Accepted Manuscript

Fuzziness in protein interactions – a historical perspective

Monika Fuxreiter

Please cite this article as: Monika Fuxreiter , Fuzziness in protein interactions – a historical perspective. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Yjmbi(2018), doi:10.1016/j.jmb.2018.02.015

Fuzziness in protein interactions – a historical perspective

Monika Fuxreiter
MTA-DE Laboratory of Protein Dynamics, Department of Biochemistry and Molecular Biology, University of Debrecen, Hungary
Correspondance: fmoni@med.unideb.hu

Abstract

The proposal that coupled folding to binding is not an obligatory mechanism for intrinsically disordered (ID) proteins was put forward 10 years ago. The notion of fuzziness implies that conformational heterogeneity can be maintained upon interactions of ID proteins, which has a functional impact either on regulated assembly or activity of the corresponding complexes. Here I review how the concept has evolved in the past decade, via increasing experimental data providing insights into the mechanisms, pathways and regulatory modes. The effects of structural diversity and transient contacts on protein assemblies have been collected and systematically analyzed (FuzDB, http://protdyn-database.org). Fuzziness has also been exploited as a framework to decipher molecular organization of higher-order protein structures. Quantification of conformational heterogeneity opens exciting future perspectives for drug discovery from small molecule-ID protein interactions to supramolecular assemblies.

Introduction

Understanding of interactions mediated by intrinsically disordered (ID) protein regions has gone through various stages of metamorphosis, from models with structured interfaces to complexes with largely heterogeneous, dynamic contacts. Advances in technology, especially in characterization of ID protein ensembles [1] provided more detailed insights into coordinated action and large-scale organization of ID proteins. In parallel, the concept of fuzziness has evolved to provide a framework for assemblies of ID proteins and their regulation by the cellular context. I review the different phases of the metamorphic process from the birth of the concept to its future perspectives.

Phase 0 – Partner recognition by ID proteins

Structural versatility of ID proteins is exploited in numerous regulatory pathways, gene-expression, cell-cycle regulation and signalling [2, 3]. These control mechanisms rely on interactions between the ID protein and its selected partner, another protein, DNA, RNA, or small metabolite. As ID proteins are represented by conformational ensembles in their native states, questions arise regarding the nature and specificity of these interactions. The first proposals limited conformational diversity to the free/unbound forms of intrinsically or natively unstructured proteins and expected a well-defined structure in the bound state [4, 5]. Termed as *coupled folding to binding*, complexes of ID proteins lose their conformational

heterogeneity as compared to their solution state [6]. This view was supported by a series of protein-peptide complexes, where the structure of an ID protein segment could be determined [7, 8]. In most cases however, the visible/observed regions were relatively short (i.e. peptides), which may not comprised the functionally relevant part of the ID protein [9], neither represented the form in the cellular context [10].

What are the factors governing binding and folding in this scenario? How does the ID protein overcome the entropic penalty of structuring? Is low-affinity an obligate consequence of the entropy loss accompanying the interactions with the partner? All these questions require a comparison of ID protein ensembles in their free forms to their structures in the bound state. Theoretical as well as experimental studies indicated that ID proteins are not random in their unbound forms [11, 12], and may possess preorganized secondary structures. Predicted secondary structure preferences of ID proteins were found to be highly correlated to those, which were observed in their complexes [13] suggesting a conformational selection mechanism. Termed as *preformed structural elements* [13], transiently populated secondary structures may represent the binding competent state towards which partner interactions shift conformational equilibrium [14] (Figure 1). Obviously, pre-structuring also lowers the entropic penalty of binding.

Preformed structural elements are straightforward to predict using secondary structure prediction algorithms [13, 15]. The ease of computations initiated a proliferation of models with a variety of names: molecular recognition elements (MoREs, [16]), molecular recognition features (MoRFs, [17]) and pre-structured motifs (PreSMos, [18]), which all are recapitulations of the original proposal. One must be very careful however, as the underlying databases may lack the required experimental evidence for the folding event [19]. Neglecting the transient nature of the preorganized elements in these models led to the overappreciation of structured states in complexes of ID proteins.

An alternative binding mechanism emerged from short, low-complexity motifs, which have been known for long to mediate protein interactions (e.g. SH3 domains are frequently targeted by proline-rich peptides) [20]. Termed as *linear motifs* (LMs), a few amino acid residues could determine partner selection [21] (Figure 1). Owing to their size and composition, LMs may not fall into regular secondary structures [22]. Despite being enriched in hydrophobic residues, the function of LMs are linked to intrinsically disordered environments [22]. This notion led to increased appreciation of LMs, which are often considered as recognition elements of ID proteins [23]. LM-based interactions however are difficult to reconcile using only the folding coupled to binding model, as they often establish heterogeneous contacts via multiple states.

Phase 1 – Proposal of fuzziness in protein interactions

The proposal of preformed structural elements, implied another interesting observation. Sequences of ID regions, which were predicted to fall outside the allowed regions of the Ramachandran map also preserved this feature upon interactions, possibly suggesting that disorder is maintained in complexes [13]. With my collaborator, Peter Tompa, we investigated this possibility and were seeking for protein complexes with considerable degree of conformational heterogeneity. As an experimental evidence, we searched for missing electron densities in crystal structures or large variations in the NMR models of ID protein assemblies as well as the lack of considerable shifts in the NMR

spectrum as compared to the unbound state. Two types of structural heterogeneity were considered (Figure 1): the same interacting elements fold into alternative structures upon binding (polymorphism)[24] or a rapid conformational exchange is observed in the bound state (dynamic disorder) [25].

Termed as a *fuzzy complex*, structural ambiguity is maintained upon protein-protein interactions (original definition in [26]). Protein complexes with structural multiplicity or dynamic disorder exhibit a structural and dynamical continuum [26]. The static or dynamic nature of these assemblies obviously depends on the spatial and temporal resolution of the applied experimental technique. Topologically, dynamically disordered regions may flank or link structured binding regions or host interaction motifs. The proposal of fuzzy complexes extended the disordered state from individual proteins to protein complexes.

Phase 2 – Functional implications of fuzziness

What is the functional consequence of fuzziness in the interactome? Structural heterogeneity in protein complexes may enable unique constellations, such as interactions with alternative partners simultaneously or consecutively, strong influence of posttranslational modifications (PTMs) on binding as well as may weaken the sequence constraints for specific partnerships [27]. The latter is reflected by the resistance of ID regions, such as transactivator domains [28, 29], histone tails [30, 31] or yeast prions [32, 33] to scrambling or truncation also referred to as 'sequence independence'. Heterogeneity may impart dynamism on interaction networks leading to considerable, yet realistic uncertanities in experimental data [34].

