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REVIEW

The protein disorder cycle

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Abstract

This mini-review represents a brief, disorder-centric consideration of the interplay between order and disorder in proteins. The goal here is to show that inside the cell, folding, non-folding, and misfolding of proteins are interlinked on multiple levels. This is evidenced by the highly heterogeneous spatio-temporal structural organization of a protein molecule, where one can find differently (dis)ordered components that can undergo local or global order-to-disorder and disorder-to-order transitions needed for functionality. This is further illustrated by the fact that at particular moments of their life, most notably during their synthesis and degradation, all proteins are at least partially disordered. In addition to these intrinsic forms of disorder, proteins are constantly facing extrinsic disorder, which is intrinsic disorder in their functional partners. All this comprises the multileveled protein disorder cycle.

Keywords Intrinsically disordered protein region · Protein function · Nascent polypeptide chain · Protein degradation · Protein biosynthesis · Protein folding · Protein misfolding

The discovery of the intrinsic disorder phenomenon and successful penetration of this concept into protein science indicated a departure from the classical description of a protein function in terms of the "lock-and-key" model. Accumulated evidence indicates that protein intrinsic disorder is ubiquitous and inexorable, and the presence of structure-less but biologically active proteins dramatically expanded the universe of functional proteins in such a way that sometimes it may seem that the grinning faces of intrinsically disordered proteins (IDPs) and proteins with IDP regions (IDPRs) peek out from every corner of modern protein science. Altogether, there is no doubt now that the fate of a newly synthesized polypeptide chain is not limited to the functional folding and assembly or pathological misfolding, as was believed for a long time, but also includes a very important "non-folding" branch (see Fig. 1). The choice between these pathways is determined by the peculiarities of the protein amino acid sequence and its environment (Uversky and Uversky 2014).

However, none of the indicated pathways (folding, non-folding, or misfolding) represent a one-way road leading to a dead-end. Instead, inside cells, proteins are involved in a constant circulation between folded, non-folded, and misfolded forms, where already folded proteins can undergo at least partial unfolding and misfold or get (partially) unfolded for new functionality; where misfolded proteins can return back to their original folded or non-folded states; where IDPs/IDPRs can partially fold or misfold; and where such transitions can be repeated several times (Uversky 2003). In other words, there is no folk-tale crossroad stone stating, "If you go left, you will lose your horse; if you go right, you will lose your life; if you go straight, you will live, but you will forget yourself," and the cellular fate of a protein is not predefined but can be rewritten (and multiple times at that). Let us examine this folding-non-folding-misfolding cycle by taking a closer look at the intrinsic disorder (non-folding) part.

The last two decades witnessed a triumph of the idea that protein functionality can be independent of the unique structure (Turoverov et al. 2010; Dyson 2011; Tompa 2011, 2012; Uversky 2013a; van der Lee et al. 2014). IDPs and hybrid proteins with ordered domains and IDPRs are abundantly present not only in all proteomes analyzed so far (Uversky et al. 2000; Dunker et al. 2000; Ward et al. 2004; Schad et al. 2011; Xue et al. 2012; Pancsa and Tompa 2012; Peng et al. 2013a, 2015) but even within the preeminent source of protein structural knowledge, the protein data bank (PDB), where the vast majority of proteins with resolved

