Intrinsically disordered proteins: Ensembles at the limits of Anfinsen's dogma [F] [SCI]

Prakash Kulkarni, Vitor B. P. Leite, Susmita Roy, et al.

COLLECTIONS

[F] This paper was selected as Featured

[SCI] This paper was selected as Scilight

View Online   Export Citation   CrossMark

Intrinsically disordered proteins: Ensembles at the limits of Anfinsen's dogma

Prakash Kulkarni,[1,a] Vitor B. P. Leite,[2] Susmita Roy,[3] Supriyo Bhattacharyya,[4] Atish Mohanty,[1] Srisairam Achuthan,[5] Divyoj Singh,[6] Rajeswari Appadurai,[7] Govindan Rangarajan,[8] Keith Weninger,[9] John Orban,[10,11] Anand Srivastava,[7] Mohit Kumar Jolly,[6] Jose N. Onuchic,[12] Vladimir N. Uversky,[13,14] and Ravi Salgia[1]

AFFILIATIONS

[1]Department of Medical Oncology and Therapeutics Research, City of Hope National Medical Center, Duarte, California 91010, USA

[2]Departamento de Física, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista (UNESP), São José do Rio Preto, São Paulo 15054-000, Brazil

[3]Department of Chemical Sciences, Indian Institute of Science Education and Research Kolkata, Mohanpur, West Bengal 741246, India

[4]Translational Bioinformatics, Center for Informatics, Department of Computational and Quantitative Medicine, City of Hope National Medical Center, Duarte, California 91010, USA

[5]Center for Informatics, Division of Research Informatics, City of Hope National Medical Center, Duarte, California 91010, USA

[6]Center for BioSystems Science and Engineering, Indian Institute of Science, Bangalore 560012, India

[7]Molecular Biophysics Unit, Indian Institute of Science, Bangalore, Karnataka, India

[8]Department of Mathematics, Indian Institute of Science, Bangalore 560012, India

[9]Department of Physics, North Carolina State University, Raleigh, North Carolina 27695, USA

[10]W. M. Keck Laboratory for Structural Biology, University of Maryland Institute for Bioscience and Biotechnology Research, Rockville, Maryland 20850, USA

[11]Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742, USA

[12]Center for Theoretical Biological Physics, Rice University, Houston, Texas 77005-1892, USA

[13]Department of Molecular Medicine, College of Medicine, Byrd Alzheimer's Institute, University of South Florida, Tampa, Florida 33620, USA

[14]Protein Research Group, Institute for Biological Instrumentation of the Russian Academy of Sciences, Pushchino, Russia

[a]Author to whom correspondence should be addressed: pkulkarni@coh.org

ABSTRACT

Intrinsically disordered proteins (IDPs) are proteins that lack rigid 3D structure. Hence, they are often misconceived to present a challenge to Anfinsen's dogma. However, IDPs exist as ensembles that sample a quasi-continuum of rapidly interconverting conformations and, as such, may represent proteins at the extreme limit of the Anfinsen postulate. IDPs play important biological roles and are key components of the cellular protein interaction network (PIN). Many IDPs can interconvert between disordered and ordered states as they bind to appropriate partners. Conformational dynamics of IDPs contribute to conformational noise in the cell. Thus, the dysregulation of IDPs contributes to increased noise and "promiscuous" interactions. This leads to PIN rewiring to output an appropriate response underscoring the critical role of IDPs in cellular decision making. Nonetheless, IDPs are not easily tractable experimentally. Furthermore, in the absence of a reference conformation, discerning the energy landscape representation of the weakly funneled IDPs in terms of reaction coordinates is challenging. To understand conformational dynamics in real time and decipher how IDPs recognize multiple binding partners with high specificity, several sophisticated knowledge-based and physics-based in silico sampling techniques have been developed. Here, using specific examples, we highlight recent advances in energy landscape visualization and molecular dynamics simulations to discern conformational dynamics and discuss how the conformational preferences of IDPs modulate their function, especially in phenotypic switching. Finally, we discuss recent progress in identifying small molecules targeting IDPs underscoring the potential therapeutic value of IDPs. Understanding structure and

function of IDPs can not only provide new insight on cellular decision making but may also help to refine and extend Anfinsen's structure/function paradigm.

