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Conditional disorder in proteins: Functional transitions between order and disorder

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Abstract

The classical view of protein function based on rigid, well-defined structures is being redefined by the emerging concept of intrinsic disorder. Conditionally disordered proteins (CDPs) represent a subset of cellular intrinsically disordered proteins (IDPs) that transition between ordered and disordered states in response to specific stimuli, such as redox changes, post-translational modifications, ligand binding, interaction with partners, or environmental stress. This review explores the diverse landscape of conditional disorder and encompasses cryptic or dormant disordered regions, redox-sensitive motifs, metamorphic proteins, and proteins exhibiting order-disorder-new order transitions. These dynamic transitions allow CDPs to perform specialized regulatory, signalling, and stress-responsive roles, which often act as interaction hubs in complex cellular networks. Importantly, conditional disorder is not an anomaly but a conserved and functionally relevant feature across many proteomes. We highlight mechanistic insights into disorder-to-order transitions and their implications for cellular plasticity, adaptability, and disease. We also discuss how the conformational heterogeneity of CDPs complicates structure-based drug design, while offering unique therapeutic opportunities. Future directions include the integration of advanced biophysical techniques, computational modelling, and profiling to map, characterize, and target CDPs with greater precision. Overall, understanding the molecular logics of the conditional disorder will open new frontiers in structural biology and offer a deeper appreciation of protein versatility beyond static structural paradigms.

Keywords

Conditional disorder; Intrinsically disordered proteins (IDPs); Metamorphic proteins; Redox-sensitive disorder; Protein conformational dynamics; Disorder-to-order transition

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1. Introduction

Intrinsically disordered proteins (IDPs) represent a distinct class of proteins that lack a stable, well-defined three-dimensional (3D) structure under physiological conditions [1]. Despite this apparent structural deficiency, IDPs are abundant in nature and play indispensable roles in diverse biological processes [2]. Unlike globular proteins, IDPs do not adopt a single, energetically favorable conformation but exist as dynamic ensembles of rapidly interconverting conformations (Figure 1) [3,4]. The sequence characteristics of IDPs are central to their structural plasticity. IDPs are typically enriched in disorder-promoting residues (e.g., proline, serine, glutamine) and depleted in order-promoting residues (e.g., aromatic residues, leucine, isoleucine, valine), which underlie their inability to fold into a stable tertiary structure [5]. This intrinsic flexibility allows IDPs to bind multiple partners with high specificity yet low affinity, often via short linear motifs (SLiMs) or molecular recognition features (MoRFs), which are disordered elements capable of disorder-to-order transition at interaction with specific partners [6].

The functional versatility of IDPs arises directly from their disordered nature. They are key players in numerous cellular processes, including signal transduction, transcriptional regulation, cell cycle control, and post-translational modifications (PTMs) such as phosphorylation [5]. Many IDPs undergo disorder-to-order transitions upon binding to specific targets, thereby achieving interaction specificity while maintaining adaptability [2]. This conformational plasticity enables IDPs to act as central hubs in cellular networks [7]. IDPs are also implicated in various pathological conditions, especially neurodegenerative diseases [8]. For example, Alzheimer's disease is marked by the accumulation of neurofibrillary tangles composed of hyperphosphorylated tau and extracellular plaques of amyloid-β (Aβ), both of which involve IDPs [9]. Their structural malleability renders IDPs prone to misfolding and aggregation, contributing to disease pathogenesis in disorders such as Parkinson's, Huntington's, and Amyotrophic lateral sclerosis (ALS). However, their disease relevance also positions them as attractive targets for therapeutic intervention [10,11].

The prevalence of intrinsically disordered regions (IDRs) in proteomes of both prokaryotes and eukaryotes is well-documented, with estimates suggesting that a significant fraction of the human proteome contains disordered segments [12]. IDRs confer functional advantages, allowing proteins to engage in multiple interactions and respond dynamically to environmental cues [11,12]. Transcription factors, for instance, frequently contain disordered effector domains (EDs) that enable multivalent interactions and the formation of transcriptional condensates [13]. The structural plasticity of IDRs also facilitates regulation via PTMs, which can fine-tune protein

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function rapidly and reversibly. IDRs further act as molecular sensors, changing conformation in response to stress or other stimuli and thereby modulating cellular signalling pathways [14]. Importantly, IDRs can drive liquid–liquid phase separation (LLPS), giving rise to membrane-less organelles (MLOs) such as nucleoli and stress granules. These dynamic condensates concentrate regulatory factors and enhance transcriptional efficiency among numerous other functionalities [15,16].

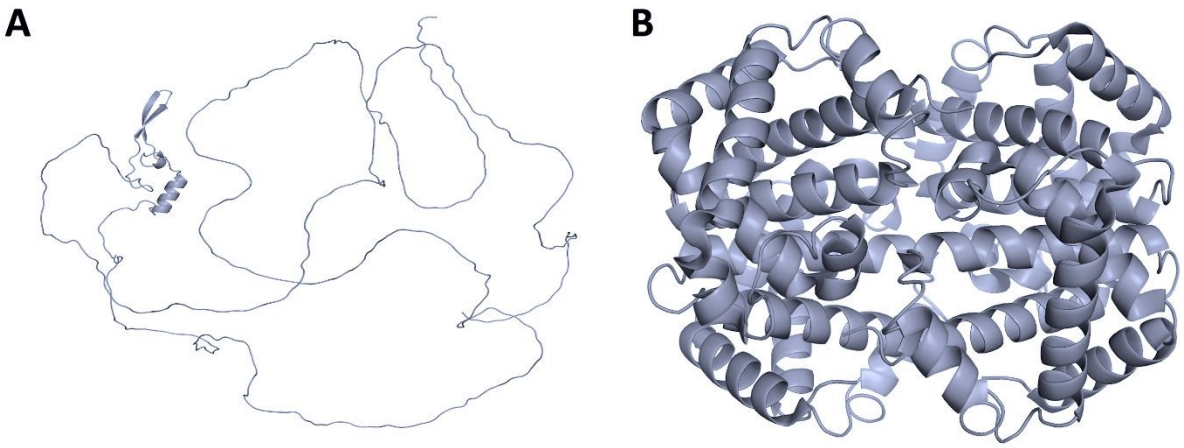

Figure 1: Disordered protein vs ordered protein. The left panel is a structural representation of human Nup98 (disordered protein) with flexible conformation, and the right panel is a representation of Haemoglobin (ordered protein), characterized by a distinct stable folded structure. The disordered region of Nup98 (residues 1-450; UniProt ID: P52948) was modelled and visualized using PyMOL. Haemoglobin structure was obtained from the Protein Data Bank (PDB ID: 4HHB) and visualized in PyMOL.

2. Effect of environment on protein disorder

Environmental factors have a major influence on the structural plasticity of proteins, particularly in modulating transitions between ordered and disordered states. Variations in the cellular environment can markedly affect protein conformation, dynamics, and function. Changes in amino acid sequence, as well as experimental conditions such as pH, temperature, macromolecular crowding, the presence of osmolytes, and ionic strength, can impact the presence, position, and length of IDRs in proteins (Figure 2) [17]. These factors affect the delicate balance of intramolecular forces governing protein conformations, thereby shifting the equilibrium between order and

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disorder [18]. Proteins may undergo substantial structural and mechanical changes in response to alterations in pH and temperature, leading to unfolding or destabilization of their native spatial organization. Such changes influence the Gibbs free energy landscape, thereby modulating the stability of folded and unfolded states [19,20]. Under specific environmental conditions, the conformational ensemble a protein adopts is shaped by its intrinsic properties (such as amino acid composition and PTMs) interacting with these external cues. Dysregulation of these interactions is frequently associated with pathological states [21].

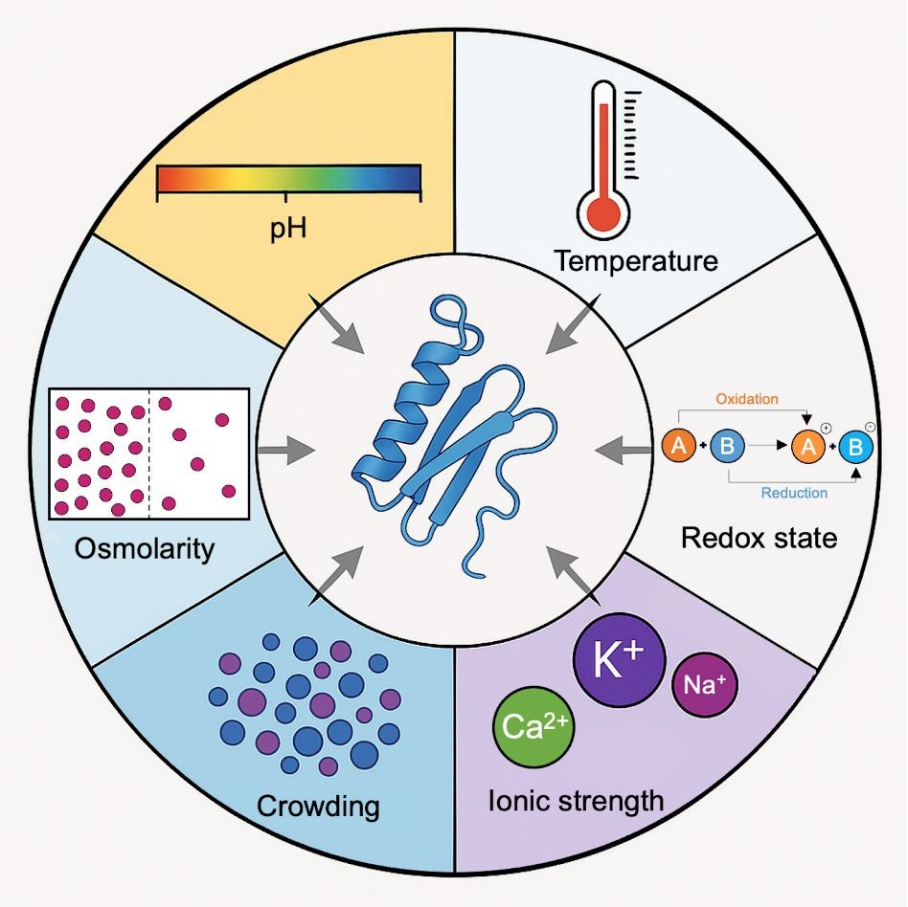

Figure 2: Effect of environmental factors on protein structure. The schematic illustrates how key external conditions, including pH, temperature, redox state, ionic strength, macromolecular crowding, and osmolarity, together impact protein conformation and stability.

