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Abstract:	Proteins may undergo adaptive structural transitions to accommodate to their cellular milieu and respond to external signals. Modulation of conformational ensembles can rewire the intra- or intermolecular interaction networks and shift between different functional states. Adaptive conformational transitions are associated with protein fuzziness, which enables i) rewiring interaction networks via alternative motifs ii) new functional features via allosteric motifs iii) functional switches upon posttranslational modifications or iv) regulation of higher-order organizations. We propose that all these context-dependent functional changes are intertwined with structural multiplicity or dynamic disorder in protein assemblies and can only be described by stochastic structure-function relationships.

Fuzziness enables context-dependence of protein interactions

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Abstract

Proteins may undergo adaptive structural transitions to accommodate to their cellular milieu and respond to external signals. Modulation of conformational ensembles can rewire the intra- or intermolecular interaction networks and shift between different functional states. Adaptive conformational transitions are associated with protein fuzziness, which enables *i)* rewiring interaction networks via alternative motifs *ii)* new functional features via allosteric motifs *iii)* functional switches upon posttranslational modifications or *iv)* regulation of higher-order organizations. We propose that all these context-dependent functional changes are intertwined with structural multiplicity or dynamic disorder in protein assemblies and can only be described by stochastic structure-function relationships.

I. The deterministic structure-function paradigm

The classical framework establishes a deterministic relationship between protein sequence and function, which are linked via a well-defined tertiary structure. According to the paradigm, a distinguished three-dimensional arrangement of the amino acid residues is a pre-requisite for a given biological activity and is unambiguously encoded in the sequence. Protein functions however, are modulated by the cellular conditions, ion or metabolite concentrations or different kinds of stress. Adaptation could be facilitated by perturbing the key functional residues (catalytic groups or binding sites) or via allosteric signals, which both involve conformational rearrangements [1, 2]. Given the deterministic connection between the sequence and function, the question is to what extent biological activity could be modulated by the cellular milieu.

II. Intrinsically disordered (ID) proteins

Disobeying the classical principle, intrinsically disordered (ID) proteins exhibit many different structures in the native state and use ensembles of conformations for function [3, 4]. The plasticity of ID proteins enables them to interact with a variety of partners consecutively or simultaneously [5, 6] and regulate signaling pathways as hubs of interaction networks [7]. Although promiscuous binding potentiates for different activities [8] it alone cannot explain how adaptive changes take place.

Combinatorial usage of short functional motifs in ID regions may respond to contextual changes [9-11]. Alternative splicing (AS) and posttranslational modifications (PTMs) for example frequently take place in intrinsically disordered protein regions and interfere with the activity of low-complexity linear motifs [12, 13]. In the following we will analyze the structural mechanisms of ID-mediated interactions.

III. Three types of structural transitions in ID interactions

ID proteins usually employ short segments for specific interactions [14], which exhibit three types of structural transitions upon contacting their partners (Figure 1). i) ID binding elements may undergo *disorder-to-order* transition upon partner interactions to adopt a stable, well-defined conformation in the bound form. This is also referred to as coupled folding to binding [15], although in most cases ordering is limited to only those secondary structure elements, which are also biased in the unbound state (e.g. PPxP motif to SH3 [16]) [17]. Partner interactions can also stabilize ID motifs, the composition of which is distinct from their flanking sequences in terms of hydrophobicity or charge [9]. The short size of the ordered regions indicates that conformational heterogeneity could be also maintained in the bound form of ID proteins. ii) Well-defined structural elements may still exhibit a conformational exchange on a ms-μs timescale [18, 19]. *Disorder-to-partial order* transitions take place in shallow, often hydrophobic binding pockets, which may require multiple ligands for optimal binding [20]. In these cases, the interface is generated by many redundant contacts and a few specificity-determining residues [18]. The resulting variable interaction patterns need to be maintained for high-affinity binding, for example in the GNC4 - Med15 complex (PDB: 2lpb) [20]. Polymorphism, or alternative structural elements/topologies can also be observed owing to alternative interaction patterns. Different prion strains, for example, are caused by alternative registers between the β-strands, which lead to different phenotypes [21]. iii) ID binding motifs can also exhibit fast conformational exchange in the bound state owing to variable or transient contacts [22]. These *disorder-to-disorder* transitions are facilitated by the neighboring segments, which often retain conformational diversity upon assembling with the partner [23, 24]. Dynamics of an ID complex may vary in a wide range depending on the truncation of the ID region and the resolution of the applied technique (Figure 1).

IV. Fuzziness in protein interactions

Both *disorder-to-partial order* (ii) and *disorder-to-disorder* (iii) transitions result in structural multiplicity or dynamic disorder in the bound state, termed as protein fuzziness [23-25]. In fuzzy protein assemblies conformational heterogeneity is demonstrated to impact function [26, 27]. Depending on the biological role of the protein, mutations, truncations or removal of regions, which preserve their structural diversity in the bound state, can affect binding affinity, specificity, transcriptional activity, cell fate, catalytic rates, half-lives, cell-cycle progress, aggregation, or a response to posttranslational modifications [24, 25]. Increasing experimental evidence corroborates the importance of structurally ill-defined regions (i.e. represented by ensembles of conformations) in protein function, as

illustrated by the annotated examples in the Fuzzy Complexes Database (FuzDB, http://protdyn-database.org) [28]. Fuzziness is an intrinsic feature of all higher-order protein organizations, ranging from static amyloids/prions, prion-like and multivalent signaling complexes as well as cytoplasmic and nuclear granules [29]. The degree of fuzziness is a critical factor for the material state (solid, hydrogel, liquid-like) of higher-order structures and may indicate pathological mutations.

We will review fuzziness-related molecular mechanisms, which enable proteins to adapt to their cellular environments and respond to incoming signals (Table 1). All these scenarios require conformational heterogeneity upon interactions with biomolecular partners.