Deriving relationships between conformational ensembles and biological roles of a protein complex requires quantification of the functional contribution by structurally heterogeneous regions [27, 35]. To this aim, we searched for biochemical data on manipulating (mutating, truncating or removing) polymorphic or disordered regions and the impact on K_D, specificity, transcriptional or enzymatic activity, half-life or any other relevant functional properties. Linking structural to biochemical evidence was tedious, as most studies did not explicitly addressed the roles of disordered regions. Nevertheless, 36 examples were found with functional relevance for protein disorder in protein complexes [27], which are involved in gene-expression, virus replication, immune response or signalling [36]. These observations led to the recognition that conformational heterogeneity in the bound state of ID regions may be directly linked to the biological activity of the assembly (Figure 1).

Phase 3 – Mechanisms of fuzzy interactions

Although their biological importance was compelling, the modes by which fuzzy regions influence protein assemblies still have remained rather unclear. Detailed structural and biochemical characterization of protein-DNA interactions offered examples to elucidate the molecular mechanisms of partner recognition by heterogeneous complexes [37]. Although fuzzy regions were highly dynamic and often invisible in structures of the protein-DNA assemblies, experimental evidence demonstrated their functional contribution in 19 complexes. Fuzzy regions mostly exerted their impact via transient interactions [37]. Both specific or nonspecific intermolecular contacts may anchor the ID protein to the partner. These increase the local concentration of the binding element near the target site and the

probability of productive contacts [38]. Intramolecular interactions may promote formation of binding-competent structural elements, frequently via relieving electrostatic repulsion to improve affinity [39]. Fuzzy regions however, may also interact with specific residues, which are involved in the binding interface leading to autoinhibitory effects [40] (Figure 1). Entropy is a key factor in heterogeneous interfaces, which could be further tuned by posttranslational modifications [41, 42]. Disordered tails may decrease stability via 'frustrating' the structured part of the protein [43] or decrease the rate of folding of the binding site [44].

All these interaction modes could be combined leading to a variety of mechanisms, which are all linked to conformational heterogeneity [35]. Formally, these scenarios could be divided into four categories [35, 37]. Termed as *conformational selection*, dynamical regions stabilize an interaction-prone structural element and shift conformational equilibrium prior to binding. Termed as *flexibility/entropy modulation*, collective motions of the protein are influenced by transient contacts thus modulate the entropy of interaction, which may increase affinity. Termed as *competitive binding*, the presence of fuzzy region causes autoinhibition via competition for the binding site (Figure 1). Termed as *tethering,* heterogeneous contacts are exploited for local concentration effects. Almost all possible combinations of mechanisms and topologies can be realized [45].

Phase 4 – Fuzziness enables context-specific regulatory features

Dynamical ensembles of protein complexes could be shifted according to the cellular environment [46]. Posttranslational modifications may influence not only charge, but also secondary structure propensities of heterogeneous regions [30, 47] to induce gradual or threshold responses [42, 48]. Interaction affinities could also be modulated by tailoring the length of fuzzy segments via alternative splicing [49]. These may reduce steric hindrance or interfere with other competitive mechanisms to improve binding [44]. A combination of AS and PTMs can generate tissue-specific 'barcodes' [50]. Transient interactions in heterogeneous protein complexes may lead to allosteric effects [51, 52]. Any of these means enables protein assemblies to respond to external signals or changes in the intracellular milieu, referred to as context-dependence [53].

Tissue-specific exons (TSE) for example, often encode protein regions without apparent structure [54]. Although direct experimental evidence is only present in a handful cases, TSE regions are predicted to remain largely heterogeneous upon interactions, and only a small fragment serves as a structured binding element (Figure 1). Tissue-specific alternative splicing thus affects specific interactions as well as transient contacts to rewire interaction networks [55]. Similar heterogeneity is also exploited in viral proteins, which often hijack the host system by mimicking short interaction motifs [56]. Given the same LM sequence patterns and target domains, the mechanism of interaction of viral and host proteins were found to be different [57]. Eukaryotic motifs tend to fold upon binding, whereas viral proteins remain heterogeneous leading to a high degree of promiscuity [58].

Phase 5 – Fuzzy Complexes Database

The proposal of fuzzy complexes [26] boosted experimental studies on conformationally heterogeneous assemblies. Structural evidence on rapid conformational exchange in the bound state of proteins started to proliferate [59-62]. These were frequently referred to as 'dynamical' complexes [63, 64], which however did not distinguish

the maintenance of the intrinsic disordered state in protein complexes from dynamical couplings in structured proteins [65]. The latter could be mediated via 'conformational waves' [66], a scenario, which is distinct from regulatory mechanisms in ID protein complexes [27, 45, 53]. Fuzziness in protein assemblies however, originates in the intimate relationship between structurally heterogeneous regions and the residues, which are directly involved in function [35, 37, 45]. That is, conformational diversity via transient or alternative interactions (Phase 3) enables molecular mechanisms, which are responsive to the cellular conditions (Phase 4). To emphasize the coupling between structural multiplicity and its biological roles, we devised the Fuzzy Complexes Database (FuzDB, http://protdyn-database.org) [67].

FuzDB contains experimentally validated examples of protein assemblies with structural evidence for alternative states or dynamical conformational exchange in the bound state as well as biochemical evidence for the functional contribution of the heterogeneous region. Conformational data may come from a variety of techniques: missing electron density in X-ray [68] or cryo-EM [69]; minor shifts in the NMR spectrum (HSQC, PRE) as compared to the unbound state [70, 71]; increased dynamics in NMR in the complex as compared to the free form (e.g. indicated by relaxation dispersion) [72, 73]; extended shapes in small-angle X-ray scattering (SAXS) [74, 75], analytical ultracentrifuge (AUC) [74, 75], size-exclusion chromatography (SEC) [60]; as well Förster resonance energy transfer (FRET) [76]. Evidence for functional contributions of conformationally heterogeneous regions is derived from a number of techniques, ranging from affinity measurements [77, 78], catalytic rates [79], transcriptional activation [80], cell-cycle progress [51], to protein degradation [81], prion formation [32], or any other cellular activities [82, 83].

The Fuzzy Complexes Database also discusses experimental data based on the original publications [67] and details those structural and biochemical results, which corroborate heterogeneity in the bound state. FuzDB defines the topological [26] and mechanistic [35, 37] categories of protein complexes, as well as provides a detailed description of the mechanism in case sufficient data is available. In addition, PTM and AS effects on ensembles of heterogeneous regions and the biological consequences are analyzed. The connection between structural diversity and function lays down the basis of stochastic structure-function relationships (Figure 1).

