FEBS Letters 589 (2015) 2533–2542

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Fuzzy complexes: Specific binding without complete folding

Rashmi Sharma [1], Zsolt Raduly [1], Marton Miskei, Monika Fuxreiter *

MTA-DE Momentum, Laboratory of Protein Dynamics, Department of Biochemistry and Molecular Biology, University of Debrecen, Hungary

ARTICLE INFO

Article history:
Received 1 June 2015
Revised 20 July 2015
Accepted 21 July 2015
Available online 27 July 2015

Edited by Wilhelm Just

Keywords:
Intrinsically disordered protein
Fuzzy complex
Transcription regulation
Protein interaction
Specificity
Signal transduction
Viral protein
Conformational selection

ABSTRACT

Specific molecular recognition is assumed to require a well-defined set of contacts and devoid of conformational and interaction ambiguities. Growing experimental evidence demonstrates however, that structural multiplicity or dynamic disorder can be retained in protein complexes, termed as fuzziness. Fuzzy regions establish alternative contacts between specific partners usually via transient interactions. Nature often tailors the dynamic properties of these segments via post-translational modifications or alternative splicing to fine-tune affinity. Most experimentally characterized fuzzy complexes are involved in regulation of gene-expression, signal transduction and cell-cycle regulation. Fuzziness is also characteristic to viral protein complexes, cytoskeleton structure, and surprisingly in a few metabolic enzymes. A plausible role of fuzzy complexes in increasing half-life of intrinsically disordered proteins is also discussed.

1. Introduction

The classical view is that molecular specificity is determined by a well-defined set of interactions, which are formed between stable, folded interfaces. Protein complexes however have to be sensitive to environmental conditions, signals, which require structural malleability and variable, tunable interactions. Intrinsically disordered (ID) proteins function as an ensemble of conformations, but are generally thought to adopt a three dimensional structure upon interacting with their partners [1,2]. Coupled folding and binding usually involves short protein regions, which are distinguished in recognition [3]. These preformed [4] or molecular recognition elements [5] have distinct secondary structure preferences, which are biased towards their bound conformation. ID protein segments, which undergo folding upon binding however are usually small peptide fragments, while a considerable fraction of the protein still retains its conformational heterogeneity in the complex. These ID regions are often overlooked as they do not mediate permanent interactions or they are simply truncated, even removed for experimental convenience (e.g. promoting crystallization or to avoid aggregation). Despite the lack of clear structural interpretation of their biological significance, biochemical data in many cases demonstrate that dynamic regions inevitably contribute to function, or can even critically influence biological activity [6]. Protein complexes, where conformational heterogeneity of ID regions is retained and is required for function are termed as fuzzy complexes [7]. The corresponding ID regions, which adopt different structures or remain disordered upon binding are called as fuzzy regions. The term is derived from fuzzy logic with variables having truth values ranging in degree between 0 and 1. This many-valued logic is in contrast to traditional binary logic, where variables only have true or false values [8,9]. In case of ID proteins, fuzzy complexes can be characterized by a range of dynamic properties, which correspond to different biological outputs [10,11].

Four topological categories of fuzzy complexes have been previously defined depending on the location of the ID region (Fig. 1) [7]: (i) *polymorphic* complexes represent static disorder, where alternative conformations are realized in the complex (e.g. WH2 domains and actin [12]); (ii) *clamps*, where fuzzy regions link globular binding domains or peptide motifs (e.g. UPF1–UPF2 [13]); (iii) *flanking* complexes, where fuzzy regions neighbor binding sites and provide additional contacts (Antennapedia-DNA complex [14]); (iv) *random* complexes, where variable short motifs are interconnected by fuzzy regions (e.g. elastin [15]). These topologies are not mutually exclusive, they can also appear in combination (e.g. in case of NLS peptides and importin α [16]). Furthermore,

* Corresponding author.
E-mail address: fmoni@med.unideb.hu (M. Fuxreiter).
[1] These two authors have equal contributions, both should be considered as first authors.

http://dx.doi.org/10.1016/j.febslet.2015.07.022
0014-5793/

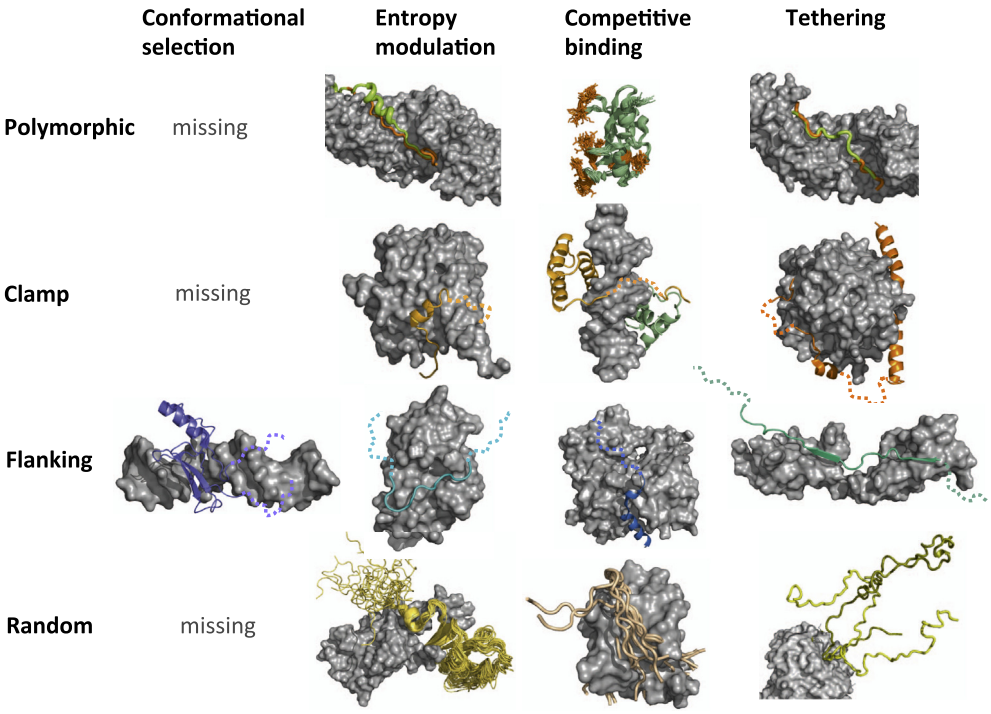

Fig. 1. Interplay between topological and mechanistic categories of fuzzy complexes. Fuzzy proteins are displayed by colored cartoons, binding partners are shown by gray solid surfaces. Dynamic fuzzy regions are represented by dotted lines. Missing designates combinations for which no experimental evidence have been found yet. Columns are described from top to bottom, rows from left to right. **Polymorphic complexes**. In the Tcf-β catenin (1jdh, lime; 1g3j, orange) complex, the multiple conformations of the transcription factor lower the entropic penalty of binding [58]. In RNase I (2k11, polymorphic regions are shown by orange) the ability of the side chains of R4, K6, R32, R39 and K102 to adopt multiple conformations is crucial upon competing for two targets, the negatively charged membrane components and RNA [124]. In the complex of NLS-importin α (1ejy, lime; 1ejl, orange) the alternative binding modes may account for diverse NLS binding by the same receptor and effective competition with mammalian NLSs [16]. These are realized via two basic clusters, which act in a synergistic manner. **Clamp complexes**. The 4E-BP2 binds to EIF4E (3am7, lightorange) in a bipartite manner, where mutations in the linker change the dynamics of the complex from microsecond to millisecond conformational exchange [111]. Binding of Ubx to DNA (1b8i, brightorange, Exd is shown by limegreen) is influenced by the interplay of three fuzzy regions. The I1 region contains a YPWM motif, which mediates communication with the Hox cofactor Extradenticle (Exd) and relieves repression of DNA binding by I1 [96]. In the complex of I2 and PP1 (2o8a, orange) the regions, which connect the recognition elements remain invisible in the electron density, yet contribute to converting the active to inactive form of the phosphatase via transient contacts [119,135]. **Flanking complexes**. In the MeCP2–DNA (3c2i, slate) complex, the presence of the fuzzy regions increase the propensity of secondary structure elements towards the binding competent form [27]. In the SF1-U2AF65 complex (1o0p, cyan) the fuzzy flanking regions tune the entropy of binding and thus improve binding affinity. Transient interactions also affect specificity for different U2AF RRMs [110]. Upon binding WH2 domains to actin (3u9d, blue) fuzziness enables two opposite functions (actin polymerization vs disassembly), which is controlled by the ionic strength via screening a single charge-charge interaction [62]. In the SfbI-Fn complex (1o9a, limegreen) the fuzzy regions flanking the consensus FnBP motifs enable combinatorial usage of the binding sites to increase binding affinity and ensure selectivity control. **Random complexes**. The UmuD′2 homodimer (1i4v, dark yellow) interchanges between many different conformations, and the degree of dynamics depends on the fuzzy N-terminal arm [114]. c-Myc binds to Bin1 SH3 (1mv0, wheat) in a multipartite manner involving two competing phosphorylation sites (T58, S62), which determine the turnover of c-Myc [48]. Sic1 has 9 suboptimal binding motifs for Cdc4, which dynamically interchange in the complex (as a courtesy of Tanja Mittag). Phosphorylation of 6 sites provides optimal binding and sets the threshold for Sic1 degradation [20].

different ranges of the structural spectrum are observed by different experimental techniques, varying between static and dynamic complexes [17]. Albeit the existence of some random complexes (e.g. T-cell receptor ξ-chain [18]) have been recently debated [19], experimental data strongly argue against global structural ordering in other cases (Sic1-Cdc4 [20], elastin [15]), where short peptide motifs [21], or posttranslational modification sites mediate variable interactions between highly disordered polymers. Weak sequence determinants (often referred as 'sequence independent binding') are also characteristic of complexes of transcription factors [22,23] or histone tails [24], and were also recently observed for proteins, which form membraneless organelles [25].