V. Rewiring interaction networks via alternative motifs

V.1. Fuzziness in tissue-specific protein isoforms

Alternative splicing (AS) expands the biological repertoire of proteins and increases regulatory complexity [30]. Alternatively spliced ID regions frequently contain functional sites, such as short linear motifs for interactions or posttranslational modification sites that can lead to distinct functionalities in the different isoforms [12]. Alternative inclusion of tissue-specific (TS) exons plays a role in maintaining tissue identity [31] and the encoded tissue-specific ID regions are frequently associated with predicted ID binding sites, which undergo *disorder-to-order* transition upon binding [32, 33]. These motifs are often located at protein-protein interaction interfaces, which almost never comprise the full TS region [32] (Table 1). This suggests that protein segments flanking/linking the contacting residues remain largely heterogeneous or dynamic in the complex (Figure 2). Conformational ensembles are modulated by the environmental conditions to rewire the interaction networks for context-dependent outcomes.

The C-terminal TS region of the phosphatidylinositol-4-phosphate 5-kinase-type 1 γ interacts with the signal-sorting domain of the AP-2 complex to mediate clathrin-mediated endocytosis in the brain, while its absence abolishes the assembly in the lymph node [34]. The same TS region is involved in interaction with Talin, to activate the kinase for focal adhesion assembly [35]. In case of the AP-2 complex only the first 13 residues (641-653, PDB:3h1z) adopt a mostly irregular secondary structure [34], whereas in the case of Talin assembly only the middle 8 residues (646-653, PDB:2g35) could be observed out of the 28 residues of the TS region [36] (Figure 3). The interfaces of both complexes comprise redundant interactions, which together with the invisible regions contribute to dynamical regulation of focal adhesion as well as endocytosis, as also indicated by the in vivo data [35].

V.2. AS and PTM generated molecular barcodes in fuzzy regions

Alternative splicing may also lead to different gene-expression patterns in different tissues or cellular locations via modulating the dynamical properties of the ID regions (Table 1). Transient interactions, which are mediated by fuzzy regions can enhance/compete for the binding interface or may also nonspecifically anchor the protein to its partner [26, 27]. Ubx is a member of the Hox family, which controls the fate of multiple body structures by context-specific gene regulation. Ubx isoforms

with differential expression of three microexons are produced in a stage- and tissue-specific manner in *Drosophila* [37]. These microexons encode fuzzy protein regions, which impact DNA binding via three different mechanisms. They can establish transient, variable interactions with the ionizable residues of the Hox domain (i), provide steric hindrance to DNA (ii) and mediate intramolecular contacts between dynamic segments of Ubx (iii), the complex interplay of which results in different DNA binding affinity and selectivity *in vivo* [38]. Taken together, contextual information for gene-expression in different stages of development or at distinct locations is derived from the transient communication among three fuzzy regions, which are further modulated by alternative splicing [26].

Posttranslational modifications in combination with alternative splicing can provide another layer of regulation to generate context-specific responses. The major isoform (18,5 kDa) of the myelin basic protein (MBP) is part of the mammalian central nervous system and plays a role in maintaining the stability and integrity of the myelin sheath (Table 1). MBP serves as a membrane-bound interaction hub for actin, tubulin, calmodulin and a variety of SH3-domain containing signaling proteins (Fyn, PSD95, cortactin, c-Src, Abl) [39]. The binding affinities with SH3 are in the mM range, which are dynamically affected by residues outside the canonical site [40]. Myelin signaling critically depends on the delicate balance between the MPB-ligand and MBP-membrane associations, which is also controlled by multi-site phosphorylation. PTMs interfere with the electrostatic interactions between the central membrane-anchoring helix and the flanking proline-rich segment, which affect its flexibility and availability for cytosolic ligands [40]. Alternatively spliced and possibly differentially phosphorylated fuzzy regions have distinct interaction partners in different tissues to reshape their interaction networks according to the cellular milieu. This generates a 'dynamical molecular barcode' to activate the myelin interaction network in a context-dependent manner.

VI. New functional features via allosteric motifs

VI.1. Allosteric coupling between fuzzy regions

Growing evidence indicates that ID region-mediated allosteric communication is present in a wide range of organisms [4, 41]. Allosteric signals in ID proteins are conveyed via dynamic linkers, which are represented by conformational ensembles in the bound states, while the coupled functional sites are assumed to fold upon binding [42]. Increasing experimental evidence illustrates however that bound ID interaction elements may still exhibit conformational exchange on the μs-ms timescale and establish redundant or polymorphic contacts with the partner (Figure 1) (e.g. GCN4 [18], Hsp90 [19]). Functional couplings between protein regions, which undergo adaptive structural transitions also provide important allosteric mechanisms [27] (Figure 2).

RSK1 is a MAPK activated protein kinase, which belongs to the calmodulin-dependent kinase (CaMK) superfamily. Along the MAPK pathway, the C-terminal RSK1 segment is required for ERK2 recruitment, but also for the autoinhibition of the RSK1 CaMK-type domain. S100B binding to RSK1 targets the C-terminal domain,

which inhibits ERK phosphorylation. Upon interacting with S100B, two short RSK1 regions (10 and 7 residues) undergo disorder-to-order transition, which are connected by a 21 AA disordered linker [43]. The structured motifs are polymorphic in the bound-state, as illustrated by a variety of secondary structures containing different topologies of helical and coil elements (Figure 3). Conformational heterogeneity of the bound RSK1 corresponds to auto-inhibited (~44% of the population) and released states, depending on the position of the C-terminal αL helix [43], which could be modulated by the context.