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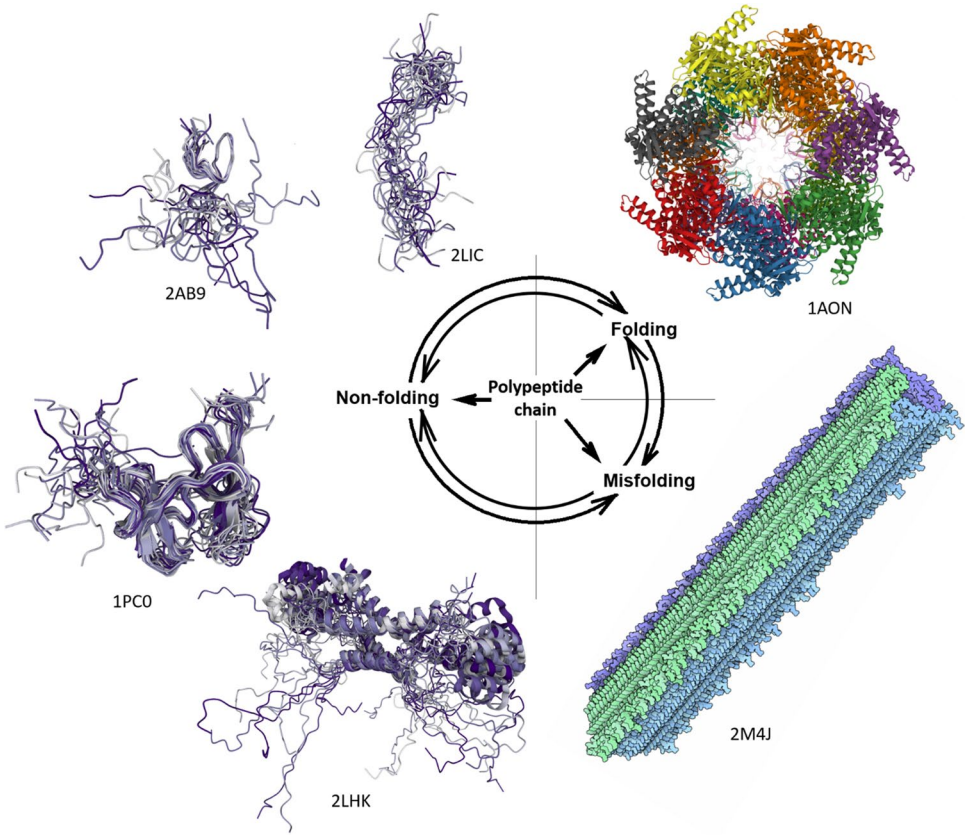


Fig. 1 Schematic representation of the fate of a polypeptide chain in the cell. In addition to the folding-misfolding-non-folding crossroad stone that seems to define the cellular fate of a protein based on the peculiarities of its amino acid sequence, the model shows that this cellular fate of a protein is not pre-defined but can be rewritten, thereby generating a perpetual folding-non-folding-misfolding cycle

X-ray structures have unobserved regions of varied length with missing electron density that frequently correspond to IDPRs (Le Gall et al. 2007; Monzon et al. 2020). Furthermore, in PDB, many protein–protein and protein-nucleic acid complexes with stable structures are in fact formed by IDPs/IDPRs as a result of the disorder-to-order transition (Gunasekaran et al. 2004; Oldfield et al. 2008; Wu et al. 2015; Zhou et al. 2020).

The levels of IDPs/IDPRs in proteomes are correlated with the evolutionary complexity of the organisms, where the more advanced species have higher IDP/IDPR counts (Dunker et al. 2000, 2015; Ward et al. 2004; Xue et al. 2012; Peng et al. 2013a, 2015). The functional range of these proteins is remarkably broad and complements functions of ordered proteins and domains (Wright and Dyson 1999; Dunker et al. 2001, 2002a, b, 2005, 2008a, b; Dunker and Obradovic 2001; Dyson and Wright 2002, 2005; Uversky 2002a, b; Uversky et al. 2005; Cortese et al. 2008; Dunker and Uversky 2008; Oldfield et al. 2008; Uversky and Dunker 2010; van der Lee et al. 2014; Oldfield and Dunker 2014).

Structurally, IDPs/IDPRs are characterized by remarkable spatio-temporal heterogeneity and can range from completely structure-less, coil-like conformational ensembles to compact (but still highly dynamic) molten globular ensembles, to proteins with a hybrid structure containing both ordered and disordered regions (Uversky and Dunker 2010; Uversky 2013a, d, e; Dunker et al. 2013). Thus, intrinsic disorder has multiple faces affecting different levels of protein structural organization, where either the whole protein or its various regions can be (dis)ordered to a different degree. As a result, while looking at such a heterogeneous structure, one can identify fragments with different structural complexity and folding complicity and admire a highly dynamic and interchanging structural mosaic containing foldons (i.e., independently foldable protein units), inducible foldons (which are IDPRs capable of at least partial folding promoted by their interactions with binding partners), morphing inducible foldons (IDPRs with the potential to fold differently due to binding to different partners), semi-foldons (regions that are always in a semi-folded state), and non-foldons (IDPRs that never fold) (Uversky 2013e, 2016a, b, c, 2019a, b). Such exceptional spatio-temporal heterogeneity of IDPs/IDPRs is translated into their multifunctionality, as differently (dis)ordered parts of a protein molecule might have different functions (Uversky 2015, 2016a).