Molecular scenarios of how fuzzy regions impact biological activities can be grouped into four categories, which have been detailed earlier [6,26]. Alternative, often transient contacts by fuzzy regions can modulate conformational equilibrium and increase probability of the binding competent form (*conformational selection*, e.g. MeCP2 [27,28]) or can influence the flexibility of the binding interface and thus decrease the entropic penalty of binding (*flexibility modulation*, e.g. Ets-1 [29,30]). Fuzzy regions can compete with the binding partner via electrostatic interactions or steric hindrance (*competitive binding*, e.g. HMGB1 [31]), but can also improve affinity by increasing the local concentration of the globular, weak-affinity binding domains (*tethering*, e.g. RPA IULD [32,33]). Fig. 1 shows the coupling between the topological categories and mechanisms of fuzzy complexes. Based on the available experimental data, flexibility/entropy modulation, competitive binding or tethering can be achieved by all four topological categories, but conformational selection is currently limited to flanking fuzzy complexes. We however cannot exclude that this scenario can also be combined to other fuzzy complex types.

In Table 1 we assemble fuzzy protein complexes, with experimental evidence for structural multiplicity or disorder in the bound state and with biochemical evidence demonstrating the impact of the fuzzy region on function. Detailed description of these examples, including structural and biochemical data can be found in the Fuzzy Complexes Database (FuzDB, http://protdyn-database.org/). In the following we discuss how structural and interaction ambiguity in protein complexes affect different cellular functions.

1.1. Fuzzy complexes in regulation of gene-expression

Eukaryotic transcription machinery relies on gigantic complexes, where different conformational states and the

Table 1
Fuzzy protein complexes with different cellular functions.

Function	Protein name	Partner	Organism	References
Gene-expression				
Chromatin structure and dynamics				
	$H1^0$ linker histone	DNA	*Mus musculus*	[24,35]
	FACT	DNA	*Drosophila melanogaster*	[36]
	MeCP2	DNA	*Homo sapiens*	[27,28]
	MBD2 NurD	DNA	*Homo sapiens*	[94]
Transcription factors				
	Max	DNA	*Homo sapiens*	[37,95]
	NKX3.1	DNA	*Drosophila melanogaster*	[40]
	ApLLP	DNA	*Aplysia kurodai*	[96]
	Neurogenin 1	DNA	*Homo sapiens*	[97]
	Ultrabithorax	DNA, Exd	*Drosophila melanogaster*	[38,44]
	HMGB1	DNA	*Rattus norvegicus*	[31]
	Oct-1	DNA	*Homo sapiens*	[39]
	Ets-1	DNA	*Mus musculus*	[29,30]
	c-Myc	Bin1 SH3 domain	*Homo sapiens*	[48]
	Nrf2	Keap1	*Mus musculus*	[98]
	Prothymosin α	Keap1	*Homo sapiens*	[99]
Coactivator interactions				
	GCN4	Med15	*Saccharomyces cerevisiae*	[46,47]
	p65 (RelA)	CBP TAZ1	*Mus Musculus*	[100]
	p53 TAD	CBP NCBD	*Homo sapiens*	[45]
	KID	KIX	*Mus musculus*	[101,102]
Interactions with the basal machinery				
	EWS	PIC	*Homo sapiens*	[41]
	SP1	TFIID	*Homo sapiens*	[103]
	GCN4	PIC	*Saccharomyces cerevisiae*	[22]
	Gal4	PIC	*Saccharomyces cerevisiae*	[104]
	PC4	PIC	*Homo sapiens*	[105]
Nuclear receptors, transport				
	PPAR-γ	DNA	*Homo sapiens*	[106]
	NLS	Importin-α	*Xenopus laevis*	[16,107]
mRNA maturation, translation				
	Cup	eIF4E	*Drosophila melanogaster*	[108]
	UPF2	UPF1	*Homo sapiens*	[13]
	RNAP II CTD	mRNA maturation factors	*Saccharomyces cerevisiae*	[109]
	SF1	$U2AF^{65}$	*Homo sapiens*	[110]
	4E-BP2	eIF4E	*Homo sapiens*	[111]
	L7/L12	Ribosome	*Escherichia coli*	[112,113]
DNA repair				
	RPA	DNA	*Homo sapiens*	[32,33]
	UmuD′2	UmuD2	*Escherichia coli*	[114]
	UvrD	DNA	*Escherichia coli*	[115]
Signaling				
	Ste5	Fus3	*Saccharomyces cerevisiae*	[56]
	Tcf3, Tcf4	β-Catenin	*Homo sapiens*	[58]
	E-cadherin	β-Catenin	*Mus musculus*	[57]
	iκBα	NFκB	*Homo sapiens*	[81]
	Calmodulin	MBP	*Homo sapiens*	[116]
	RLP1	SH3	*Gallus gallus*	[117]
	I2	PP1	*Mus musculus*	[118,119]
Cell-cycle regulation				
	$p27^{Kip1}$	Cdk2/cyclin	*Homo sapiens*	[60]
	$p21^{WAF1/CIP1}$	Cdk2/cyclin	*Homo sapiens*	[59]
	Sic1	Cdc4	*Saccharomyces cerevisiae*	[20,61]
Cytoskeleton structure				
	Thymosin β4	Actin	*Bos taurus*	[62]
	Ciboulot	Actin	*Drosophila melanogaster*	[12]
	Myelin basic protein	Actin	*Mus musculus*	[63]
	Dynein IC	NudE	*Drosophila melanogaster*	[120]
Viral proteins				
	Nucleoprotein	Phosphoprotein	*Measles virus*	[64]
	Nucleoprotein	Phosphoprotein	*Henipah virus*	[65,67]
	Nucleoprotein	Phosphoprotein	*Hendra virus*	[65,66]
	E1A	CBP TAZ2	*Human adenovirus*	[121]

(*continued on next page*)

Table 1 (*continued*)

Function	Protein name	Partner	Organism	References
	NS5A	SH3	*Hepatitis C virus*	[71]
	preS1	γ2-Adaptin	*Hepatitis B virus*	[72]
	NS5B	VAPC	*Hepatitis C virus*	[122]
	Nucleoprotein	VP35	*Ebola virus*	[123]
Enzymes				
	Cellulase E	Cellulose	*Humicola insolens*	[73]
	Thymine–DNA glycosylase	DNA	*Homo sapiens*	[74]
	Anhydrin	DNA	*Aphelenchus avenae*	[75]
	RNase I	RNase inhibitor	*Homo sapiens*	[124]
Endocytosis/adhesion				
	LigB	Fibronectin	*Leptospira interrogans*	[125]
	SfbI	Fn3	*Streptococcus pyogenes*	[126]
	AP180	Clathrin	*Mus musculus*	[127]
Chaperones				
	Hsp90	Ppp5 TRP	*Homo sapiens*	[17]
	Hsp25	α-Lactalbumin	*Homo sapiens*	[128,129]
	αA-crystallin	HMM	*Bos taurus*	[130,131]
Self-assembly, aggregation				
	Sup35	Sup35 prion amyloid	*Saccharomyces cerevisiae*	[132]
	Ure2	Ure2 prion amyloid	*Saccharomyces cerevisiae*	[133]
	Elastin	Elastin	*Bos taurus*	[15]
	α-Synuclein	Membrane	*Homo sapiens*	[134]

corresponding alternative interaction patterns result in gene-specific activation or repression. Conformational heterogeneity of fuzzy regions contribute to regulation of transcription at all levels.

