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Review

# Intrinsically Disordered Proteins: Critical Components of the Wetware

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Cite This: *Chem. Rev.* 2022, 122, 6614–6633

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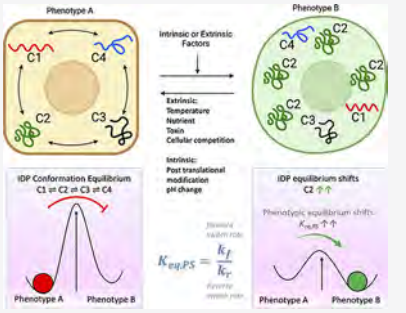
**ABSTRACT:** Despite the wealth of knowledge gained about intrinsically disordered proteins (IDPs) since their discovery, there are several aspects that remain unexplored and, hence, poorly understood. A living cell is a complex adaptive system that can be described as a wetware—a metaphor used to describe the cell as a computer comprising both hardware and software and attuned to logic gates—capable of "making" decisions. In this focused Review, we discuss how IDPs, as critical components of the wetware, influence cell-fate decisions by wiring protein interaction networks to keep them minimally frustrated. Because IDPs lie between order and chaos, we explore the possibility that they can be modeled as attractors. Further, we discuss how the conformational dynamics of IDPs manifests itself as conformational noise, which can potentially amplify transcriptional noise to stochastically switch cellular phenotypes. Finally, we explore the potential role of IDPs in prebiotic evolution, in forming proteinaceous membrane-less organelles, in the origin of multicellularity, and in protein conformation-based transgenerational inheritance of acquired characteristics. Together, these ideas provide a new conceptual framework to discern how IDPs may perform critical biological functions despite their lack of structure.



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## 1. INTRODUCTION

### 1.1. Dark Matter in Biology

The plethora of proteins from all of the extinct and extant organisms is defined as the protein universe, a concept introduced by István Ladunga in the 1990s.[1] While a significant fraction of the protein universe is well-studied and the structures of thousands of proteins have been determined, the nature of the remainder of this universe remains poorly understood. The latter fraction of proteins with unknown structures is referred to as the dark proteome, which is a constituent of the biological dark matter.[2] Indeed, significant

Received: October 1, 2021
Published: February 16, 2022





https://doi.org/10.1021/acs.chemrev.1c00848
*Chem. Rev.* 2022, 122, 6614–6633

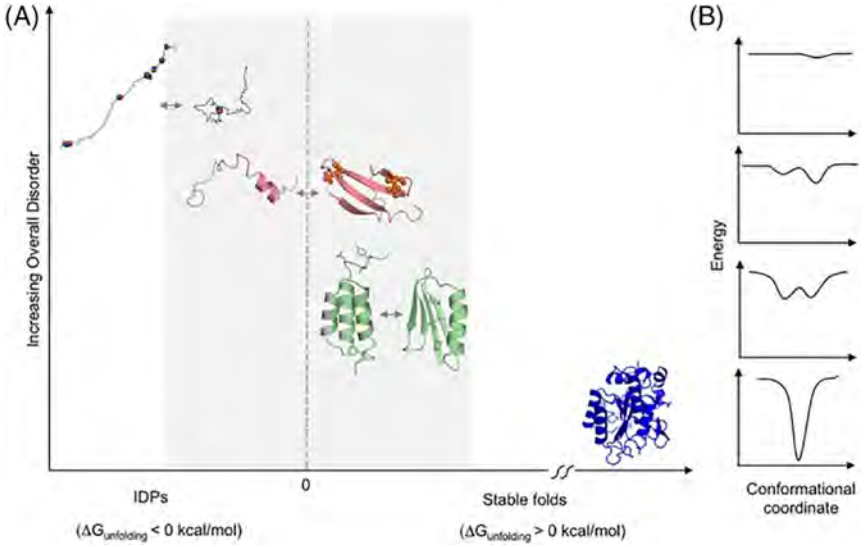


**Figure 1.** Proteins on the brink of stability can undergo a continuum of order/disorder transitions. (A) Examples of transitions from top left to bottom right: transition between the extended and collapsed disordered states of prostate-associated gene 4 (PAGE4), which is modulated by phosphorylation; disorder-to-order transition of 4E-BP2 induced by phosphorylation; order-to-order fold switching between $G_{A98}$ and $G_{B98}$, which is triggered by single amino acid changes or ligand binding. In contrast, stable proteins such as subtilisin (shown in dark blue) do not undergo such changes. (B) Approximate energy-well diagrams for each protein from PAGE4 (top) to subtilisin (bottom). Reproduced with permission from ref 253. Copyright 2018 Wiley.

fractions of the proteomes of archaea and bacteria, and almost half of the eukaryotic and viral proteomes, are estimated to be dark.[3] Like the dark matter in the physical universe, the dark matter in biology is not easily detectable by the traditional approaches of structural biology. However, the dark matter is essential to life, is involved in performing functions that are perceptible, and complements the functions performed by their ordered (visible) counterparts.

Several computational studies on the molecular evolution of the protein universe[3−6] suggest that the protein universe is expanding, and proteins with common ancestors billions of years ago are diverging in their molecular composition.[7] Further, they also reveal that, within the dark proteome, even the foldable domains have specific features. For example, they are generally shorter in length and have unique distributions of hydrophobic residues when compared to known domain families. More importantly, the studies indicate that the constituents of the dark proteome exhibit a higher propensity for intrinsic disorder.[6] Indeed, intrinsically disordered proteins (IDPs) (and intrinsically disordered protein regions within ordered proteins, or IDPRs) that lack a 3-dimensional structure under physiological conditions[8−10] comprise a large fraction of the dark matter in the protein universe.[4,8,11−16]

In line with these observations, bioinformatics analyses reveal that the proteomes of organisms across kingdoms, including viral proteomes, are considerably enriched in IDPs/IDPRs.[8,11−16] Furthermore, the length and frequency of IDPRs appear to positively correlate with organismal complexity. For example, ∼33% of eukaryotic proteins contain long IDPRs, and ∼10% of proteins in eukaryotes are fully disordered.[17] In other studies, noticeably broader penetrance of intrinsic disorder into the various proteomes was indicated, where fractions of disordered residues ranged from 12 to 21% and from ∼18% to ∼28% in archaea and bacteria, increasing to 20−40% and 35−45% in viruses and eukaryotes, respectively.[11] Whole genome analyses from several organisms from bacteria to mammals revealed that their genomes encode proteins that are predicted to be highly disordered.[18] Furthermore, it was recently shown that intrinsic disorder is not only correlated with organismal complexity but also appears to contribute to clade-specific functions.[19]

### 1.2. IDPs and Their Interactions

IDPs lack a rigid structure and exist as diverse conformational ensembles. These ensembles are malleable (dynamic), which facilitates the interactions of IDPs with multiple partners in response to the changing environment.[20,21] All of these interactions form the interaction networks (PINs) that are scale-free[22,23] and channelize the flow of information within the cell. Consistent with this role, PIN organization and properties are evolutionarily conserved.[24] IDPs occupy hub positions in the PINs[25−30] and play important roles in transcription, translation, signal transduction,[13,31−36] cell cycle regulation,[37,38] circadian rhythmicity,[39−42] and regulation of phenotypic plasticity.[11,43,44] Furthermore, IDPs tend to engage in promiscuous interactions, which can lead to pathological states.[45−48] Therefore, it is unsurprising that overexpression of IDPs has emerged as a major cause for several chronic human diseases, e.g., diabetes, cancer, and neurodegenerative illnesses.[32,49−56]

With regard to the molecular mechanisms underlying the functions of IDPs, it is generally held that at least some IDPs transition from disordered conformational ensembles to (at least partially) ordered structures upon interacting with their targets, a process referred to as coupled folding and binding.[57] Possible mechanisms that underlie this phenomenon[58] include the induced-fit hypothesis, which suggests that folding occurs after binding to the target, and the conformational selection hypothesis, which envisages that potential conformations pre-exist in the unbound state and the target selects the most appropriate one. However, the binding mechanisms of many IDPs possibly involve some combination of these two models. On the other hand, many IDPs retain significant intrinsic disorder in their bound states,[59−65] with some of them being


https://doi.org/10.1021/acs.chemrev.1c00848
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capable of forming ultrahigh-affinity but highly disordered complexes (see later). Finally, some IDPs have been observed to stochastically switch conformational states while remaining intrinsically disordered.[66]

Together, these observations suggest that some IDPs are on the verge of structural instability and can readily adopt functional conformations to become biologically active. This is conceptually analogous to the conformational (fold) switching events seen in some metastable folded proteins (referred to as metamorphic proteins) subsequent to mutagenesis or environmental perturbations, giving rise to new functions (Figure 1).[67] On the other hand, some IDPs remain mostly disordered even when they interact with other proteins.[60,68−70] In fact, recent computational studies seem to indicate that less compact protein structures are essential for biological activity due to their capacity of weak yet dynamic interactions with the target proteins.[71,72]