IDPs/IDPRs behave as highly frustrated systems with no single thermodynamically stable state. This is reflected in their free energy landscapes, which are relatively flat, do not have deep energy minima seen in the free energy landscapes of ordered globular proteins, and instead represent a hilly plateau with multiple shallow local minima corresponding to the allowed conformations separated by low hills indicating

forbidden conformations (Uversky et al. 2008; Turoverov et al. 2010; Fisher and Stultz 2011). Such a flattened energy landscape is extremely sensitive to different environmental changes and can be modified in a number of different ways. Depending on the peculiarities of the environment, some energy minima can be deepened, while some energy barriers can increase. This explains the conformational plasticity of IDPs/IDPRs, their extreme sensitivity to changes in the environment, the ability to specifically interact with many partners of different nature, and to fold differently as a result of these interactions (Uversky 2013e).

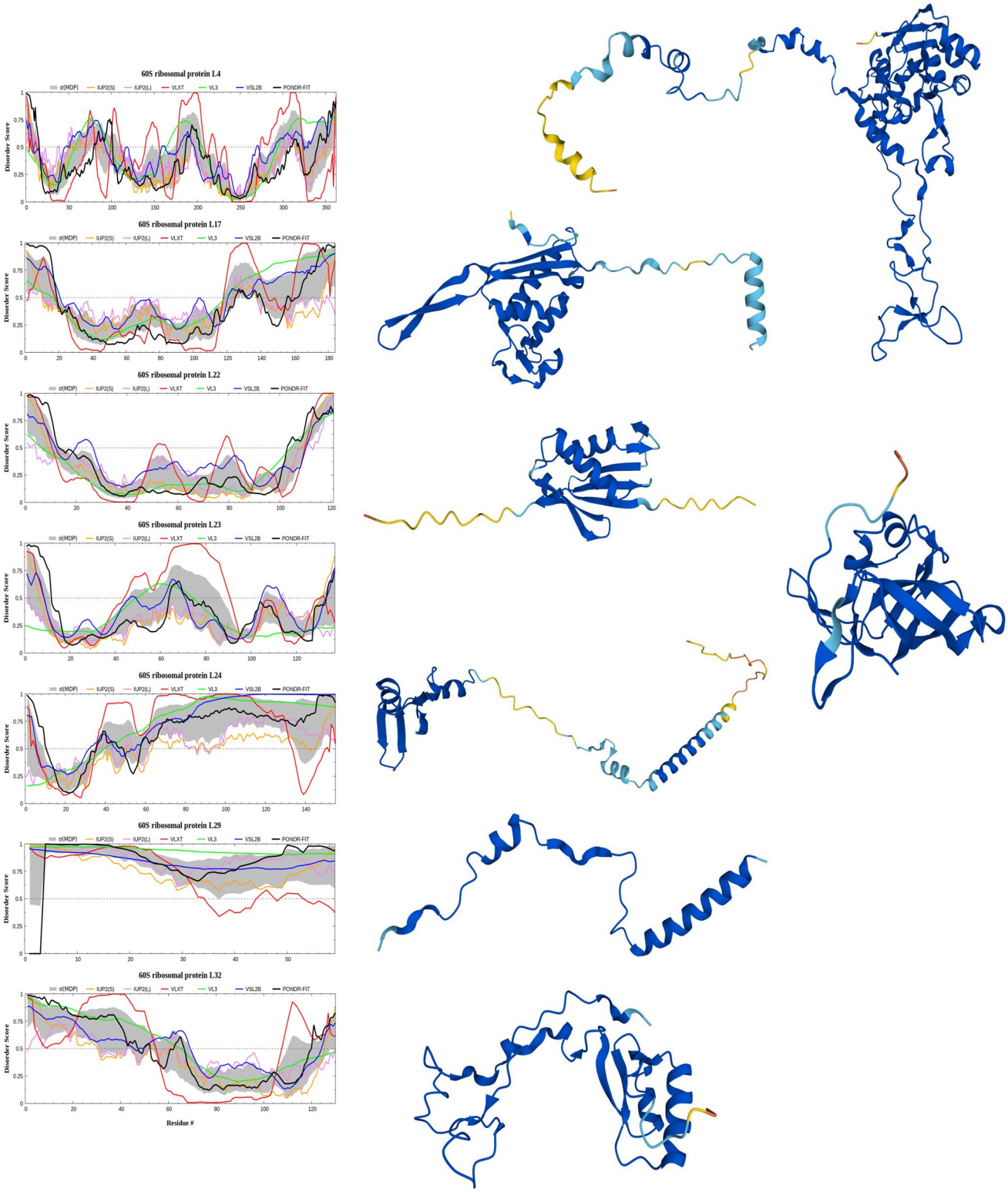
One should also remember that there is no actual boundary between order and disorder. Instead, structures of proteins can be presented as a continuous spectrum of differently structured/disordered conformations that extends from the fully ordered to completely structure-less species, with everything in between (Uversky 2013e; DeForte and Uversky 2016). Such complex, highly heterogeneous spatiotemporal organization of the protein structural space forms the foundation of the structure–function continuum concept (Uversky 2016a, b, 2019a, b), where instead of the classical "one gene-one protein-one structure-one function" model, any protein is considered as a highly dynamic conformational ensemble with a broad spectrum of structural features, and as such the structural heterogeneity and conformational plasticity of IDPs define their remarkable multifunctionality and binding promiscuity (Uversky 2016b, 2019b; Fonin et al. 2019).