VI.2. Evolution of novel regulatory features in fuzzy assemblies

Emergence of allosteric sites can also result in novel regulatory features. Functional cooperativity of HoxA11 and Foxo1 is required to activate the expression of the prolactin gene in mammalian endometrial stromal cells. The physical interaction between the two transcription factors has evolved before their functional connection, although the homeodomain interface remained conserved from marsupials to placental mammals [44]. The derived cooperativity between HoxA11 and Foxo1 is due to derived allosteric regulation of HoxA11 by Foxo1 [45] (Table 1). The N-terminal ID region of HoxA11 can establish autoinhibitory interactions within the activation domain via a 20AA linear motif. Deletion of the negative regulatory peptide leads to Foxo1-independent activation of the ancestral HoxA11 [45]. Conditional cooperativity of HoxA11 could be developed by introducing four mutations: two replacements in an interaction motif with the KIX domain of CBP/p300 and two proline residues leading to derived phosphorylation sites. NMR studies corroborated that the N-terminal IDR binds to the KIX domain of CBP/p300 and establishes a fuzzy complex [45]. Phospho-mimicking mutations improve the weak mM affinity, suggesting that derived phosphorylation sites are required for more efficient interactions with the transcriptional coactivator. Taken together, Foxo1 binding unmasks the repressive intramolecular interactions within the activation domain via a complex array of interactions between linear motifs. Here fuzziness enables the evolution of an allosteric switch within the pre-existing HoxA11- Foxo1 complex to functionally respond to Foxo1 for transcriptional activation.

VII. Functional switches via posttranslational modifications

VII.1. Activity modulation by PTMs targeting fuzzy assemblies

PTM-induced conformational biases can interfere with long-range communication of fuzzy regions to generate alternative functional states (Figure 2). Upon ER stress, Ire1 catalyzes the splicing of mRNAs to mature master regulators of unfolded protein response. The ribonuclease and kinase domains of Ire1 are functionally linked, as the cofactor and substrate binding is facilitated by the kinase activity. Ire1 is a monomer in the resting state, and accumulation of unfolded proteins in ER stimulates its dimerization. In the dimeric form, a dynamic activation loop of the other molecule may reach the kinase active site and initiates trans-autophosphorylation [46] (Figure 3). Phosphorylation induces structuring of the fuzzy activation loop, which further stabilizes the dimer interface. In this manner,

trans-autophosphorylation locks the assembly-prone conformation of Ire1 and can induce further oligomerization, which also increases the local concentration of the kinase/RNase domains [47]. Binding of the nucleotide cofactor requires the open form of the kinase, which is present in both dimeric and oligomeric forms. Although the mechanistic connection to ribonuclease catalysis has not been unambiguously elucidated, dimerization induced phosphorylation of the fuzzy kinase activation loop is a key event to activate Ire1 RNase and start the unfolded protein response program [47]. In innate immune response, phosphorylation of IRF-3 by TBK-1 similarly takes place in a dimeric form to convert the autoinhibitory configuration into a domain-swapped active form [48] (Table 1). A pLxIS motif, which plays a role in this process is also critical for the recruitment of IRF-3 by STING, MAVS or TRIF adaptor proteins.

Phosphorylation induced ordering may also significantly decrease affinity of binding, as in case of 4E-BP:eIF4E interactions, which enable eIF4G to access eIF4E and initiate translation [49] (Table 1). Here phosphorylation provides a regulatory mechanism via modulating the dynamic landscape of 4E-BP. Phosphorylation of the activating loops however does not always induce disorder-to-order transition in protein assemblies. Phosphorylation of MLKL by RIP3 is required for downstream signaling in programmed necrosis. Although phosphorylation stabilizes the association, the phosphorylation sites in MLKL are still invisible in the complex structure [50] (Table 1).

VII.2. Modulation of protein turnover within fuzzy assemblies

Phosphorylation in protein assemblies can also impact protein turnover. The short half-life of c-Myc oncoprotein is critical for cell proliferation and is controlled by phosphorylation at two residues. Phosphorylation of S62 increases Myc stability, whereas subsequent phoshorylation of T58 bias for degradation by promoting dephosphorylation of S62 [51]. Perturbation of any of these PTM events leads to aggressive lymphomas. Upon interacting with Bin1, the entire N-terminal transactivation region of c-Myc remains largely heterogeneous, exhibiting a multipartite binding via short, transiently structured regions [52] (Table 1). Fuzziness of the complex enables a conformational exchange of the binding sites on the ms timescale. This not only couples the PTM sites, but also triggers protein phosphatase 2A (PP2A) to approach S62.

A more complex interplay between phosphorylation and degradation takes place in Wnt signaling. Phosphorylation of APC downregulates β-catenin and also protects it from destruction (Table 1). Owing to the heterogeneous conformation of the phosphorylated APC in the complex, it becomes accessible for the axin bound PP2A phosphatase [53]. In turn, the dephosphorylated APC controls β-catenin release. In the absence of Wnt, β-catenin is sequestered by only phosphorylated APC, while in the presence of Wnt the higher concentration of β-catenin binds to both higher and low affinity forms of APC. It was proposed that fuzziness enables β-catenin binding to the ubiquitination machinery without completely dissociating from the destruction complex to prevent the unnecessary entry of β-catenin to the nucleus [53].

VIII. Regulation of higher-order protein organizations

VIII.1. Fuzzy regions regulate assembly/disassembly

Higher-order assemblies modulate signaling amplitudes and reduce signaling noise by increasing avidity for low-affinity substrates [54, 55]. All higher-order organizations are generated via nucleated polymerization [29], which is usually induced via allosteric motif interactions or via relieving autoinhibition (Figure 2).

The AIM2 inflammasome is assembled via three different oligomerization mechanisms to defend against bacterial and viral DNA. In the absence of the signal, an inactive form is stabilized by electrostatic intramolecular interactions between the PYD and HIN domains, which is promoted by a ~50 residue highly charged linker (Table 1). Recognition of the foreign dsDNA by the HIN domain relieves autoinhibition and triggers PYD-PYD interactions. The oligomerized AIM2 PYDs serve as a platform to nucleate ASC PYD filaments [56-58]. The ~25 residue extended linker between the ASC PYD and the CARD domains stretches out the filament and aligns the CARD domains (Figure 3). The ASC CARDs may interact with each other and nucleate caspase-1 filaments via CARD-CARD interactions [56]. All these different mechanisms are mediated by dynamic linkers, which also preserve heterogeneity in the assembly. Fuzziness enables to shift the conformational ensemble between autoinhibited and extended forms and can also transmit allosteric signals between the different domains to promote oligomerization (Figure 2). Similarly, the 200-residue dynamic linker in the p62 autophagy scaffolding protein allosterically regulates the disassembly of the helical filament upon recognition of the ubiquitinated cargo by the exposed UBA domain [59] (Table 1). Furthermore, the fuzzy linker facilitates delivery of the substrates to the autophagosome via the embedded LC3 interaction motifs.

