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COMMENTARY

Paradoxes and wonders of intrinsic disorder: Complexity of simplicity

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ABSTRACT

At first glance it may seem that intrinsically disordered proteins (IDPs) and IDP regions (IDPRs) are simpler than ordered proteins and domains on multiple levels. However, such multilevel simplicity equips these proteins with the ability to have very complex behavior.

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This article continues a series of short comments on the paradoxes and wonders of the protein intrinsic disorder phenomenon by introducing the "complexity of simplicity" paradox. Intrinsically disordered proteins (IDPs) are simpler than their ordered counterparts at various levels, ranging from reduced amino acid alphabet to redundancy of amino acid sequences (manifested in various ways, such as in the common presence of multiple sequence repeats), and to structural "primitivity" (i.e., an inability to gain well-ordered structures in isolation). Despite this multilevel simplicity, IDPs are very complex creatures characterized by an expanded sequence space, binding promiscuity, and conformational plasticity and polymorphism.

Reduced amino acid alphabet and expanded sequence space

The first lesson one learn about IDPs/IDPRs is that they lack ordered structure because of specific amino acid biases, as they are typically depleted in order-promoting residues (Cys, Trp, Tyr, Phe Ile, Leu, Val, and Asn) and enriched in disorder-promoting residues (Pro, Arg, Gly, Gln, Ser, Glu, Lys, and Ala) (see Figure 1A),[1-8] and have amino acid sequences that commonly contain repeats. In other words, when compared to ordered proteins and domains, the overall alphabet that IDPs and IDPRs utilize in their amino acid sequences is somehow reduced. Therefore, the informational content of the amino acid sequences encoding IDPs/IDPRs is also reduced, making these proteins and domains simpler than their ordered counterparts at the sequence level. However, this sequence simplicity is translated into a vastly expanded sequence space and related structural complexity. Furthermore, the sequence space of IDPs/IDPRs is further increased due to the removal of restrictions posed to ordered proteins and domains created by the need to spontaneously fold into a unique ordered structure.[9]

Using an assumption that any of the normally occurring 20 amino acids can be found in a protein with equal probability (which is a drastic oversimplification), the amino acid sequence space for a protein of 100 amino acids was estimated to be 20^{100} (10^{130}).[10] Obviously, this number serves as the upper-most limit of the sequence space for a 100 residues-long protein, and in reality this space is noticeably smaller, especially for foldable or ordered proteins that require unique structures in order to be functional. The reduction of the space originates from the inequality of the natural abundance of 20 amino acids in proteins, and from a simple observation that not all sequences can fold. Theoretical and statistical analyses based on the existing variation of protein sequences revealed that the actual identity of the majority of amino acids is irrelevant for protein folding, indicating that not all 20