Condensation and dynamics of chromatin fibers is modulated by malleable regions, in High Mobility Group (HMG) proteins, chromatin remodeling complexes and histone tails [34]. $H1^0$ linker histones for example affect linker DNA structures and play a role in stabilization of higher-order chromatin assemblies via their disordered 100 AA C-terminal domain (CTD) [35]. Owing to the high positive charge of H1 CTD, it was thought to function through an electrostatic mechanism. Detailed mutagenesis and scrambling experiments however showed that only two discontinuous stretches of CTD (98–122 and 147–170) mediate interactions with DNA and facilitate chromatin fiber condensation irrespective of their exact order and primary sequence [24]. Stabilization of chromatin structure was dependent only on the amino acid composition of these two regions, which is conserved in all H1 isoforms and their distance from the globular domain. In addition to chromatin, H1°CTD is also capable to interact with various other nuclear proteins, for example DFF40 apoptotic nuclease. Any H1°CTD peptides, which are longer than 47 amino acids can activate the enzyme in a sequence-independent, but composition-dependent manner [35]. Mutants, with altered amino acid compositions indicate that different CTD-mediated functions require different degrees of disorder to order transition, but are incompatible with a completely folded structure. Sequence-independent interactions of the H1 isoform CTDs that are mediated by variable pattern of α-helical elements enable redundant functions, where posttranslational modifications or further protein–protein interactions impart isoform-specific effects on gene-expression. The chromatin remodeling factor FACT (facilitates chromatin transcription) uses HMG domains for DNA interactions to displace histone H2A/H2B dimers from nucleosomes. The globular domains are flanked by two disordered regions of opposite charge, which do not gain structure upon DNA binding [36]. The negatively charged segment establishes intramolecular interactions with the positive residues of the HMG domain as well as the ID region and competes for DNA. Multiple phosphorylation of this negative ID region increases the inhibitory effect by masking the nucleotide-binding residues. Indeed, FACT was shown to be dephosphorylated in early embryogenesis. Methyl CpG binding protein 2 (MeCP2) is involved in deciphering epigenetic information by recognizing DNA methylation patterns [27]. Mutations in disordered linkers, which connect specific DNA-binding elements of MeCP2 are diagnosed in Rett-syndrome. The N-terminal domain (NTD) remains substantially disordered in complex with DNA with marginal increase (7%) in secondary structure elements as compared to the unbound form. On the other hand, the MeCP2 NTD improves binding affinity to DNA by 10-fold by inducing conformational changes in the globular methylated DNA binding domain (MBD) via transient interactions, which increase population of binding-competent states [28].

Transcription factors (TFs) bind to specific DNA sequences to activate or repress gene-expression, while their disordered transactivation domains (TADs) are involved in interactions with other proteins of the transcription machinery. Numerous examples illustrate that conformational heterogeneity of TADs can be used to fine-tune DNA-binding affinity or specificity. Transient intramolecular interactions could be established (i) with the charged DNA-binding residues and screen/mask positive charges (e.g. HMGB1 [31]), (ii) with the binding interface and modulate its flexibility (e.g. Ets-1 [30]), (iii) with the globular domains to perturb their conformational equilibrium (e.g. Max [37]), (iv) with other disordered regions to provide steric hindrance (e.g. Ultrabithorax [38]). Fuzzy regions can also mediate variable intermolecular contacts and provide anchor sites to the DNA target (e.g. Oct-1 [39] or NK-2 homeodomains [40]). Although fuzzy segments are often distantly located from the binding interface, their impact on DNA binding could be significant, modulating affinity even up to 3 orders of magnitude [29].

Structural heterogeneity implies weak sequence constraints and compositional bias for TAD interactions, as it was observed in case of GCN4 [22] or EWS [41]. Interactions between aromatic residues and phosphoserine (π–cation interactions) or simple electrostatic patterns can also drive the formation of dynamic assemblies (e.g. Ets-1 [42], EWS [43]). Alternative splicing often tailors the length or the embedded binding motifs of the fuzzy TAD regions resulting

in context-dependent gene regulation. Different Ubx isoforms for example are expressed in a stage- and tissue-specific manner and are spliced in the fuzzy linker, which connects the homeodomain (HD) to the Extradenticle binding YPWM motif [44].

Communication of TADs with other parts of the transcription machinery is also facilitated by ambiguous interactions. Binding of the tumor suppressor p53 TAD to the coactivator CBP/p300 is mediated by two α-helices, while residues between these structured elements remain largely disordered in the complex [45]. Despite that no persistent contacts of the linker region with CBP were observed, its presence improves binding by 10-fold, mostly via tethering the low-affinity acidic region to the target. The GCN4 transcription activator binds the Gal11/Med15 Mediator subunit via multiple, low affinity interactions, which additively contribute to transcription activation [46]. The highly specific complex has a simple interface, which is mostly formed by hydrophobic residues. GCN4 contacts Gal11/Med15 in multiple conformations and orientations, which enable rapid sampling of multiple Gal11 binding domains to recruit Mediator to the promoter. In accord, multimerization of transcription factor DNA-binding sites increases transcriptional activity. Interestingly, a high-affinity binding to Gal11/Med15 could be achieved by optimizing residues around the short, conserved sequence-specific motif of GCN4 resulting in a more ambiguous ('fuzzier') interface [47]. This interaction mode also facilitates specific recognition of other activator binding domains, such as the TFIID subunit 12. Binding of 1–88 residues of c-Myc oncoprotein TAD to Bin1 SH3 domain is also mediated by multivalent interactions and results in a highly dynamic complex [48]. Fuzziness facilitates probing of the phosphorylation state of S62, which is critical for c-Myc cellular stability. This is achieved by simultaneously sampling an ensemble of adjacent interactions, enabling a rapid exchange between the primary site and weaker adjacent binding sites.

A lower resolution structural characterization has been obtained on how fuzziness contributes to the activity of multiprotein *transcriptional coactivators*. Signal transmission from activators/repressors to the core machinery is often coupled to significant conformational rearrangements. A series of experimental evidence support conformational heterogeneity of the Mediator of RNA polymerase II (RNAPII) [49]. Mediator is a complex of ~26 subunits (the exact composition varies by species), which activates gene-expression via interacting with gene-specific transcription factors. Binding to different TFs however, does not reduce the conformational ensemble to a single state, instead, it only shifts the conformational equilibrium [50]. The p53 TAD for example triggers different structures upon contacting different Mediator subunits, which have different impacts on RNAPII activity [51]. Similarly, upon binding to the core machinery the conformation of the Mediator Head module is shifted towards an open state, which facilitates more extensive, yet heterogeneous interactions with RNAP CTD and promotes its phosphorylation [52]. Ambiguity of Mediator interactions enables dynamic exchange at the actively transcribed genes, as the activator induced structural changes facilitate dissociation from the promoter and switch to the elongation state [53].

1.2. Fuzzy complexes in signal transduction

Signaling requires an orchestrated action of multiple short motifs [54], which are usually embedded in disordered regions [55]. The relatively simple pattern of residues, which determines binding affinity or specificity results in weak structural constraints on the flanking segments even within the context of the partner. A series of examples illustrate that regions outside the motifs remain disordered upon contacting the target, yet affect thermodynamics or kinetics of complex formation. This is achieved by transient electrostatic or hydrophobic interactions mediated by fuzzy regions, which can provide non-specific contacts with the partner. On the other hand, dynamic regions can also contribute to specificity by coordinating the action of multiple motifs.

In yeast pheromone response pathway the Ste5 scaffold protein binds its mitogen-activated protein kinase partner Fus3 in a bipartite manner, while the linking segment remains invisible in the complex by X-ray crystallography [56]. The individual Ste5 binding sites have no measurable affinity for Fus3, while the affinity of the construct with both sites is comparable to that of other docking peptides (e.g. Ste7). Multiple independent recruitment sites for Fus3 were shown to allosterically activate the kinase and mediate phosphorylation events thus quantitatively modulate pathway output.

Multiple, quasi-independent interactions govern β-catenin binding to E-cadherin and Tcf transcription factors in the Wnt signaling pathway. In this manner the different interaction sites are controlled independently, which result in combinatorial regulation via integration of multiple signals. All these complexes are characterized by structural multiplicity, where a minimal interface is defined, while the rest of the contacts are variable or dynamic. The interaction of the cytoplasmic domain of E-cadherin with β-catenin for example is gradually modulated by phosphorylation, where each of the posttranslational modifications induce local structure ordering and leaves the rest of the interface unperturbed [57]. On the other hand, phosphorylation of β-catenin by GSK-3β has an opposite effect and decrease affinity for E-cadherin without completely eliminating the interaction between the two proteins owing to the disordered nature and extensive interaction interface. The phosphoserine interactions of E-cadherin are thought to mimic the amphipathic helix binding of Tcf3, which contacts the same site on β-catenin. Tcf3 binds β-catenin via two salt bridges, also denoted as charge buttons, mediated by a cluster of negatively charged residues. Although only one of them is sufficient to mediate high-affinity interactions with β-catenin, these multiple negative residues are conserved in the Tcf family [58]. Upon interacting with β-catenin the negatively charged residues adopt multiple conformations (α-helix in Tcf4 and β-strand in Tcf3), and mutating any of them reduces binding affinity. Structural multiplicity has been proposed to lower the entropic penalty of binding, but it can also be more effective in competing with E-cadherin at different phosphorylation stages.

1.3. Fuzzy complexes in cell-cycle regulation

Tight regulation of cell division is assisted by the dynamics of the corresponding cell-cycle kinase complexes with their inhibitors. $p21^{WAF1/CIP1}$ and $p27^{Kip1}$ interact with the Cdk2/cyclin complex in a similar fashion, where regions binding to cyclin (D1) and Cdk2 (D2) are connected by an α-helical segment (LH). Although the helical conformation of LH is transiently populated in the unbound state, it is not fully stabilized upon interacting with Cdk/cyclin complexes [59]. Instead, LH remains dynamic and serves as an adaptable linker to specifically recognize different Cdk/cyclin structures using conserved D1 and D2 interfaces. Albeit LH does not establish direct contacts with the targets and provides only negligible contribution to the binding free energy, it affects cell-cycle regulation. Indeed, changing the length of LH of p21 by 3 residues (+/−) impairs Cdk/cyclin binding and thus impacts the G1 → S transition. Alteration of the LH subdomain also affects binding promiscuity in vivo.