Therefore, multifunctionality of proteins is based on the constant order–disorder-order and disorder-order–disorder cycles. This is reflected in the crucial dependence of the functionality of many ordered proteins on the existence of "unfoldons," i.e., ordered regions that must undergo an order-to-disorder transition to make the protein active (Uversky 2013e), or, more generally, in a dormant (transient, conditional, or cryptic) disorder phenomenon (Jakob et al. 2014; Creamer 2013; Bardwell and Jakob 2012). On the other hand, the functionality of IDPs/IDPRs depends on the presence of disorder-based binding sites, called molecular recognition features (MoRFs), which are interaction-prone disordered regions that can fold at binding to specific partners (Cheng et al. 2007; Vacic et al. 2007; Mohan et al. 2006; Oldfield et al. 2005). The presence of such MoRFs (inducible foldons) or morphing MoRFs defines the exceptional binding plasticity and promiscuity of IDPs/IDPRs (Uversky 2011b, 2013c), where a single IDPR can bind to multiple partners gaining very different structures in the bound state (Oldfield et al. 2008; Hsu et al. 2012, 2013; Alterovitz et al. 2020), and explains the abundance of intrinsic disorder among hub proteins and their binding partners in various protein–protein interaction networks (Dunker et al. 2005; Patil and Nakamura 2006; Haynes et al. 2006; Ekman et al. 2006; Dosztanyi et al. 2006; Singh et al. 2006).

Furthermore, the lack of stable structure defines the exceptional sensitivity of IDPs/IDPRs (which represent the edge of chaos systems) to even small environmental changes that can trigger very different structural and functional outputs, thereby serving as illustrative examples of the butterfly effect (Uversky 2013e, 2019a, b). Finally, since the formation of the pathological aggregates, including amyloid fibrils, is critically dependent on the presence of partially folded aggregation-prone species, protein misfolding, by default, relies on the order-(partial) disorder or disorder-(partial) disorder transitions. For example, it was emphasized that amyloidogenic conformations must be relatively unfolded (but not completely unfolded or coil-like), as the inherent flexibility of such pre-molten globular intermediates defines their capability to undergo conformational rearrangements necessary to form amyloid fibrils (Uversky and Fink 2004). It is likely that similar conformational constrains are applicable to proteins involved in the formation of functional amyloids (Deshmukh et al. 2018; Christensen et al. 2019; Jain and Chapman 2019; Rubel et al. 2020; Sergeeva and Galkin 2020; Daskalov et al. 2021; Levkovich et al. 2021).

Although the aforementioned examples serve as illustrations of the folding-non-folding-misfolding circle, it seems that any given protein constantly faces disorder throughout its entire life within the cell, and this disorder can be of intrinsic or extrinsic nature (i.e., intrinsic disorder in functional partners) (Uversky 2013b). Even proteins with the most ordered structures are synthesized as linear polymers that need to undergo disorder-to-order transitions to gain their functional structure. A newly synthesized nascent polypeptide chain passes through the 100-Å long, 10–28-Å-wide ribosomal exit tunnel (Gabashvili et al. 2001), which, at the exit site, contains a ring of 7 ribosomal proteins (L4, L17, L22, L23, L24, L29, and L32) and which regulates translation via interaction of L22 with specific nascent chains (Wilson and Nierhaus 2005). Figure 2 shows that all these proteins contain significant levels of disorder, as evidenced by their per-residue intrinsic disorder profiles and by their unusual structural shapes, which (with the exception of L22) are not consistent with simple globular structure, suggesting that, similar to the majority of other ribosomal proteins, these proteins fold at binding (Peng et al. 2013b) and might preserve significant levels of disorder even in the bound state, forming so-called fuzzy complexes (Uversky 2011b; Tompa and Fuxreiter 2008; Fuxreiter and Tompa 2012; Sharma et al. 2015; Miskei et al. 2017; Fuxreiter 2020).