Phase 6 – Fuzziness in higher-order protein organizations

Increasing complexity from 'traditional' protein complexes to supramolecular organizations is paralleled by increasing contribution of fuzziness to regulated assembly [84]. PTMs of dynamical regions may shift conformational equilibrium towards aggregation-prone states [85, 86] or promote disassembly by diminishing stabilizing transient contacts [87]. Membrane interactions may also modulate ensembles and influence polymerization [88]. Heterogeneity is present even in the most static higher-order assemblies either as alternatively folded states exemplified in different prion strains [89] or in regions exhibiting a rapid conformational exchange [90]. Dynamical regions could promote assembly by interfering with autoinhibition [91] (Figure 1); could be responsible for allosteric effects [92, 93], or generate context-specific responses via PTMs [94]. Taken together, fuzziness is an intrinsic and ubiquitous feature of supramolecular protein organizations, as conformational diversity influences regulated formation or activity of the different kinds of higher-order assemblies [84].

Membraneless cellular organelles are non-membrane-bound compartments, which are formed via phase transition of proteins, induced by weak interactions amongst redundant binding motifs [95]. Multivalency [96] and intrinsic disorder [97] are considered as two key factors driving this process, other molecular determinants have yet to be elucidated. Dynamics of the granules is determined by the multitude of interaction patterns/binding topologies, which altogether create a heterogeneous conformational ensemble. Indeed, high-resolution structural data suggests that conformational heterogeneity of Fus is not reduced in the RNP granule as compared to its unbound form [98]. Familial mutations could impair fuzziness by limiting the combinations of interactions, which leads to reduced number of microstates and fibril formation [84]. Pathological conversions in the material state and dynamics of the assembly results in neurological disorders such as amyotrophic lateralsclerosis or dementia [99, 100].

Phase 7 – Small molecule interactions may generate fuzzy complexes

The involvement of ID proteins in key regulatory processes raises a pharmaceutical interest [101]. One of the challenges for the field of intrinsically disordered proteins is thus to open new areas for drug research [102]. Indeed, small molecules inhibiting protein-protein interactions usually target dynamic interfaces [103]. A handful studies addressed the problem of specifically designing inhibitors for ID regions [104-107] using different screening technologies. NMR results indicate that small molecules often lack a well-defined binding site and visit an ensemble of states in the bound form generating a fuzzy complex [106, 108]. Entropy seems to be a key factor of selectivity [108, 109]. Interpretation and prediction of such heterogeneous assemblies however, require stochastic structure-function relationships.

Phase 8 – Fuzziness provides a mathematical framework for heterogeneous protein interactions

The term fuzziness is derived from the mathematical concept of fuzzy sets [110]. This concept describes the existing uncertainties in our observations by using a continuum of membership functions (in the range of [0,1]) instead of crisp categories [111]. A certain value of a measure may belong to multiple categories, but to different extents. In the protein world, functionally different landscapes could be defined by distinct sets of conformations. The formalism of fuzziness enables us to define the contributions of certain structures to different states, leading to quantitative relationships of the ensemble. To demonstrate the power of this, we employed the fuzzy framework to describe biological phase transition.

Formation of liquid granules is accompanied by generation of large system-spanning networks exchanging intra- for intermolecular interactions [112]. Polymer physics approaches have been applied to account for sequence features of proteins undergoing liquid-liquid phase transition [96, 113]. Although the multitude of interaction patterns could be realized between redundant motifs, all models were based on one-to-one binding. Considering fuzziness as a critical feature in phase transition [84], we incorporated the option of multivalent binding (i.e. interactions with multiple sites) into the simulations using a fuzzy mathematical framework [114]. The fuzzy simulations could more efficiently recapitulate the impact of valency on phase transition than the one-to-one binding model. We could also perform a systematic analysis of the linker dynamics and motif affinity and analyse their interplay. We showed how these two factors provide alternative scenarios

leading to assemblies with markedly different dynamic properties. Although the model systems were simple, by balancing between the dynamics of the multivalent contacts we could tune the rate and entropy of polymerization [114]. This illustrates that simulations based on the fuzzy framework have a potential in modelling biological phase transitions and other complex systems.

Phase 9 – Future perspectives

The advances of structural determination approaches, especially EM and NMR provide higher resolution view of our molecular systems on rapidly increasing scales. These measurements witness on the intrinsic heterogeneity of protein machines and protein-based cellular organelles. The biological roles and direct consequences of such structural diversity however, are far from being trivial. At the same time, highly dynamic protein regions interact with small molecules using heterogeneous binding modes, which has an unforeseen potential in targeting regulatory proteins by drug candidates. The proposal of **fuzziness in protein interactions links conformational heterogeneity to its functional contributions** [26, 35] and provides a wealth of experimental evidence for how structural diversity operates in assemblies of proteins [67]. In the past decade, our understanding on the role of transient interactions and conformational exchange in protein complexes has largely evolved and enabled interpretation of context-specific cellular responses [45, 53].

On the other hand, fuzziness is derived from a mathematical concept, when a boundary or a value of an application (function) change according to the context. This mathematical framework could be applied to heterogeneous protein assemblies and may lead to quantitative structure-function relationships. The fuzzy framework can tackle redundant binding modes or polymorphism in case of multivalent complexes or alternative, co-existing states in case of rapid conformational exchange. An illustration has been provided for biological phase transition recently [114], but the formalism can be exploited for any other biological problems related to heterogeneous states.

Our description of protein assemblies has been largely refined in the past two decades and dynamical interactions are becoming integral part of our understanding. Future work need to elaborate the relationship between conformational ensembles and biological activities. Fuzziness in protein interactions may play a key role in this endeavor by laying down the basis of stochastic structure-function relationships. Coming years will bring us the excitement of quantifying this new paradigm and probe its validity in specific cellular systems.

References

[1] Jensen MR, Zweckstetter M, Huang JR, Blackledge M. Exploring free-energy landscapes of intrinsically disordered proteins at atomic resolution using NMR spectroscopy. Chem Rev. 2014;114:6632-60.

[2] Oldfield CJ, Dunker AK. Intrinsically disordered proteins and intrinsically disordered protein regions. Annu Rev Biochem. 2014;83:553-84.

[3] Wright PE, Dyson HJ. Intrinsically disordered proteins in cellular signalling and regulation. Nat Rev Mol Cell Biol. 2015;16:18-29.

[4] Dunker AK, Garner E, Guillot S, Romero P, Albrecht K, Hart J, et al. Protein disorder and the evolution of molecular recognition: theory, predictions and observations. Pac Symp Biocomputing. 1998;3:473-84.

[5] Wright PE, Dyson HJ. Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. J Mol Biol. 1999;293:321-31.

[6] Wright PE, Dyson HJ. Linking folding and binding. Curr Opin Struct Biol. 2009;19:31-8.