Notwithstanding the multiplicity of mechanisms, both coupled folding and binding[73] and the degree of disorder in the fuzzy complexes[62] constitute the majority of the atomistic mechanisms underlying the interactions promoted by IDPs. Indeed, IDPs can interact with very high affinity ($K_d$ = picomolar range)[74] while remaining disordered and maintaining their flexibility and dynamic natures.[74,75] Interactions of the above kind were observed in IDPs with regions of considerable opposite net charges in their sequences. Protein−protein interactions between these IDPs are governed by the complementarily charged, unstructured regions (long-range electrostatic attraction) rather than specific interresidue contacts or binding sites within folded domains.[74] Because eukaryotic proteomes are enriched in proteins with high net charge,[32,76] it is possible that such an interaction mechanism is quite abundant in nature.[74] Consistent with this reasoning, it is now increasingly evident that IDPs are key functional constituents of proteinaceous membrane-less organelles (PMLOs). The opposite charge interactions are likely crucial in IDP-mediated liquid−liquid phase separation forming these organellies.[77−82]

### 1.3. Scope of This Focused Review

Despite this wealth of knowledge regarding IDPs since they were first discovered >20 years ago,[83] as well as the mechanisms by which they accomplish their biological functions in the absence of apparent structure, there are several unique aspects that remain relatively unexplored and poorly understood. In this focused Review, we examine IDPs from a dynamical systems perspective because this unique aspect of the IDPs, although acknowledged, has not been investigated in sufficient detail. First, we examine how IDPs, as complex systems that self-organize, may influence cell-fate decisions by wiring PINs to drive the system toward a stable attractor. Next, we discuss how the conformational dynamics of IDPs could potentially manifest itself as conformational noise, which in turn can amplify transcriptional noise, resulting in PIN rewiring, phenotypic plasticity, and adaptive evolution. Finally, we highlight the potential role of IDPs in the origin and evolution of life from a primordial, chemoton-like entity[84,85] to the last universal common ancestor (LUCA), and eventually through the major evolutionary transitions,[86] to multicellular forms and beyond. In concluding, we note that a deeper understanding of the IDPs that likely predated life as we know it can shed new light on how these constituents of the dark matter of biology evolved as critical components of the wetware and guided cellular decision making.

## 2. IDPS, SELF-ORGANIZATION, AND SCALE-FREE NETWORKS

### 2.1. Self-organization

Living systems, and many nonliving systems, fall in the category of complex systems that follow two primary characteristics: (1) the ability to self-organize and (2) the manifestation of nonlinear dynamics.[87] Self-organization is defined as a process by which global order emerges from local interactions between the components of a system that is initially disordered or chaotic. Such systems are open systems that reside far from thermodynamic equilibrium.[88] Furthermore, self-organized systems exhibit emergent properties that are different from those of their individual components and are collectively governed by the interactions among the constituent parts within a global topology. Emergent properties are not predictable based on the behavior of the individual components unless their mutual interactions are considered as well. Further, systems with emergent properties respond to external perturbations in nonlinear ways, leading to complex coordinated dynamics.[89]

### 2.2. Self-organizing Properties of Scale-Free Networks

Previously, it was generally held that biological networks follow a random architecture, where the nodes interact with one another with a fixed probability that is independent of the other edges in the network.[90] The modern concept of scale-free networks was introduced by Barabasi and co-workers,[22,23,91] who discovered that the degree distribution (probability $P(k)$ of a random network node to be connected to $k$ other nodes, with degree being the number of connections) of biological networks often follows a power law. Specifically, $P(k) \approx k^{-\gamma}$ (exponent $\gamma$ is a constant), where most of the network connectivity is concentrated among a few of the nodes (hubs) while the rest of the nodes have a very low degree. Furthermore, these insightful studies also revealed that scale-free networks are resilient and remain functional even in response to the failure of random nodes. However, scale-free networks are vulnerable and can become suboptimal in response to the failure of hubs.[92]

As self-organizing systems, scale-free networks are composed of self-repeating patterns (fractals) across diverse length scales.[93] Curiously, the same principles are also applicable to individual proteins because the spatiotemporal structural organization can be envisaged as an intraprotein network, wherein the individual amino acids participate in interactions that are transient or stable.[94] In fact, it was recently shown that a protein molecule may be visualized as a complex system existing as a dynamic, multilevel network of networks analogous to a nesting doll (Matryoshka). In this metaphor, one can think of the different regions of the polypeptide chain as forming secondary structure elements through local networks of interresidue contacts as the lowest level or organization. Interactions between the elements of a secondary structure would give rise to the next level to form foldons, nonfoldons, unfoldons, etc. These interactions give rise to higher levels of the network, namely, proteins domains and a network including interdomain and between-domain interactions to result in second-tier networks. Therefore, from this Russian nesting doll perspective, a typical PIN represents a highly dynamic and multilevel network of networks.[94]


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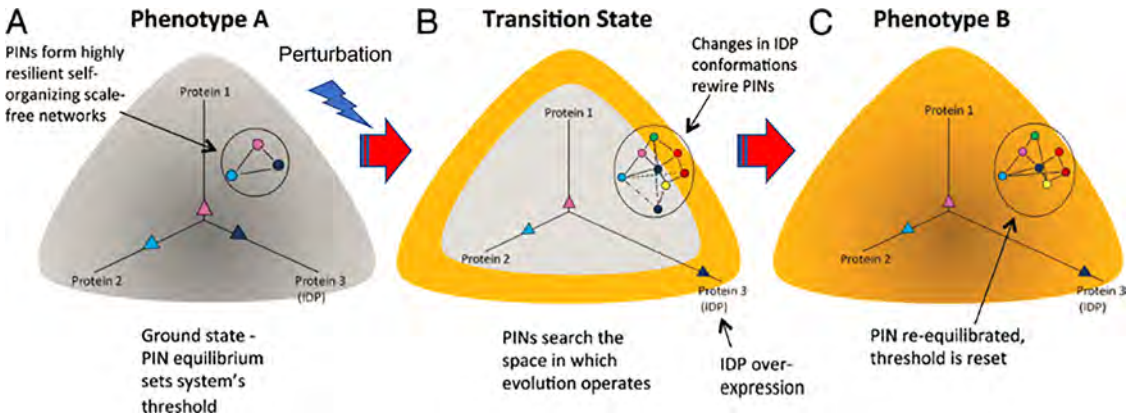


**Figure 2.** Rewiring of protein networks facilitates state switching by activating latent pathways. (A) State of a cell with phenotype A depicted in gray, showing a simple protein network with three proteins (1−3), of which one is an IDP (indicated in dark blue) and is expressed at different levels represented by the three vectors. This configuration represents the protein network's ground-state threshold. (B) Depiction of the transition state. A perturbation causes increased IDP expression (protein 3). Overexpression of the IDP results in promiscuity and the protein network explores the network search space shown by the various dashed lines. This transition state is depicted in yellow around the gray area. (C) State of the cell after it has transitioned to phenotype B from phenotype A, represented in yellow. A particular configuration of the protein network that increased its fitness is selected and now represents the new ground state. Reproduced with permission from ref 95. Copyright 2013 Taylor & Francis Online.

## 3. IDPS, CONFORMATIONAL NOISE, AND PHENOTYPIC SWITCHING

### 3.1. Conformational Noise

On the basis of the characteristics discussed in section 2, a model was proposed[95] that is known as the MRK hypothesis.[96,97] The authors postulated that the conformational dynamics of IDPs may contribute to differential signaling noise in cells. The authors referred to this noise as conformational noise to distinguish it from the well-recognized transcriptional noise. Thus, in contrast to transcriptional noise, the MRK hypothesis states that conformational noise may be defined by the randomness in the confirmations that an IDP ensemble populates. Although, interconversion of IDP conformations exhibits fast exchange, covalent modifications that occur post-translationally, or changes in their environment can enable the ensemble to preferentially occupy particular conformations and/or conformational dynamics. Thus, variations in the conformational ensembles can have significant physiological effects.[98] Furthermore, conformational noise can promote promiscuous interactions of the IDPs.[48] Given that many transcription factors (TFs) are IDPs,[99−102] the authors of the MRK hypothesis posited that conformational noise can be a fundamental aspect of transcriptional variation, and IDPs could potentially propagate or enhance the stochasticity of the cellular response in reaction to intrinsic and/or extrinsic perturbations.

### 3.2. IDPs and Phenotypic Switching

As per the MRK hypothesis, conformational noise due to stochastic IDP interactions in conjunction with intrinsic or extrinsic stimuli would enable the system to explore the broader network configurational space. Therefore, this heuristic enables network rewiring and, consequently, phenotypic switching, giving rise to phenotypic heterogeneity. Stated differently, the hypothesis implies that IDPs unmask latent network configurations to cause phenotypic switching to alternate states (Figure 2).[95] Indeed, stochastic phenotypic switching is observed during differentiation,[103] somatic cell reprogramming (induced pluripotent (iPS) cell generation),[104−109] and cancer cells with stem cell-like properties.[110,111]

Of note, the MRK model implied that information encoding the cellular phenotype resides in the PIN configuration, and every cell has the potential to switch its phenotype in response to a specific input. Per this model, although the network is relatively flexible to physiological changes, it is robust in resisting nonphysiological perturbations. More importantly, per the MRK model IDPs can rewire the network, unmasking latent network configurations (and, consequently, alternative phenotypes) in response to stress. However, the model envisaged that the PIN can return to the normal (default) setting when the stress is relieved.