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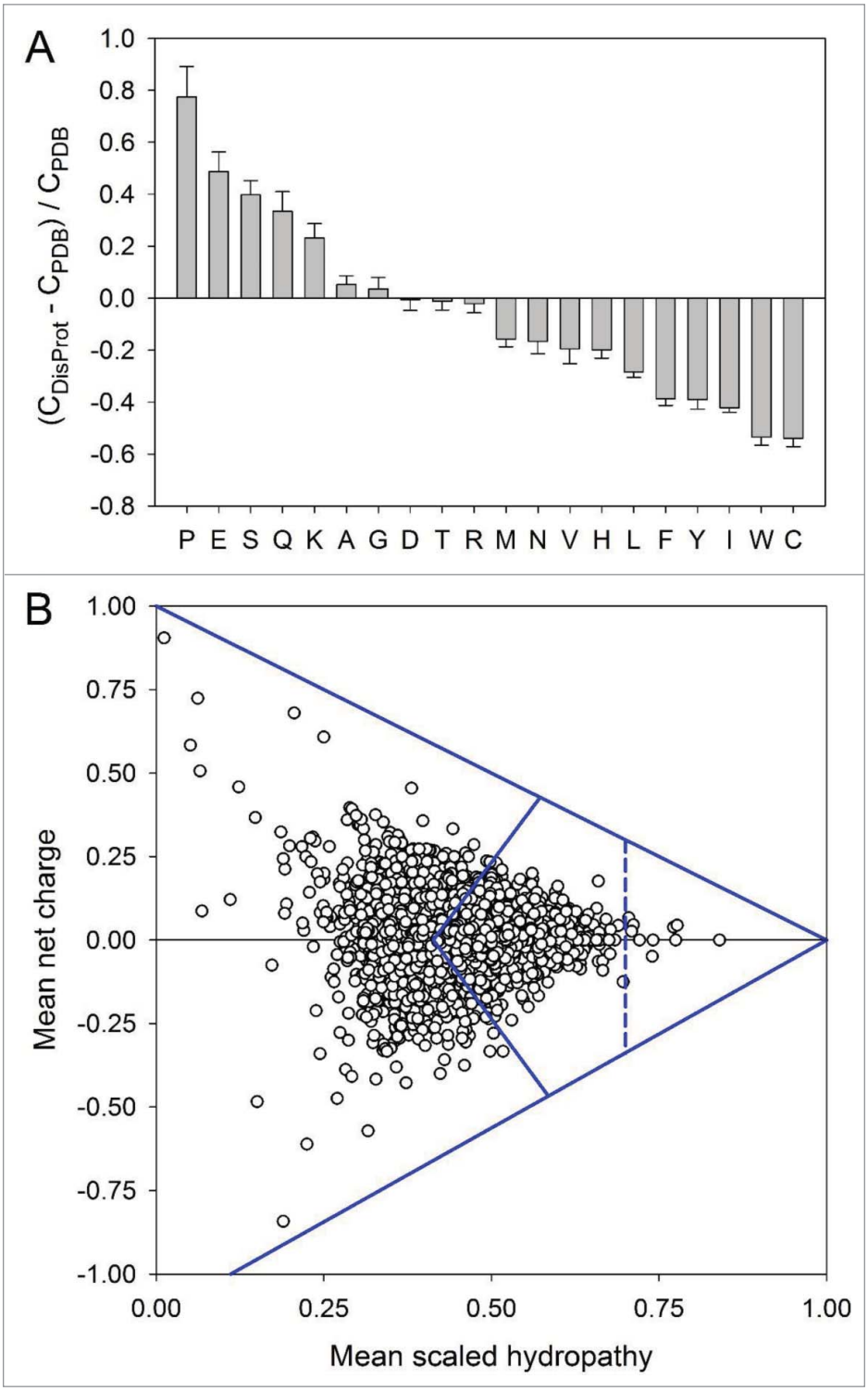

Figure 1. Peculiarities of the amino acid sequences of intrinsically disordered proteins. (A) Amino acid determinants defining structural and functional differences between the ordered and intrinsically disordered proteins. Fractional difference in the amino acid composition (compositional profile) between the typical IDPs from the DisProt database[56] and a set of completely ordered proteins[57] calculated for each amino acid residue. The fractional difference was evaluated as $(C_{DisProt}-C_{PDB})/C_{PDB}$, where $C_{DisProt}$ is the content of a given amino acid in a DisProt database,[56] and C_{PDB} is the corresponding content in the dataset of fully ordered proteins from PDB select 25.[57] Positive bars correspond to residues found more abundantly in IDPs, whereas negative bars show residues, in which IDPs are depleted. Amino acid types were ranked according to their decreasing disorder-promoting potential.[58] (B) Evaluation of the charge-hydropathy space available for mouse proteins. In this plot, areas accessible to sequences encoding compact proteins and extended IDPs are separated by a set of boundaries described in the text.

residues are equally needed for this process to occur.[11-18] These observations indicated that the size of the amino acid 'alphabet' that defines protein folding is noticeably smaller than 20 natural amino acids,[10] resulting in a dramatic reduction of the 'foldable' sequence space. Assuming that only the surface of the protein is important for its function, the size of functional sequence space was estimated to be approximately 2^{33} (10^{10}).[19] The lowest limit of the number of 'foldable" sequences for a protein of 100 residues is 2^{100} (10^{30}), which can be evaluated based on the hypothesis that only 2 types of amino acid were needed to form a protein structure, namely hydrophilic and hydrophobic.[19] However, even a reduced amino acid alphabet should be sufficient for producing all the protein folds (which is believed to be in the range of a few thousand folds[20]) and for generating scaffolds needed to support all protein functions.[10]