pH plays a particularly critical role in determining protein conformation. It alters the ionization states of amino acid side chains, thereby affecting intramolecular hydrophobic and

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electrostatic interactions and potentially triggering phase separation and coacervation behaviors [22]. Changes in pH can alter the shape, molecular size, and adhesion properties of proteins, with extreme pH levels often decreasing structural stability [20]. Even at a fixed pH, proteins with multiple ionizable residues can exist in various charge states, leading to a heterogeneous distribution of conformers. This heterogeneity is especially relevant to IDPs, whose conformational flexibility is highly sensitive to local charge environments [23]. Histidine residues, which can exist in both neutral and positively charged forms, exemplify this sensitivity and can modulate IDP structure through pH-induced charge interactions [24]. At acidic pH, IDPs often exhibit enhanced formation of α-helices and compactness, which can affect their aggregation propensity and functional behavior [25]. By altering surface charge and electrostatic forces, pH exerts a significant impact on protein-protein interactions, which in turn affects the protein phase behavior and the tendency to aggregate [26]. Electrostatic interactions are central to protein fold stability, binding specificity, and condensation processes, all of which are essential for cellular function and phase separation [27].

Temperature, a fundamental thermodynamic variable, directly influences the conformational stability of proteins. Elevated temperatures tend to promote unfolding by favoring conformational entropy over enthalpic stabilization of the folded state [28]. This process is often associated with the disruption of hydrophobic core interactions, decreasing protein compactness, and increasing disorder [29]. For IDPs, temperature shifts can alter their functional organization and capacity to undergo phase separation. Their marginal stability makes them particularly responsive to such thermal perturbations, which is essential for their roles in dynamic cellular processes [19,20]. One should also remember that, due to the temperature dependence of hydrophobic interactions, which become stronger at higher temperatures, extended IDPs (those classified as native coils and native pre-molten globules) exhibit a so-called “turned out” response to heat and may undergo partial folding when exposed to elevated temperatures [30].

Redox conditions also regulate protein structure. Redox-sensitive residues such as cysteines, through reversible disulfide bond formation, can induce order-disorder transitions [31,32]. A notable example is the conditionally disordered protein CP12 from *Arabidopsis thaliana*, which undergoes a transition from a completely disordered state to a less disordered state upon oxidation due to the formation of disulfide bonds [33].

Ionic strength of a solution profoundly affects IDP structure and interaction potential. Changes in ionic environment modulate the structure, charge distribution, and electrostatic repulsion within IDPs, thereby influencing their conformational landscape and binding affinities [34].

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Environmental factors such as salts have a major impact on nanopore gating and stoichiometry during cargo translocation by modulating protein behavior through the Hofmeister effect [35]. The Hofmeister effect explains how specific ions impact the stability and solubility of proteins by altering hydration shells and electrostatic interactions, with kosmotropes generally stabilizing and chaotropes destabilizing protein conformations [36]. Single-molecule studies have demonstrated that kosmotropic and chaotropic ions differentially regulate the conformational states and interactions of a protein by altering electrostatic forces and hydration layers, thereby regulating transmembrane channel gating and the efficiency of cargo transport [35]. Ionic strength further modulates IDPs: fluctuations in charge distribution can induce dipole formation and conformational changes [37]. Elevated ionic strength often promotes structural opening and reduces electrostatic repulsion, thereby enhancing the interactions of IDPs with binding partners [38]. In addition, metal ions can coordinate with IDRs, stabilizing them into more ordered conformations and modifying their functional interactions. Such metal-induced folding is essential for a variety of cellular processes, including signalling, phase separation, and enzyme activation [39].

Liquid-liquid phase separation (LLPS) is a fundamental physicochemical process underlying the formation of membrane-less organelles (MLOs) and biomolecular condensates [40–42]. Due to their low sequence complexity and promiscuous binding potential, IDPs and proteins with large IDRs are ideally suited to drive LLPS. These condensates create specialized microenvironments that regulate biochemical reactions and cellular responses [41]. IDPs play central roles in the assembly and function of MLOs, such as under stress conditions, where they contribute to the formation of stress granules that protect genetic material [43]. Conditional disorder enhances the regulatory potential of proteins by allowing them to switch between functional states in response to environmental signals [19]. The presence of IDPs across diverse cellular compartments further underscores their importance in facilitating complex protein interactions and dynamic communication [44].

Macromolecular crowding further complicates the conformational behavior of IDPs. Crowding can alter protein-protein and protein-ligand interactions by modulating the excluded volume and available conformational space [45]. IDPs, which often rely on disorder-to-order transitions upon binding, are particularly sensitive to crowding [46]. Depending on their folding response, they can be classified as foldable, unfoldable, or non-foldable. Crowding can also promote LLPS, leading to the formation of MLOs that concentrate biomolecules and alter the structural behavior of IDPs [47]. In such environments, amyloidogenic peptides may aggregate more readily, influencing the kinetics and morphology of pathological aggregates relevant to

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neurodegenerative diseases [48,49]. The crowded intracellular milieu thus provides a more physiologically relevant context to study IDP function, revealing behaviors that are not observable in dilute solutions [50,51].

Osmolytes, small water-soluble molecules, also influence protein disorder [52]. They promote the formation of secondary structure and stabilize IDPs under osmotic stress, which enables adaptation to varying environmental conditions [30,52]. Osmolytes modulate the thermodynamic balance between folded and unfolded states of a protein, thereby contributing to cellular resilience [46]. Depending on their chemical nature, osmolytes can either stabilize or destabilize protein conformations by altering water structure and hydration shells [53,54].

3. Binding-induced disorder-to-order transitions

3.1. Mechanisms of coupled folding

IDPs challenge the classical structure-function paradigm by functioning without a fixed 3D structure under physiological conditions [55,56]. Instead, they exist as dynamic ensembles of rapidly interconverting conformations, sampling a wide range of structural possibilities [57]. A hallmark feature of many IDPs is their ability to undergo binding-induced disorder-to-order transitions, also referred to as coupled folding and binding, where a conformational shift is stabilized upon interaction with a specific target [58]. One common mechanism driving this process is the induced-fit model, wherein the IDP adopts an ordered conformation only after engaging with its binding partner. For instance, the phosphorylated kinase-inducible domain (pKID) of cAMP-response element binding (CREB) undergoes a transition from a disordered state to an α-helical structure upon binding to the KIX domain of CREB-binding protein (CBP) (Figure 3) [59]. This process involves the formation of weak native interactions that stabilize the folded state and promote rapid compaction of the protein structure [60]. An alternative mechanism is the conformational selection model, where the binding partner selects a pre-existing, native-like conformation from the IDP's ensemble [17]. In this model, the protein transiently samples ordered structures even before interaction. The c-Myb activation domain, for example, binds to KIX primarily in a pre-folded state [59]. Simulations of pKID binding to KIX have also demonstrated a nucleation-condensation mechanism, wherein the formation of a local native-like fragment acts as a nucleation point, driving the transition to a fully ordered complex [60].

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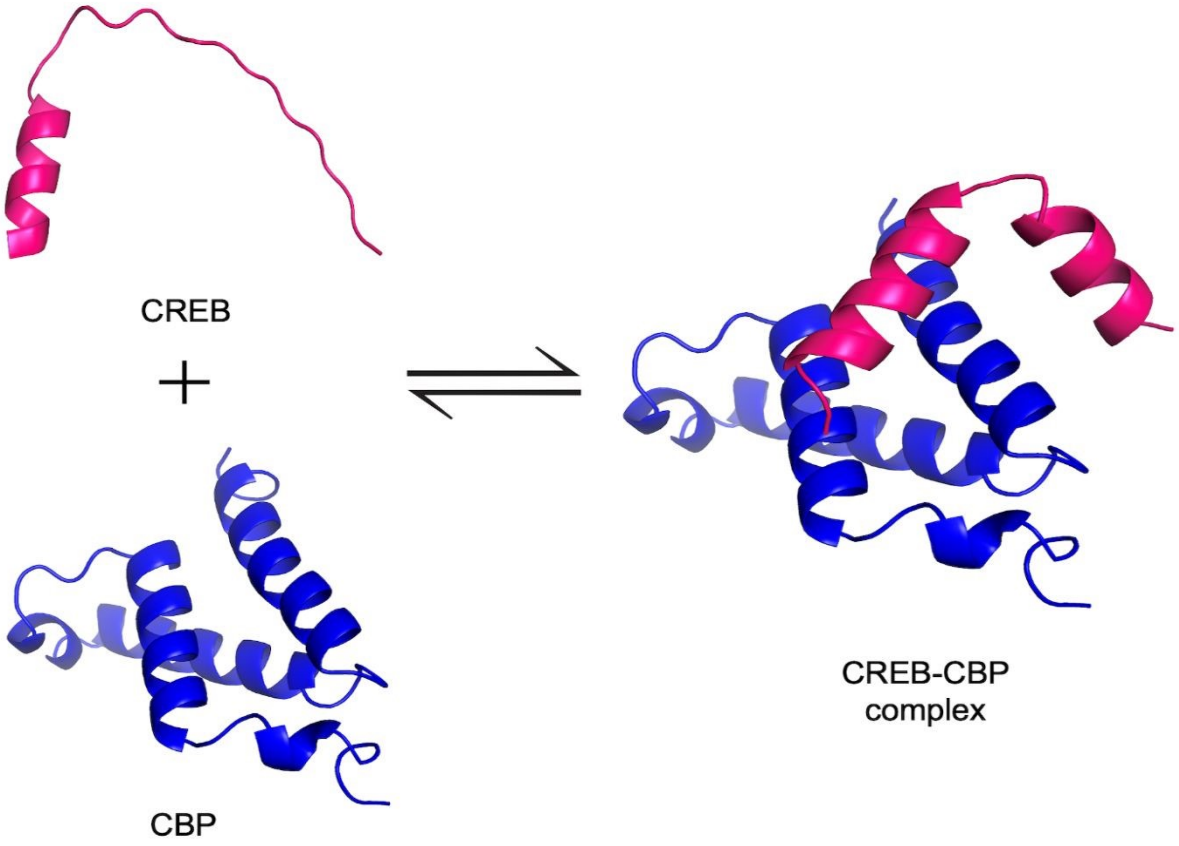