TABLE OF CONTENTS

I. INTRODUCTION

For well over half a century, Anfinsen's dogma enunciated that a protein sequence/function paradigm constituted the foundation of our understanding of the protein universe. Anfinsen postulated that under favorable conditions, a protein will fold consistently into a native state structure, that is, effectively encoded in its amino acid sequence. The contextual nature (*under favorable conditions*) was implicit in the proclamation, "*the native conformation is determined by the totality of interatomic interactions and hence by the amino acid sequence, in a given environment*" (Anfinsen *et al.,* 1961 and Anfinsen, 1973). Per this view, proteins have unique tertiary structures characterized by the fixed positions of their atoms and backbone dihedral angles that vary slightly around their equilibrium positions because of the low-amplitude thermal fluctuations.

However, the discovery that a significant portion of the proteome in all domains of life and all viral proteomes examined comprise intrinsically disordered proteins (IDPs) (or regions within ordered proteins, referred to as intrinsically disordered regions or IDRs) that lack rigid structure in the native state has drastically changed our perception of proteins (Ward *et al.,* 2004; Uversky, 2010; Schad *et al.,* 2011; Dyson, 2011; Xue *et al.,* 2012; Pancsa and Tompa, 2012; Midic and Obradovic, 2012; Korneta and Bujnicki, 2012; Hegyi and Tompa, 2012; Di Domenico *et al.,* 2013; van der Lee *et al.,* 2014; and Peng *et al.,* 2015). Furthermore, numerous computational studies have also revealed that the proportion of disorder increases with organism complexity (Dunker *et al.,* 2001; Ward *et al.,* 2004; Uversky, 2010; and Xue *et al.,* 2012). Thus, while the fraction of sequences predicted to have long IDPRs ($\geq$30 residues) is approximately equal in bacteria and archaea, it is significantly higher in eukaryotes (Dunker *et al.,* 2000; Ward *et al.,* 2004; Xue *et al.,* 2010; Xue *et al.,* 2012; Na *et al.,* 2013; and Peng *et al.,* 2015).

Despite the ubiquitous presence and evolutionary conservation, the IDP field was met with skepticism in the early years. In fact, the then unique, perhaps, tantalizing, observation that some proteins lacked ordered structures in isolation was considered as a mere artifact. On the contrary, it was tacitly assumed that, in the crowded cellular environment, such proteins would assume the native state. Thus, disorder was hardly considered as being important in orchestrating several of the molecular events in cell and developmental biology (Uversky and Dunker, 2010; Dyson and Wright, 2019; Fusco and Gianni, 2021; and for an interesting historical perspective, see Uversky and Kulkarni, 2021). Furthermore, because of the "apparent" lack of structure, IDPs are often misinterpreted by some as posing a challenge to Anfinsen's dogma (Potenza *et al.,* 2015; Das *et al.,* 2018; and Baul *et al.,* 2019). However, contrary to this view, IDPs abide by the Anfinsen's postulate albeit at its extreme limits (Vila, 2020 and Kulkarni, 2021). Therefore, despite lacking a single, well-defined equilibrium structure, IDPs exist as heterogeneous ensembles whose conformational properties do not abide by a single set of coordinates or backbone Ramachandran angles (Dunker *et al.,* 2013).

A. Order and disorder represent a structural and dynamic continuum rather than binary states

The description of proteins as ordered (folded) or disordered (unfolded) is predicated on their conformational ensembles. Ordered proteins tend to have thermally accessible states that resemble the ensemble average; however, disordered proteins sample an ensemble of dissimilar conformations during their biological lifetime (Wright and Dyson, 1999). Thus, while the native state of an ordered protein corresponds to a global energy minimum, that is, distinct from a large number of high energy states, disordered proteins display energy surfaces that contain multiple local energy minima that are separated by