VIII.2. PTMs in fuzzy regions can relief autoinhibition

PTMs could promote assembly or disassembly by up- or downregulation of respective kinases in a context-dependent manner. RIP1 regulates cell fate according to the cellular contexts to activate the NF-κB pathway, apoptosis or programmed necrosis. In the TNF-induced programmed necrosis pathway, the RIP1 and RIP3 kinases form a functional amyloid complex using their RIP homotypic interaction motifs (RHIMs) [60]. Formation of the higher-order structure induces kinase autophosphorylation, leading to activation of downstream molecules responsible for necrosis execution (Table 1). In their inactive states, the core RHIM sequences may be concealed by the disordered flanking sequences of RIP1 and RIP3 to create an autoinhibitory configuration. Therefore, only the RIP1 and RIP3 RHIM fragments, but not the full-length RIP1 and RIP3, induced spontaneous clustering in cells without stimulation [60]. Kinase activation and the resultant hyperphosphorylation may create charge repulsion to expose the RHIM core to enhance complex formation. In feedforward mechanism, complex formation further potentiates kinase activation to propagate the pronecrotic signal. Cryo-EM and solid-state NMR confirmed the presence of short peptide sequences in the amyloid core, which are flanked by highly dynamic regions [60]. Mutations in these fuzzy segments are defective in programmed necrosis as they block the switch from autoinhibited to aggregation-prone state.

VIII.3. Fuzzy regions in phase separation

Tandem interaction domains/motifs interspersed by fuzzy linkers enable combinatorial crosslinks in multivalent signaling complexes [29, 61]. The resulting assemblies are highly dynamic, reversible and may undergo liquid-liquid phase separation [62]. Liquid droplets of ribonucleoprotein (RNP) complexes are metastable higher-order structures, which could easily be perturbed by PTMs. Electrostatic attraction between oppositely charged motifs, charge-π or dipole-dipole interactions in RNP granules sensitize to modification of charge [63-67]. Redundant, low-affinity contacts between patterns of FG and RG motifs in the ID N-terminal region of DDx4 drive formation of nudge granules [63]. Phase separation is regulated via arginine methylation by PRMT1 according to the context of the cellular microenvironment (Table 1). Along these lines, arginine methylation influences the localization and toxicity of Fus, as inhibition of PRMT1 reduces the tendency of ALS causing Fus mutants to associate with cytoplasmic stress granules [68]. Despite the fact that degenerate, mostly hydrophobic interactions are critical for phase separation of Fus, phosphorylation can also promote its shuttling to the cytoplasm [64]. Owing to sensitivity to PTMs, DYRK3 kinase activation upon cellular stress could induce dissolution of stress granules to initiate mTORC1 signaling [69] (Table 1). Although high-resolution structural characterization of these dynamic assemblies is a bottleneck owing to their structural heterogeneity and redundancy of weak contacts, NMR data on Fus [64] and FRET results on Nup153 [70] are consistent with the intrinsic fuzziness of these higher-order systems.

IX. Stochastic structure-function relationship

Adaptation to the environment is a vital task for all cellular entities, which requires efficient communication between the different components of signalling pathways. Four scenarios for context-dependence have been discussed: *i)* reorganization of interaction networks in distinct cellular locations via alternative inclusion of binding elements, *ii)* emergence of functional links between distinct domains/sites via allosteric coupling, *iii)* PTM-induced activity switches and *iv)* regulation of higher-order organizations to modulate signalling amplitudes. We demonstrated that all these mechanisms are intertwined with adaptive structural transitions of the underlying protein segments. Interactions mediated by fuzzy regions can influence adjacent binding elements, transmit allosteric signals, promote autoinhibition via intramolecular contacts or increase local concentration to improve binding [24, 27]. We have shown that structural multiplicity or dynamic disorder persisting in the bound state of protein assemblies leads to polymorphism and alternative functional states, which could be influenced by the environmental conditions.

These observations call to revise the classical structure-function paradigm. Instead of a deterministic link between protein sequence $\rightarrow$ structure$\rightarrow$ function, a stochastic model needs to emerge, which relates generalized ensembles of functional motifs/modules to structural ensembles of proteins and their assemblies. The generalized sequence ensemble corresponds to a set of sequences, which

generate the same interaction patterns via redundant contacts. Polymorphism increases with system complexity and dynamics, and is critical for biological phase transitions [29, 61]. Conformational ensembles are composed of different sub-populations, the weights of which are modified by contextual changes leading to alternative interaction patterns and different functional outcomes. Fuzziness is central to the stochastic model, as structural diversity of proteins and assemblies generates alternative connections between the functional modules, which in turn impact activities according to the cellular milieu. Importantly, the functional space is also expanded in the model, as different activities within the functional ensemble are associated with the different combinations of the conformational sub-populations.

X. Concluding remarks

Although protein dynamics has been considered as an important element of protein function, its critical role in protein adaptation has been overlooked thus far. Within the framework of the stochastic structure-function relationship, modulation of conformational ensembles can rewire the underlying interaction networks and shift towards alternative functional states. Fuzzy regions, which are often invisible in complexes or higher-order assemblies enable different contact topologies according to the environment or incoming signals. Careful characterization of ensemble populations and their biochemical connections are required to shed light on how adaptation takes place and how it could possibly be influenced.