Figure 3: Binding-induced Disorder-to-order transition in CREB. The illustration depicts the disordered region of CREB (shown in pink; residues 119–146; UniProt: P15337) in its unbound, extended state and the KIX domain of CBP (shown in blue; PDB ID: 1KDX). Upon binding, the CREB region adopts an ordered conformation as part of the complex, illustrating the disorder-to-order transition. Structures were visualized using PyMOL.

IDPs achieve functionality through these intricate binding mechanisms, which often involve cooperative transitions between disordered and ordered states [61]. The induced-fit and conformational selection models are not mutually exclusive but may operate concurrently or in tandem, depending on the context and partner interaction [46]. For example, Zika virus protease adopts a significantly different conformation upon ligand binding, illustrating an induced-fit mechanism, whereas interaction of the dengue virus (DENV) protease (the NS2B-NS3 protease complex) with its ligands follows a conformational selection mechanism, where ligands bind to a pre-existing structural state of this protease [62].

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The biological relevance of binding-induced folding lies in its contribution to the functional plasticity of IDPs. This structural adaptability allows IDPs to engage in a diverse range of cellular activities and ensures context-dependent specificity [63]. The kinetic and thermodynamic aspects of this transition are governed by the interplay between conformational entropy (lost during folding) and binding affinity (gained in the ordered state) [64]. Protein folding is fundamentally determined by a balance between enthalpic (ΔH) and entropic (TΔS) contributions, which often compensate for one another in a phenomenon known as enthalpy-entropy compensation (EEC), resulting in a narrow range of Gibbs free energy (ΔG) values [65]. Interestingly, proteins can sometimes be stabilized despite enthalpic losses if the folded state gains configurational entropy. For example, engineered variants of acylphosphatase exhibited increased entropy in the folded state, which enhanced stability even though enzymatic activity was reduced [66]. This balance allows IDPs to fine-tune their structural ensemble for optimized binding, retaining a degree of conformational heterogeneity while avoiding misfolding [67].

3.2. Examples of IDPs undergoing disorder-to-order transitions

IDPs often undergo disorder-to-order transitions upon interaction with specific binding partners, stabilizing into structured conformations that are critical for their function. These transitions exemplify the functional adaptability of IDPs in diverse biological contexts. Molecular recognition features (MoRFs) are disordered segments that undergo structural transitions upon binding. MoRFs are categorized into α-MoRFs (that form α-helices), β-MoRFs (that form β-strands), and ι-MoRFs (that form irregular structures). Structural analysis of 258 complexes revealed 62 α-MoRFs, 20 β-MoRFs, and 176 ι-MoRFs, underscoring their role in specific molecular recognition events [68].

One notable example is Prostate-associated gene 4 (PAGE4), an IDP implicated in prostate cancer. Phosphorylation induces distinct disorder-to-order transitions in PAGE4, modulating its structural dynamics and interactions with the AP-1 signalling axis. These changes influence cell phenotype and therapeutic response, highlighting the functional consequences of structural switching [69]. Similarly, hybrid proteins combining IDPs with globular domains demonstrate how disorder-to-order transitions in the IDP component can significantly alter the overall structural and functional properties of the fusion construct [70,71]. The Hahellin protein, a member of the βγ-crystallin family, is intrinsically disordered in its apo form. Upon binding of Ca^{2+},

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it adopts a well-ordered βγ-crystallin fold, exemplifying ion-induced structural stabilization [72]. The N-terminal transactivation domain of p53, intrinsically disordered in its free state, forms an α-helix upon binding to MDM2, serving as a regulatory checkpoint for the activity and degradation of p53 protein [73]. The C-terminal region of this protein is able to fold differently upon interaction with various partners: it adopts an α-helical structure when binding to S100Bββ, forms a β-strand in a complex with sirtuin, takes on a β-turn when complexed with the CBP bromodomain, and exhibits an irregular structure with diverse morphologies in complexes with the histone methyltransferase Set9 and cyclin A/cyclin-dependent protein kinase 2 [74–76]. The linker histone H1 illustrates how binding to DNA can trigger structural rearrangement: its disordered N-terminal domain adopts a helical form upon nucleosome interaction, enhancing its affinity for DNA [77]. In neurons, tau protein stabilizes microtubules through a disorder-to-order transition upon binding, which is essential for maintaining cytoskeletal integrity [78]. Small molecules can also induce structural transitions in IDPs. For example, the disordered 'lid' region of MDM2 becomes structured upon binding to the small molecule AM-7209, significantly enhancing binding affinity and illustrating a mechanism of pharmacological stabilization [79]. Likewise, α-synuclein, associated with Parkinson's disease, undergoes ordering when bound to lipid membranes, facilitating its role in synaptic vesicle trafficking [78].

The transcription factor c-Myc undergoes a disorder-to-order transition upon dimerization with its partner Max, forming a stable DNA-binding complex that regulates genes involved in cell proliferation and apoptosis [2]. Other transcription factors, such as Antennapedia (Antp) and thyroid transcription factor-1 (TTF-1), also experience such transitions upon DNA binding. Their disordered N-terminal tails play crucial roles in DNA recognition, enhancing specificity and affinity by modulating the folding–binding coupling [80]. In the high-mobility group (HMG) family, the transcription factor Lymphoid enhancer-binding factor-1 (LEF-1) is only partially folded in the absence of DNA but adopts a more ordered structure upon binding to target sequences, demonstrating context-dependent structural rearrangement [81].

Among viral proteins, the C-terminal domain of the measles virus nucleoprotein (NTAIL) interacts with the folded X domain of the viral phosphoprotein (XD), undergoing a folding transition essential for viral replication [82]. In prion proteins (PrP), the intrinsically disordered N-terminal domain binds nucleic acids with high affinity. The conformational state of nucleic acid aptamers regulates the phase separation behavior and aggregation propensity of the Y145Stop variant of PrP, which influences transitions from liquid-like droplets to amyloid-like aggregates [83].

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Other examples include BtuB, a β-barrel protein involved in nutrient transport, which undergoes a calcium-induced disorder-to-order transition to facilitate function [84]. In *Neisseria meningitidis*, the outer membrane protein OpcA exhibits structural reorganization at its receptor-binding site upon ligand binding, critical for host-cell interactions [85]. Additionally, analysis of purine nucleotide-binding proteins reveals that over 22% contain segments undergoing disorder-to-order transitions, highlighting their widespread importance in nucleotide recognition and signalling pathways [86].

3.3. Functional implications of binding-induced folding

When IDPs interact with their binding partners, they often undergo substantial conformational changes, transitioning from disordered to ordered states, which can significantly enhance their functional capabilities [87,88]. This phenomenon exemplifies the dynamic nature of protein-protein interactions and is notably represented in the concept of mutual synergistic folding, where two disordered proteins fold together into a stable, structured complex [87]. A classic example is the bacterial protein BirA, which undergoes a ligand-induced disorder-to-order transition that facilitates dimerization and stabilizes the overall structure, an essential step for its function [89]. Similarly, in the case of the KIX domain binding to the transcription factor **c-**Myb, the binding transition state exhibits a considerable amount of native-like structure, implying that intrinsic disorder can fine-tune molecular recognition by enhancing binding specificity [90]. The sequence-function relationship in binding-induced folding remains complex. Despite considerable sequence variation, many IDPs retain functional integrity, which highlights that modifications, particularly in hydrophobic side chains, can profoundly impact folding pathways and interaction dynamics without abolishing function [89]. Thus, there is a delicate balance between order and disorder in protein function: while some proteins require preservation of disorder for activity, others rely on disorder-to-order transitions for their function [8].

Unlike globular proteins, which depend on pre-formed tertiary structures for activity, IDPs leverage their structural plasticity to achieve specificity through folding upon binding [46,63]. This coupled binding and folding mechanism enables IDPs to interact with diverse partners with high specificity under context-dependent conditions, while preserving the adaptability needed for promiscuous interactions in complex signalling and regulatory networks [91]. IDPs use transient conformations to fine-tune interactions, which allow them to adopt distinct structural states tailored

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to different targets. This functional plasticity enhances their interaction versatility and contributes to regulatory complexity in cellular pathways [92].

From an evolutionary perspective, disordered regions offer significant advantages. The ability to switch between disordered and ordered states enables proteins to optimize binding affinity and specificity while remaining adaptable to novel partners. Evolutionary processes such as alternative splicing, domain shuffling, and modular recombination are enriched by the inherent flexibility of IDPs, which facilitates functional innovation without compromising protein integrity [93]. The evolutionary benefit of transient disorder is clearly evident in the coupled folding-binding mechanism, where rapid conformational changes mediated by disordered segments enable swift and efficient molecular recognition [84]. Such properties are particularly critical for proteins involved in signal transduction and regulatory pathways, where adaptability and responsiveness are key. By enabling a broad range of binding modes, IDPs promote evolvability, which allows organisms to develop complex multicellular functions while remaining robust to genetic variation [94,95]. Cryo-EM studies reveal that the intrinsically disordered tau protein forms fibrils through a compact, structured C-terminal core that nucleates aggregation [96]. Recent developments in label-free nanopore technology have further improved diagnostic potential by enabling highly sensitive detection of Alzheimer's disease–related protein aggregates and biomarkers [97].