In all the aforementioned considerations, which were based on the assumption that proteins need to fold for performing their function,[10] IDPs/IDPRs were obviously ignored. However, it is clear now that many biological functions of IDPs/IDPRs do not require protein folding.[5,21-27] Furthermore, by preserving significant amounts of disorder in their bound states, IDPs are known to often be engaged in the formation of fuzzy complexes, which makes disorder-based interactions very different from interactions involving ordered proteins.[28, 29] All this suggests that, due to the lack of restrictions posed by the need to gain ordered structure, the sequence space of IDPs is noticeably greater than that of ordered proteins.[9] In fact, assuming that all the amino acids are important for IDP function, the size of the sequence space for an IDP of 100 amino acids returns to the original estimate of 20^{100} (10^{130}) a. Even for IDPs that do not have major order-promoting residues, e.g., C, W, F and Y, the potentially available sequence space is still gigantic, 16^{100} ($\sim 10^{120}$).

Figure 1B shows the oversimplified sequence space for the mouse proteome in a form of the 'modified' charge-hydropathy plot. Here, each protein is represented by a single point calculated based on a protein's mean net charge and mean net hydropathy. This plot differs from the traditional CH-plot by showing both positive and negative mean net charge values instead of absolute mean net charges. The area accessible to sequences encoding ordered and disordered proteins are defined by several boundaries: (i) the known boundary separating compact proteins and extended IDPs ($<R> = 2.785 <H> - 1.151$, where $<R>$ and $<H>$ correspond to the absolute mean charge and mean hydropathy, respectively[30]); (ii) the mirror image of this boundary ($<R> = -2.785 <H> + 1.151$, where $<R>$ and $<H>$ correspond to the mean charge and mean hydropathy, respectively), which is included to consider negatively charged proteins; (iii) 2 boundaries showing logical limits of the CH-space ($<R> = -1.125 + 1.125 <H>$ and $<R> = 1.00 - <H>$), evaluated for a series of hypothetical polypeptides containing different proportions of Ile (which is, according to the Kyte and Doolittle scale, is the most hydrophobic residue with the normalized hydropathy of 1, ref.[31]) and a negatively charged Asp (which is characterized by the normalized Kyte and Doolittle hydropathy of 0.1111, ref.[31]) or a positively charged Arg (which is characterized by the normalized Kyte and Doolittle hydropathy of 0.0, ref.[31]); and (iv) the boundary line within the area corresponding to compact proteins to separate soluble and membrane proteins, since proteins whose hydropathy in the normalized Kyte and Doolittle scale exceeds 0.7 are unlikely to be soluble. A comparison of the areas accessible to soluble compact proteins and to extended IDPs clearly shows that the IDP sequence space is significantly larger than that accessible to the compact proteins. In reality, this difference is even bigger, since area assigned to compact proteins also includes compact IDPs.[30,32]

Reduced structural content and structural heterogeneity

From the viewpoint of their spatial organization and structural content, IDPS/IDPRs are also simpler than ordered proteins and domains. It is well-accepted that 3 levels with increased structural complexity are typically used to represent the structural hierarchy of an ordered monomeric protein: a simple primary structure, a more complex secondary structure, and a highly complex tertiary structure. Due to their inability to fold in isolation, IDPs/IDPRs definitely lack the ability to reach the highest level of this hierarchy, and many of these proteins/regions either do not have an ordered secondary structure or contain very limited amounts of such structure. This is illustrated by Figure 2 (left side), which shows characteristic examples of an ordered, rigid like a rock, and a completely disordered, noodle-like, protein.