These observations indicate that the cradle of a nascent polypeptide chain, being enriched in IDPs/IDPRs, is soft and fluffy. Even chaperones and nanny-proteins, which guard a newly synthesized polypeptide chain before it properly folds and matures, are IDPs or hybrid proteins containing ordered domains and functional IDPRs (Tompa


60S ribosomal protein L4
60S ribosomal protein L17
60S ribosomal protein L22
60S ribosomal protein L23
60S ribosomal protein L24
60S ribosomal protein L29
60S ribosomal protein L32
σ(MDP) IUP2(S) IUP2(L) VLXT VL3 VSL2B PONDR-FIT
Disorder Score
Residue #

◀ **Fig. 2** Order and disorder in ribosomal proteins L4, L17, L22, L23, L24, L29, and L32 forming the ring at exit site of the ribosomal exit tunnel. The left panels represent intrinsic disorder profiles generated by these proteins by a set of commonly used disorder predictors, whereas the corresponding structures of these proteins modeled by AlphaFold (Jumper et al. 2021) are shown on the right side. Disorder profiles were assembled by the DiSpi web crawler that aggregates the results from PONDR® VLXT (Romero et al. 2001), PONDR® VL3 (Peng et al. 2006), PONDR® VLS2B (Peng et al. 2005), PONDR® FIT (Xue et al. 2010), IUPred2 (Short) and IUPred2 (Long) (Dosztányi et al. 2005; Meszaros et al. 2018). The outputs of the evaluation of the per-residue disorder propensity (PRDP) by these tools are represented as real numbers between 1 (ideal prediction of disorder) and 0 (ideal prediction of order). Thresholds of $0.15 \leq PRDP < 0.5$ and $PRDP \geq 0.5$ were used to identify flexible and disordered residues and regions. AlphaFold-predicted structures are colored based on the per-residue confidence scores (pLDDT) that range between 0 and 100: blue, cyan, yellow, and orange colors correspond to regions with very high ($pLDDT > 90$), high ($90 > pLDDT > 70$), low ($70 > pLDDT > 50$), and very low ($pLDDT < 50$) per-residue confidence scores. Some regions with low pLDDT may be unstructured in isolation

and Csermely 2004; Kovacs et al. 2009; Tompa and Kovacs 2010; Uversky 2011a; Tsvetkov et al. 2009; Kovacs and Tompa 2012). In its mature form, a protein is placed within the aforesaid perpetual folding-non-folding-misfolding circle, and even on its deathbed it faces disorder. In fact, both proteolytic digestion and proteasomal degradation are dependent on intrinsic disorder. Proteolysis of disordered substrates by numerous proteases is extremely fast (Dunker et al. 2001; Iakoucheva et al. 2001; Fontana et al. 1986, 1997a, b, 1993), and ATP-dependent, active unfolding of protein substrates (likely by mechanical pulling of the polypeptide chain into their channel) represents a crucial functional step of proteasomes and their prokaryotic and archaeal analogues (Weber-Ban et al. 1999; Kim et al. 2000; Van Melderen et al. 1996; Navon and Goldberg 2001; Lee et al. 2001; Prakash and Matouschek 2004; Prakash et al. 2004).

In conclusion, intrinsic disorder represents a crucial part of the functional and dysfunctional life of any given protein, and no single protein can avoid some form of disorder (intrinsic, induced, or extrinsic) during its lifetime. In fact, some proteins are always at least partially disordered. Others, being mostly ordered, possess transient disorder and have to undergo at least partial unfolding to become functional. The functions of many proteins depend on extrinsic disorder, as these proteins are controlled, regulated, and activated via utilization of the functional intrinsic disorder of their partners. Furthermore, the birth and death of all proteins are inevitably associated with disorder, since protein biosynthesis involves generation of a linear polypeptide chain and proteasomal degradation relies on the induced unfolding of a degradation target.

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Declarations

Conflict of interest The author declares no competing interests.

Ethical approval Not applicable

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