4. Proteins with cryptic disorder

4.1. Concept of cryptic disorder in ordered proteins

Moonlighting proteins, a class of multifunctional proteins, are capable of performing two or more distinct yet physiologically relevant biochemical or biophysical functions using a single polypeptide chain. A subset of these, known as cryptic enzymes, possesses hidden or dormant functions that are not evident under normal physiological conditions [98]. While IDPs lack stable tertiary structures under physiological environments, many conventionally ordered proteins contain short disordered regions that remain inactive or cryptic (or dormant or latent) until triggered by specific stimuli [56]. The concept of cryptic disorder challenges the classical structure–function paradigm by suggesting that intrinsic disorder can be a latent property even in folded proteins [99]. This idea adds a layer of complexity to our understanding of protein dynamics, implying that ordered proteins may not exist in a single, rigid conformation but rather in an ensemble of conformational states [28]. Cryptically disordered segments are often buried within the folded architecture and

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remain inaccessible to molecular interactions until conformational shifts, environmental changes, or binding events expose them [100].

Cryptic disorder confers conditional functionality, which enables proteins to undergo conformational transitions in response to cellular cues. This adaptability enhances the functional repertoire of a protein, including regulatory interactions and allosteric modulation. Such flexibility allows proteins to respond to spatial and temporal signals, contributing to dynamic cellular processes [101,102]. The presence of variably ordered or disordered regions equips proteins with the ability to modulate their structures and perform context-specific functions [103]. As a result, proteins with cryptic disorder often exhibit binding promiscuity and functional plasticity. Controlled unfolding of ordered regions, facilitated by cryptic disorder, allows for activation under distinct conditions. This underscores the broader functional relevance of conditional disorder in enhancing interaction versatility, even in the absence of a stable, ordered conformation [101,104]. The capacity of IDPs and IDRs to adopt diverse conformations is vital to their roles in signalling and regulation [103]. However, while cryptic disorder enhances biological versatility, it also increases the risk of misfolding and aggregation, which has been linked to the pathogenesis of several protein conformational diseases [105].

4.2. Activation of functional sites via partial unfolding

A key mechanism for activating otherwise inaccessible functional regions involves partial unfolding events that transiently expose critical motifs embedded within the native fold (Figure 3) [106]. PTMs, such as phosphorylation and acetylation, can modulate electrostatic interactions, leading to local destabilization and promoting partial unfolding of specific regions [107]. Similarly, genetic mutations may alter the protein folding landscape, resulting in misfolding, gain or loss of function, or altered activity levels [108]. Environmental stressors, such as changes in pH or temperature, can destabilize native structures, enhance conformational flexibility, and expose hidden functional sites. Some proteins, termed metamorphic proteins (discussed later in section 6), illustrate the functional importance of partial unfolding by reversibly switching between multiple folded states in response to environmental stimuli, each conformation associated with distinct biological roles [109]. In contrast, misfolding often exposes hydrophobic residues typically buried in the protein core, leading to aggregation and formation of non-native conformations [110].

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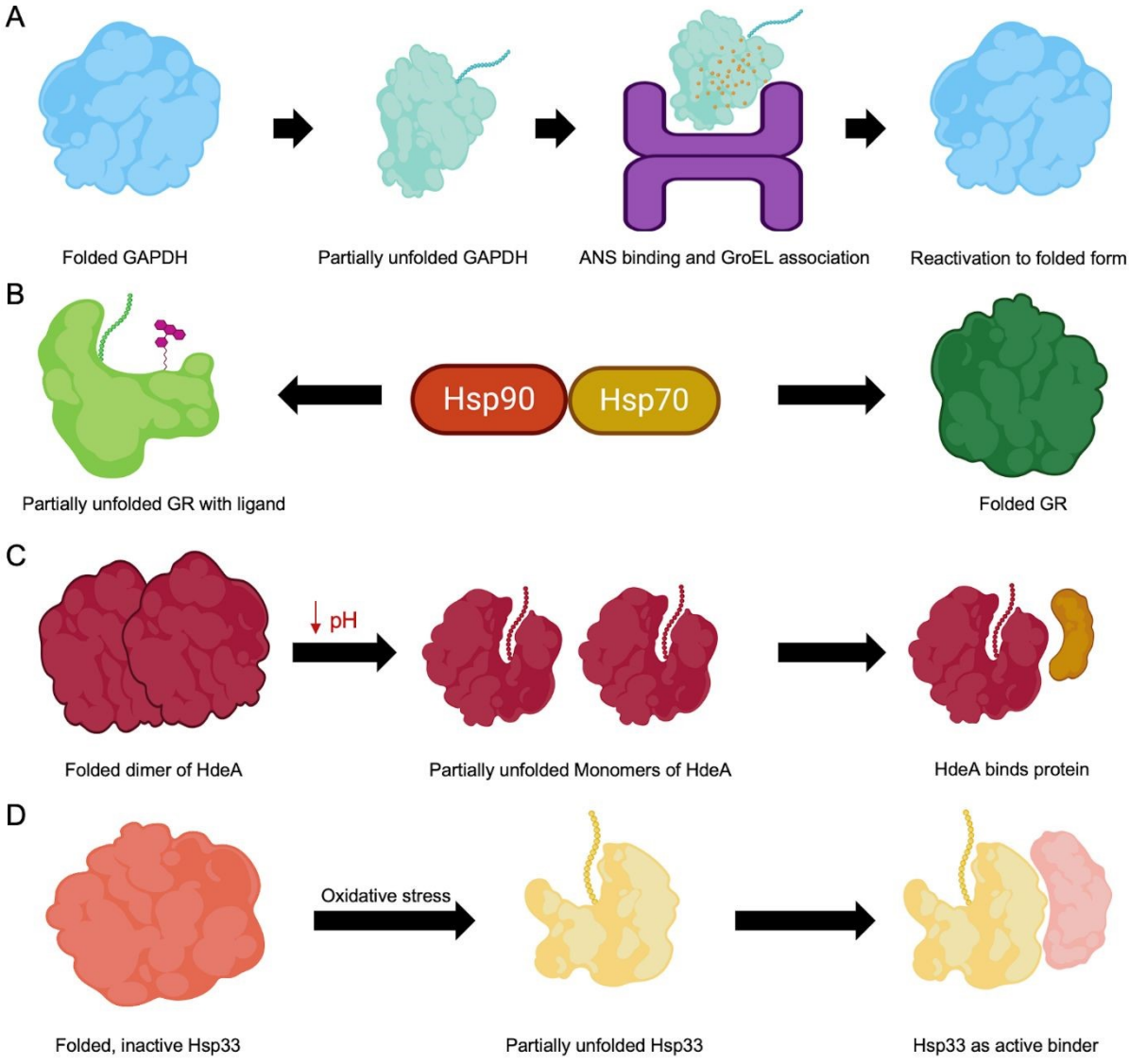

Figure 4: Partial unfolding and refolding of conditionally disordered proteins. (A): GAPDH transitions from its native folded state to a partially unfolded intermediate that binds ANS and associates with the GroEL chaperone, resulting in its reactivation. (B) The glucocorticoid receptor (GR) undergoes a cycle of partial unfolding and refolding facilitated by Hsp70 and Hsp90, enabling ligand binding. (C) HdeA exists as a folded dimer at neutral pH and, at low pH, dissociates into monomers that become partially unfolded and bind client proteins. (D) Hsp33 switches from an inactive folded form to a partially unfolded active state that interacts with client proteins.

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Experimental evidence supports the functional relevance of such transiently unfolded states. For instance, D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH), when partially unfolded in dilute guanidine hydrochloride in the presence of ATP, forms a folding intermediate that is reactivated by the chaperonin GroEL. This intermediate binds 8-anilino-1-naphthalenesulphonic acid (ANS) and exhibits enhanced fluorescence, consistent with a partially unfolded, reactivatable conformation (Figure 4A) [111]. The glucocorticoid receptor (GR) is another classic example: its activation depends on the chaperone machinery of heat shock proteins (Hsps) involving Hsp90 and Hsp70. GR undergoes cycles of partial unfolding during maturation, enabling ligand binding and proper folding. These dynamic transitions within the chaperone complexes are crucial for functional activation (Figure 4B) [112]. The bacterial chaperone HdeA demonstrates pH-triggered partial unfolding. In *E. coli*, HdeA exists as an inactive dimer at neutral pH but becomes active at acidic pH by monomerizing and partially unfolding, which allows it to prevent substrate protein aggregation under stress conditions (Figure 4C) [113]. Similarly, Hsp33 relies on partial unfolding of its central linker region to transition from a self-inhibited state to a client-binding-competent form, which underscores the role of partial unfolding in molecular chaperone function (Figure 4D) [114].

In the realm of IDPs, ligand binding can drive disorder-to-order transitions, which are often essential for allosteric regulation. These interactions are frequently "fuzzy" in nature, which retains a degree of disorder to support regulatory flexibility [115]. This conformational plasticity enables IDPs to participate in complex biological processes such as signal transduction and cellular regulation - roles often inaccessible to rigid, globular proteins [116]. Furthermore, as discussed earlier, disordered regions can drive LLPS, leading to the formation of biomolecular condensates that organize biochemical activities in space and time within cells [117]. Molecular dynamics (MD) simulations and structural studies have revealed the existence of cryptic sites as transient surface pockets hidden in unbound states by side chains or loops. These sites can emerge during partial unfolding and enable induced-fit binding. Tools such as FTMap offer valuable insights into the structural dynamics of cryptic sites and help understand their functional significance [118].