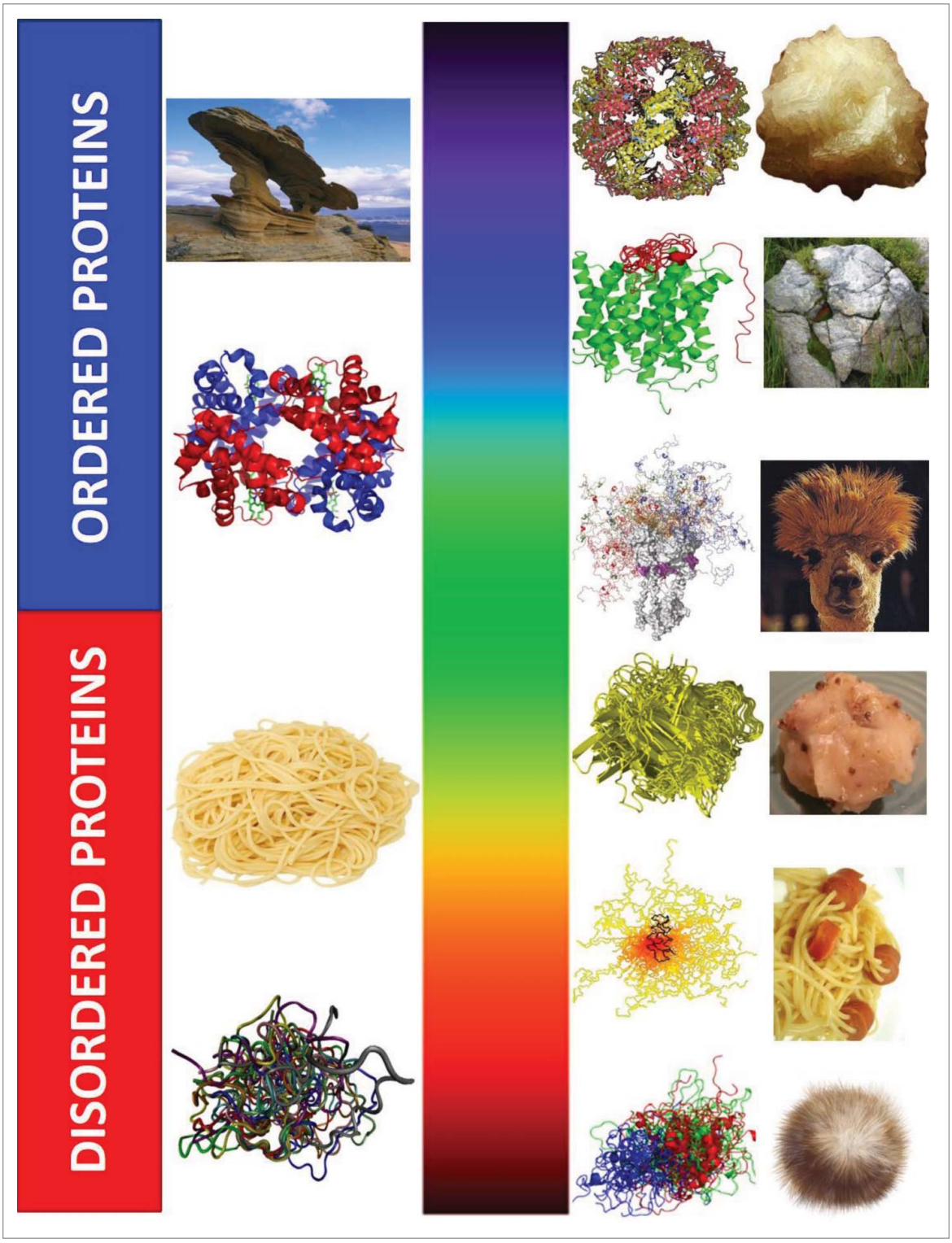

Figure 2. Structural heterogeneity of IDPs. Left side. Bi-colored, oversimplified representation of functional fully ordered proteins (blue) and fully disordered, completely structure-less proteins (red). Right side. A continuous emission spectrum illustrates exceptional structural heterogeneity of functional proteins ranging from fully ordered to completely structure-less proteins, with everything in between. Here, intrinsic disorder can have multiple faces, can affect different levels of protein structural organization, and whole proteins, or various protein regions can be disordered to a different degree.

On the other hand, due to their lack of stable ordered structure, IDPs/IDPRs are characterized by the exceptional structural heterogeneity, which, at least in part, is defined by the highly inhomogeneous distribution of order- and disorder-promoting residues within their sequences. It was pointed out that a

sequence of an IDP represents a very complex mosaic and typically contains a multitude of potentially foldable, partially foldable, differently foldable, or not foldable at all segments.[9] Furthermore, it seems that intrinsic disorder can have multiple faces, can affect different levels of protein structural organization, and whole proteins, or various protein regions, can be disordered to a different degree. This is illustrated by Figure 2 which shows that instead, of a highly polarized bi-colored picture with ordered and disordered proteins being homogeneously painted in blue and red (see left side of Figure 2), the actual structural space of a protein can be described as a continuous spectrum of differently disordered conformations extending from fully ordered to completely disordered proteins, with everything in-between (see right side of Figure 2).[9]