low energy barriers. This helps ensure the rapid exchange between dissimilar states during the lifetime of the protein (Csermely et al., 2010; Jensen et al., 2014; Burger et al., 2016; Schneider et al., 2019; and Adamski et al., 2019). Furthermore, the disorder spectrum covers a range of different entities from almost completely disordered and molten globules, to folded domains connected by disordered linkers and folded proteins flanked by disordered tails (Dyson and Wright, 2005; Uversky, 2013a; and van der Lee et al., 2014). Therefore, although order and disorder are typically thought of as binary states, they form a continuum (DeForte and Uversky, 2016). Thus, in contrast to the lock-and-key analogy that represents a protein as highly ordered molecule, in reality, a protein molecule represents a complex system with remarkable spatiotemporal heterogeneity. Thus, a protein is likely to embody fragments with different structural complexities and folding complicities, such as foldons, inducible foldons, morphing inducible foldons, semi-foldons, and non-foldons (Uversky, 2013b; Uversky, 2016a; Uversky, 2016b; and Uversky, 2019b). This spatiotemporal heterogeneity of IDPs/IDRs is manifested in their multifunctionality with different (dis)ordered regions engaging in different functions (Uversky, 2015 and Uversky, 2016a). It also defines a structure-function continuum concept (Uversky, 2016b; Uversky, 2016c; Uversky, 2019a; and Uversky, 2019b); instead of the "one gene–one protein–one structure–one function" model, a protein molecule is a highly dynamic conformational ensemble with remarkable multifunctionality and binding promiscuity (Kulkarni et al., 2018; Fonin et al., 2019; Uversky, 2016b; and Uversky, 2019b).

The continuum also includes the specificity of interaction. This may explain the larger interactomes of the IDPs that are enabled by their flexibility and propensity to engage in promiscuous interactions. These properties allow IDPs to explore novel mechanisms such as facilitated exchange through trimer formation and ultra-sensitivity via threshold effects, and ensemble redistribution (Teilum et al., 2021). Indeed, emerging evidence indicates that, like their ordered counterparts that exhibit allostery wherein the binding of the ligand stabilizes specific states and shifts the conformational ensemble (Kern and Zuiderweg, 2003; Gunasekaran et al., 2004; and Tsai and Nussinov, 2014), IDPs can also exhibit allosteric effects (Garcia-Pino et al., 2010; Motlagh et al., 2012; Tompa, 2012; Ferreon et al., 2013; Motlagh et al., 2014; Krishnan et al., 2014; Choi et al., 2015; Garcia-Pino et al., 2016; Zhang et al., 2018; Li et al., 2018; Berlow et al., 2018; and Rehman et al., 2019). The glucocorticoid receptor that contains a large IDR characteristic of the hormone receptor family of proteins further illuminates the degree of sophistication adopted by IDPs. In this case, genetically tunable "energetic frustration" controls allostery. Here, energetic frustration is defined the activity of the protein results from the combination of the opposing interactions, while the term genetically tunable refers to the splice variants with varying degrees of intrinsic disorder. Furthermore, the disordered regions can have opposing effects on other regions of the protein. Thus, energetic frustration can be envisaged as a "tug-of-war" whereby protein activity is predicated on a combination of the opposing interactions (Li et al., 2017).

B. Structural plasticity enhances functionality

Because of the enormous conformational plasticity, IDPs occupy key nodal (hub) positions in cellular protein interaction networks (PINs) (Dunker et al., 2005; Haynes et al., 2006; Gsponer and Babu, 2009; Patil et al., 2010; and Hu et al., 2017). PINs that adopt a scale-free architecture serve as a conduit for channeling information flow within the cell (Barabasi and Albert, 1999; Barabási and Oltvai, 2004; and Barabási, 2009). However, because IDPs engage in promiscuous interactions when overexpressed (Vavouri et al., 2009), they can also rewire PINs affording the system a robust degree of plasticity (Buljan et al., 2013). It is, therefore, not surprising that the organization and properties of the PINs appear to be evolutionarily conserved (Rangarajan et al., 2015).