Beyond the Cdk2 binding region, the ~100 AA C-terminal domain of $p27^{Kip1}$ remains dynamically disordered in the complex [60]. Owing to conformational heterogeneity, the CTD dimensions vary in a wide range between extended and rather compact forms. The latter enable T187 at the C-terminus to contact the Cdk2 active site. Phosphorylation of T187 initiates degradation of p27 and progress of the cell cycle, as it will be detailed below.

Ultrasensitive regulation of the cell cycle is illustrated by the CDK inhibitor Sic1, multiple phosphorylation of which sets the threshold for the onset of DNA replication. Sic1 binds to the Cdc4 subunit of the SCF ubiquitin ligase in a phosphorylation dependent manner via 9 sub-optimal CPD (Cdc4 phosphodegron) motifs. Although only a single target site is available, binding becomes optimal after phosphorylation of six or more sites [61]. A single high-affinity CPD motif can also recruit Sic1 to Cdc4, but leads to premature degradation of Sic1 and genome instability. Sic1 remains intrinsically disordered upon phosphorylation with only local ordering [20]. Surprisingly, the multiple phosphorylated sites on Sic1 were shown to be in dynamic equilibrium upon interacting with Cdc4. Although electrostatics is crucial for binding, the intrinsically disordered state also helps to span the distance to the catalytic center of Cdc34 subunit of the ubiquitin ligase.

1.4. Fuzzy complexes in organization of cytoskeleton structure

Upon various stimuli, the actin cytoskeleton has to undergo substantial rearrangements, which involves partial disassembly of actin filaments and then re-assembly of new filament arrays. Proteins with multiple WASP-homology 2 (WH2) domains, such as thymosin β4, Ciboulot, WASP or WAVE serve as actin regulators [12]. WH2 domains are disordered in isolation and adopt different structures upon binding to actin. The C-terminal regions however, remain disordered in the complex, dynamic interactions of which determine whether actin assembly or sequestration takes place. Increasing dynamics of the C-terminal region of thymosin β4 supports unidirectional assembly, whereas decreased dynamics result in disassembly of the filament [62]. The degree of dynamics of the WH2 C-terminal region critically depends on a single salt bridge close to the LKKT/V motif. At physiological ionic strength this induces sequestration of G-actin, whereas at higher salt concentrations the weaker electrostatic interaction increases k_{off} and leads to elongation of actin filaments.

The myelin basic protein (MBP) also promotes G-actin polymerization and assembly of F-actin fibers into bundles to provide links between cytoskeleton and the myelin sheath. Cytoskeleton interactions can be reversed by calcium-dependent binding of MBP to calmodulin and are also regulated by a variety of posttranslational modifications of MBP. Association with actin induces formation of secondary structure elements in MBP, but the complex exhibits polymorphism and some degree of disorder [63]. Fuzziness is likely required for simultaneous interactions of MBP with actin and the myelin membrane as well as a series of signaling proteins.

1.5. Viral fuzzy complexes

Interaction between nucleoprotein (N) encapsidating viral RNA and the viral polymerase phosphoprotein (P) initiates transcription and replication of viral genomes. N-P complexes have been extensively characterized in Measles [64], Hendra [65,66] and Nipah [65,67] viruses. N is anchored to P via an α-helical element, which is located within the disordered N_{TAIL}. In the context of the full Measles virus nucleocapsid, regions connecting the molecular recognition element (MoRE) of N_{TAIL} to the globular domain (90 AA) remain largely dynamic [68]. The disordered character of N_{TAIL} facilitates transient interactions between the MoRE and the capsid surface, which provides spatial constraints for polymerase interactions. A series of experimental evidence support conformational heterogeneity of N_{TAIL} in complex with P [65]. Hydrophobic residues in fuzzy regions of Measles virus N_{TAIL} establish transient interactions with the target and contribute to binding affinity [69]. Highly dynamic character of N_{TAIL} was consistent with Hendra [66] and Nipah virus [67] P interactions. These were proposed to facilitate the access of the polymerase without major rearrangements of the nucleocapsid.

The hepatitis C virus (HCV) core protein only partially undergoes disorder to order transition upon multimerization, i.e. formation of the nucleocapsid. The fuzzy regions are required for a repertoire of dynamic interactions with viral RNA, membranes as well as with protein components of the viral replication machinery and of various cellular pathways [70]. HCV core functions are tightly regulated and fuzziness contributes to promiscuous interactions of this protein.

Viral motif-mimicry often involves multivalent, ambiguous interactions. The PxxP motifs of HCV non-structural protein 5A (NS5A) are recognized by SH3 domains of the Src kinase family. In addition to the canonical binding site, NS5A embeds multiple low-affinity motifs, which can interact with SH3 domains in a mutually exclusive manner [71]. Upon interacting with Bin1 SH3 domain, for example conformational heterogeneity of the non-canonical motifs increases, which provides positive entropic contribution and results in 2–3-fold increase in binding affinity. Alternative interaction patterns by multiple motifs can also help to alleviate functional constraints owing to the small genome size of the virus. The preS1 domain of hepatitis B virus (HBV) establishes multivalent, variable contacts with the cell-surface receptor γ2-adaptin, but remains largely disordered in the complex [72].

1.6. Fuzziness in enzymes

Intrinsic disorder is abundant in regulatory proteins, but generally devoid of proteins with metabolic functions. Although enzymes usually require a well-defined structure for efficient catalysis, conformational heterogeneity has been observed in a few enzyme–substrate complexes. In case of cellulase E two catalytic modules are connected by an 88 residue disordered linker, which dimensions can vary from 10 to 80 Å. Plasticity of the linker enables the catalytic modules to act independently for more efficient hydrolysis of cellulose [73]. Once hydrolysis has completed, the catalytic module can diffuse away from the substrate, while the other module still remains attached to the cellulose fiber. This results in a caterpillar-like motion, which is propelled by the dynamism of the linker region.

Thymine DNA glycosylase (TDG) removes GT and GU mismatches in the base excision repair (BER) pathway. TDG has two catalytic states ('open' and 'closed' form), which correspond to different activities. The regulatory domain (RD) of the NTD establishes dynamic interactions with the catalytic domain in the 'closed' form and non-specific, competitive interactions with double-stranded DNA in the 'open' form [74]. The RD is disordered in both the free and substrate-bound states and controls the transition between the 'open' and 'closed' conformations thereby regulating the GT and GU repair activities. In addition, the fluctuating C-terminal domain provides a steric hindrance in the complex and imparts length-dependent regulation on substrate-binding affinity.

Anhydrin is a fully intrinsically disordered plant protein, which has been identified as a novel endonuclease [75]. It was shown to remain unfolded when bound to DNA, albeit the molecular mechanism of how fuzziness contributes to DNA cleavage has not been revealed yet. Anhydrin also has a chaperone like activity, which protects aggregation of client proteins upon dehydration stress.

We must also note that modern enzymology studies are also consistent with conformational heterogeneity of enzymes. In case of chorismate mutase for example, the shallow folding landscape corresponds to alternative pathways with comparable reorganization energies [76].

1.7. Fuzzy complexes can affect protein half-life

Intrinsically disordered regions could be degraded 'by default' in a ubiquitin-independent pathway by the 20S proteasome [77]. Accordingly, the presence of long intrinsically disordered regions were shown to decrease protein half-life [78]. Furthermore, variations in the length and number of disordered segments can be utilized in evolution to generate phenotypes with different protein turnover. These results raise the question of how ID proteins can survive in vivo. A plausible mechanism is provided by the 'nanny' model, when interacting partners protect newly synthesized ID proteins by masking the disordered regions [79]. A key point of the proposal is that nannies are not chaperones as they do not fold ID proteins. Instead, disordered regions remain dynamic in nanny-client interactions.

Fuzziness of ID protein-nanny complexes is also supported by experimental data. Free IκBα is rapidly degraded via a ubiquitin-independent pathway and its half-life is less than 10 min. Binding to NFκB results in a high-affinity (picomolar) complex and increases the IκBα half-life to many hours [80]. These interactions inhibit nuclear localization of NFκB and its transcriptional activity. IκBα has 6 ankyrin repeats (ARs), out of which AR1 provides major contribution (~8 kcal/mol) to binding, while retains its dynamic character with fluctuations on the millisecond timescale [81]. AR1 dynamics is required for the tight control of the NFκB interactions, as fluctuations enable ubiquitination and subsequent degradation of IκBα and thus the NFκB release for nuclear translocation.