[7] Kussie PH, Gorina S, Marechal V, Elenbaas B, Moreau J, Levine AJ, et al. Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. Science. 1996;274:948-53.

[8] Russo AA, Jeffrey PD, Patten AK, Massague J, Pavletich NP. Crystal structure of the p27Kip1 cyclin-dependent-kinase inhibitor bound to the cyclin A-Cdk2 complex. Nature. 1996;382:325-31.

[9] Selenko P, Gregorovic G, Sprangers R, Stier G, Rhani Z, Krämer A, et al. Structural basis for the molecular recognition between human splicing factors U2AF65 and SF1/mBBP. Mol Cell. 2003;11:965-76.

[10] Leuenberger P, Ganscha S, Kahraman A, Cappelletti V, Boersema PJ, von Mering C, et al. Cell-wide analysis of protein thermal unfolding reveals determinants of thermostability. Science. 2017;355.

[11] Tsai C, Ma B, Sham YY, Kumar S, Nussinov R. Structured disorder and conformational selection. Proteins. 2001;44:418-27.

[12] Bienkiewicz EA, Adkins JN, Lumb KJ. Functional consequences of preorganized helical structure in the intrinsically disordered cell-cycle inhibitor p27(Kip1). Biochemistry. 2002;41:752-9.

[13] Fuxreiter M, Simon I, Friedrich P, Tompa P. Preformed structural elements feature in partner recognition by intrinsically unstructured proteins. J Mol Biol. 2004;338:1015-26.

[14] Sivakolundu SG, Bashford D, Kriwacki RW. Disordered p27Kip1 exhibits intrinsic structure resembling the Cdk2/cyclin A-bound conformation. J Mol Biol. 2005;353:1118-28.

[15] Ptitsyn OB, Finkelstein AV. Theory of protein secondary structure and algorithm of its prediction. Biopolymers. 1983;22:15-25.

[16] Oldfield CJ, Cheng Y, Cortese MS, Romero P, Uversky VN, Dunker AK. Coupled folding and binding with alpha-helix-forming molecular recognition elements. Biochemistry. 2005;44:12454-70.

[17] Mohan A, Oldfield CJ, Radivojac P, Vacic V, Cortese MS, Dunker AK, et al. Analysis of molecular recognition features (MoRFs). J Mol Biol. 2006;362:1043-59.

[18] Lee SH, Kim DH, Han JJ, Cha EJ, Lim JE, Cho YJ, et al. Understanding Pre-Structured Motifs (PreSMos) in Intrinsically Unfolded Proteins. Curr Protein Pept Sci. 2011.

[19] Pancsa R, Fuxreiter M. Interactions via intrinsically disordered regions: what kind of motifs? IUBMB Life. 2012;64:513-20.

[20] Yu H, Chen JK, Feng S, Dalgarno DC, Brauer AW, Schreiber SL. Structural basis for the binding of proline-rich peptides to SH3 domains. Cell. 1994;76:933-45.

[21] Puntervoll P, Linding R, Gemund C, Chabanis-Davidson S, Mattingsdal M, Cameron S, et al. ELM server: A new resource for investigating short functional sites in modular eukaryotic proteins. Nucleic Acids Res. 2003;31:3625-30.

[22] Fuxreiter M, Tompa P, Simon I. Local structural disorder imparts plasticity on linear motifs. Bioinformatics. 2007;23:950-6.

[23] Tompa P, Davey NE, Gibson TJ, Babu MM. A million peptide motifs for the molecular biologist. Mol Cell. 2014;55:161-9.

[24] Graham TA, Ferkey DM, Mao F, Kimelman D, Xu W. Tcf4 can specifically recognize beta-catenin using alternative conformations. Nat Struct Biol. 2001;8:1048-52.

[25] Mittag T, Orlicky S, Choy WY, Tang X, Lin H, Sicheri F, et al. Dynamic equilibrium engagement of a polyvalent ligand with a single-site receptor. Proc Natl Acad Sci U S A. 2008;105:17772-7.

[26] Tompa P, Fuxreiter M. Fuzzy complexes: polymorphism and structural disorder in protein-protein interactions. Trends Biochem Sci. 2008;33:2-8.

[27] Fuxreiter M, Tompa P. Fuzzy complexes: a more stochastic view of protein function. Adv Exp Med Biol. 2012;725:1-14.

[28] Sigler PB. Transcriptional activation. Acid blobs and negative noodles. Nature. 1988;333:210-2.

[29] Hope IA, Mahadevan S, Struhl K. Structural and functional characterization of the short acidic transcriptional activation region of yeast GCN4 protein. Nature. 1988;333:635-40.

[30] Hansen JC, Lu X, Ross ED, Woody RW. Intrinsic protein disorder, amino acid composition, and histone terminal domains. J Biol Chem. 2006;281:1853-6.

[31] Lu X, Hamkalo B, Parseghian MH, Hansen JC. Chromatin condensing functions of the linker histone C-terminal domain are mediated by specific amino acid composition and intrinsic protein disorder. Biochemistry. 2009;48:164-72.

[32] Ross ED, Baxa U, Wickner RB. Scrambled prion domains form prions and amyloid. Mol Cell Biol. 2004;24:7206-13.

[33] Ross ED, Edskes HK, Terry MJ, Wickner RB. Primary sequence independence for prion formation. Proc Natl Acad Sci U S A. 2005;102:12825-30.

[34] Welch GR. The 'fuzzy' interactome. Trends Biochem Sci. 2009;34:1-2; author reply 3.

[35] Fuxreiter M. Fuzziness: linking regulation to protein dynamics. Mol Biosyst. 2012;8:168-77.

[36] Fuxreiter M, Tompa P. Fuzziness: Structural Disorder in Protein Complexes Austin, New York: Landes BioScience/Springer; 2011.

[37] Fuxreiter M, Simon I, Bondos S. Dynamic protein-DNA recognition: beyond what can be seen. Trends Biochem Sci. 2011;36:415-23.

[38] Vise PD, Baral B, Latos AJ, Daughdrill GW. NMR chemical shift and relaxation measurements provide evidence for the coupled folding and binding of the p53 transactivation domain. Nucleic Acids Res. 2005;33:2061-77.

[39] Pursglove SE, Fladvad M, Bellanda M, Moshref A, Henriksson M, Carey J, et al. Biophysical properties of regions flanking the bHLH-Zip motif in the p22 Max protein. Biochem Biophys Res Commun. 2004;323:750-9.

[40] Watson M, Stott K, Thomas JO. Mapping intramolecular interactions between domains in HMGB1 using a tail-truncation approach. J Mol Biol. 2007;374:1286-97.