A case in point is the N-terminal oligomerization domain of nucleophosmin (Npm-N), which can transition from a folded pentameric state to a disordered monomer in response to changes in ionic strength or PTMs. This reversible switch reflects the evolution of Npm to toggle between structured and disordered conformations as a regulatory strategy [119]. Moreover, the presence of cryptic amyloidogenic regions (CARs) in IDPs, which are normally masked under native conditions, has been linked to functional protein-protein interactions. These CARs appear to have

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evolved to enhance protein adaptability and function under varying cellular conditions [120]. The ability of proteins to transition between conformational states enables precise regulation of key biological processes, including centrosome duplication, signalling, and stress response. Thus, cryptic disorder and partial unfolding emerge as central themes in protein function modulation [121].

5. Redox-sensitive disordered regions

5.1. Influence of redox conditions on protein structure

Redox conditions play a pivotal role in modulating protein structure, which influences conformational states and functional activities across diverse biological systems [122]. The cellular redox environment, which is defined by the balance between oxidized and reduced states, critically affects proteins containing redox-sensitive residues, especially cysteines [32]. Oxidation of cysteine thiol groups leads to the formation of disulfide bonds, which can drastically alter protein folding, stability, and intermolecular interactions [123]. Shifts in the redox environment can trigger conformational transitions, driving proteins from ordered to disordered states or vice versa [31]. This redox-responsive structural plasticity underscores the importance of integrating redox regulation into our understanding of protein function [18]. Furthermore, reactive oxygen species (ROS) and reactive nitrogen species (RNS) mediate PTMs, which regulate proteolytic enzymes and contribute to cellular homeostasis [124]. Dysregulation of redox balance is implicated in a wide range of pathological conditions, highlighting the need to understand the complex interplay between redox signalling, protein structure, and cellular function [125].

Many redox-sensitive proteins possess conditionally disordered regions that respond dynamically to oxidative stress. These regions may undergo structural transitions upon exposure to ROS, altering protein stability, activity, and interaction networks [57]. For instance, the *Arabidopsis thaliana* protein CP12 becomes prevalently disordered upon oxidation, a conformational shift that is essential for its role in regulating photosynthesis (Figure 5) [33]. Similarly, oxidation-induced structural changes can expose otherwise hidden regions, enabling processes such as ubiquitination and proteasomal degradation. For example, oxidative modification of peroxiredoxin 2 (Prx2) facilitates its breakdown via proteasomes and autophagy pathways [126]. Redox-sensitive conformational switching also influences protein-protein interactions and cellular signalling. The reversible formation of disulfide bonds, as seen in proteins like the human SH3

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domain (hSH3), serves as a molecular switch that fine-tunes protein function in response to oxidative cues [127].

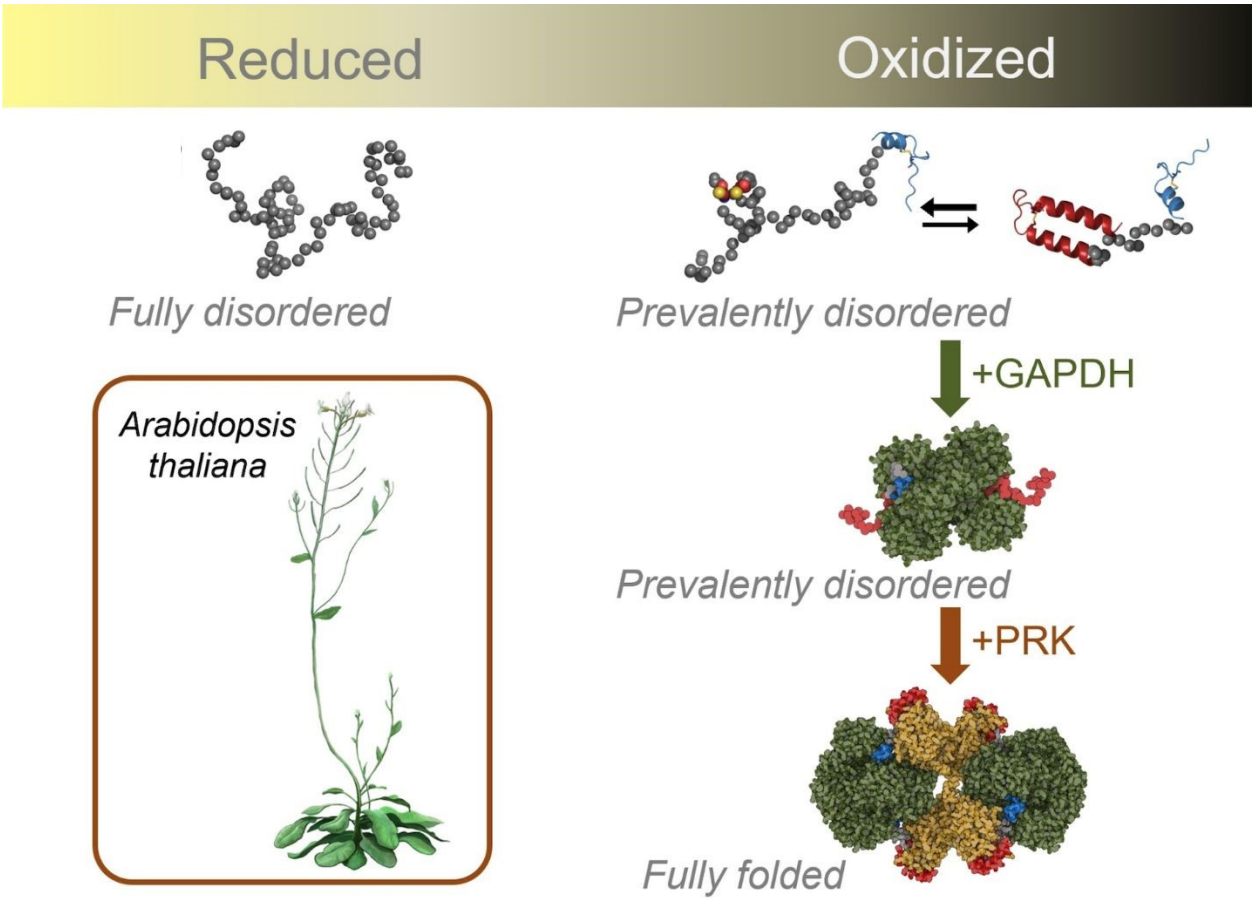

Figure 5: Schematic representation of conformational forms of redox-sensitive conditionally disordered protein, CP12. In the reduced state, CP12 is disordered, and upon oxidation, it adopts a prevalently disordered conformation. This redox-induced change affects how CP12 binds to its binding partners GAPDH and PRK, helping assemble a fully folded complex [33]. Figure adapted under open access and terms of the CC BY 4.0 licence.

5.2. Mechanisms of redox-induced disorder

Redox-induced disorder in proteins primarily arises from chemical modifications to redox-sensitive amino acid residues, particularly cysteine, methionine, and tyrosine [128]. Oxidation of cysteine residues can lead to disulfide bond formation, which may stabilize protein structure or, paradoxically, introduce local or global disorder depending on the structural context and redox

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state [100,129]. These modifications can modulate protein dynamics, disrupt existing intra- or inter-molecular interactions, and promote partial or complete unfolding of structured regions. Alterations in the redox environment also influence the protonation states of amino acid side chains, affecting hydrogen bonding and electrostatic interactions essential for maintaining the protein's conformational stability. Such changes are a fundamental mechanism by which redox states regulate cellular signalling and protein function. The inherent susceptibility of proteins to oxidative PTMs offers a versatile strategy for regulating their activity in response to environmental and physiological cues [130].

Disulfide bonds typically confer rigidity and structural stability to proteins. Their reduction, however, can destabilize protein structure and promote conformational flexibility or disorder [131]. Redox-sensitive regions often harbour cysteine residues that stabilize structure upon oxidation; their reduction can lead to disorder through unfolding or exposure of previously buried regions [57,132]. Dysregulation of these redox dynamics and disulfide bond formation/breakage has been implicated in various pathological conditions, including cancer and neurodegenerative diseases such as Parkinson's disease [132]. Redox-sensitive cysteine residues are particularly crucial in mediating disorder-to-order transitions. Upon oxidation, these residues can form disulfide bonds that stabilize protein conformation, driving structural ordering from a disordered state. For example, the activation of Hsp33 and COX17 involves redox-induced structural changes that confer functional regulation [31].

Granulins, a family of small (~6 kDa) multifunctional proteins derived from the proteolytic processing of their precursor progranulin, provide an illustrative example of proteins with high disulfide bond density. These proteins contain 12 conserved cysteines forming six intramolecular disulfide bonds. In their oxidized forms, granulins adopt structures dominated by irregular loops stabilized solely by disulfide bonds. Despite the absence of regular secondary structure, they exhibit remarkable thermal stability. However, reduction of the disulfide bonds renders them completely disordered [133]. A recent review provides an in-depth analysis of the intricate interplay between intrinsic disorder and cysteines in proteins, highlighting how disordered sequences with interspersed cysteines can modulate protein function under stress and varying environmental conditions [134].

In plant calmodulin (CaM), oxidation of methionine residues (especially Met-144 and Met-145) disrupts the structural linkage between the domains of CaM, resulting in a disordering effect that prevents activation of target proteins such as the PMCA calcium pump. Similarly, methionine

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oxidation contributes to helical unfolding in proteins involved in amyloidosis, exacerbating disease pathology through conformational destabilization [135]. Peroxiredoxin 2 (Prx2) provides another compelling example of oxidation-induced structural transitions in redox-sensitive proteins. Under oxidative stress, Prx2 undergoes a conformational change, exposing its C-terminal region, facilitating Lys191 ubiquitination, a key step in proteasomal and autophagic degradation pathways. Structural changes are further supported by elevated hydrogen/deuterium exchange rates in the GGLG and YF motifs, suggesting dynamic rearrangement upon oxidation [126].