Another salient feature of this model is that information generated by network rewiring may operate across different temporal scales. Therefore, while transient information resides within the PIN, information related to slower changes (e.g., evolutionary processes) is transferred to the genome, accounting for transgenerational inheritance. Alternatively, the model postulated that a mechanism similar to genetic assimilation of the acquired character, as suggested by Waddington, may be involved.[112] Furthermore, it was postulated that the macroscopic behaviors of a system, such as phenotype switching and adaptive evolution, are driven by PIN rewiring initiated by the IDPs.[95−97,113]

From the foregoing it follows that cell-fate specification need not be only deterministic but can be stochastic and thus account for reversible phenotypic switching as seen in epithelial−mesenchymal transition (EMT) and mesenchymal−epithelial transition (MET), which are the switching of a drug-sensitive cell to one that is drug-resistant or vice versa[108,114−117] or the malignant transformation of a normal cell and its reversal to a nonmalignant phenotype.[118,119] Interestingly, many transcription factors involved in EMT and/or MET[43] and in drug resistance such as paxillin[120] are IDPs. A theoretical study elucidating how transformation of a normal cell into a cancer cell and its reversal may be actuated by modulating the levels of the oncogene c-Myc, an illustrative IDP,[121] further supports many aspects of the MRK hypothesis.


https://doi.org/10.1021/acs.chemrev.1c00848
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The significance of IDPs in PIN rewiring warrants the development of statistical or dynamical models to further elucidate such mechanisms. The complexity in developing any such model is contributed by the multiple cellular processes (e.g., protein dynamics, PTMs, intracellular diffusion, and gene expression) manifested at various spatiotemporal levels. Therefore, one has to come up with a suitable multiscale approach that integrates the different time and length scales using appropriate models and parametrizations. Development of such approaches has been the topic of several reviews.[122,123]

## 4. IDPS AS COMPLEX DYNAMICAL SYSTEMS

### 4.1. IDPs As Complex or Edge-of-Chaos Systems

In dynamical systems theory pioneered by Poincaré[124] that describes complex systems, an attractor may be defined as a set of values of the variables toward which the system tends to evolve from diverse initial states (Box 1). As discussed earlier, the configuration of the PIN (in conjunction with the environment) defines a cell's phenotype.[95] A PIN could be represented as a dynamical system in an appropriate configuration space. It could then be thought of as starting from a context-dependent initial configuration, evolving in time due to mutual interactions, and eventually settling down into an attractor, defined here as a stable cell phenotype.[43,125] The different steady states that a PIN can potentially occupy can be predicted by modeling its dynamics. Therefore, at steady state, the probability that the system will occupy an attractor is proportional to the stability of the PIN configuration of the attractor. Thus, a set of attractors and their probabilities of being occupied by the system represent a high-dimensional landscape as envisioned by Waddington in the epigenetic landscape metaphor (Figure 3).[126]

**Box 1**

Attractor can be described as a stable state toward which a system would tend to get attracted in the long run. A strange attractor is strange because it has a fractal structure. Often, but not always, they are chaotic in nature. A chaotic attractor is very sensitive to the initial conditions. It means that two very close initial conditions inside a chaotic attractor can lead to two locally diverging trajectories, thus showing local instability. However, once the trajectory is inside its basin of attraction, it will not depart from the attractor, which shows global stability.

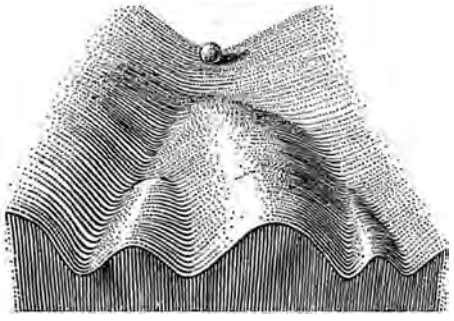

**Figure 3.** Schematic illustration of Waddington's epigenetic landscape. Adapted from ref 126. Reproduced with permission from ref 97. Copyright 2020 MDPI Publishers.

The concept of an "attractor" has been adopted in cancer as well.[127−129] Here, cancer attractors, defined as stable states of latent PIN configurations not occupied by normal cells, drive a normal cell to switch to a malignant phenotype. Access to cancer attractors can be determined by genetic or nongenetic mechanisms in which the IDPs play a crucial role, especially as occupants of key hubs in the PIN.

To elaborate further, complex systems are thought to have some of the following properties:[130]

(a) They contain many nonlinearly interacting heterogeneous components. Thus, the behavior of complex systems may not be defined by the sum of its parts.
(b) There is interdependency of constituents.
(c) They constitute a nested entity that encompasses multiple components (each of which can be complex systems themselves) spanning diverse scales.
(d) They are capable of emergent behavior.
(e) The system enables a dynamic exchange between order and chaos (disorder).
(f) They can involve cooperation as well as positive (reinforcing) and negative (damping) feedbacks.
(g) They are likely to have a memory (hysteresis). Previous states may influence future states.

Thus, it follows that IDPs could be considered as complex systems.[131] For example, IDPs are heterogeneous (see earlier) with constituents that are autonomous or dependent on each other and can interact nonlinearly. The functional misfolding may be thought of as a product of coupled competition and cooperation. The fact that the IDPs can undergo excursions between order and disorder underscores their spatiotemporal complexity. IDPs exhibit conformational changes in response to various stimuli to exhibit the butterfly effect where minute thermodynamic perturbations might produce significant conformational and eventually functional changes (Figure 4). Finally, IDPs display emergent behavior. Under certain conditions (e.g., post-translational modifications or contact with other proteins), they can self-organize by undergoing disorder-to-order transitions.[131]

### 4.2. IDPs: Strange Attractors

From the foregoing, it is tempting to conjecture that, in an appropriate space, the dynamics of the constantly changing IDP network configuration can be described as a chaotic system,[131,132] where the system neither converges to a steady state nor diverges to infinity; it will stay in a bounded region with chaotic motion in that space (Figure 5, Box 1). Under some conditions, it is likely that the system trajectory could be analogous to a strange attractor, i.e., small changes in the initial conditions can cause significant changes in the outcome (butterfly effect).[133]

To make matters more concrete, consider the Lorenz system. The Lorenz attractor is a nonlinear dynamical system that was developed to describe atmospheric convection in response to perturbations in gravity and temperature.[134,135] Lorenz and his co-workers modeled this system using coupled differential equations involving three independent variables, $x$, $y$, and $z$. This system exhibits a strange attractor behavior (Figure 5). The time evolution of an independent variable in the Lorenz system (Figure 5B) shows a striking resemblance to the conformational dynamics trajectory of an IDP, which is represented by the FRET characteristics plotted over time (Figure 5A). The donor−acceptor distance stochastically


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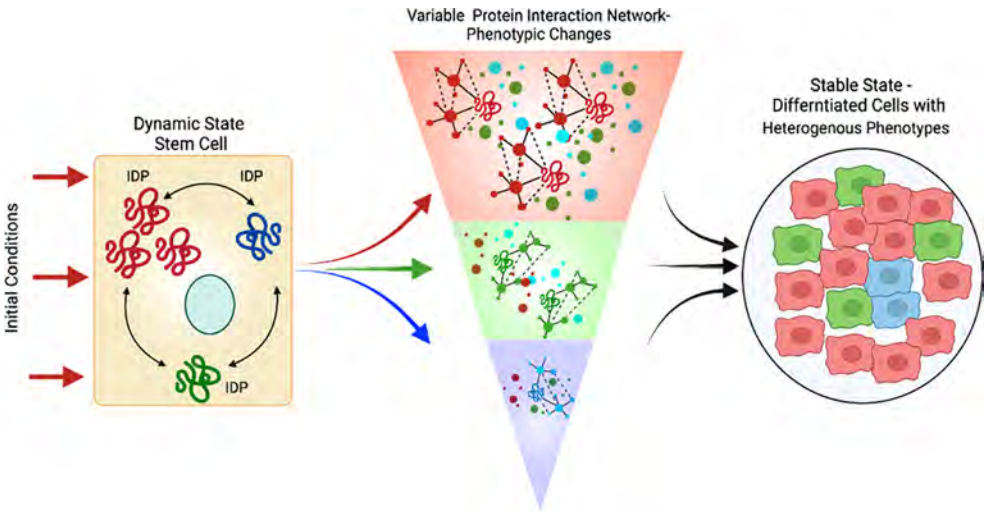


**Figure 4.** IDPs, attractors, and stable states. A key IDP is expressed in a stem cell that exhibits a high degree of conformational dynamics. The various conformations are shown in red to blue to green. If the initial conditions favor the red conformation more than the green or blue, the red conformation induces a specific protein interaction that leads to the differentiation of the stem cell (e.g., the red phenotype). In addition, the initial conditions favor the green conformation partially followed by the blue conformation. The green and blue conformations initiate a distinct interaction and give rise to the green and blue phenotypic state. The net result is a heterogeneous population with a mixture of phenotypes. This figure was made using the BioRender software.