The C-terminal region (134–164 amino acids) of $p21^{WAF1/CIP1}$ interacts with the C8 subunit of the 20S proteasomes and promotes its degradation via ubiquitin-independent mechanism [82]. This segment overlaps with residues that interact with PCNA, binding to which results in 2 h increase in half-life of $p21^{WAF1/CIP1}$. PCNA thus masks the degradation signal for the 20S proteasome and increase stability of $p21^{WAF1/CIP1}$. In the high-affinity complex with PCNA (PDB code: 1axc) however the N-terminal region of the $p21^{WAF1/CIP1}$ peptide remains to be disordered [83]. The recognition motif for Cdk2-cyclin complex has also been proposed to inhibit proteasome binding to the degron and hence contribute to protecting $p21^{WAF1/CIP1}$.

Fuzzy complexes can also rescue ID proteins from ubiquitination and decrease turnover. The c-Myc oncoprotein has a short half-life of 20–30 min, which is tightly regulated by phosphorylation of TAD residues S62 and T58 [84]. S62 phosphorylation transiently increases cellular stability of c-Myc and stabilizes its active state. Dephosphorylation of S62 recruits SCF-Fbwx7 ubiquitin ligase and induces degradation of c-Myc. Bin1 is a negative regulator of c-Myc, which preferably binds to the S62 unphosphorylated state. The 1–88 TAD of c-Myc was shown to be dynamically disordered in complex with Bin1, which enables efficient screening of both S62 and T58 phosphorylation states and facilitates rapid dissociation of c-Myc [48].

In contrast to c-Myc, $p27^{Kip1}$ turnover is increased upon binding to Cdk2/cyclin. In this case however, enzymatic function of Cdk2 promotes phosphorylation of T187 $p27^{Kip1}$, which serves as a recognition signal for SCF^{SKP2} ubiquitin ligase [85]. Indeed, a $p27^{Kip1}$ variant which is disabled to interact with the Cdk2/cyclin has an increased half-life. Dynamics of the 100 AA C-terminal region of the bound $p27^{Kip1}$ (in complex with Cdk2/cyclin) is required for phosphorylation of T187, as it was described above [60]. Thus in this case Cdk2/cyclin facilitates ubiquitin-dependent degradation of $p27^{Kip1}$ rather than serving as a 'nanny' to protect from the degradation by the 20S proteasome.

2. Conclusion

A variety of examples illustrate that structural ambiguity can be maintained in protein complexes resulting in alternative, often transient interactions. Despite the central dogma – specificity requires a unique set of interactions – ambiguity in interaction patterns can still result in specific complexes. Furthermore, ambiguous interactions could provide manifold benefits for a range of biological functions. Alternative contacts at the binding interface improve binding entropy and thereby increase affinity [58]. Additional transient interactions, which are established by more distant regions can contribute to specificity [74]. Albeit protein disorder has recently been concluded to reduce changes in binding free energy as compared to ordered protein complexes [86], various experimental examples demonstrate that the presence of fuzzy regions increases selectivity in vivo [44,87]. In addition to modulating affinity, transient buttressing interactions often improve kinetics by serving as non-specific anchors to the target [88]. Multivalent interactions may also impart dynamisms on recognition [89], for example multisite electrostatic and aromatic interactions can drive liquid–liquid phase separation [90], formation of protein-dense droplets or hydrogels [91]. These simple interaction patterns – similarly to what was seen in sequence-independent binding of transcription factors or histone tails – can result in regulated, membraneless organelles in the cell [25], which are responsive to different cellular conditions. Taken together, the utmost benefit of fuzzy regions is the ease of regulation, by shifting the conformational equilibrium according to different environmental conditions or signals.

The concept of fuzziness is used in various fields (e.g. mathematics [92], computer science, linguistics, psychology, economy, sociology [9]) to describe that the boundaries of an application vary according to the conditions [8]. In case of disordered proteins, the degree of disorder and the propensity of different conformations can be altered in context-specific manner. Thus fuzziness, e.g. disorder in protein complexes gains a definite meaning when the context is specified. Fuzzy regions for example can be used to fine-tune affinity or alter specificity e.g. via posttranslational modifications (on/off switches or rheostats), or via alternative splicing to change the length of fuzzy regions or the embedded motifs resulting in tissue-specificity [93]. Fuzzy regions also enable combinatorial usage of motifs and thereby initiating different pathways upon different inputs. Experimental characterization of fuzzy complexes have uncovered various mechanisms by which transient interactions are mediated by bound disordered regions and how they impact biological outputs of the complex. We are however just at the beginning to explore these regime of molecular interactions, which open numerous new opportunities to interfere with functionalities of protein complexes.

Acknowledgements

The support of the Momentum program (LP2012-41) of the Hungarian Academy of Sciences and the Hungarian Science Fund program (OTKA NN 106562) is gratefully acknowledged (M.F.). M.F. is also grateful to Dr Bela Katai-Toth for fruitful discussions.

References

[1] Wright, P.E. and Dyson, H.J. (1999) Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. J. Mol. Biol. 293, 321–331.
[2] Wright, P.E. and Dyson, H.J. (2009) Linking folding and binding. Curr. Opin. Struct. Biol. 19, 31–38.
[3] Pancsa, R. and Fuxreiter, M. (2012) Interactions via intrinsically disordered regions: what kind of motifs? IUBMB Life 64, 513–520.