5.3. Functional significance of redox-sensitive disorder

Redox-sensitive disordered regions (RSDRs), often associated with cysteine-containing motifs and disulfide bonds, are widely present across proteomes, particularly in multicellular organisms. These regions endow proteins with the ability to dynamically alter their conformation and function in response to fluctuations in ROS, thereby serving as vital regulatory elements [57]. Functioning as sensors of oxidative stress, RSDRs undergo conformational transitions that can activate signalling cascades or initiate antioxidant defense mechanisms. Notably, many signalling proteins harbour RSDRs that modulate their interactions with other molecules, enabling rapid, reversible, and finely tuned responses to shifts in cellular redox conditions [136]. IDRs, due to their structural flexibility, enhance the interaction potential and functional diversity of proteins. Like structured domains, IDRs contribute to the assembly of protein complexes and higher-order structures [137]. The environmental sensitivity of IDRs allows them to act as modulators of protein-protein interactions and activity. Importantly, redox dysregulation (especially involving cysteine oxidation) has been linked to protein misfolding and aggregation in several neurodegenerative diseases, such as ALS and Parkinson's disease [138,139].

While redox regulation is essential for normal cellular physiology, its imbalance can lead to pathological outcomes. Elevated ROS levels, characteristic of oxidative stress, can cause irreversible protein damage and contribute to conditions like cancer, cardiovascular disorders, and muscular dystrophies [140]. Transcription factors such as OCT4, p53, and NRF2 are tightly regulated by redox mechanisms. For example, oxidation of cysteine residues can alter their DNA-binding capabilities and transcriptional output, thereby influencing processes like stem cell pluripotency, tumorigenesis, and apoptosis. KEAP1, which normally retains NRF2 in the cytoplasm, undergoes cysteine oxidation under oxidative stress, allowing NRF2 to translocate to

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the nucleus and activate the expression of antioxidant genes [141]. Similarly, the activity of proto-oncogenes (e.g., c-FOS, c-JUN) and tumor suppressors (e.g., p53) is redox-dependent.

Redox-active enzymes, such as thioredoxins and NADPH oxidases, regulate ROS production and modulate protein function through reversible oxidation of cysteine residues. The thioredoxin family plays a key role in immune signalling and inflammatory responses by catalyzing thiol-disulfide exchange reactions [21].

In prokaryotes, proteins such as Hsp33 serve as redox-regulated chaperones. Upon oxidative stress, Hsp33 forms disulfide bonds, undergoes partial unfolding, and binds to unfolded substrates, enhancing its chaperone activity. Analogous mechanisms exist in eukaryotic systems, where 2-Cys peroxiredoxins transition into chaperones via sulfinic acid formation [142]. The pathological importance of redox-sensitive disorders is further underscored by the oxidative modification of cytoskeletal proteins (e.g., actin, tubulin) and nuclear regulators like histone deacetylases (HDACs), which influence chromatin structure and gene expression during neural differentiation [143].

6. Metamorphic proteins

6.1. Definition and characteristics of metamorphic proteins

Metamorphic proteins represent a unique deviation from the traditional “one sequence, one structure” paradigm of protein folding [144]. These proteins can adopt multiple distinct, stable conformations from a single amino acid sequence (Figure 6) [109]. This structural plasticity equips them with functional versatility, making them the so-called *"Janus proteins"* of structural biology [145]. Key features include:

1. **Multiple stable conformations**: Unlike canonical proteins that fold into a single native structure, metamorphic proteins exist in two or more thermodynamically stable and functional conformations [109].

2. **Environmental sensitivity**: External factors such as pH, temperature, and ligand binding can shift the equilibrium between different structural states [109].

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3. **Diverse functionality**: Each structural form can mediate distinct biological functions, allowing the same protein to participate in different pathways or interact with diverse molecular partners [145].

4. **Conformational plasticity**: A rugged, multifunnel energy landscape enables these proteins to switch between folds, often without the need for cofactors or ligands [145,146].

Metamorphic proteins, such as XCL1, demonstrate how a single sequence can encode multiple structural states by modulating intramolecular contacts, oligomerization interfaces, and structural constraints (Figure 6A) [147]. This contradicts classical folding theories and suggests that co-evolutionary pressures, particularly in host-pathogen dynamics, may promote metamorphism as an adaptive strategy [145]. For instance, RfaH exemplifies how a metamorphic switch enables bacteria to regulate virulence and conjugation genes in response to environmental cues (Figure 6B) [148].

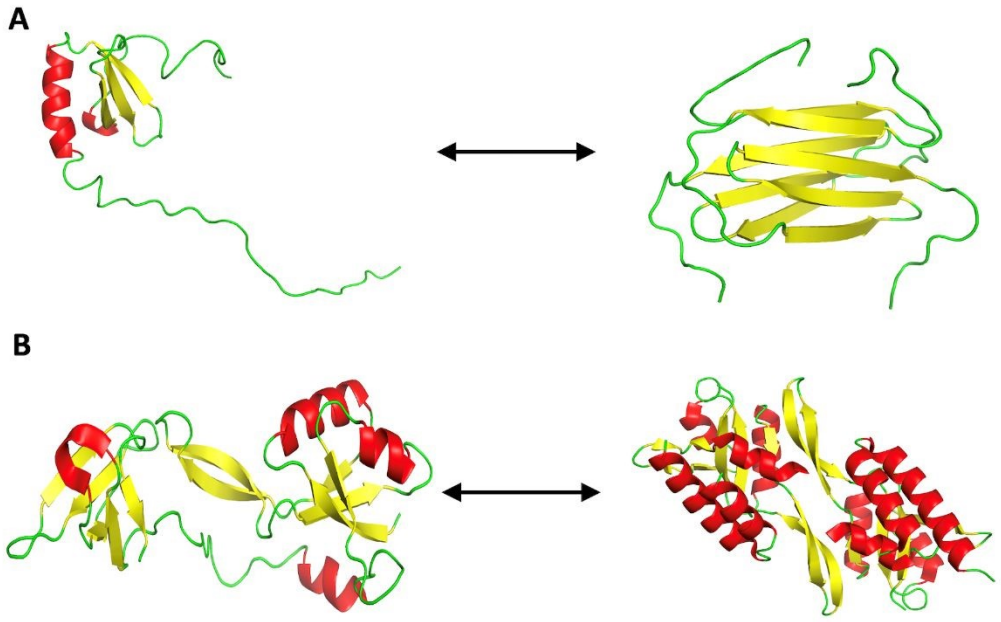

Figure 6. Conformational metamorphism in proteins. (A) Lymphotactin shows metamorphism by existing in two different folds. The structure on the left depicts the canonical monomeric chemokine fold (PDB ID: 1J9O) that binds to XCR1 receptor, favored at low temperature and high salt conditions. The structure on the right presents the dimeric all β-sheet fold (PDB ID: 2JP1), which facilitates glycosaminoglycan (GAG) binding, favored at high temperature and low salt conditions. (B) RfaH, a bacterial transcription factor, exhibits metamorphic folding by interconverting between two distinct conformations. The structure on the left shows the α-helical

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autoinhibited form (PDB ID: 5OND) and the one on the right shows the active β-fold (PDB ID: 6C6S) that engages ribosome interactions. The alpha helices are labelled in red, the beta sheets in yellow, and the coils are represented in green.

6.2. Examples of proteins with multiple stable folds

Approximately 90 naturally occurring metamorphic proteins are currently known [149], and metamorphism has been successfully engineered into several of the most commonly occurring protein folds [150]. Several well-characterized examples illustrate the phenomenon of metamorphic folding:

1. **Lymphotactin (Ltn/XCL1)**: A chemokine that interconverts between two structurally unrelated native folds - one being a monomeric α/β chemokine-like structure and the other a dimeric β-sheet fold. This switching is rapid and reversible under physiological conditions [151].

2. **Staphylococcal nuclease**: Exhibits at least two interconvertible native states with independent folding and unfolding pathways, as revealed by magnetization transfer NMR experiments [152].

3. **ThreeFoil protein**: A computationally designed, symmetric protein with high kinetic stability and long-range interactions, demonstrating the potential for engineered metamorphic behavior [153].

4. **RfaH**: A bacterial elongation factor that switches from an α-helical to a β-barrel structure, regulating operons associated with virulence and conjugation [148].

5. **Prion protein (PrP)**: Exists in a normal cellular form and a misfolded, pathogenic conformation associated with neurodegenerative disorders. Its ability to switch forms exemplifies pathological metamorphism [145].

6. **Amyloid-β peptides**: Demonstrate structural plasticity through fibrillogenesis, relevant to Alzheimer's disease pathology [145].

7. **NusG-like transcription factors**: Structurally homologous to RfaH, these proteins adapt their folds to modulate gene expression in response to environmental changes [148].

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6.3. Biological roles and evolutionary advantages

The ability of metamorphic proteins to adopt multiple conformations expands their functional repertoire without requiring multiple genes or extensive sequence changes [109,144]. Each conformation can serve a distinct biological role, which offers adaptive advantages:

- Signal modulation and pathway integration: Conformational switching enables dynamic regulation of signalling cascades by altering interaction profiles [130].
- Environmental responsiveness: Proteins like RfaH and KaiB (a clock protein) use fold-switching to modulate their activity in response to environmental or circadian changes [148,154].
- Resource conservation: Transitioning to an inactive conformation under stress or crowded cellular conditions may serve as an energy-saving mechanism [154].
- Molecular switching: The reversible fold-switching of metamorphic proteins allows for rapid adaptation to stressors - critical for cellular survival in fluctuating environments [155].
- Biotechnological potential: Metamorphic proteins hold promise for the development of biosensors, responsive biomaterials, and switchable therapeutic agents [129].