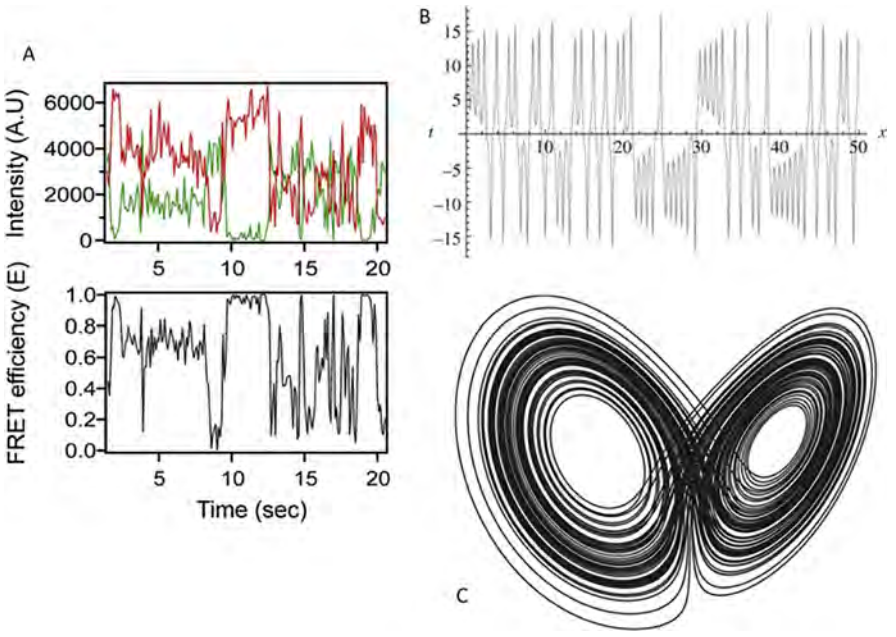


**Figure 5.** Similarity of the dynamic conformational behavior of an IDP with the behavior of a typical chaotic system (the Lorenz attractor). (A) Single-molecule FRET trajectory of the intrinsically disordered neuroligin cytoplasmic domain as a representation of conformational dynamics. The top plot depicts the time evolution of donor (green) and acceptor (red) intensities. The bottom plot shows the FRET efficiency over time. A.U. = arbitrary unit. (B) Dynamics trajectory of a chaotic system, i.e., the Lorenz attractor, which shows a qualitative resemblance with the conformational dynamics of an IDP. The Lorenz system is composed of three independent variables and coupled differential equations to describe their dynamics. Here, the time evolution of one of the independent variables is depicted. (C) Phase-space representation of one of the variables of the Lorenz system. The variable is plotted against its rate of change. The overlapping loops represent the attractor basins of the strange attractor, where the system does not converge to a single state nor diverge to an infinitely large space but rather hovers within a defined domain of the attractor basin. Reproduced with permission from ref 131. Copyright 2013 Elsevier.

fluctuates as the IDP adopts various conformations over time, leading to different FRET efficiencies.

How IDP dynamics can be modeled in a similar way using nonlinear differential equations to reproduce chaotic behavior (analogous to the Lorenz system) is currently unclear. However, we want to provide the readers with some possible avenues for the investigation in this direction. At first glance, the analogy between the two systems (weather and IDP) is not at all apparent. One is a model for fluid convection and atmospheric changes over time, while the other is about the changes in protein conformation over time. However, upon closer inspection, one can see that the two systems indeed have certain common aspects.


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Like the weather model, proteins are multidimensional systems involving numerous variables (e.g., the coordinates of thousands of protein atoms and the surrounding solvent and ions). Due to the frequent interatomic interactions, the dynamics of many of these variables are correlated, thus making dimensionality reduction feasible.[136] It is the coupled dynamics of correlated variables that makes it possible to represent complex systems like the weather using simpler mathematical models, such as the one devised by Lorenz and co-workers. Notably, the strange attractor was also recently discussed in the context of cellular networks (which also involve thousands of interacting variables) that drive diseases such as cancer.[137] Protein dynamics features resulting from methods like molecular dynamics can be approximated by linear models in reduced dimensions using approaches such as principal component analysis or time-lagged independent component analysis.[138,139] This raises several important questions that warrant further investigation. For example, can the IDP dynamics involving millions of atoms be expressed as a mathematical model in reduced dimensions, analogous to the system of ordinary differential equations (ODEs), that exemplifies the strange attractor? Could certain thresholds in the parameters of this model represent the transition from the nearly deterministic behavior of a structured protein to the chaotic behavior of an IDP?

It is likely that a mathematical model involving IDPs may not be feasible using only three independent variables that were invoked to create the Lorenz attractor. Instead of two attractors of the Lorenz system, most IDPs will likely transition among multiple attractor basins. Perhaps it may be possible to develop a mathematical system involving a greater number of dimensions. However, whether such a model is feasible for describing IDP dynamics is not presently known. We leave it to the enthusiastic researchers of this and the future generation to figure it out.

**Box 2**

A key property of dynamical systems, irrespective of the complexity, is that they can be expressed as a combination of deterministic and random elements. Indeed, Pinsker[140] surmised that most, if not all, dynamical systems are a fusion of a stochastic dynamical system and a deterministic one (the Pinsker conjecture). This idea was challenged by Ornstein.[254] However, Thouvenot suggested that Pinsker's conjecture may be valid if the deterministic component in Pinsker's original description had some residual randomness. This led to the weak Pinsker conjecture. Recently, the weak Pinsker conjecture was mathematically proven by Austin[141] on the foundation of the "stationary stochastic process" (where a dynamical system is described as a sequence of events that are individually random but with fixed probabilities). The uncertainty of each event in the process (i.e., it is decoupled from the past events) can be quantified by entropy, where zero entropy makes the system entirely deterministic. Austin's proof analyzes the self-clustering (deterministic) property of a dynamical system trajectory on a Hamming cube by discretizing it in space and time, simultaneously resolving the inherent randomness within each cluster. This shows that the deterministic component of the system is always associated with some randomness that varies depending on the system characteristics.

### 4.3. IDP Dynamics: A Combination of Deterministic and Random Components

It is well-known that some dynamical systems can be completely deterministic. Therefore, if one knows the system's position a priori, one can predict its position in the future. However, some dynamical systems can be completely random. In such cases, even if one knows the history of the system up to a certain point in time a priori, that information cannot precisely predict the behavior of the system in the future. Most dynamical systems, however, fall somewhere in between these

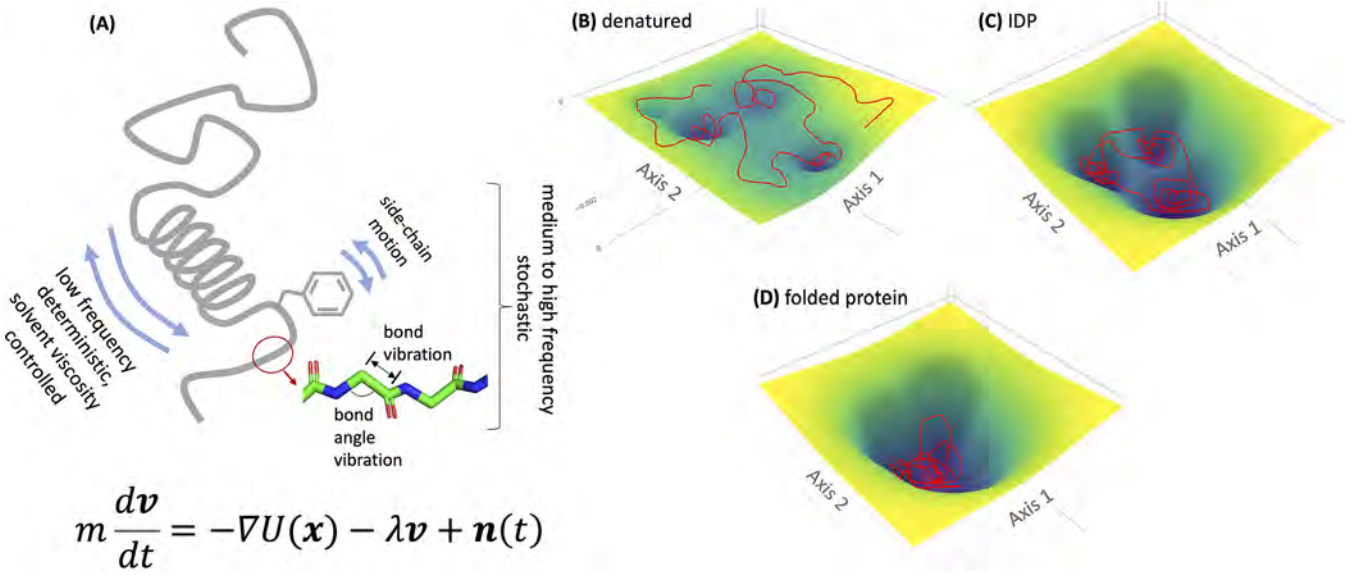


**Figure 6.** (A) Schematic of IDP with random coil and transient secondary structures. Zoomed-in view of the protein backbone is represented as sticks, with carbon (green), oxygen (red) and nitrogen (blue). The Langevin equation modeling the deterministic and stochastic components of protein dynamics is shown below. (B–D) Model attractor landscapes for denatured, disordered, and folded proteins, respectively. The protein conformational trajectories over time are shown as red scribbles.



