S.C., Struhl, K., 1990. Folding transition in the DNA-binding domain of GCN4 on specific binding to DNA. *Nature* 347, 575–578.
- Wells, J.A., McClendon, C.L., 2007. Reaching for high-hanging fruit in drug discovery at protein–protein interfaces. *Nature* 450, 1001–1009.
- Wells, M., Tidow, H., Rutherford, T.J., Markwick, P., Jensen, M.R., Mylonas, E., Svergun, D.I., Blackledge, M., Fersht, A.R., 2008. Structure of tumor suppressor p53 and its intrinsically disordered N-terminal transactivation domain. *Proc. Natl. Acad. Sci. U.S.A.* 105, 5762–5767.
- Williams, R.M., Obradovic, Z., Mathura, V., Braun, W., Garner, E.C., Young, J., Takayama, S., Brown, C.J., Dunker, A.K., 2001. The protein non-folding problem: amino acid determinants of intrinsic order and disorder. *Pac. Symp. Biocomput.* 89–100.
- Worrall, J.A., Gorna, M., Pei, X.Y., Spring, D.R., Nicholson, R.L., Luisi, B.F., 2007. Design and chance in the self-assembly of macromolecules. *Biochem. Soc. Trans.* 35, 502–507.
- Wright, P.E., Dyson, H.J., 1999. Intrinsically unstructured proteins: re-assessing the protein structure–function paradigm. *J. Mol. Biol.* 293, 321–331.
- Wright, P.E., Dyson, H.J., 2009. Linking folding and binding. *Curr. Opin. Struct. Biol.* Xie, H., Vucetic, S., Iakoucheva, L.M., Oldfield, C.J., Dunker, A.K., Obradovic, Z., Uversky, V.N., 2007a. Functional anthology of intrinsic disorder. 3. Ligands, post-translational modifications, and diseases associated with intrinsically disordered proteins. *J. Proteome Res.* 6, 1917–1932.
- Xie, H., Vucetic, S., Iakoucheva, L.M., Oldfield, C.J., Dunker, A.K., Uversky, V.N., Obradovic, Z., 2007b. Functional anthology of intrinsic disorder. 1. Biological processes and functions of proteins with long disordered regions. *J. Proteome Res.* 6, 1882–1898.
- Yang, X.J., 2005. Multisite protein modification and intramolecular signaling. *Oncogene* 24, 1653–1662.
- Yao, T.P., Oh, S.P., Fuchs, M., Zhou, N.D., Ch'ng, L.E., Newsome, D., Bronson, R.T., Li, E., Livingston, D.M., Eckner, R., 1998. Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell* 93, 361–372.
- Yu, H., Braun, P., Yildirim, M.A., Lemmens, I., Venkatesan, K., Sahalie, J., Hirozane-Kishikawa, T., Gebreab, F., Li, N., Simonis, N., Hao, T., Rual, J.F., Dricot, A., Vazquez, A., Murray, R.R., Simon, C., Tardivo, L., Tam, S., Svrikapa, N., Fan, C., de Smet, A.S., Motyl, A., Hudson, M.E., Park, J., Xin, X., Cusick, M.E., Moore, T., Boone, C., Snyder, M., Roth, F.P., Barabasi, A.L., Tavernier, J., Hill, D.E., Vidal, M., 2008. High-quality binary protein interaction map of the yeast interactome network. *Science* 322, 104–110.
- Zeev-Ben-Mordehai, T., Rydberg, E.H., Solomon, A., Toker, L., Auld, V.J., Silman, I., Botti, S., Sussman, J.L., 2003. The intracellular domain of the *Drosophila* cholinesterase-like neural adhesion protein, gliotactin, is natively unfolded. *Proteins* 53, 758–767.
- Zor, T., Mayr, B.M., Dyson, H.J., Montminy, M.R., Wright, P.E., 2002. Roles of phosphorylation and helix propensity in the binding of the KIX domain of CREB-binding protein by constitutive (c-Myb) and inducible (CREB) activators. *J. Biol. Chem.* 277, 42241–42248.