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Table 1 Examples of fuzzy regions with context-dependent activities

Protein	Partner	Fuzzy region and binding site (PDB)	Function	Impact	Posttranslational modification	FuzDB ID	Reference
Tissue- or cell-type specific alternative splicing							
PIP5K1C	AP-2	641-668 (3h1z)	endocytosis	Interaction with AP-2 in brain and not lymph nodes.			[34]
PIP5K1C	Talin FERM domain	641-668 (2g35)	cell migration	Kinase activation to affect focal adhesion assembly. Synaptic vesicle recycling at nerve terminals in brain and not in heart	phosphorylation		[36]
MeCP2	CpG DNA	1-75 (3c2i)	transcription	Different gene expression patterns in brain.		FC0043	[71]
Max	DNA	1-18 (1hlo)	transcription	Increasing DNA binding affinity in normal vs malignant bronchial epithelial cells	phosphorylation	FC0042	[72]
Ubx	Exd, DNA	238-286 (1b8i)	transcription	Modulating DNA binding affinity and selectivity in tissue and development-specific manner.		FC0053	[38]
MBP	Fyn SH3	38-107	myelin sheath development	Affecting myelin signalling via modulating partner interactions.	phosphorylation	FC0105	[40]
MBD2	DNA	212-273, 317-361	gene expression	Relieving repression in embryonic stem cells.		FC0087	[73]

Novel regulatory features by allosteric motifs							
RSK1	S100B, ERK	685-731 (5csf, 5csi, 5csj, 5csn)	cell growth	Allosteric inhibition of RSK1 phosphorylation.	phosphorylation	FC0091	[43]
HoxA11	Foxo1, CBP/p300	1-120	gene-expression	Allosteric switch to activate prolactin gene expression in Foxo1 dependent manner in placental mammals.	phosphorylation		[45]
E1A	TAZ2 (CBP/p300), Rb	92-126 (2kje)	repression of gene-expression	Allosteric coupling between CBP and Rb to reprogram host cell transcription.		FC0083	[74]
ORF57	ALYREF, RNA	81-120 (2yka)	viral mRNA export	Allosteric regulation of ALYREF to bind viral RNA via formation of the ternary complex.		FC0102	[75]
Ets-1	DNA	244-300 (1mdm)	transcription	Tuning DNA binding affinity via allosteric transient interactions.	phosphorylation, AS	FC0047	[76]
LRRC16A	CP	971-1035 (3lk3)	cytoskeleton organization	Dynamic regulation of actin assembly via an allosteric region.		FC0071	[77]
PSD95	SH3-GK	396-429	molecular trafficking	Allosteric regulation of PSD95 length for dynamic remodelling of postsynaptic density.		FC0095	[78]
Hsp90	Ppp5	727-732 (2bug)	protein folding	Allosteric relief of the phosphatase auto-inhibition.		FC0003	[19]

Functional switches by PTMs of the assembly							
Ire1	Ire1	838-851 (3fvb)	protein homeostasis	Trans-auto-phosphorylation activates ribonuclease to initiate the unfolded protein response.	phosphorylation		[46, 47]
IRF3	CBP	344-379 (5jej)	Innate immunity	Phosphorylation of the dimer converts to the active form available to adaptor proteins.	phosphorylation		[48]
MLKL	RIP3	345-357 (4m69)	programmed necrosis	Phosphorylation stabilizes the complex and activates downstream signaling	phosphorylation		[50]
4E-BP2	eIF4E	1-65 (3am7)	translation	Phosphorylation n allosterically regulates eIF4E binding to mRNA cap and increases sensitivity for rapamycin.	phosphorylation	FC0072	[49, 79]
c-Myc	Bin	1-88 (1mv0)	transcription	Phosphorylation induced ubiquitination regulates half-life.	phosphorylation, ubiquitination	FC0074	[52]
p27	Cdk2/cyclin	105-198 (1jsu)	cell-cycle regulation	Phosphorylation initiates ubiquitination-mediated degradation allowing the progress to the S phase.	phosphorylation ubiquitination	FC0036	[80]
APC	β-catenin	1499-1528	Wnt signaling	Phosphorylation modulates β-catenin binding and degradation.	phosphorylation	FC0037	[53]

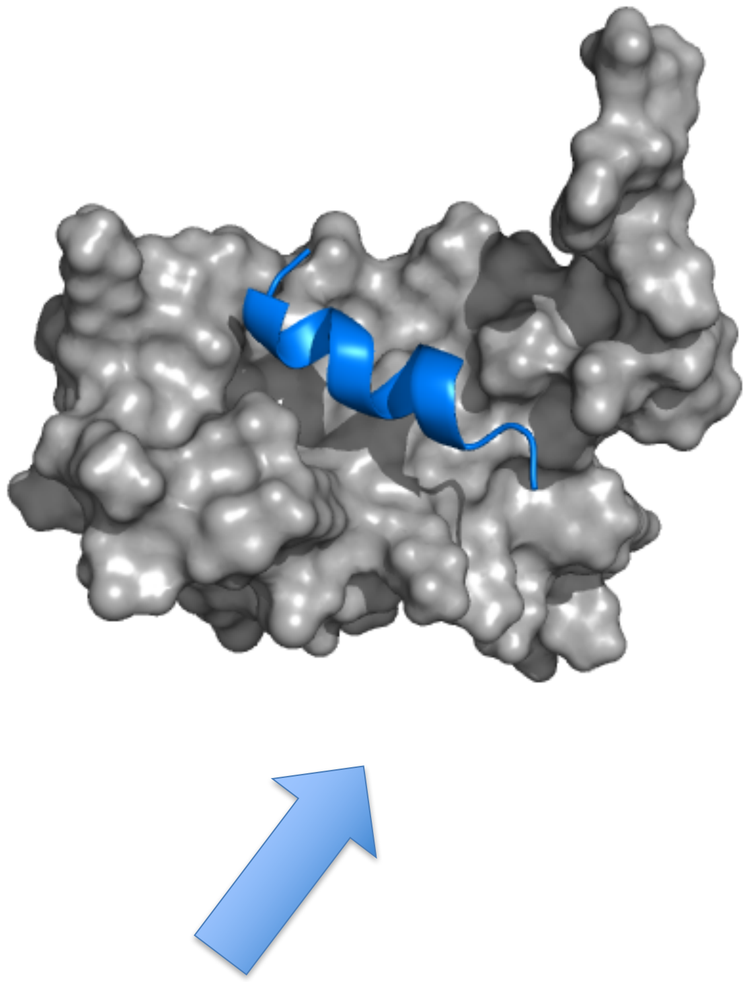
Regulation of higher-order assemblies							
AIM2/PYD	AIM2/HIN	88-137	innate immunity	Promotes autoinhibiton via electrostatic interactions and facilitates PYD oligomerization.			[81]
ASC/PYD	ASC/CARD	92-116	apoptosis	Mediates allosteric signals between oligomerized PYD and CARD.			[56]
p62/PB1	p62/UBA	168-388	autophagosome	Transmits allosteric signals between PB1 and UBA to initiate disassembly upon substrate recognition.	ubiquitination	FC0092	[59]
RIP1	RIP3	496-524, 556-583	programmed necrosis	Switches between autoinhibited and aggregation-prone forms to activate necrosis.	phosphorylation		[60]
Ddx4	Ddx4	1-236	germline generation	Mediates low-affinity interactions between patterns of RG and FG motifs to drive granule formation.	methylation (Arg)		[63]
Fus	Fus	1-237	RNA processing	Drives granule formation and co-localizes with nuclear or cytoplasmic stress granules.	methylation (Arg), phosphorylation	FC0097	[64]
DYRK3	Stress granule	1-188	cellular stress	Regulates stress granule assembly/disassembly via kinase activity.	phosphorylation		[69]