https://doi.org/10.1021/acs.chemrev.1c00848
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two extremes, where some aspects of the dynamics can be described as deterministic and the rest can be described as stochastic. Recently, the weak Pinsker conjecture, which states that all ergodic stationary processes can be expressed as a combination of an almost deterministic component and a completely random component (Box 2), was proven by Austin.[140,141] Such endeavors, and the promising development of artificial intelligence in reproducing chaotic behavior,[142,143] have rekindled the interest in understanding complex systems by deconvolving their dynamics into elementary components.

Because IDPs are complex dynamical systems[97] that exist as flexible ensembles in their physiological state, a logical question is whether it is possible to understand the complex motion of IDPs in more systematic ways by elucidating the dynamics using statistical theories of dynamical systems. Protein motion, in general, is a combination of dynamics at multiple lengths and time scales ranging from bond vibrations in the picoseconds to domain motions (of ordered proteins) in the micro- to millisecond time scales.[144] This complex multiscale dynamics is a result of solvent viscosity that influences the slower motions of entire protein domains through friction, as well as thermal noise that affects the moderate to high-frequency components of protein dynamics, such as side-chain fluctuations and bond and angle vibrations. Consequently, all proteins exist as ensembles of conformations with frequent exchanges in real time. The difference between folded/ordered proteins and IDPs is that, for folded proteins/domains, this ensemble of conformations is tightly restricted to a small region of the conformational space, whereas in the case of IDPs, the conformational ensemble is highly diverse.

The ensemble of protein conformations can be visualized via their corresponding free-energy landscapes (Figure 6),[145] which is analogous to Waddington's epigenetic landscape originally proposed for studying cellular phenotypes.[126] The energy landscapes provide a mapping of possible states of the dynamical system, which, in the context of proteins, describe all possible conformations and their corresponding stabilities. While the free energy landscape of a structured protein typically shows a single deep attractor basin (also known as the folding funnel, Figure 6C), the energy landscape of an IDP can have multiple attractors within a large overall basin (Figure 6D). Therefore, the dynamics of an IDP can be envisaged as excursions to different attractors over time. Within each attractor well, the protein traverses a trajectory of closely related conformations before transitioning to a neighboring well. However, the overall IDP dynamics is bounded within a well-defined region of the conformational space (the wider basin in Figure 6D that includes the three attractors). In contrast, the free energy landscape of a completely unfolded (random coil-like) polypeptide can be thought of as relatively flat (Figure 6B). Of note, because of the enormous spatiotemporal heterogeneity, some IDPs can also be characterized by nearly flat free energy landscapes.

How can such complex dynamics be broken into elementary components, for example, nearly deterministic and stochastic components? Although we envision that more research will be required in this direction, we will simply conjecture here that the IDP excursions between different attractor wells can be described in terms of stochastic dynamics. Under steady state, the probability of finding the IDP in a given attractor well is governed by the nature of the energy landscape. However, at a sufficiently long time scale, the transitions between these wells can be considered to be completely random. In contrast, the motion of an IDP within each attractor well can be described as clusters of closely related trajectories and, therefore, nearly deterministic. As a concrete but simplistic example, a hybrid system consisting of deterministic and random components can be modeled using the Langevin equation,[146]

$$m\frac{\mathrm{d}v}{\mathrm{d}t} = -\nabla U(x) - \lambda v + n(t) \tag{1}$$

where $m$ is the mass of a protein particle (usually a coarse-grain cluster of protein atoms), $v$ is the velocity vector, $-\nabla U(x)$ is the force-field-dependent term that describes intra- and intermolecular forces (i.e., the gradient of potential energy $U$, which is a function of the protein atomic/coarse-grain particle coordinates, represented by $x$), the term $\lambda v$ represents the viscous force due to the solvent, and $n(t)$ is a noise term describing the stochastic component (Figure 6A). Models, such as those described by the Langevin equation and similar but more complicated realistic models, are suitable for modeling protein and polymer dynamics because they include both a deterministic component (i.e., $-\nabla U(x)$ and $\lambda v$) and a random component ($n(t)$). By varying the strength of the noise term, it is possible to adjust the contribution of the random component in such hybrid models. Inclusion of a protein conformation-dependent component ($-\nabla U(x)$) allows for excursions of the different attractor wells.

Such approaches in modeling IDP dynamics could have multiple applications. Deconvoluting the IDP dynamics into underlying components that are easier to conceptualize allows us to complement existing computationally intensive methods for predicting IDP dynamics (e.g., molecular dynamics) with novel approaches. Such approaches have practical applications in understanding how the IDPs alter their conformations in response to cellular environments and interact with other proteins as well as in designing therapeutics. One potential application is the deconvolution of the ensemble-average experimental properties of IDPs, such as NMR shifts or small-angle X-ray scattering intensities, to derive the underlying conformational ensemble. For example, the intermolecular contacts made by the IDPs can be encoded by a Hopfield network to model the free energy landscape, where each attractor is characterized by a different cluster of intermolecular contacts.[147] The resulting network can be trained using existing data to predict conformational ensembles in new cases, even with incomplete or noisy data. Such an approach has been applied in modeling Waddington's epigenetic landscape, where the inner architecture of gene regulatory networks (GRNs) could be derived by training against single-cell transcriptomics data.[148] Therefore, such approaches could also prove highly advantageous in modeling the IDP conformational landscapes. However, one should keep in mind that, as a complex dynamical system, IDPs are not easily subjected to the reductionist approach. In fact, as follows from the general understanding of such systems, their heterogeneous components are interconnected and interact nonlinearly, indicating that a perturbation does not necessarily cause a proportional effect and that the system's behavior is not equal to the sum of its parts. Clearly, further studies along these lines are needed to shed new light on the remarkable correlation between complexity and intrinsic disorder; they could also reveal why IDPs, as complex dynamical systems, occupy key nodes in PINs and, hence, were selected during evolution.


https://doi.org/10.1021/acs.chemrev.1c00848
Chem. Rev. 2022, 122, 6614−6633

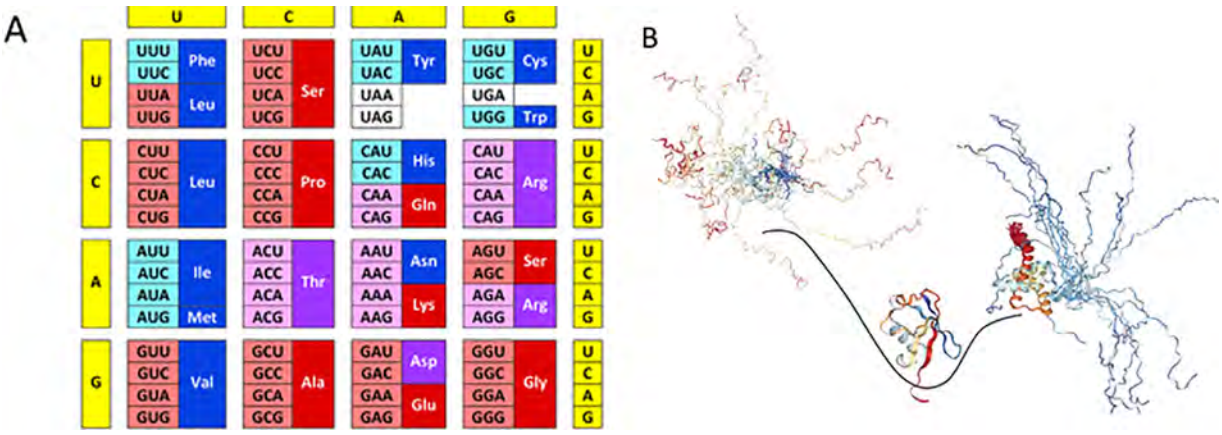


**Figure 7.** Intrinsic disorder and protein evolution. (A) Modern genetic code with information on the early and late codons (shown by light pink and light cyan colors, respectively) and disorder- and order-promoting residues (shown by dark red and dark blue colors, respectively). Codons with intermediate ages are shown by a light violet color. Disorder-neutral residues are shown by a dark violet color. (B) Evolution of intrinsic disorder in proteins with a characteristic wavy pattern. The *x*-axis represents evolutionary time, and the *y*-axis shows global disorder content in proteins at a given evolutionary time point. Here, primordial proteins are expected to be mostly disordered (left-hand side of the plot), proteins in LUA likely are mostly structured (center of the plot), whereas many proteins in eukaryotes are either totally disordered or represent hybrids containing both ordered and disordered regions (right-hand side of the plot). Reproduced with permission from ref 150. Copyright 2018 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