Despite their advantages, characterizing metamorphic proteins remains challenging due to their dynamic nature, which defies traditional structural biology techniques [156].

6.4. Transitions between metamorphic states

Metamorphic proteins are distinguished by their ability to reversibly transition between distinct folded conformations, often in response to environmental cues [109,145]. Their secondary structures are inherently flexible, allowing for large-scale conformational changes that can be triggered by factors such as pH, temperature, and ionic strength. These transitions occur within a polymorphic ensemble of conformations, where even minor perturbations can drastically reshape the energy landscape of the protein [107]. In some cases, global unfolding is necessary to facilitate interconversion between native states, and reversible switching between folds has been observed

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under changes in temperature [157]. This ability to morph between structures is closely linked to specific sequence features that promote flexibility and enable conformational transitions [158]. Modifications in the dimer interface, along with dynamic residue contact networks and structural pliability, collectively contribute to metamorphic folding behavior [109]. Ligand binding can also modulate the equilibrium between different conformational states, thereby fine-tuning the structural and functional repertoire of a protein [147].

For instance, Mad2 transitions between open and closed states via a denatured intermediate, although the presence of stable intermediates can hinder this switch by slowing or redirecting the transition pathway [159]. Similarly, Ltn requires large-scale unfolding to switch between its native states, a process that underscores the importance of unfolding in metamorphic transitions [158]. In these systems, different conformations are often thermodynamically comparable in stability, allowing reversible transitions to occur under physiological conditions [159]. The kinetics of such interconversions are sensitive to environmental parameters, with temperature influencing the free energy of transition states and modulating the rates at which these transitions proceed [158].

A recent comprehensive analysis of well-characterized metamorphic proteins revealed that temperature plays a crucial role in their structural transitions [160]. In many cases, this temperature sensitivity is associated with partial cold denaturation, as the low-temperature state of these proteins tends to have a smaller hydrophobic core and is more disordered than their high-temperature state [160].

7. Order-disorder-new order transitions

7.1. Pathways and mechanisms of structural transitions

Protein function depends on transitions between ordered, disordered, and newly ordered states, especially in IDPs. Upon interaction with physiological partners, IDPs often undergo disorder-to-order transitions. These transitions typically follow cooperative folding mechanisms, similar to those in structured proteins. However, due to the lack of a fixed folding nucleus, the folding pathways of IDPs are highly heterogeneous and flexible [46]. For example, in Npm-N, phosphorylation can induce disorder under certain ionic conditions, thereby modulating its folding and assembly [161]. Mutual folding occurs when disordered proteins bind each other, leading to the formation of structured complexes that are functionally active [87]. The biological roles of proteins

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undergoing order–disorder transitions are closely linked to their enhanced conformational diversity. Upon ligand binding, more than 60% of such proteins become more structured, thereby enhancing their functional roles [162].

The functions of ordered proteins are dependent on the acquisition of specific structures through folding on physiological timescales. Ordered proteins may exist as partially organized intermediates under both equilibrium and non-equilibrium conditions. These intermediates typically possess some features of the fully folded state and are crucial in the folding pathway. In the presence of specific binding partners, such intermediates (commonly referred to as molten globules) can undergo disorder-to-order transitions, resulting in well-folded conformations [163–166]. These reversible structural changes are essential for cellular signalling and function [167].

Metamorphic proteins, due to their marginal stability, can undergo large-scale conformational shifts and respond to environmental cues such as pH, temperature, and redox conditions by switching between distinct fold topologies [107]. In proteins like IscU, N-terminal order-disorder transitions play a key role in metamorphic regulation, influencing their interaction networks and contributing to structural heterogeneity [168].

In Npm-N, phosphorylation and partner binding orchestrate a sequential series of disorder-mediated structural rearrangements. Phosphorylation can disrupt the coupled folding and assembly process by inducing disorder, whereas binding to physiological partners can counteract this effect by stabilizing the ordered state [161]. This dynamic process involves a rapid collapse into a disordered intermediate, followed by a gradual conversion into a folded monomer, and ultimately assembly into a folded pentamer [161]. The significance of binding in order–disorder transitions is also evident in mutual synergistic folding, where two disordered proteins form a stable, structured complex [87]. Proteins commonly populate partially structured intermediates such as molten globules, which can transform into fully ordered conformations upon ligand binding. This transition is energetically favorable and enables proteins to reach their lowest energy structural states [167].

7.2. Thermodynamic and kinetic considerations

Proteins exist along an energy continuum, from hyperstable folded states to hyperdynamic disordered conformations. This continuum is governed by both the amino acid sequence and environmental conditions, such as solvent composition, temperature, and ionic strength [169].

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Disordered proteins can transition into ordered structures through structural rearrangements that modulate free energy. During such transitions, the flexible peptide backbone acts as an entropic reservoir, balancing interaction enthalpy against entropy-driven conformational variability [170].

Statistical mechanics offers a robust framework to understand these transitions, suggesting that proteins can adopt ordered conformations under specific thermodynamic variables like temperature and interaction energy variance [171]. Frequently, these order-disorder transitions are triggered by protein–protein or protein–ligand interactions. Disordered proteins, for instance, can form structured complexes through mutual synergistic folding, a process involving dynamic cooperative transitions [87]. However, due to the fleeting nature of intermediate states, studying these transitions is challenging. In adenylate kinase (Adk), a unique "order-disorder-order" mechanism has been observed, where a segment within the ATP-binding subdomain locally unfolds and refolds during catalysis [172]. Similarly, glucokinase exhibits glucose-induced disorder-to-order transitions that underlie its kinetic cooperativity and allosteric regulation [173].

Experimental techniques like atomic force microscopy and X-ray scattering have revealed that order-disorder transitions in thin films follow linear time-dependent kinetics, typically initiated at defect sites with lower energy barriers [174]. These transitions are best visualized through the free energy landscapes, which describe the thermodynamic stability of protein conformations and the energetic pathways connecting them [175]. Entropic and enthalpic factors shape these landscapes and define activation barriers for transitions between states [176]. A case in point is the glutamine-binding protein (GlnBP), whose conformational dynamics is reflected in its free energy landscape, with distinct basins corresponding to open and closed states [177]. Transition states, representing the highest energy barriers, are critical points where folding decisions are made. By modelling the structural distribution within these states, folding mechanisms can be quantitatively predicted [178].

External factors, such as temperature, further influence these landscapes by reshaping energy barriers and altering conformational minima. For example, in bovine serum albumin, thermal variation affects the balance between ordered and disordered states, reinforcing the idea that proteins often exist in dynamic equilibrium [179]. This conformational plasticity is essential for functional versatility and highlights the role of protein interactions in driving order–disorder transitions, including mutual synergistic folding [87].

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7.3. Case studies illustrating order-disorder-new order scenarios

Several proteins exemplify the dynamic transitions between ordered, disordered, and newly ordered conformations, often in response to ligand binding, redox changes, or PTMs (Figure 7). For instance, ligand binding near aromatic amino acid residues can induce changes in the tertiary structure of proteins[180]. IDPs are uniquely characterized by their structural plasticity [181]. Upon binding to their physiological partners, these proteins frequently undergo disorder-to-order transitions [46]. A well-documented example is Hsp33, a heat shock protein from *E. coli*, which undergoes a disorder-to-order transition under reducing conditions, thereby activating its chaperone function [57]. Another case is adenylate kinase, which displays an "order-disorder-order" transition involving local unfolding and refolding in its ATP-binding subdomain [172]. Similarly, the C-terminal domain of troponin I exhibits order-disorder transitions, potentially impacting its role in cardiac regulation and disease [182].

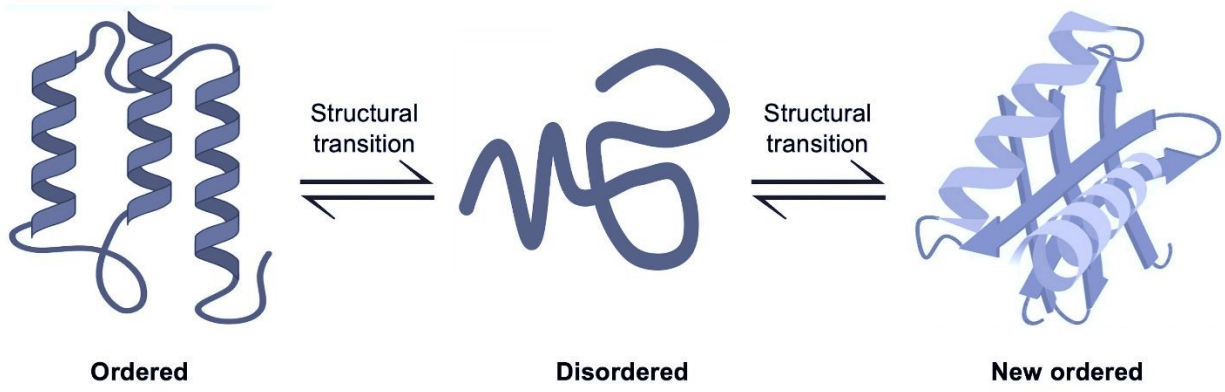

Figure 7: Schematic illustration of order-disorder-new order transition. Proteins can adopt a stable ordered conformation (left), switch to a disordered conformation (middle), and then refold into a new ordered conformation (right) under varying conditions.