Figure legends

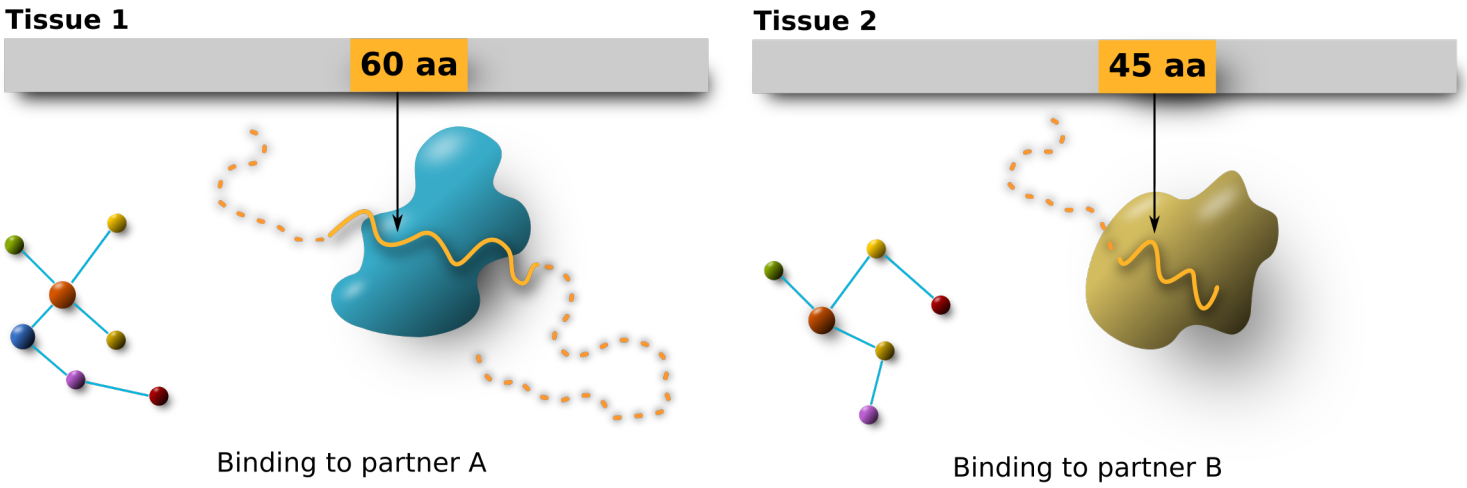
Figure 1 Structural transitions of ID regions upon partner interactions generate a structural and dynamical continuum of ID complexes. *Disorder-to-order* transition (upper panel) usually involves ID regions, with a conformational bias in the unbound state. *Disorder-to-partial order* transition can originate from ms-μs dynamics of ordered structure elements, or by the dynamic disorder of the flanking regions (middle panels). *Disorder-to-disorder* transitions result in polymorphic states, with fast conformational exchange in the bound state (lower panel). The representative conformations are generated from the different models of the GCN4-Med15 complex (PDB:2lpb) [20].

Figure 2 Different mechanisms of context-dependent responses by fuzzy regions. (**A**) Rewiring protein interaction networks by changing the length and dynamics of the fuzzy region via alternative splicing. (**B**) Long-range communication within fuzzy region can lead to allosteric mechanisms. (**C**) PTMs within fuzzy regions can provide functional switches to activate proteins/pathways. (**D**) Shifting the conformational equilibrium of fuzzy regions can initiate higher-order assemblies by converting the auto-inhibited to assembly-prone forms. Fuzzy regions are shown by orange, dotted lines.