[41] Pufall MA, Lee GM, Nelson ML, Kang HS, Velyvis A, Kay LE, et al. Variable control of Ets-1 DNA binding by multiple phosphates in an unstructured region. Science. 2005;309:142-5.

[42] Lee GM, Pufall MA, Meeker CA, Kang HS, Graves BJ, McIntosh LP. The affinity of Ets-1 for DNA is modulated by phosphorylation through transient interactions of an unstructured region. J Mol Biol. 2008;382:1014-30.

[43] Toth-Petroczy A, Simon I, Fuxreiter M, Levy Y. Disordered tails of homeodomains facilitate DNA recognition by providing a trade-off between folding and specific binding. J Am Chem Soc. 2009;131:15084-5.

[44] Gruet A, Dosnon M, Blocquel D, Brunel J, Gerlier D, Das RK, et al. Fuzzy regions in an intrinsically disordered protein impair protein-protein interactions. The FEBS journal. 2016;283:576-94.

[45] Sharma R, Raduly Z, Miskei M, Fuxreiter M. Fuzzy complexes: Specific binding without complete folding. FEBS Lett. 2015;589:2533-42.

[46] Rosenzweig R, Sekhar A, Nagesh J, Kay LE. Promiscuous binding by Hsp70 results in conformational heterogeneity and fuzzy chaperone-substrate ensembles. eLife. 2017;6.

[47] Bah A, Vernon RM, Siddiqui Z, Krzeminski M, Muhandiram R, Zhao C, et al. Folding of an intrinsically disordered protein by phosphorylation as a regulatory switch. Nature. 2015;519:106-9.

[48] Borg M, Mittag T, Pawson T, Tyers M, Forman-Kay JD, Chan HS. Polyelectrostatic interactions of disordered ligands suggest a physical basis for ultrasensitivity. Proc Natl Acad Sci U S A. 2007;104:9650-5.

[49] Liu Y, Matthews KS, Bondos SE. Multiple intrinsically disordered sequences alter DNA binding by the homeodomain of the Drosophila hox protein ultrabithorax. J Biol Chem. 2008;283:20874-87.

[50] Polverini E, Rangaraj G, Libich DS, Boggs JM, Harauz G. Binding of the proline-rich segment of myelin basic protein to SH3 domains: spectroscopic, microarray, and modeling studies of ligand conformation and effects of posttranslational modifications. Biochemistry. 2008;47:267-82.

[51] Galea CA, Nourse A, Wang Y, Sivakolundu SG, Heller WT, Kriwacki RW. Role of intrinsic flexibility in signal transduction mediated by the cell cycle regulator, p27 Kip1. J Mol Biol. 2008;376:827-38.

[52] Nnamani MC, Ganguly S, Erkenbrack EM, Lynch VJ, Mizoue LS, Tong Y, et al. A Derived Allosteric Switch Underlies the Evolution of Conditional Cooperativity between HOXA11 and FOXO1. Cell reports. 2016;15:2097-108.

[53] Miskei M, Gregus A, Sharma R, Duro N, Zsolyomi F, Fuxreiter M. Fuzziness enables context dependence of protein interactions. FEBS Lett. 2017;591:2682-95.

[54] Buljan M, Chalancon G, Eustermann S, Wagner GP, Fuxreiter M, Bateman A, et al. Tissue-Specific Splicing of Disordered Segments that Embed Binding Motifs Rewires Protein Interaction Networks. Mol Cell. 2012;46:871-83.

[55] Buljan M, Chalancon G, Dunker AK, Bateman A, Balaji S, Fuxreiter M, et al. Alternative splicing of intrinsically disordered regions and rewiring of protein interactions. Curr Opin Struct Biol. 2013;23:443-50.

[56] Davey NE, Trave G, Gibson TJ. How viruses hijack cell regulation. Trends Biochem Sci. 2011;36:159-69.

[57] Duro N, Miskei M, Fuxreiter M. Fuzziness endows viral motif-mimicry. Mol Biosyst. 2015;11:2821-9.

[58] Longhi S. Structural disorder within paramyxoviral nucleoproteins. FEBS Lett. 2015;589:2649-59.

[59] Communie G, Habchi J, Yabukarski F, Blocquel D, Schneider R, Tarbouriech N, et al. Atomic resolution description of the interaction between the nucleoprotein and phosphoprotein of Hendra virus. PLoS pathogens. 2013;9:e1003631.

[60] Habchi J, Blangy S, Mamelli L, Jensen MR, Blackledge M, Darbon H, et al. Characterization of the interactions between the nucleoprotein and the phosphoprotein of Henipavirus. J Biol Chem. 2011;286:13583-602.

[61] Jensen MR, Communie G, Ribeiro EA, Jr., Martinez N, Desfosses A, Salmon L, et al. Intrinsic disorder in measles virus nucleocapsids. Proc Natl Acad Sci U S A. 2011;108:9839-44.

[62] Milles S, Mercadante D, Aramburu IV, Jensen MR, Banterle N, Koehler C, et al. Plasticity of an Ultrafast Interaction between Nucleoporins and Nuclear Transport Receptors. Cell. 2015;163:734-45.

[63] Andresen C, Helander S, Lemak A, Fares C, Csizmok V, Carlsson J, et al. Transient structure and dynamics in the disordered c-Myc transactivation domain affect Bin1 binding. Nucleic Acids Res. 2012;40:6353-66.

[64] Berlow RB, Dyson HJ, Wright PE. Hypersensitive termination of the hypoxic response by a disordered protein switch. Nature. 2017;543:447-51.

[65] Tzeng SR, Kalodimos CG. Dynamic activation of an allosteric regulatory protein. Nature. 2009;462:368-72.

[66] Gruber R, Levitt M, Horovitz A. Sequential allosteric mechanism of ATP hydrolysis by the CCT/TRiC chaperone is revealed through Arrhenius analysis. Proc Natl Acad Sci U S A. 2017;114:5189-94.

[67] Miskei M, Antal C, Fuxreiter M. FuzDB: database of fuzzy complexes, a tool to develop stochastic structure-function relationships for protein complexes and higher-order assemblies. Nucleic Acids Res. 2017;45:D228-D35.

[68] Bhattacharyya RP, Remenyi A, Good MC, Bashor CJ, Falick AM, Lim WA. The Ste5 scaffold allosterically modulates signaling output of the yeast mating pathway. Science. 2006;311:822-6.

[69] Rock RS, Ramamurthy B, Dunn AR, Beccafico S, Rami BR, Morris C, et al. A flexible domain is essential for the large step size and processivity of myosin VI. Mol Cell. 2005;17:603-9.

[70] Brzovic PS, Heikaus CC, Kisselev L, Vernon R, Herbig E, Pacheco D, et al. The acidic transcription activator Gcn4 binds the mediator subunit Gal11/Med15 using a simple protein interface forming a fuzzy complex. Mol Cell. 2011;44:942-53.