## 5. IDPS AND EVOLUTION

### 5.1. IDPs and Prebiotic Evolution

Starting from the prebiotic origins of life to the last universal common ancestor (LUCA), which gave rise to all living beings, several evolutionary innovations occurred that are thought to have been guided by self-organization.[149] Furthermore, it was postulated that short, unstructured peptides (possible precursors to IDPs) may have been the key components of a flexible cellular network with the capacity to readily evolve.[150] The inclusion of primitive IDPs into discrete membranous structures may have given rise to a self-sustaining unit that could replicate itself. Such a hypothetical entity is conceptually similar to the chemoton (or chemical automaton, a basic entity satisfying the sufficient, necessary conditions for sustaining life).[84,85] Thus, it is quite likely that IDPs had self-templating activity. In subsequent steps, interactions between IDPs and other biopolymers, through self-organization, may have formed an interaction network. Through many rounds of selection, such a structure may have given rise to the predecessor of LUCA.[151,152]

Consistent with their involvement in prebiotic evolution, IDPs across lineages are enriched in polar and charged amino acids but show a paucity in hydrophobic residues.[76,153−155] Furthermore, the amino acids are thought to be added to the genetic code in a temporal order: G/A, V/D, P, S, E/L, T, R, N, K, Q, I, C, H, F, M, Y, and W. Notably, the first few amino acids to emerge (until S in the above list) are disorder-inducing, while the later amino acids are order-promoting (e.g., Y, F, W, and C), suggesting that protein disorder played a critical evolutionary role in early life (Figure 7).[156,157] These observations were confirmed by Brooks et al., who estimated the distributions of different amino acids in the last universal ancestral genomes.[158]

It is generally held that the primordial IDPs in prebiotic evolution may not have catalytic activity.[159] However, it is equally unlikely that the transition from disordered primordial polypeptides to modern enzymes with highly ordered domains occurred in quick succession during evolution. Thus, it is possible that some primordial IDPs evolved limited catalytic functions. In fact, the Janus challenge[160] seeks to address this particular facet of IDPs. Indeed, it has been reported that some modern enzymes or their mutated variants behave as molten globular structures (i.e., structures with a loose tertiary topology but lacking intricate interresidue contacts).[161−164] It is also possible that primordial enzymes were more disordered than the molten globule state. In line with this argument, a ligase evolved in vitro lacked a hydrophobic core synonymous with structured enzymes; instead, it contained a flexible, catalytic loop that was supported by a polar core with zinc atoms.[165] Indeed, emerging evidence suggests that so-called classical enzymes may lack a unique structure and yet be functional.[163,164,166−172] Therefore, the idea that prototypical IDPs could have had catalytic activity seems plausible, and the chances that the Janus challenge will be met in the near future are high.

Yet another salient feature of the prototypic IDPs that may be of significant relevance to the origin of life is the potential for liquid−liquid phase transitions (LLPTs) or liquid−liquid phase separation (LLPS) or coacervation, resulting in protein-enriched liquid phases. Proteins, together with nucleic acids or by themselves, can phase-separate, resulting in proteinaceous membrane-less organelles (PMLOs). PMLOs exist as liquid droplets and are found in chloroplasts.[77,173−179] Recently, it was pointed out that LLPS serves as a fundamental mechanism of compartmentalizing intracellular space and biomembranes, thus enhancing the cellular adaptation to environmental variations.[180] Furthermore, it seems that LLPS can take place in 1D, 2D, and 3D, where the 3D condensation is associated with the PMLO biogenesis, 2D films may be formed near membrane surfaces, and 1D phase separation occurs on DNA and/or the cyto- and nucleoskeleton.[180] PMLOs serve many important biological functions such as protein degradation, phosphorylation, splicing, and transcriptional regulation.[79] Thus, it is conceivable that PMLO functionality is predetermined by the nature of its constituent IDPs.[79] Alternatively, it is also possible that the properties of PMLOs could enable the functions of their constituents.[79]


https://doi.org/10.1021/acs.chemrev.1c00848
Chem. Rev. 2022, 122, 6614−6633

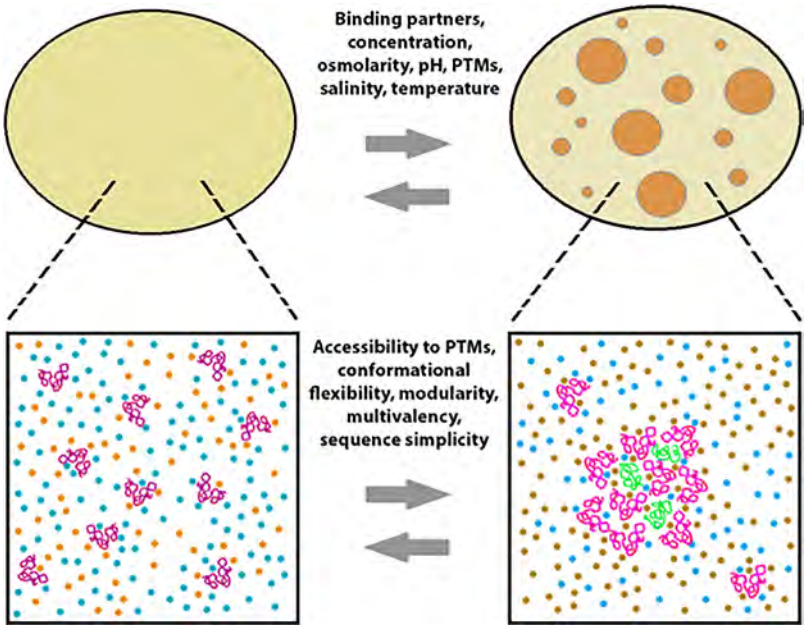


**Figure 8.** Thermodynamic factors (top) and disorder-related features controlling liquid−liquid phase transitions in protein solutions.

PMLOs may also act as microreactors that bring the RNA and protein molecules in close proximity to accelerate cytoplasmic reactions.[178,181,182] Thus, we speculate that primordial IDPs equipped with the ability to phase separate gave rise to protein-enriched phases[183] (Figure 8). This conjecture is consistent with the model proposed by Oparin in 1922.[184] Indeed, the demonstration of peptide-based synthons can form catalytically active coacervates, lending further credence to this hypothesis.[185] For a recent article on the conformational plasticity of IDPs from a physical chemistry standpoint, see a recent comprehensive review by Mukhopadhyay.[186]

Additionally, it was recently hypothesized that spaces between mica sheets may have been potential sites for the origin of life, as they may have provided shelters to the primitive PMLOs.[187] Taken together, these examples underscore the critical role of IDPs in life's origin and evolution on the earth,[188] which ultimately led to the evolution of multicellular forms and their expansion.

### 5.2. IDPs and Multicellularity

One of the key milestones in the timeline of evolution is the emergence of multicellularity. It is estimated that multicellularity has evolved multiple times independently in evolutionary distinct lineages from bacterial biofilms to plants and metazoans with the most ancient eukaryotic instances dating back to ∼1.6 billion years ago.[189] It is thought to be driven by the life history trade-offs between survival and reproduction in response to stress and/or predation. Adaptive changes, such as an increase in size, a division of labor, and an increase in complexity, enable the multicellular organisms to escape from predators and cope with environmental and nutritional stresses. Comparative and functional genomics data suggest that a recurring theme in the evolution of multicellularity is the cooption of ancestral pathways from unicellular organisms.[190−192] However, the key question is how were the ancestral pathways and genes adopted for the new functions of the multicellular organisms? The fact that IDPs, by virtue of their conformation flexibility and propensity for post-translational modification (e.g., phosphorylation), can rewire the PINs and several of the stress response proteins are IDPs, suggest that they can be a source of the adaptive plasticity required for bringing about this evolutionary transition.