Another level of increased complexity originating from simplicity is present in the form of the spatio-temporal heterogeneity of IDPs.[9] In fact, due to the lack of fixed 3D-structure, different parts of an IDP are ordered (or disordered) to a different degree and this distribution is constantly changing with time. As a result, at any given moment, a protein molecule has a structure which is different from its structure seen at another moment, and the structure of one molecule in the conformational ensemble is significantly different from the structure of another member of this ensemble. In other words, a given segment of a protein molecule will have different structures at different time points. Therefore, IDPs act as 4D-proteins,[33] whose structural description requires time as a crucial component, since their structures are not fixed, as is generally the case for "3D proteins," but rather defined by time and space, and since a given structure in an IDP is seen at a given time only.[9]

Simplified energy landscape, induced folding, and functional complexity

For a protein molecule, an energy landscape can be created, with a topography that describes the probability of each achievable conformation.[34] Such energy landscapes are very different for ordered and disordered proteins. Figure 3A shows that the energy landscape of an ordered protein has a complex funnel-like shape and possesses a well-defined global energy minimum,[35, 36] which corresponds to a unique well-folded state. However, it is worth noting that the bottom of this funnel-like energy landscape is rugged due to the fact that even ordered proteins possess noticeable structural flexibility[37] and might have numerous conformational substates (or nearly isoenergetic conformations).[38-40] On a functional side, the presence of this roughness defines the protein's allosteric regulation, where functional response is achieved via energetic coupling of the remote sites, with the ligand binding at one site modulating the structure and dynamics of a distant binding site.[41-49] Such roughness also defines the capability of an ordered protein to undergo a conformational change at ligand binding.[50]

The free energy of an extended IDP that exists as the dynamic ensemble of a large number of interconverting conformations lacks a deep energy minimum seen in the landscape of an ordered protein. Instead, the corresponding energy landscape is dramatically simpler, relatively flat, and represents a "hilly plateau" (Figure 3B), with hills corresponding to the forbidden conformations.[34,51,52] The lack of a global energy minimum and the presence of numerous local energy minima force an IDP to behave as a highly frustrated system without single folded state.

On the other hand, this flattened energy landscape is highly sensitive to environmental changes and explains the conformational plasticity of an IDP. In fact, different environmental factors might affect the energy landscape in a number of very different ways, making some energy minima deeper and some energy barriers higher (see Figure 3C). This gives some logical explanations to the ability of an IDP to specifically interact with many ligands of different nature, and to fold differently as a result of these interactions. Here, the interaction with a particular binding partner affects the IDP folding landscape in a unique way, promoting the formation of a specific structure on a template-dependent manner.[9]

Importantly, due to the aforementioned multi-level heterogeneity of IDPs, their actual energy landscapes are essentially more complex than shown in Figure 3B. In fact, individual energy landscapes should be used to describe different parts of an IDP, with each of these sub-landscapes capable of responding differently to different environmental changes.[9] This heterogeneity of the energy landscape defines the ability of IDPs to form fuzzy complexes, where a significant part of a protein preserves its intrinsically disordered state even in the bound conformation.[28,29,53,54]