[4] Fuxreiter, M., Simon, I., Friedrich, P. and Tompa, P. (2004) Preformed structural elements feature in partner recognition by intrinsically unstructured proteins. J. Mol. Biol. 338, 1015–1026.
[5] Oldfield, C.J., Cheng, Y., Cortese, M.S., Romero, P., Uversky, V.N. and Dunker, A.K. (2005) Coupled folding and binding with alpha-helix-forming molecular recognition elements. Biochemistry 44, 12454–12470.
[6] Fuxreiter, M. (2012) Fuzziness: linking regulation to protein dynamics. Mol. BioSyst. 8, 168–177.
[7] Tompa, P. and Fuxreiter, M. (2008) Fuzzy complexes: polymorphism and structural disorder in protein–protein interactions. Trends Biochem. Sci. 33, 2–8.
[8] Haack, S. (1996) Deviant logic, fuzzy logic: beyond the formalism, University of Chicago Press, Chicago.
[9] Tanaka, K. (1996) An Introduction to Fuzzy Logic for Practical Applications, Springer.
[10] Fuxreiter, M. and Tompa, P. (2012) Fuzzy complexes: a more stochastic view of protein function. Adv. Exp. Med. Biol. 725, 1–14.
[11] Fuxreiter, M. and Tompa, P. (2011) Fuzziness: Structural Disorder in Protein Complexes Landes BioScience/Springer, Austin, New York.
[12] Renault, L., Bugyi, B. and Carlier, M.F. (2008) Spire and Cordon-bleu: multifunctional regulators of actin dynamics. Trends Cell Biol. 18, 494–504.
[13] Clerici, M. et al. (2009) Unusual bipartite mode of interaction between the nonsense-mediated decay factors, UPF1 and UPF2. EMBO J. 28, 2293–2306.
[14] Billeter, M., Qian, Y.Q., Otting, G., Muller, M., Gehring, W. and Wuthrich, K. (1993) Determination of the nuclear magnetic resonance solution structure of an Antennapedia homeodomain–DNA complex. J. Mol. Biol. 234, 1084–1093.
[15] Pometun, M.S., Chekmenev, E.Y. and Wittebort, R.J. (2004) Quantitative observation of backbone disorder in native elastin. J. Biol. Chem. 279, 7982–7987.
[16] Fontes, M.R., Teh, T. and Kobe, B. (2000) Structural basis of recognition of monopartite and bipartite nuclear localization sequences by mammalian importin-alpha. J. Mol. Biol. 297, 1183–1194.
[17] Cliff, M.J., Harris, R., Barford, D., Ladbury, J.E. and Williams, M.A. (2006) Conformational diversity in the TPR domain-mediated interaction of protein phosphatase 5 with Hsp90. Structure 14, 415–426.
[18] Sigalov, A., Aivazian, D. and Stern, L. (2004) Homooligomerization of the cytoplasmic domain of the T cell receptor zeta chain and of other proteins containing the immunoreceptor tyrosine-based activation motif. Biochemistry 43, 2049–2061.
[19] Nourse, A. and Mittag, T. (2014) The cytoplasmic domain of the T-cell receptor zeta subunit does not form disordered dimers. J. Mol. Biol. 426, 62–70.
[20] Mittag, T. et al. (2008) Dynamic equilibrium engagement of a polyvalent ligand with a single-site receptor. Proc. Natl. Acad. Sci. U.S.A. 105, 17772–17777.
[21] Davey, N.E. et al. (2012) Attributes of short linear motifs. Mol. BioSyst. 8, 268–281.
[22] Hope, I.A., Mahadevan, S. and Struhl, K. (1988) Structural and functional characterization of the short acidic transcriptional activation region of yeast GCN4 protein. Nature 333, 635–640.
[23] Ma, J. and Ptashne, M. (1987) Deletion analysis of GAL4 defines two transcriptional activating segments. Cell 48, 847–853.
[24] Lu, X., Hamkalo, B., Parseghian, M.H. and Hansen, J.C. (2009) Chromatin condensing functions of the linker histone C-terminal domain are mediated by specific amino acid composition and intrinsic protein disorder. Biochemistry 48, 164–172.
[25] Nott, T.J. et al. (2015) Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles. Mol. Cell 57, 936–947.
[26] Fuxreiter, M., Simon, I. and Bondos, S. (2011) Dynamic protein–DNA recognition: beyond what can be seen. Trends Biochem. Sci. 36, 415–423.
[27] Adams, V.H., McBryant, S.J., Wade, P.A., Woodcock, C.L. and Hansen, J.C. (2007) Intrinsic disorder and autonomous domain function in the multifunctional nuclear protein, MeCP2. J. Biol. Chem. 282, 15057–15064.
[28] Ghosh, R.P., Nikitina, T., Horowitz-Scherer, R.A., Gierasch, L.M., Uversky, V.N., Hite, K., Hansen, J.C. and Woodcock, C.L. (2010) Unique physical properties and interactions of the domains of methylated DNA binding protein 2. Biochemistry 49, 4395–4410.
[29] Pufall, M.A., Lee, G.M., Nelson, M.L., Kang, H.S., Velyvis, A., Kay, L.E., McIntosh, L.P. and Graves, B.J. (2005) Variable control of Ets-1 DNA binding by multiple phosphates in an unstructured region. Science 309, 142–145.
[30] Lee, G.M., Pufall, M.A., Meeker, C.A., Kang, H.S., Graves, B.J. and McIntosh, L.P. (2008) The affinity of Ets-1 for DNA is modulated by phosphorylation through transient interactions of an unstructured region. J. Mol. Biol. 382, 1014–1030.
[31] Stott, K., Watson, M., Howe, F.S., Grossmann, J.G. and Thomas, J.O. (2010) Tail-mediated collapse of HMGB1 is dynamic and occurs via differential binding of the acidic tail to the A and B domains. J. Mol. Biol. 403, 706–722.
[32] Daughdrill, G.W., Narayanaswami, P., Gilmore, S.H., Belczyk, A. and Brown, C.J. (2007) Dynamic behavior of an intrinsically unstructured linker domain is conserved in the face of negligible amino acid sequence conservation. J. Mol. Evol. 65, 277–288.
[33] Vise, P.D., Baral, B., Latos, A.J. and Daughdrill, G.W. (2005) NMR chemical shift and relaxation measurements provide evidence for the coupled folding and binding of the p53 transactivation domain. Nucleic Acids Res. 33, 2061–2077.
[34] Fuxreiter, M., Tompa, P., Simon, I., Uversky, V.N., Hansen, J.C. and Asturias, F.J. (2008) Malleable machines take shape in eukaryotic transcriptional regulation. Nat. Chem. Biol. 4, 728–737.
[35] Hansen, J.C., Lu, X., Ross, E.D. and Woody, R.W. (2006) Intrinsic protein disorder, amino acid composition, and histone terminal domains. J. Biol. Chem. 281, 1853–1856.
[36] Tsunaka, Y., Toga, J., Yamaguchi, H., Tate, S., Hirose, S. and Morikawa, K. (2009) Phosphorylated intrinsically disordered region of FACT masks its nucleosomal DNA binding elements. J. Biol. Chem. 284, 24610–24621.
[37] Pursglove, S.E., Fladvad, M., Bellanda, M., Moshref, A., Henriksson, M., Carey, J. and Sunnerhagen, M. (2004) Biophysical properties of regions flanking the bHLH-Zip motif in the p22 Max protein. Biochem. Biophys. Res. Commun. 323, 750–759.
[38] Liu, Y., Matthews, K.S. and Bondos, S.E. (2008) Multiple intrinsically disordered sequences alter DNA binding by the homeodomain of the Drosophila hox protein ultrabithorax. J. Biol. Chem. 283, 20874–20887.
[39] van Leeuwen, H.C., Strating, M.J., Rensen, M., de Laat, W. and van der Vliet, P.C. (1997) Linker length and composition influence the flexibility of Oct-1 DNA binding. EMBO J. 16, 2043–2053.
[40] Ju, J.H., Maeng, J.S., Lee, D.Y., Piszczek, G., Gelmann, E.P. and Gruschus, J.M. (2009) Interactions of the acidic domain and SRF interacting motifs with the NKX3.1 homeodomain. Biochemistry 48, 10601–10607.
[41] Ng, K.P., Potikyan, G., Savene, R.O., Denny, C.T., Uversky, V.N. and Lee, K.A. (2007) Multiple aromatic side chains within a disordered structure are critical for transcription and transforming activity of EWS family oncoproteins. Proc. Natl. Acad. Sci. U.S.A. 104, 479–484.
[42] Desjardins, G., Meeker, C.A., Bhachech, N., Currie, S.L., Okon, M., Graves, B.J. and McIntosh, L.P. (2014) Synergy of aromatic residues and phosphoserines within the intrinsically disordered DNA-binding inhibitory elements of the Ets-1 transcription factor. Proc. Natl. Acad. Sci. U.S.A. 111, 11019–11024.
[43] Song, J., Ng, S.C., Tompa, P., Lee, K.A. and Chan, H.S. (2013) Polycation-pi interactions are a driving force for molecular recognition by an intrinsically disordered oncoprotein family. PLoS Comput. Biol. 9, e1003239.
[44] Liu, Y., Matthews, K.S. and Bondos, S.E. (2009) Internal regulatory interactions determine DNA binding specificity by a Hox transcription factor. J. Mol. Biol. 390, 760–774.
[45] Lee, C.W., Martinez-Yamout, M.A., Dyson, H.J. and Wright, P.E. (2010) Structure of the p53 transactivation domain in complex with the nuclear receptor coactivator binding domain of CREB binding protein. Biochemistry 49, 9964–9971.
[46] Brzovic, P.S. et al. (2011) The acidic transcription activator Gcn4 binds the mediator subunit Gal11/Med15 using a simple protein interface forming a fuzzy complex. Mol. Cell 44, 942–953.
[47] Warfield, L., Tuttle, L.M., Pacheco, D., Klevit, R.E. and Hahn, S. (2014) A sequence-specific transcription activator motif and powerful synthetic variants that bind Mediator using a fuzzy protein interface. Proc. Natl. Acad. Sci. U.S.A. 111, E3506–E3513.
[48] Andresen, C. et al. (2012) Transient structure and dynamics in the disordered c-Myc transactivation domain affect Bin1 binding. Nucleic Acids Res. 40, 6353–6366.
[49] Taatjes, D.J., Schneider-Poetsch, T. and Tjian, R. (2004) Distinct conformational states of nuclear receptor-bound CRSP-Med complexes. Nat. Struct. Mol. Biol. 11, 664–671.
[50] Ebmeier, C.C. and Taatjes, D.J. (2010) Activator-Mediator binding regulates Mediator-cofactor interactions. Proc. Natl. Acad. Sci. U.S.A. 107, 11283–11288.
[51] Meyer, K.D., Lin, S.C., Bernecky, C., Gao, Y. and Taatjes, D.J. (2010) P53 activates transcription by directing structural shifts in Mediator. Nat. Struct. Mol. Biol. 17, 753–760.
[52] Cai, G., Chaban, Y.L., Imasaki, T., Kovacs, J.A., Calero, G., Penczek, P.A., Takagi, Y. and Asturias, F.J. (2012) Interaction of the mediator head module with RNA polymerase II. Structure 20, 899–910.
[53] Bernecky, C., Grob, P., Ebmeier, C.C., Nogales, E. and Taatjes, D.J. (2011) Molecular architecture of the human Mediator-RNA polymerase II-TFIIF assembly. PLoS Biol. 9, e1000603.
[54] Gibson, T.J. (2009) Cell regulation: determined to signal discrete cooperation. Trends Biochem. Sci. 34, 471–482.
[55] Fuxreiter, M., Tompa, P. and Simon, I. (2007) Local structural disorder imparts plasticity on linear motifs. Bioinformatics 23, 950–956.
[56] Bhattacharyya, R.P., Remenyi, A., Good, M.C., Bashor, C.J., Falick, A.M. and Lim, W.A. (2006) The Ste5 scaffold allosterically modulates signaling output of the yeast mating pathway. Science 311, 822–826.
[57] Huber, A.H. and Weis, W.I. (2001) The structure of the beta-catenin/E-cadherin complex and the molecular basis of diverse ligand recognition by beta-catenin. Cell 105, 391–402.
[58] Graham, T.A., Ferkey, D.M., Mao, F., Kimelman, D. and Xu, W. (2001) Tcf4 can specifically recognize beta-catenin using alternative conformations. Nat. Struct. Biol. 8, 1048–1052.
[59] Wang, Y. et al. (2011) Intrinsic disorder mediates the diverse regulatory functions of the Cdk inhibitor p21. Nat. Chem. Biol. 7, 214–221.
[60] Galea, C.A., Nourse, A., Wang, Y., Sivakolundu, S.G., Heller, W.T. and Kriwacki, R.W. (2008) Role of intrinsic flexibility in signal transduction mediated by the cell cycle regulator, p27 Kip1. J. Mol. Biol. 376, 827–838.
[61] Borg, M., Mittag, T., Pawson, T., Tyers, M., Forman-Kay, J.D. and Chan, H.S. (2007) Polyelectrostatic interactions of disordered ligands suggest a physical basis for ultrasensitivity. Proc. Natl. Acad. Sci. U.S.A. 104, 9650–9655.