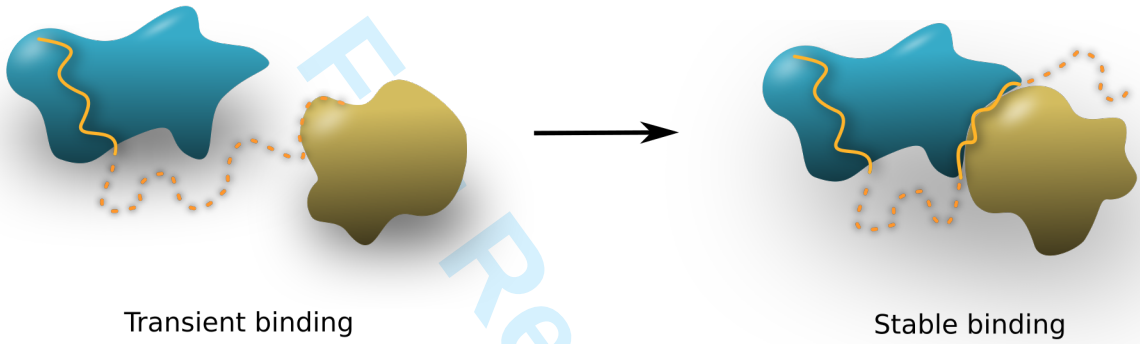
Figure 3 Examples of context-dependent responses by fuzzy regions. (**A**) Tissue-specific splicing of PIPK1γ generates alternative responses in brain and lymph node [34]. The TS region may interact with AP-2 (PDB:3h1z) or Talin (PDB:2g35) [36] with different degrees of ordering. (**B**) Allosteric sites of the RSK1 in S100B complex (PDB:5csi, 5csj, 5csn) exhibit different secondary structures, which are connected by a dynamic linker [43]. Polymorphism is resulted by the auto-inhibited and released states. (**C**) Trans-autophosphorylation of a fuzzy activation loop in the Ire dimer (PDB:2rio) leads to increased stability and further oligomerization (PDB:3fbv) [46, 47]. (**D**) Phosphorylation can induce assembly of ASC by shifting from auto-inhibited to aggregartion-prone states (PDB:2kn6). The conformational ensemble of the dynamic linker between in PYD and CARD domains will prefer the extended conformations owing to charge repulsion [56]. Fuzzy regions are shown by orange, dotted lines.


disorder-to-order
disorder-to-partial order
disorder-to-disorder

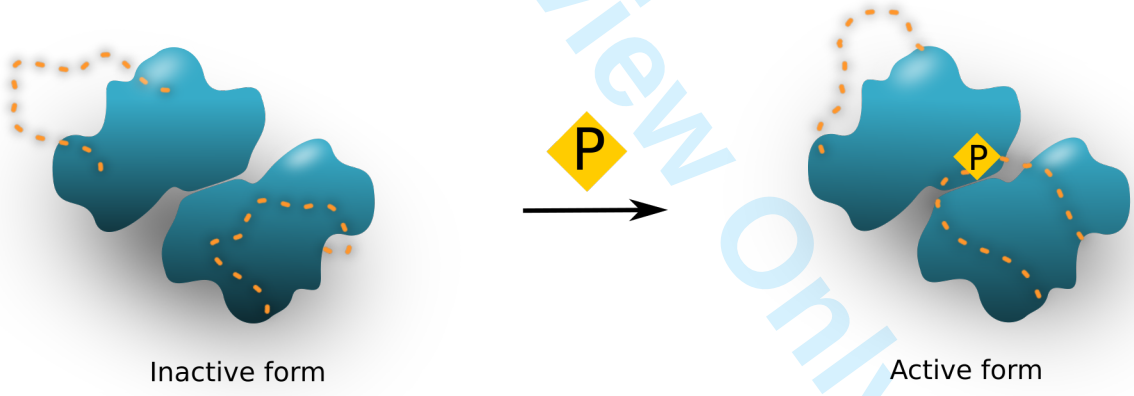
A Rewiring interaction networks by alternative splicing



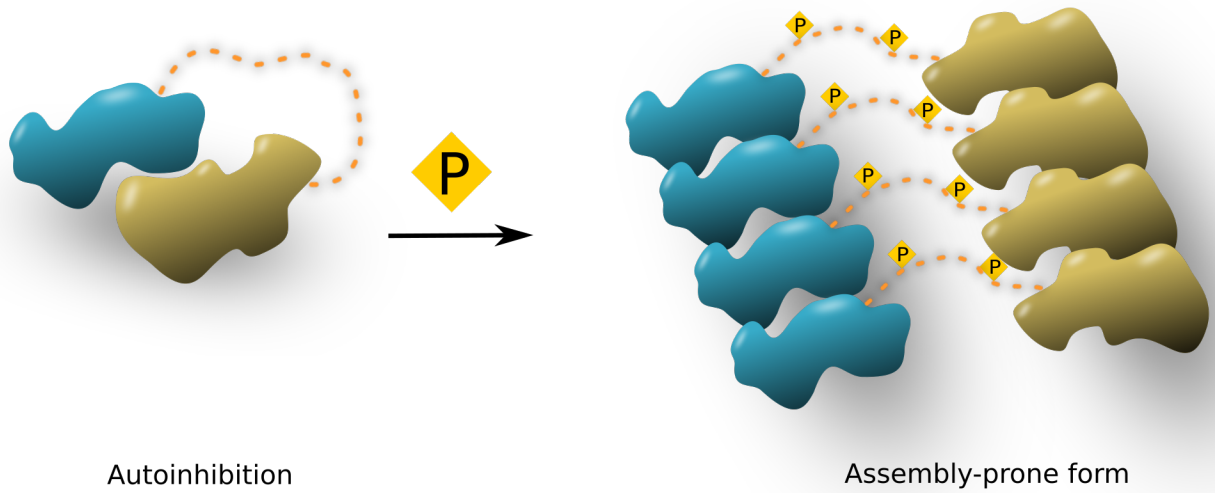
B New functional features by allosteric motifs



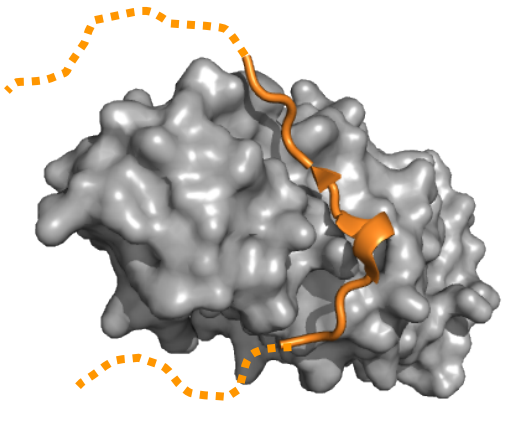
C Functional switches by PTMs



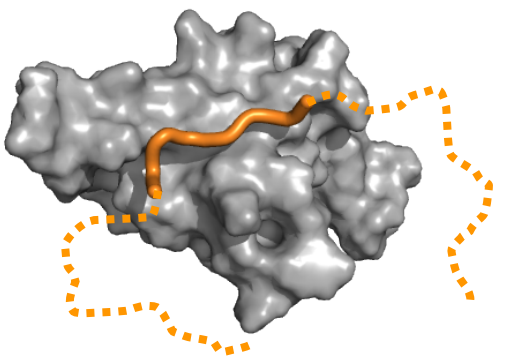
D Regulation of higher-order organizations