Cell specification and specialization is thought to be facilitated by modification of the GRNs,[193] involving differential gene expression and cell signaling. TFs and signaling molecules are important components of the GRNs, and many of them are IDPs.[36,194−196] IDPs and hybrid proteins with ordered domains and IDPRs can regulate cell-specific transcription by forging promiscuous interactions with different partners via changes in conformations in the IDPs/IDPRs. In addition to the stochastic changes in conformations, the PTMs of IDPs can cause changes in their conformational ensembles, resulting in interactions with different partners. IDPs can also change protein interaction networks via the addition, deletion, or modification of binding sites by means of alternate splicing of pre-mRNA-coding IDPRs.[9,197,198] Transcriptional noise originating from the inherent stochasticity of gene expression can result in heterogeneity in isogenic cellular populations. Therefore, IDP overexpression in response to a specific extrinsic perturbation (e.g., stress) results in amplification of transcriptional noise and rewiring of PINs, resulting in modification of the GRNs to actuate phenotypic switching and facilitate adaptive evolution.[97,98]

Several proteins that are key to the development of innovations required for multicellularity, such as extracellular matrix expansion, cell adhesion, cell communication, cell-cycle modifications, asymmetric cell division, and cell differentiation, are IDPs. For example, cadherins, which play important roles in cell segregation and boundary formation during development, are IDPs[199] and were coopted from the unicellular progenitor of animals to function as adhesion receptors in epithelia. Similarly, retinoblastoma (RB) and retinoblastoma-related proteins (RBRs) that regulate the cell cycle are IDPs.[200] RBs and RBRs are transcriptional repressors that modulate cell-cycle-regulated gene expression through binding to E2F-DP transcription factors. The disordered linker domain


https://doi.org/10.1021/acs.chemrev.1c00848
Chem. Rev. 2022, 122, 6614−6633

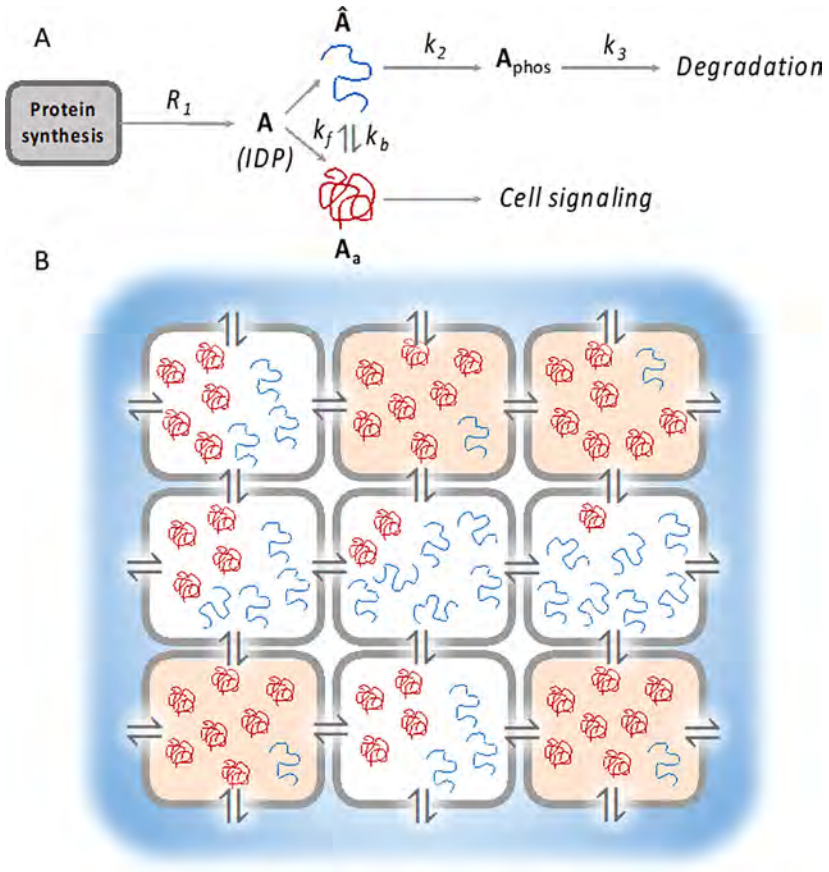


**Figure 9.** Model for IDP conformational noise contributing to multicellularity. (A) Conceptual reaction network where conformational noise leads to fluctuating levels of IDP conformation. In this model, the IDP *A* (where *A* also denotes its concentration in the cell) can assume two alternative conformations $A_a$ (denoted by the red string, which participates in cellular signaling) and $\hat{A}$ (blue string, which is phosphorylated and subsequently degraded). *A* stochastically switches between $A_a$ and $\hat{A}$, leading to fluctuating levels of the two conformations over time. (B) Schematic representing an array of cells with varying levels of the IDP *A*. High level of the functional conformation $A_a$ allows the cells to switch to a different phenotype, represented in orange. The level of $A_a$ is further modulated by the exchange of diffusible factors with neighboring cells and the environment (indicated by the ⇌ symbol). Such a system can give rise to spatiotemporal patterns and cooperative development of cellular clusters as a precursor to multicellularity.

of the RB-like protein, which is phosphorylated by cyclin-CDK,[201] is different between *Chlamydomonas*, a unicellular organism. and *Gonium* and *Volvox*, which are multicellular,[192] suggesting that different protein−protein interactions of RBs and their interacting partners regulate the cell cycle in these unicellular and multicellular organisms. HSP70, a chaperone protein that regulates asymmetric cell division in *Volvox*, is also an IDP,[202] and so is hydroxyproline-rich glycoprotein (HRGP), a constituent of the extracellular matrix (ECM), which is also highly disordered.[203] Disorder in the ECM interactome could provide the structural flexibility required for the ECM to interact with membrane proteins and soluble proteins in more complex organisms.

Interestingly, some HRGPs of both multicellular and unicellular algal forms have evolved into pheromones deployed for sexual signaling.[204−207] The HRGPs are also present in much greater numbers in *Volvox* than in unicellular algae. Thus, it is likely that during evolution intrinsically disordered ECM/cell wall proteins diversified to adapt to developmental processes. Therefore, these proteins may represent a source of adaptive plasticity that is specifically observed in the volvocine algae.[190] The increase in the disordered residues in proteomes from bacteria to single-celled and multicellular eukaryotes lends further credence to the involvement of IDPs in the origin of multicellularity.[19,208−210]

For unicellular organisms to exist as stable multicellular clusters, the individual cells need to adapt to changing environments and cooperate with neighboring cells. We envision that IDPs, due to their unique structural and dynamic properties, facilitated the necessary phenotypic plasticity and adaptation that were critical to the origin of multicellularity. It is well-known that IDPs can couple with multiple binding partners, and these promiscuous interactions could have been advantageous in PIN rewiring and switching of phenotypes. Moreover, IDPs alternate between multiple structures in a stochastic manner, as evidenced from the time-resolved Förster resonance energy transfer (FRET) measurements.[211] This conformational noise coupled with their interaction with multiple signaling proteins could produce the phenotypic diversity that enabled the multicellular clusters to exist.

While a sophisticated model connecting IDP conformational noise with PIN rewiring is beyond the scope of this Review, we present a simple conceptual framework elucidating the role of IDPs, where conformational noise acts as intrinsic noise in the cellular network and phenotypic transitions are controlled by the level of disorder in the cell (Figure 9A). Consider that within the cell the IDP *A* is expressed at low levels (at any


https://doi.org/10.1021/acs.chemrev.1c00848
Chem. Rev. 2022, 122, 6614−6633

time, the expression of $A$ is denoted by $A$ as well) and is produced at a rate $R_1$. For simplicity, we will assume $R_1$ to be constant. We also propose that $A$ stochastically switches between two alternative conformations $A_a$ and $\hat{A}$ (this is a simplified description because IDPs typically will adopt multiple conformational states rather than just two). While the conformation $A_a$ couples with other proteins to carry out cell signaling and phenotypic transition, the conformation $\hat{A}$ gets phosphorylated and degraded. This model bears similarity to the PAGE4 circuit described previously, where the IDP PAGE4 is degraded upon hyperphosphorylation.[212] Due to the structural flexibility of IDPs, the level of $\hat{A}$ in the cell will fluctuate with time, and these fluctuations will be more prominent at low protein concentrations, which is typical for many IDPs in the cell.[213] The time-averaged $\hat{A}$ concentration will depend on the depth of the attractor well-representing $\hat{A}$ in the IDP-free energy landscape. Under these assumptions, the temporal rate of change of the IDP concentration can be expressed as

$$\frac{dA}{dt} = R_1 - k_2\hat{A} \tag{2}$$

$$\frac{dA_{phos}}{dt} = k_2\hat{A} - k_3A_{phos} \tag{3}$$

Here, $k_2$ is the rate constant for converting $\hat{A}$ to $A_{phos}$, the phosphorylated form of $A$, by a kinase. In general, such enzymatic reactions will follow Michaelis−Menten kinetics,[214] but at low substrate concentrations, the reaction rate can be approximated to be linearly proportional to the substrate concentration. Also, we model the degradation rate as linearly proportional to the level of phosphorylated $A$ with a rate constant $k_3$. Because $\hat{A}$ fluctuates due to intrinsic, conformational noise, the discussed equations are stochastic in nature.