[71] Lukhele S, Bah A, Lin H, Sonenberg N, Forman-Kay JD. Interaction of the eukaryotic initiation factor 4E with 4E-BP2 at a dynamic bipartite interface. Structure. 2013;21:2186-96.

[72] Schwarten M, Solyom Z, Feuerstein S, Aladag A, Hoffmann S, Willbold D, et al. Interaction of nonstructural protein 5A of the hepatitis C virus with Src homology 3 domains using noncanonical binding sites. Biochemistry. 2013;52:6160-8.

[73] Kurzbach D, Schwarz TC, Platzer G, Hofler S, Hinderberger D, Konrat R. Compensatory adaptations of structural dynamics in an intrinsically disordered protein complex. Angewandte Chemie. 2014;53:3840-3.

[74] von Ossowski I, Eaton JT, Czjzek M, Perkins SJ, Frandsen TP, Schulein M, et al. Protein disorder: conformational distribution of the flexible linker in a chimeric double cellulase. Biophys J. 2005;88:2823-32.

[75] Gogl G, Alexa A, Kiss B, Katona G, Kovacs M, Bodor A, et al. Structural Basis of Ribosomal S6 Kinase 1 (RSK1) Inhibition by S100B Protein: MODULATION OF THE EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK) SIGNALING CASCADE IN A CALCIUM-DEPENDENT WAY. J Biol Chem. 2016;291:11-27.

[76] Lamboy JA, Kim H, Dembinski H, Ha T, Komives EA. Single-molecule FRET reveals the native-state dynamics of the IkappaBalpha ankyrin repeat domain. J Mol Biol. 2013;425:2578-90.

[77] Hurley TD, Yang J, Zhang L, Goodwin KD, Zou Q, Cortese M, et al. Structural basis for regulation of protein phosphatase 1 by inhibitor-2. J Biol Chem. 2007;282:28874-83.

[78] Warfield L, Tuttle LM, Pacheco D, Klevit RE, Hahn S. 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. 2014;111:E3506-13.

[79] Smet-Nocca C, Wieruszeski JM, Chaar V, Leroy A, Benecke A. The thymine-DNA glycosylase regulatory domain: residual structure and DNA binding. Biochemistry. 2008;47:6519-30.

[80] Khan H, Cino EA, Brickenden A, Fan J, Yang D, Choy WY. Fuzzy complex formation between the intrinsically disordered prothymosin alpha and the Kelch domain of Keap1 involved in the oxidative stress response. J Mol Biol. 2013;425:1011-27.

[81] Komives EA. Consequences of fuzziness in the NFkappaB/IkappaBalpha interaction. Advances in experimental medicine and biology. 2012;725:74-85.

[82] Bourhis JM, Receveur-Brechot V, Oglesbee M, Zhang X, Buccellato M, Darbon H, et al. 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. 2005;14:1975-92.

[83] Ferreon AC, Ferreon JC, Wright PE, Deniz AA. Modulation of allostery by protein intrinsic disorder. Nature. 2013;498:390-4.

[84] Wu H, Fuxreiter M. The Structure and Dynamics of Higher-Order Assemblies: Amyloids, Signalosomes, and Granules. Cell. 2016;165:1055-66.

[85] Li J, McQuade T, Siemer AB, Napetschnig J, Moriwaki K, Hsiao YS, et al. The RIP1/RIP3 Necrosome Forms a Functional Amyloid Signaling Complex Required for Programmed Necrosis. Cell. 2012;150:339-50.

[86] Lu A, Magupalli VG, Ruan J, Yin Q, Atianand MK, Vos MR, et al. Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. Cell. 2014;156:1193-206.

[87] Ciuffa R, Lamark T, Tarafder AK, Guesdon A, Rybina S, Hagen WJ, et al. The selective autophagy receptor p62 forms a flexible filamentous helical scaffold. Cell reports. 2015;11:748-58.

[88] Fusco G, De Simone A, Gopinath T, Vostrikov V, Vendruscolo M, Dobson CM, et al. Direct observation of the three regions in alpha-synuclein that determine its membrane-bound behaviour. Nature communications. 2014;5:3827.

[89] Krishnan R, Lindquist SL. Structural insights into a yeast prion illuminate nucleation and strain diversity. Nature. 2005;435:765-72.

[90] Schwarz-Romond T, Fiedler M, Shibata N, Butler PJ, Kikuchi A, Higuchi Y, et al. The DIX domain of Dishevelled confers Wnt signaling by dynamic polymerization. Nat Struct Mol Biol. 2007;14:484-92.

[91] Greenwald J, Buhtz C, Ritter C, Kwiatkowski W, Choe S, Maddelein ML, et al. The mechanism of prion inhibition by HET-S. Mol Cell. 2010;38:889-99.

[92] Korennykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, Shokat KM, et al. The unfolded protein response signals through high-order assembly of Ire1. Nature. 2009;457:687-93.

[93] Lee KP, Dey M, Neculai D, Cao C, Dever TE, Sicheri F. Structure of the dual enzyme Ire1 reveals the basis for catalysis and regulation in nonconventional RNA splicing. Cell. 2008;132:89-100.

[94] Wippich F, Bodenmiller B, Trajkovska MG, Wanka S, Aebersold R, Pelkmans L. Dual specificity kinase DYRK3 couples stress granule condensation/dissolution to mTORC1 signaling. Cell. 2013;152:791-805.

[95] Brangwynne CP, Eckmann CR, Courson DS, Rybarska A, Hoege C, Gharakhani J, et al. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science. 2009;324:1729-32.

[96] Li P, Banjade S, Cheng HC, Kim S, Chen B, Guo L, et al. Phase transitions in the assembly of multivalent signalling proteins. Nature. 2012;483:336-40.

[97] Lin Y, Protter DS, Rosen MK, Parker R. Formation and Maturation of Phase-Separated Liquid Droplets by RNA-Binding Proteins. Mol Cell. 2015;60:208-19.

[98] Burke KA, Janke AM, Rhine CL, Fawzi NL. Residue-by-Residue View of In Vitro FUS Granules that Bind the C-Terminal Domain of RNA Polymerase II. Mol Cell. 2015;60:231-41.

[99] Molliex A, Temirov J, Lee J, Coughlin M, Kanagaraj AP, Kim HJ, et al. Phase Separation by Low Complexity Domains Promotes Stress Granule Assembly and Drives Pathological Fibrillization. Cell. 2015;163:123-33.

[100] Patel A, Lee HO, Jawerth L, Maharana S, Jahnel M, Hein MY, et al. A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. Cell. 2015;162:1066-77.