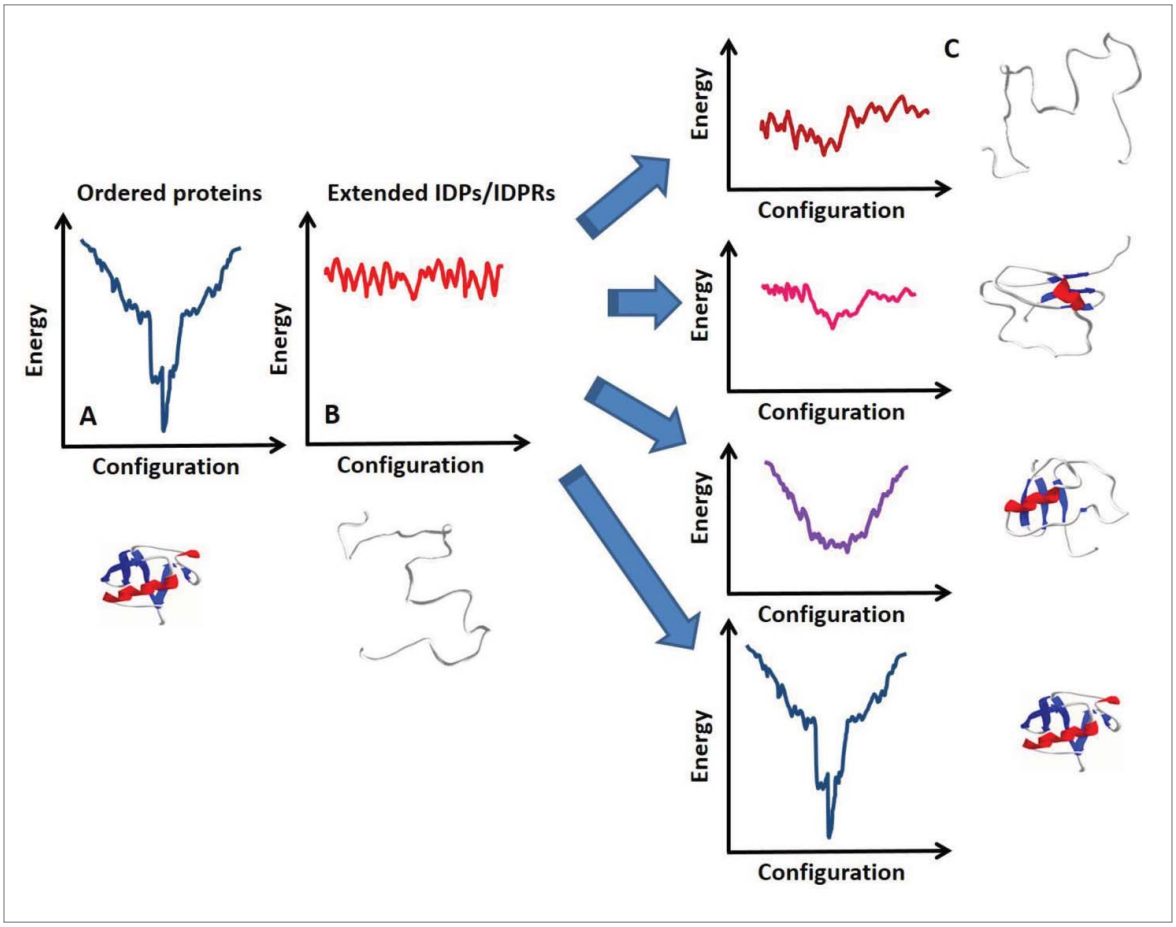

Figure 3. Energy landscape of ordered proteins and IDPs. A diagram showing the folding energy landscapes of a typical globular protein (A) and of a typical natively unfolded protein in the absence (B) or presence of different binding partners (C). These landscapes are depicted schematically in one-dimensional cross-section. Illustrative examples of corresponding structures are also shown. Reprinted from *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, Vol. 1834 (5), Uversky V.N., Unusual biophysics of intrinsically disordered proteins, Pages No. 932–951, Copyright (2013), with permission from Elsevier.

Furthermore, there is a principle difference between the spontaneously foldable ordered proteins and IDPs undergoing binding-induced folding. In fact, although all information required for an ordered protein to spontaneously fold to its functional state is encoded in its sequence, a significant portion of such folding code is missing in IDPs. Since this missing portion of the folding code (or a part of it) can be supplemented by a binding partner(s), many IDPs can partially fold at binding to their partners. Importantly, the folding fate and the final folded state of an IDP/IDPR are not strictly defined and depend on the partner. As a result, a given IDP/IDPR can bind to multiple partners and gain very different structures in the bound state.[55]

Finally, an interplay should be mentioned between the complex 'anatomy' of IDPs/IDPRs, which are highly heterogeneous entities containing multiple relatively short functional elements that are folded/disordered to different degree and are able to respond differently to changes in their environment, and the unique 'physiology' of these proteins; i.e., their ability to interact, regulate, and control, and be regulated and controlled by multiple structurally unrelated partners.[54]

In summary, the exceptional functional complexity and structural heterogeneity of IDPs/IDPRs would not be possible without the multilevel sequential, structural, and spatiotemporal simplicity of these proteins and without their simplified energy landscapes.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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