[62] Didry, D. et al. (2011) How a single residue in individual beta-thymosin/WH2 domains controls their functions in actin assembly. EMBO J..
[63] Ahmed, M.A., Bamm, V.V., Shi, L., Steiner-Mosonyi, M., Dawson, J.F., Brown, L., Harauz, G. and Ladizhansky, V. (2009) Induced secondary structure and polymorphism in an intrinsically disordered structural linker of the CNS: solid-state NMR and FTIR spectroscopy of myelin basic protein bound to actin. Biophys. J. 96, 180–191.
[64] Bourhis, J.M. et al. (2005) The intrinsically disordered C-terminal domain of the measles virus nucleoprotein interacts with the C-terminal domain of the phosphoprotein via two distinct sites and remains predominantly unfolded. Protein Sci. 14, 1975–1992.
[65] Habchi, J. et al. (2011) Characterization of the interactions between the nucleoprotein and the phosphoprotein of Henipavirus. J. Biol. Chem. 286, 13583–13602.
[66] Communie, G. et al. (2013) Atomic resolution description of the interaction between the nucleoprotein and phosphoprotein of Hendra virus. PLoS Pathog. 9, e1003631.
[67] Baronti, L., Erales, J., Habchi, J., Felli, I.C., Pierattelli, R. and Longhi, S. (2015) Dynamics of the intrinsically disordered C-terminal domain of the nipah virus nucleoprotein and interaction with the x domain of the phosphoprotein as unveiled by NMR spectroscopy. ChemBioChem 16, 268–276.
[68] Jensen, M.R. et al. (2011) Intrinsic disorder in measles virus nucleocapsids. Proc. Natl. Acad. Sci. U.S.A. 108, 9839–9844.
[69] D'Urzo, A. et al. (2015) Molecular basis for structural heterogeneity of an intrinsically disordered protein bound to a partner by combined ESI-IM–MS and modeling. J. Am. Soc. Mass Spectrom. 26, 472–481.
[70] Ivanyi-Nagy, R. and Darlix, J.L. (2012) Fuzziness in the core of the human pathogenic viruses HCV and HIV. Adv. Exp. Med. Biol. 725, 142–158.
[71] Schwarten, M., Solyom, Z., Feuerstein, S., Aladag, A., Hoffmann, S., Willbold, D. and Brutscher, B. (2013) Interaction of nonstructural protein 5A of the hepatitis C virus with Src homology 3 domains using noncanonical binding sites. Biochemistry 52, 6160–6168.
[72] Jurgens, M.C. et al. (2013) The hepatitis B virus preS1 domain hijacks host trafficking proteins by motif mimicry. Nat. Chem. Biol. 9, 540–547.
[73] von Ossowski, I. et al. (2005) Protein disorder: conformational distribution of the flexible linker in a chimeric double cellulase. Biophys. J. 88, 2823–2832.
[74] Smet-Nocca, C., Wieruszeski, J.M., Chaar, V., Leroy, A. and Benecke, A. (2008) The thymine–DNA glycosylase regulatory domain: residual structure and DNA binding. Biochemistry 47, 6519–6530.
[75] Chakrabortee, S., Meersman, F., Kaminski Schierle, G.S., Bertoncini, C.W., McGee, B., Kaminski, C.F. and Tunnacliffe, A. (2010) Catalytic and chaperone-like functions in an intrinsically disordered protein associated with desiccation tolerance. Proc. Natl. Acad. Sci. U.S.A. 107, 16084–16089.
[76] Roca, M., Messer, B., Hilvert, D. and Warshel, A. (2008) On the relationship between folding and chemical landscapes in enzyme catalysis. Proc. Natl. Acad. Sci. U.S.A. 105, 13877–13882.
[77] Asher, G., Reuven, N. and Shaul, Y. (2006) 20S proteasomes and protein degradation "by default". BioEssays 28, 844–849.
[78] van der Lee, R. et al. (2014) Intrinsically disordered segments affect protein half-life in the cell and during evolution. Cell Rep 8, 1832–1844.
[79] Tsvetkov, P., Reuven, N. and Shaul, Y. (2009) The nanny model for IDPs. Nat. Chem. Biol. 5, 778–781.
[80] Bergqvist, S., Croy, C.H., Kjaergaard, M., Huxford, T., Ghosh, G. and Komives, E.A. (2006) Thermodynamics reveal that helix four in the NLS of NF-kappaB p65 anchors IkappaBalpha, forming a very stable complex. J. Mol. Biol. 360, 421–434.
[81] Lamboy, J.A., Kim, H., Dembinski, H., Ha, T. and Komives, E.A. (2013) Single-molecule FRET reveals the native-state dynamics of the IkappaBalpha ankyrin repeat domain. J. Mol. Biol. 425, 2578–2590.
[82] Touitou, R., Richardson, J., Bose, S., Nakanishi, M., Rivett, J. and Allday, M.J. (2001) A degradation signal located in the C-terminus of p21WAF1/CIP1 is a binding site for the C8 alpha-subunit of the 20S proteasome. EMBO J. 20, 2367–2375.
[83] Gulbis, J.M., Kelman, Z., Hurwitz, J., O'Donnell, M. and Kuriyan, J. (1996) Structure of the C-terminal region of p21(WAF1/CIP1) complexed with human PCNA. Cell 87, 297–306.
[84] Yeh, E. et al. (2004) A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. Nat. Cell Biol. 6, 308–318.
[85] Sabile, A., Meyer, A.M., Wirbelauer, C., Hess, D., Kogel, U., Scheffner, M. and Krek, W. (2006) Regulation of p27 degradation and S-phase progression by Ro52 RING finger protein. Mol. Cell. Biol. 26, 5994–6004.
[86] Huang, Y. and Liu, Z. (2013) Do intrinsically disordered proteins possess high specificity in protein–protein interactions? Chemistry 19, 4462–4467.
[87] Wang, Y. et al. (2011) Intrinsic disorder mediates the diverse regulatory functions of the Cdk inhibitor p21. Nat. Chem. Biol. 7, 214–221.
[88] Toth-Petroczy, A., Simon, I., Fuxreiter, M. and Levy, Y. (2009) Disordered tails of homeodomains facilitate DNA recognition by providing a trade-off between folding and specific binding. J. Am. Chem. Soc. 131, 15084–15085.
[89] Chen, T., Song, J. and Chan, H.S. (2015) Theoretical perspectives on nonnative interactions and intrinsic disorder in protein folding and binding. Curr. Opin. Struct. Biol. 30, 32–42.
[90] Li, P. et al. (2012) Phase transitions in the assembly of multivalent signalling proteins. Nature 483, 336–340.
[91] Kato, M. et al. (2012) Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. Cell 149, 753–767.
[92] Kaushal, P., Mohan, N., Parvinder, S. and Sadhu, S. (2010) Relevancy of fuzzy concept in mathematics. Inter. J. Innov. Manag. Technol. 1, 312–315.
[93] Buljan, M., Chalancon, G., Eustermann, S., Wagner, G.P., Fuxreiter, M., Bateman, A. and Babu, M.M. (2012) Tissue-specific splicing of disordered segments that embed binding motifs rewires protein interaction networks. Mol. Cell 46, 871–883.
[94] Desai, M.A., Webb, H.D., Sinanan, L.M., Scarsdale, J.N., Walavalkar, N.M., Ginder, G.D. and Williams Jr., D.C. (2015) An intrinsically disordered region of methyl-CpG binding domain protein 2 (MBD2) recruits the histone deacetylase core of the NuRD complex. Nucleic Acids Res. 43, 3100–3113.
[95] Naud, J.F., McDuff, F.O., Sauve, S., Montagne, M., Webb, B.A., Smith, S.P., Chabot, B. and Lavigne, P. (2005) Structural and thermodynamical characterization of the complete p21 gene product of Max. Biochemistry 44, 12746–12758.
[96] Liu, J. and Song, J. (2008) A novel nucleolar transcriptional activator ApLLP for long-term memory formation is intrinsically unstructured but functionally active. Biochem. Biophys. Res. Commun. 366, 585–591.
[97] Aguado-Llera, D., Goormaghtigh, E., de Geest, N., Quan, X.J., Prieto, A., Hassan, B.A., Gomez, J. and Neira, J.L. (2010) The basic helix-loop-helix region of human neurogenin 1 is a monomeric natively unfolded protein which forms a "fuzzy" complex upon DNA binding. Biochemistry 49, 1577–1589.
[98] Fukutomi, T., Takagi, K., Mizushima, T., Ohuchi, N. and Yamamoto, M. (2014) Kinetic, thermodynamic, and structural characterizations of the association between Nrf2-DLGex degron and Keap1. Mol. Cell. Biol. 34, 832–846.
[99] Khan, H., Cino, E.A., Brickenden, A., Fan, J., Yang, D. and Choy, W.Y. (2013) Fuzzy complex formation between the intrinsically disordered prothymosin alpha and the Kelch domain of Keap1 involved in the oxidative stress response. J. Mol. Biol. 425, 1011–1027.
[100] Mukherjee, S.P., Behar, M., Birnbaum, H.A., Hoffmann, A., Wright, P.E. and Ghosh, G. (2013) Analysis of the RelA:CBP/p300 interaction reveals its involvement in NF-kappaB-driven transcription. PLoS Biol. 11, e1001647.
[101] Radhakrishnan, I., Perez-Alvarado, G.C., Parker, D., Dyson, H.J., Montminy, M.R. and 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.
[102] Zor, T., Mayr, B.M., Dyson, H.J., Montminy, M.R. and 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.
[103] Gill, G., Pascal, E., Tseng, Z.H. and Tjian, R. (1994) A glutamine-rich hydrophobic patch in transcription factor Sp1 contacts the dTAFII110 component of the Drosophila TFIID complex and mediates transcriptional activation. Proc. Natl. Acad. Sci. U.S.A. 91, 192–196.
[104] Sigler, P.B. (1988) Transcriptional activation. Acid blobs and negative noodles. Nature 333, 210–212.
[105] Jonker, H.R., Wechselberger, R.W., Boelens, R., Kaptein, R. and Folkers, G.E. (2006) The intrinsically unstructured domain of PC4 modulates the activity of the structured core through inter- and intramolecular interactions. Biochemistry 45, 5067–5081.
[106] Chandra, V., Huang, P., Hamuro, Y., Raghuram, S., Wang, Y., Burris, T.P. and Rastinejad, F. (2008) Structure of the intact PPAR-gamma-RXR-nuclear receptor complex on DNA. Nature 456, 350–356.
[107] Fontes, M.R., Teh, T., Toth, G., John, A., Pavo, I., Jans, D.A. and Kobe, B. (2003) Role of flanking sequences and phosphorylation in the recognition of the simian-virus-40 large T-antigen nuclear localization sequences by importin-alpha. Biochem. J. 375, 339–349.
[108] Kinkelin, K., Veith, K., Grunwald, M. and Bono, F. (2012) Crystal structure of a minimal eIF4E-Cup complex reveals a general mechanism of eIF4E regulation in translational repression. RNA 18, 1624–1634.
[109] Proudfoot, N.J., Furger, A. and Dye, M.J. (2002) Integrating mRNA processing with transcription. Cell 108, 501–512.
[110] Selenko, P., Gregorovic, G., Sprangers, R., Stier, G., Rhani, Z., Krämer, A. and Sattler, M. (2003) Structural basis for the molecular recognition between human splicing factors U2AF65 and SF1/mBBP. Mol. Cell 11, 965–976.
[111] Lukhele, S., Bah, A., Lin, H., Sonenberg, N. and Forman-Kay, J.D. (2013) Interaction of the eukaryotic initiation factor 4E with 4E-BP2 at a dynamic bipartite interface. Structure 21, 2186–2196.
[112] Mulder, F.A., Bouakaz, L., Lundell, A., Venkataramana, M., Liljas, A., Akke, M. and Sanyal, S. (2004) Conformation and dynamics of ribosomal stalk protein L12 in solution and on the ribosome. Biochemistry 43, 5930–5936.
[113] Bubunenko, M.G., Chuikov, S.V. and Gudkov, A.T. (1992) The length of the interdomain region of the L7/L12 protein is important for its function. FEBS Lett. 313, 232–234.
[114] Simon, S.M., Sousa, F.J., Mohana-Borges, R. and Walker, G.C. (2008) Regulation of Escherichia coli SOS mutagenesis by dimeric intrinsically disordered umuD gene products. Proc. Natl. Acad. Sci. U.S.A. 105, 1152–1157.
[115] Manelyte, L., Guy, C.P., Smith, R.M., Dillingham, M.S., McGlynn, P. and Savery, N.J. (2009) The unstructured C-terminal extension of UvrD interacts with UvrB, but is dispensable for nucleotide excision repair. DNA Repair (Amst) 8, 1300–1310.
[116] Nagulapalli, M. et al. (2012) Recognition pliability is coupled to structural heterogeneity: a calmodulin intrinsically disordered binding region complex. Structure 20, 522–533.
[117] Yu, H., Chen, J.K., Feng, S., Dalgarno, D.C., Brauer, A.W. and Schreiber, S.L. (1994) Structural basis for the binding of proline-rich peptides to SH3 domains. Cell 76, 933–945.