GRASP proteins display multiple disorder-to-order transitions that can be modulated by changes in the dielectric constant [183]. In calcineurin, a key phosphatase involved in calcium signalling, interaction with calmodulin leads to disorder-to-order transitions in its regulatory domain. This event enables the displacement of the autoinhibitory domain from the active site, thus activating the enzyme [184]. The human copper chaperone Cox17 provides another example of a conditionally disordered protein. It adopts a defined structured conformation upon forming a disulfide bond in the mitochondrial intermembrane space. This conformational change is essential

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for its role in copper transport and enzyme activation [185]. Disorder-to-order transitions are also widespread among transcription factors, which allow them to remodel their structure for interactions with diverse targets. For example, phosphorylation of FoxM1 at Ser715 by Plk1 and Cdk kinases results in the release of its transactivation domain (TAD) into a disordered state. This transition permits binding by co-activators like CBP, thereby enhancing transcription during mitosis [186]. Npm-N exhibits complex order-disorder transitions modulated by phosphorylation and partner interactions. These transitions influence its ability to assemble into ordered pentameric assemblies or remain disordered, depending on ionic strength and binding partners [161]. A particularly striking case is RfaH, which undergoes a dramatic fold switch from an α-helical domain to a β-barrel upon DNA binding. This structural transformation shifts its role from promoting transcription elongation to facilitating translation initiation, thereby tightly coupling the two processes [187].

Such adaptability underlies the function of many IDPs as dynamic switches in signalling networks [188]. PAGE4, an IDP associated with prostate cancer, exemplifies this principle: it undergoes phosphorylation-dependent structural changes that alter its conformational dynamics and interaction with the AP-1 signalling axis. These transitions give rise to distinct cellular phenotypes and differential therapeutic sensitivities, highlighting the regulatory versatility encoded in the disorder of PAGE4 [69]. In *E. coli*, the biotin repressor BirA illustrates how effector binding triggers disorder-to-order transitions. Upon interaction with biotinoyl-5′-AMP, BirA dimerizes and becomes competent for DNA binding, thereby repressing transcription. This transition supports long-range allosteric regulation, demonstrating how intrinsic disorder enables precise gene control mechanisms [189].

8. Functional implications of conditional disorder

Conditional disorder enables proteins to adopt specific conformations in response to specific environmental cues or PTMs [19]. This structural adaptability allows proteins to reversibly fold or unfold, providing an effective mechanism for modulating function under changing physiological conditions [8]. Many essential biological processes are regulated by such reversible transitions, especially in response to redox conditions, temperature fluctuations, or phosphorylation events.

Proteins exhibiting conditional disorder play diverse and critical roles in biology. This phenomenon is widespread across proteomes and is particularly enriched in multicellular

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organisms, where it contributes to the formation of specialized functional domains. Redox-sensitive conditional disorder is a notable mechanism in many biological systems, influencing regulatory and stress response pathways [57]. For instance, under reducing environments, the *E. coli* heat shock protein Hsp33 undergoes a transition from a disordered to an ordered state, enabling its function in protecting cells from oxidative stress [57]. Similarly, some molecular chaperones exist in partially disordered forms under basal conditions and become fully active upon stress-induced unfolding, thereby enabling the recognition and binding of a wide array of aggregation-prone client proteins [100]. Conditional disorder allows proteins to regulate their activity dynamically by toggling between ordered and disordered conformations. This reversible switching facilitates context-dependent molecular recognition, interaction, and signalling [8,19]. IDPs enriched in signalling pathways act as dynamic hubs, enhancing the sensitivity and adaptability of cellular responses [190]. These proteins often engage in interactions characterized by low affinity but high specificity, which are crucial for the precision of signalling cascades [191].

Moreover, CDPs are well-suited to function as central nodes in protein–protein interaction networks. Their structural flexibility permits binding with multiple partners, enabling participation in a variety of biological processes [191,192]. The presence of functional motifs within disordered regions can also increase the complexity of protein interaction networks, particularly in pathological conditions [192]. Despite their functional versatility, CDPs present challenges for drug discovery, particularly when they are involved in diseases such as cancer or neurodegenerative disorders. Their lack of a stable structure complicates rational drug design and target validation [193]. The dynamic structural patterns of CDPs can now be explored because of the recent advances in AI-driven protein structure prediction tools such as AlphaFold [194]. By providing detailed insights into protein functions and interactions, the precisely predicted structures of AlphaFold have accelerated drug discovery. This is especially important for addressing diseases like Parkinson's and Alzheimer's that are closely associated with IDPs [195].

9. Conclusions and future prospects

The study of CDPs marks a paradigm shift in our understanding of the structure–function relationship in biology. It is now evident that function does not necessarily rely on a fixed, well-folded 3D structure. Proteins exhibiting cryptic disorder, redox-sensitive disordered regions, metamorphic transitions, and intrinsic disorder challenge the classical structure-centric dogma

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(Figure 8). These proteins exploit their conformational plasticity to engage in a wide range of biological processes, including signalling, regulation, stress response, and molecular recognition. The dynamic behavior of CDPs, manifested through disorder-to-order transitions upon binding or environmental cues, enables them to fine-tune their interactions in a temporally and spatially controlled manner. In particular, transitions involving "order-disorder-new order" states highlight the ability of these proteins to undergo complex structural rearrangements that expand their functional repertoire. Moving forward, it is imperative to deepen our molecular understanding of the mechanisms that govern conditional disorder.

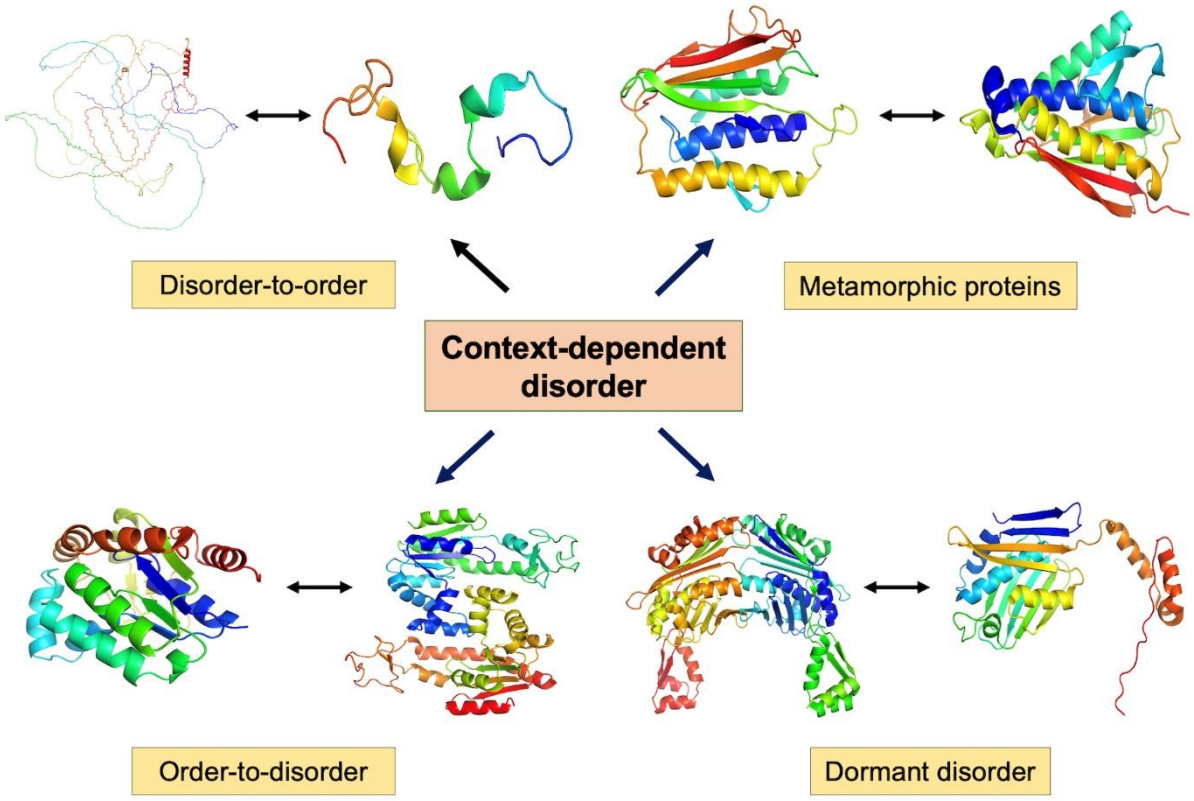

Figure 8. Dynamic protein structures. A schematic representation of context-dependent disorder illustrating four key transitions – Disorder-to-order, Metamorphism, Order-to-disorder, and Dormant disorder. In the disorder-to-order transition, full-length Tau protein (AlphaFold predicted structure AF-P10636-F1) adopts an ordered conformation upon binding to microtubules, shown by Tau fragment bound to microtubules (PDB ID: 2MZ7). In the metamorphism representation, MAD2 protein switches between two stable folded conformations (PDB ID: 1DUJ & 2V64). In the order-to-disorder transition, Adenylate kinase undergoes partial

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unfolding depending on conditions (PDB ID: 1AKY & 4AKE). In Dormant Disorder representation, Hsp33 transitions under stress (PDB ID: 1VZY & 1HW7).

Future research should aim to: i) Elucidate the conformational ensembles of CDPs using high-resolution structural techniques such as NMR spectroscopy, cryo-EM, single-molecule FRET, and hydrogen–deuterium exchange mass spectrometry, ii) Leverage computational simulations and AI-based structure prediction tools to model dynamic disorder-order transitions and predict functional states under diverse conditions, iii) Explore the role of conditional disorder in disease mechanisms, especially in cancer, neurodegeneration, and infection, where misregulation of IDPs and CDPs is often observed, iv) Identify and target transient or condition-specific structural states for therapeutic interventions—an emerging but challenging frontier in drug discovery, v) Integrate systems-level and proteomics approaches to map the distribution, dynamics, and interaction networks of CDPs across cellular and developmental contexts, vi) Ultimately, understanding how proteins leverage conditional disorder to balance structural flexibility with functional precision will offer profound insights into the fundamental principles of protein biology and provide new avenues for biomedical innovation.

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Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study