A

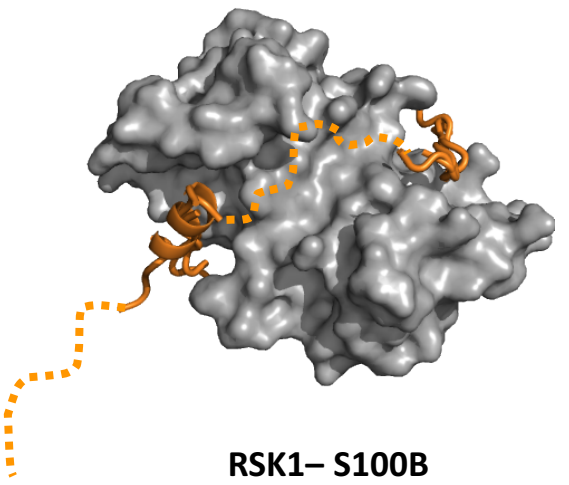


PIPK1γ – AP2



PIPK1γ – talin

B

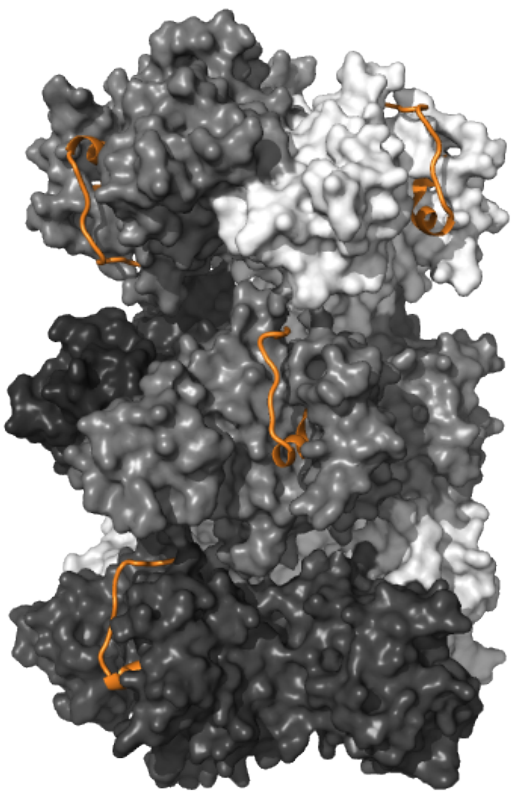


RSK1– S100B

C

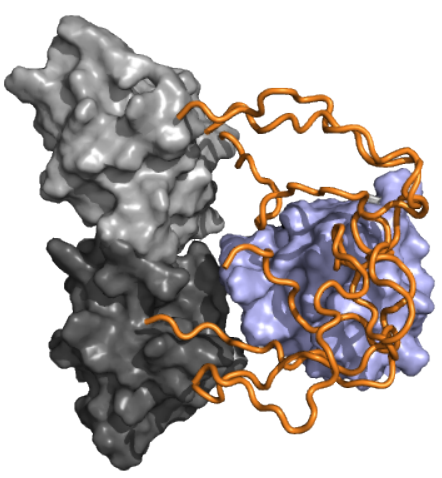
Ire dimer

P



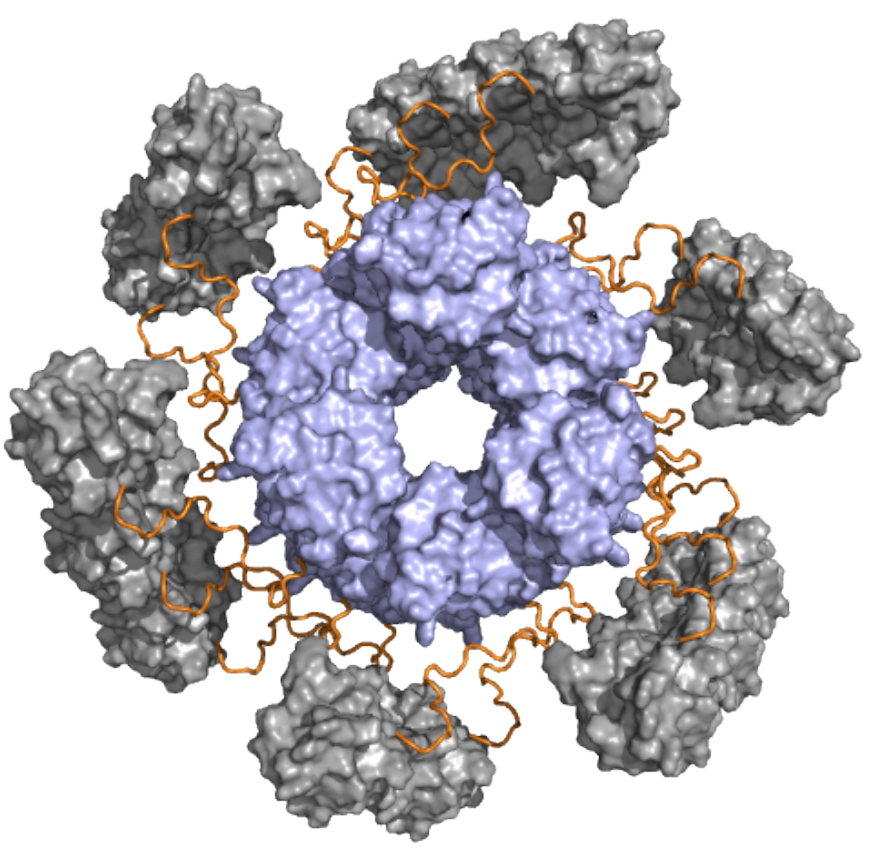
Ire oligomer

D



ASC monomer

P



ASC oligomer