[118] Park, I.K. and DePaoli-Roach, A.A. (1994) Domains of phosphatase inhibitor-2 involved in the control of the ATP-Mg-dependent protein phosphatase. J. Biol. Chem. 269, 28919–28928.
[119] Hurley, T.D., Yang, J., Zhang, L., Goodwin, K.D., Zou, Q., Cortese, M., Dunker, A.K. and DePaoli-Roach, A.A. (2007) Structural basis for regulation of protein phosphatase 1 by inhibitor-2. J. Biol. Chem. 282, 28874–28883.
[120] Nyarko, A., Song, Y. and Barbar, E. (2012) Intrinsic disorder in dynein intermediate chain modulates its interactions with NudE and dynactin. J. Biol. Chem. 287, 24884–24893.
[121] Ferreon, A.C., Ferreon, J.C., Wright, P.E. and Deniz, A.A. (2013) Modulation of allostery by protein intrinsic disorder. Nature 498, 390–394.
[122] Goyal, S., Gupta, G., Qin, H., Upadya, M.H., Tan, Y.J., Chow, V.T. and Song, J. (2012) VAPC, an human endogenous inhibitor for hepatitis C virus (HCV) infection, is intrinsically unstructured but forms a "fuzzy complex" with HCV NS5B. PLoS One 7, e40341.
[123] Shi, W. et al. (2008) A filovirus-unique region of Ebola virus nucleoprotein confers aberrant migration and mediates its incorporation into virions. J. Virol. 82, 6190–6199.
[124] Kover, K.E., Bruix, M., Santoro, J., Batta, G., Laurents, D.V. and Rico, M. (2008) The solution structure and dynamics of human pancreatic ribonuclease determined by NMR spectroscopy provide insight into its remarkable biological activities and inhibition. J. Mol. Biol. 379, 953–965.
[125] Lin, Y.P., Greenwood, A., Nicholson, L.K., Sharma, Y., McDonough, S.P. and Chang, Y.F. (2009) Fibronectin binds to and induces conformational change in a disordered region of leptospiral immunoglobulin-like protein B. J. Biol. Chem. 284, 23547–23557.
[126] Schwarz-Linek, U., Pilka, E.S., Pickford, A.R., Kim, J.H., Hook, M., Campbell, I.D. and Potts, J.R. (2004) High affinity streptococcal binding to human fibronectin requires specific recognition of sequential f1 modules. J. Biol. Chem. 279, 39017–39025.
[127] Zhuo, Y., Ilangovan, U., Schirf, V., Demeler, B., Sousa, R., Hinck, A.P. and Lafer, E.M. (2010) Dynamic interactions between clathrin and locally structured elements in a disordered protein mediate clathrin lattice assembly. J. Mol. Biol. 404, 274–290.
[128] Lindner, R.A. et al. (2000) Mouse Hsp25, a small shock protein. The role of its C-terminal extension in oligomerization and chaperone action. Eur. J. Biochem. 267, 1923–1932.
[129] Carver, J.A., Esposito, G., Schwedersky, G. and Gaestel, M. (1995) 1H NMR spectroscopy reveals that mouse Hsp25 has a flexible C-terminal extension of 18 amino acids. FEBS Lett. 369, 305–310.
[130] Lindner, R.A., Kapur, A., Mariani, M., Titmuss, S.J. and Carver, J.A. (1998) Structural alterations of alpha-crystallin during its chaperone action. Eur. J. Biochem. 258, 170–183.
[131] Smulders, R., Carver, J.A., Lindner, R.A., van Boekel, M.A., Bloemendal, H. and de Jong, W.W. (1996) Immobilization of the C-terminal extension of bovine alphaA-crystallin reduces chaperone-like activity. J. Biol. Chem. 271, 29060–29066.
[132] Ross, E.D., Edskes, H.K., Terry, M.J. and Wickner, R.B. (2005) Primary sequence independence for prion formation. Proc. Natl. Acad. Sci. U.S.A. 102, 12825–12830.
[133] Ross, E.D., Baxa, U. and Wickner, R.B. (2004) Scrambled prion domains form prions and amyloid. Mol. Cell. Biol. 24, 7206–7213.
[134] Fusco, G., De Simone, A., Gopinath, T., Vostrikov, V., Vendruscolo, M., Dobson, C.M. and Veglia, G. (2014) Direct observation of the three regions in alpha-synuclein that determine its membrane-bound behaviour. Nat. Commun. 5, 3827.
[135] Marsh, J.A., Dancheck, B., Ragusa, M.J., Allaire, M., Forman-Kay, J.D. and Peti, W. (2010) Structural diversity in free and bound states of intrinsically disordered protein phosphatase 1 regulators. Structure 18, 1094–1103.