Coupled differential equations have been widely used in the literature to model transcriptional regulation and catalytic events such as phosphorylation involving multiple partners, potentially in a feedback loop. Equation 3 demonstrates how such models can be extended to incorporate the stochastic effect of IDP conformational fluctuations that may lead to distinct cellular states. Figure 9 demonstrates how IDP conformational fluctuations in conjunction with environmental perturbations may stabilize distinct phenotypes in a multicellular environment. We suggest that single-cell models exemplified by eq 3 can be coupled together to numerically simulate such multicellular systems and analyze the emergence of stable phenotypes. Quantitative demonstration of such models is an exciting idea, but unfortunately it is beyond the scope of this Review. One of the overarching goals of this Review is to stimulate exploration in this direction by aspiring scientists.

It would be an interesting exercise to extend this model further by imagining a cluster of cells, with each exhibiting a similar set of reactions. We can also introduce additional terms in the rate equations to represent the influence of soluble factors exchanged with neighboring cells or the effect of the environment. Such a model is schematically depicted in Figure 9B. It remains to be seen whether the solution to the discussed system of differential equations can yield multiple stable states, where each stable state is represented by a different proportion of $A_a$ to $\hat{A}$. Cells that achieve a stable state with an $A_a$ level beyond a certain threshold may undergo transition to a different phenotype (Figure 9B). Additionally, conformational noise can make these phenotypic transitions more feasible. The discussed model can be easily extended to incorporate multiple functional conformations instead of just two, which may lead to increased phenotypic heterogeneity. We may envision, therefore, that increased disorder may lead to more stable states in the phenotypic landscape. Thus, in a multicellular system such as the one described earlier, IDP conformational noise, in conjunction with a variable environment, can enable the cells to adopt different phenotypes. Such heterogeneity superposed with intercellular signaling may lead to interesting spatiotemporal patterns or cooperative phenotype development in an organized community of cells.

### 5.3. IDPs and Inheritance of Acquired Characteristics

Yet another discovery that is of immense fundamental importance but appears to challenge the central dogma of molecular biology[215] is the facilitation of transgenerational information transfer by IDPs. Emerging evidence[216] that prions (proteins that are capable of self-replication by inducing prion-like conformations in other proteins), apart from nucleic acids, can drive organismal phenotypes by self-templating conformations and thereby serve as vehicles of inheritance. Consistent with this observation, it was recently reported that in the yeast several IDPs resemble prions from a functional perspective.[68] Indeed, the transient overexpression of several of these proteins yielded heritable traits even after their expressions returned to normal levels. Most intriguingly, these proteins were not identified as prions previously, and they did not form amyloids. However, they contained hallmarks of nucleic acid-binding proteins with long IDPRs and were evolutionarily conserved.[68] Taken together, the data established a general form of protein-based inheritance wherein IDPs gave rise to new traits and adaptive opportunities.

At least one of these prion-like proteins was reported to drive self-assembly, giving rise to gel-like condensates.[217] Nonetheless, such protein-rich particles did not form amyloid fibrils but were infectious, apparently using a protein-based epigenetic element. Cells expressing these proteins were found to repress gene expression patterns in order to facilitate improved growth when the nutrient supply was limited. Thus, these nonamyloid proteins appeared to modulate a form of nonchromosomal epigenetics to modulate the expression of genes that are heritable over significantly long biological time scales. Because IDPs undergo LLPTs or coacervation that gives rise to PMLOs,[79,174] it may be expected that the prion-like particles self-assemble into gel-like condensates. In line with the observations in yeast, the IDP from *C. elegans*, called PGL-1, was observed to form aggregate-like structures in the germ cells. Amazingly enough, such aggregates were found to be inherited for multiple generations even after the original mutation triggering their formation was not observed any more. Therefore, these observations suggest that, even in animals, IDPs generate self-propagating aggregates that may serve as vehicles for transgenerational inheritance.[255]

## 6. CONCLUSIONS AND FUTURE DIRECTIONS

Despite several excellent reviews on IDPs that appeared in just this past year alone that cover many interesting aspects of these multifaceted proteins with pleotropic functions,[218−232] very little is known about the IDPs from a dynamical systems perspective.[43,97,125,131,233,234] However, from the foregoing, it follows that a rigorous theoretical foundation addressing how


https://doi.org/10.1021/acs.chemrev.1c00848
Chem. Rev. 2022, 122, 6614−6633

interactions between an organism and its environment can shape its phenotype,[125,235] as well as how IDPs may modulate the attractor landscape in driving the phenotypic states[131] (i.e., guide cellular decisions), warrants further investigations. Although the idea that a cell (or a protist) is capable of making decisions is often met with skepticism, and perhaps may even be bantered, several tantalizing reports suggest that cells can anticipate, and even learn from, environmental fluctuations and survival hurdles.[236−243] Together with the demonstration that noise plays an important role in cellular decision making,[244] these observations should inspire new research in this next frontier. The wetware (i.e., the combined hardware and software in biology) metaphor should help invigorate scientists from across disciplines to study these fascinating molecules that are critical yet poorly understood.

From an experimental perspective, elucidating the structure and dynamics of an IDP ensemble, as well as how the preferences are shaped by the environment, may help to better understand the mechanism(s) by which IDPs interact with their partners to carry out their respective functions. With the recognition of the IDPs and the critical roles they perform, a new frontier in the biology, biochemistry, and biophysics of proteins has dawned. Furthermore, recent developments in targeting IDPs that were previously marginalized as undrug-gable", with small-molecule inhibitors/activators,[71,245−252] have ushered in a new era in biomedical research.

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◆P.K. and S.B. contributed equally to this work. P.K. and V.U. conceived the idea and designed the study outline.

### Notes

The authors declare no competing financial interest.

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Supriyo Bhattacharya, Ph.D., is a Research Assistant Professor at City of Hope. After obtaining his B.Tech. degree from the Indian Institute of Technology, Kharagpur, he did his Ph.D. in Chemical Engineering from North Carolina State University and completed his postdoctoral training in computational biophysics at City of Hope. He has more than 15 years of experience in computational science, statistics, algorithms development, data visualization, statistical analysis of biological data using linear regression, multivariate analysis, innovative clustering, and stochastic methods. His research interests are diverse, ranging from protein dynamics to evolutionary theories. One of his major contributions was in the development of algorithms to analyze allosteric communication pipelines in proteins from molecular dynamics simulations.

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https://doi.org/10.1021/acs.chemrev.1c00848
Chem. Rev. 2022, 122, 6614−6633

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Atish Mohanty, Ph.D., is a Research Assistant Professor at City of Hope. He obtained his Ph.D. in molecular cell biology from the University of Tokyo and completed his postdoctoral training in oncology at City of Hope. His main research interests are to understand the molecular mechanism of drug resistance and identify key targets that can suppress the evolution of resistant clones. He identified integrin $\beta 4$ and paxillin as not only being required for adhesion-associated signaling but also being essential for platinum resistance. His studies revealed that the disordered region in the cytoplasmic tail of integrin $\beta 4$ drives the interaction with paxillin, for which the amino terminus is intrinsically disordered.

Govindan Rangarajan, Ph.D., is Professor of Applied Mathematics, JC Bose National Fellow, and Director, Indian Institute of Science, Bangalore. He obtained an Integrated M.Sc. (Hons) degree from the Birla Institute of Technology and Science, Pilani, and a Ph.D. in physics from the University of Maryland. He completed his postdoctoral training at the Lawrence Berkeley Lab, University of California, Berkeley. Prof. Rangarajan's research interests include nonlinear dynamics and chaos and time series analysis, as well as mathematical biology. He is currently the Director of the Indo-French Centre for Applied Mathematics (IFCAM). He is also a Fellow of the Indian Academy of Sciences and the National Academy of Sciences, India. He was awarded the Chevalier dans l'Ordre des Palmes Academiques by the Government of France and is a recipient of the Homi Bhabha Fellowship, Government of India.

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## ACKNOWLEDGMENTS

S.B. is part of Integrative Genomics Core at City of Hope, which is supported by the National Cancer Institute of the National Institutes of Health (Bethesda, MD) under grant number P30CA033572. M.K.J. was supported by Ramanujan Fellowship (SB/S2/RJN-049/2018) awarded by SERB (Science and Engineering Research Board), DST (Department of Science & Technology), and Government of India.

## ABBREVIATIONS

| | |
|---|---|
| ECM | extracellular matrix |
| EMT | epithelial−mesenchymal transition |
| FRET | Förster resonance energy transfer |
| GRNs | gene regulatory networks |
| HRGP | hydroxyproline-rich glycoprotein |
| IDPs | intrinsically disordered proteins |
| IDPRs | intrinsically disordered protein regions |
| iPSCs | induced pluripotent stem cells |
| LLPTs | liquid−liquid phase transitions |
| LLPS | liquid−liquid phase separation |
| LUCA | last universal common ancestor |
| MET | mesenchymal−epithelial transition |
| MRK | Mahmoudabadi, Rangarajan, Kulkarni hypothesis |
| ODEs | ordinary differential equations |
| PAGE4 | prostate-associated gene 4 |
| PINs | protein interaction networks |
| PMLOs | proteinaceous membrane-less organelles |
| PTMs | post-translational modifications |
| RB | retinoblastoma |
| RBRs | retinoblastoma-related proteins |
| TFs | transcription factors |

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