[101] Metallo SJ. Intrinsically disordered proteins are potential drug targets. Curr Opin Chem Biol. 2010;14:481-8.

[102] Cheng Y, LeGall T, Oldfield CJ, Mueller JP, Van YY, Romero P, et al. Rational drug design via intrinsically disordered protein. Trends Biotechnol. 2006;24:435-42.

[103] Arkin MR, Wells JA. Small-molecule inhibitors of protein-protein interactions: progressing towards the dream. Nat Rev Drug Discov. 2004;3:301-17.

[104] Follis AV, Hammoudeh DI, Wang H, E.V. P, Metallo SJ. Structural rationale for coupled binding and unfolding of the c-Myc oncoprotein by small molecules. Chem Biol. 2008;15:1149-55.

[105] Iconaru LI, Ban D, Bharatham K, Ramanathan A, Zhang W, Shelat AA, et al. Discovery of Small Molecules that Inhibit the Disordered Protein, p27(Kip1). Scientific reports. 2015;5:15686.

[106] Radic-Sarikas B, Tsafou KP, Emdal KB, Papamarkou T, Huber KV, Mutz C, et al. Combinatorial Drug Screening Identifies Ewing Sarcoma-specific Sensitivities. Mol Cancer Ther. 2017;16:88-101.

[107] Nishikawa JL, Boeszoermenyi A, Vale-Silva LA, Torelli R, Posteraro B, Sohn YJ, et al. Inhibiting fungal multidrug resistance by disrupting an activator-Mediator interaction. Nature. 2016;530:485-9.

[108] Heller GT, Aprile FA, Bonomi M, Camilloni C, De Simone A, Vendruscolo M. Sequence Specificity in the Entropy-Driven Binding of a Small Molecule and a Disordered Peptide. J Mol Biol. 2017;429:2772-9.

[109] Heller GT, Sormanni P, Vendruscolo M. Targeting disordered proteins with small molecules using entropy. Trends Biochem Sci. 2015;40:491-6.

[110] Zadeh LA. Fuzzy sets. Information and Control. 1965;8:338-53.

[111] Singh H, Gupta MM, Meitzler T, Hou Z-G, Garg KK, Solo AMG, et al. Real-Life Applications of Fuzzy Logic. Advances in Fuzzy Systems. 2013;2013:e581879.

[112] Semenov AN, Rubinstein M. Thermoreversible Gelation in Solutions of Associative Polymers. 1. Statics. Macromolecules 1998;31:1373-85.

[113] Harmon TS, Holehouse AS, Rosen MK, Pappu RV. Intrinsically disordered linkers determine the interplay between phase separation and gelation in multivalent proteins. eLife. 2017;6.

[114] Tuu-Szabo B, KOczy L, Fuxreiter M. Simulations of higher-order protein assemblies using a fuzzy framework. BioRxiv. 2018. doi: 10.1101/248062.

[115] Didry D, Cantrelle FX, Husson C, Roblin P, Moorthy AM, Perez J, et al. How a single residue in individual beta-thymosin/WH2 domains controls their functions in actin assembly. Embo J. 2012;31:1000-13.

[116] Stott K, Watson M, Howe FS, Grossmann JG, Thomas JO. 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. 2010;403:706-22.

[117] Kahlfeldt N, Vahedi-Faridi A, Koo SJ, Schafer JG, Krainer G, Keller S, et al. Molecular basis for association of PIPKI gamma-p90 with clathrin adaptor AP-2. J Biol Chem. 2010;285:2734-49.

[118] Di Paolo G, Pellegrini L, Letinic K, Cestra G, Zoncu R, Voronov S, et al. Recruitment and regulation of phosphatidylinositol phosphate kinase type 1 gamma by the FERM domain of talin. Nature. 2002;420:85-9.

[119] Xie T, Peng W, Yan C, Wu J, Gong X, Shi Y. Structural insights into RIP3-mediated necroptotic signaling. Cell reports. 2013;5:70-8.

[120] Morales RA, MacRaild CA, Seow J, Krishnarjuna B, Drinkwater N, Rouet R, et al. Structural basis for epitope masking and strain specificity of a conserved epitope in an intrinsically disordered malaria vaccine candidate. Scientific reports. 2015;5:10103.

Figure legend

Figure 1 Understanding of ID protein interactions and complexes. *Recognition elements.* The tumor suppressor p53 interacts with the ubiquitin ligase Mdm2 via a 15-residue helix of the transactivator domain, while residues flanking the *preformed element* remain disordered in the complex [7]. The cell cycle kinase inhibitor p27[Kip1] is anchored to cyclin-Cdk2 complex via a short RNLF motif [8]. *Fuzzy complexes.* Upon interaction of the MAPK 7 kinase with JNK1, *alternative conformations* are observed reflecting different binding mechanisms and pathways [75]. In the MAPK cascade, Ste5 interacts with mitogen activated kinase Fus3 in a bipartite manner. The linking region is invisible in the structure of the complex and exhibits a *rapid conformational exchange*, yet its length critically affects Fus3 autophosphorylation and activation of mating response in yeast [68]. *Functional implications of fuzzy interactions.* To initiate pre-mRNA target site selection, spicing factor SF1 interacts with U2AF[65] via a 10-residue interface. Residues without apparent physical contacts *increase binding affinity*, which is further improved by the dynamical flanking region in the full-length protein [9]. Actin polymerization is assisted by disordered WH2 domains. Dynamics of transiently interacting regions is regulated by the ionic-strength, which balances the system between *assembly and sequestration* [115]. *Molecular mechanisms of fuzzy interactions.* The serine-arginine rich (SRR) region of the Ets-1 transcription factor does not adopt a stable structure in complex with DNA, yet its interactions with the autoinhibitory helix impact binding affinity in length and PTM dependent manner. Transient contacts of SRR were demonstrated to influence *flexibility of the interface* and binding entropy [41, 42]. The negatively charged tail of the high-mobility group protein (HMG) B1 exerts autoinhibition on DNA binding via *competitive interactions* with the target site. The conformational equilibrium between the closed and open form of the globular regions determines the affinity for the binding site [116]. *Context-dependence of fuzzy interactions.* Tissue-specific alternative splicing of phosphatidylinositol-4-phosphate-5-kinase (PIPK1c) affects a short, structured recognition motif flanked by fuzzy regions, the presence of which mediates assembly with AP-2 in brain, but not in lymph node [117]. A subset of these residues may bias for synaptic vesicle recycling via interactions with talin [118]. PTM-driven interactions between RIP3 kinase and MLKL initiate necroctopic signalling. The RIP3 activation loop is partly disordered in the complex, yet mediates species-specific interactions via different phosphorylation patterns [119]. *Stochastic structure-function relationships.* Multiple, weak interactions contribute to the interface of GCN4 transcription factor with the coactivator Med15, resulted by multiple co-existing conformations in the complex [70]. *Transcription by synthetic activators increase with fuzziness* of the interface owing to redundancy of hydrophobic, weak interactions [78]. Malaria antigens of merozoite surface protein 2 (MSP2) contact with their respective antibodies via a short, structured interface, while transient interactions by the flanking *fuzzy regions enable strain-specific binding* [120]. *Fuzziness in higher-order assemblies.* Oligomerization of the dual enzyme Ire1 is required for unfolded protein response [92, 93]. Assembly is driven by trans-autophosphorylation of the kinase activation loop, which induces its stabilization within the complex. Fuzzy regions balance between the autoinhibitory conformation of the PYD and CARD domains of the ASC inflammasome. Phosphorylation of the dynamical linker induces the extended form and promotes prion-like polymerization [86].

Periods refer to development of the original proposal, formulated in the references. ID proteins are salmon, regions exhibiting a rapid conformational exchange in the assembly are shown by dotted lines. Alternative structures (polymorphism) are salmon, cyan and yellow. Phosphorylation sites are shown by yellow spheres. Partners are represented by grey surfaces. PDB codes for the structures are shown in parenthesis.
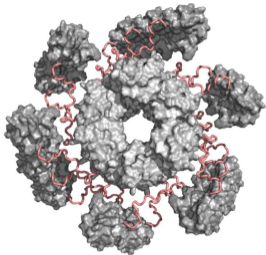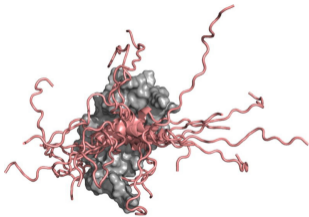
Graphical abstract
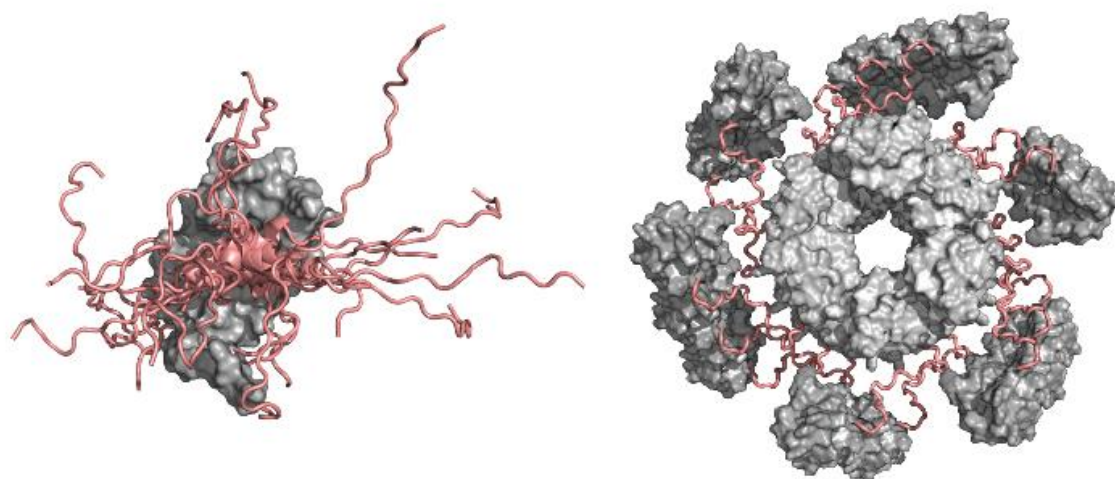
Fuzziness enables stochastic structure-function relationships from binary to higher-order protein assemblies

Research highlights

- conformational heterogeneity can be maintained in ID protein complexes
- heterogeneous regions have important functional contributions
- fuzziness enables context-dependent regulatory features
- fuzziness is a ubiquitous feature of higher-order protein assemblies
- fuzziness provides the basis for stochastic structure-function relationships

Fuzziness enables stochastic structure-function relationships from binary to higher-order protein assemblies

Graphics Abstract
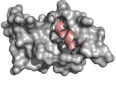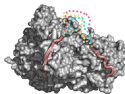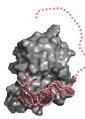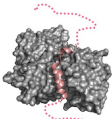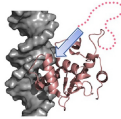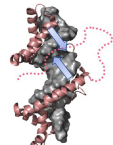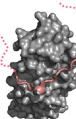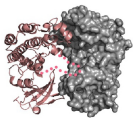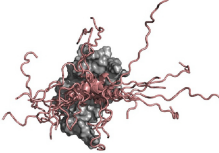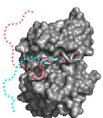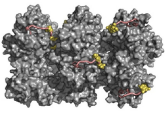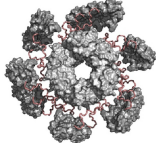
Period	Proposal	Representative structures		References
Phase 0 (2004 – 2007)	Short recognition elements for ID interactions	**p53 – Mdm2** (1ycq) *preformed structural element*	**p27**[Kip1] **– Cyclin** (1su) *linear motif*	[13], [22]
Phase 1 (2008)	Fuzzy complexes: conformational heterogeneity in protein interactions	**MKK7 – JNK1** (5csf,5csi,5csj) *polymorphism*	**Ste5 – Fus3** (2f49) *dynamic disorder*	[26]
Phase 2 (2008 – 2012)	Functional implications of fuzziness	**SF1 – U2AF**[65] (1o0p) *affinity improvement*	**WH2 – actin** (3u9d) *polymerization, sequestration*	[27], [36]
Phase 3 (2011 – 2012)	Molecular mechanisms of fuzzy interactions	**Ets-1 – DNA** (1mdm) *entropy modulation*	**HMGB1 – DNA** (2gzk) *competitive binding*	[35], [37]
Phase 4 (2012 – 2017)	Context-dependence of fuzzy interactions	**PiP5K1c – AP-2** (3h1z) *tissue-specificity (AS)*	**RIP3 – MLKL** (4m69) *programmed necrosis (PTM)*	[53], [54]
Phase 5 (2015 – 2017)	Stochastic structure-function relationships	**GCN4 – Med15** (2lpb) *fuzzy interface increases transcription by synthetic activator domains*	**MSP2 – mAB6D8** (4qy8, 4qxt) *transient interactions enable strain-specificity of malaria antigens*	[45], [67]
Phase 6 (2016 – 2018)	Fuzziness in higher-order protein assemblies	**Ire1 oligomer** (3fbv) *autophosphorylation driven oligomerization*	**ASC inflammasome** (3j63) *shift from autoinhibition to polymerization*	[84],[114]

Figure 1