In addition to channeling information, IDPs play important roles in numerous biological processes, including transcriptional regulation, splicing, signaling and development, and differentiation (Uversky et al., 2008; Wright and Dyson, 1999; Dunker et al., 2002; Uversky and Dunker, 2010; Xue et al., 2012; Oldfield and Dunker, 2014; Uversky, 2015; Wright and Dyson, 2015; Berlow et al., 2015; Fung et al., 2018; Deiana et al., 2019; and Csermely et al., 2020). The physical characteristics of IDPs such as interactions characterized by high specificity but low affinity, and kinetic advantages in signaling, allow for an exquisite level of control of cellular signaling processes (Pontius, 1993; Wright and Dyson, 1999; Dyson and Wright, 2005; Oldfield et al., 2005; and Wright and Dyson, 2015). Post-translational modifications (PTMs), for example, phosphorylation, further fine-tune the functions of IDPs to behave as sophisticated and sensitive switches and rheostats in the regulatory circuits they modulate (Dyson and Wright, 2005; Gsponer and Babu, 2009; Lee et al., 2010; Van Roey et al., 2012; and Van Roey et al., 2013).

In addition, IDPs play important roles in many cellular processes such as regulation of the cell division cycle (Galea et al., 2008; Yoon et al., 2012; Mitrea et al., 2012; Buske et al., 2015; and Tsytlonok et al., 2019), circadian rhythmicity (Hurley et al., 2013; Dong et al., 2016; Pelham et al., 2018; Pelham et al., 2020; and Diernfellner and Brunner, 2020), stress response (Boothby et al., 2017), and phenotypic plasticity (Mooney et al., 2016; Jia et al., 2017; and Kulkarni et al., 2020). Furthermore, several IDPs are reported to prion-like functions to actuate protein-based molecular memories underlying the emergence and inheritance of biological traits (Chakrabortee et al., 2016), underscoring their importance in phenotype switching. Indeed, based on these observations, IDPs have also been implicated as playing a role in multicellularity, a cornerstone in major evolutionary transitions (Kulkarni and Uversky, 2018a and Kulkarni, 2021). Moreover, when dysregulated, IDPs can also cause pathological states (Iakoucheva et al., 2002; Uversky et al., 2008; Uversky, 2014; and Uversky et al., 2014) (Vavouri et al., 2009 and Marcotte and Tschansky, 2009). Consistent with these observations, IDPs are dysregulated in several chronic human diseases, including cancer, diabetes, neurodegenerative diseases, and several genetic diseases (Uversky et al., 2008; Babu et al., 2011; Uversky, 2014; Kulkarni and Uversky, 2019; Santofimia-Castaño et al., 2020; Brocca et al., 2020; Midic et al., 2009; and Uversky et al., 2009). It is, therefore, not surprising that cellular IDP levels are tightly regulated from synthesis to degradation (Gsponer et al., 2008 and Edwards et al., 2009).

Paradoxically, however, some IDPs are important in protein folding (Lermyte, 2020). For example, several stress-response proteins and chaperone proteins are IDPs (Tompa and Kovacs, 2010; Uversky, 2011; and Webster et al., 2019). Consistent with a chaperone function, deleting a disordered 23-residue C-terminal portion of GroEL that faces the central cavity of the bacterial GroEL-GroES complex in which folding occurs compromises chaperone function (Machida et al., 2008). In tardigrades that can endure extreme conditions, IDPs

are found to respond to these challenges (Janis *et al.*, 2018 and Hesgrove and Boothby, 2020). Similarly, several stress response proteins in plants (Covarrubias *et al.*, 2017; Balcerowicz, 2020; and Rae *et al.*, 2014) and proteins that mediate plant immune responses to pathogens are IDPs (Sun *et al.*, 2014). Furthermore, IDPs play a role in regulation of plant growth (Sun *et al.*, 2010), development, and signaling, often by integrating signals from multiple plant growth regulatory inputs (Sun *et al.*, 2011 and Sun *et al.*, 2013). Similarly, in the human, IDRs are found in the small heat shock proteins Hsp22 and αB-crystallin (Kazakov *et al.*, 2009 and Sudnitsyna *et al.*, 2012), highlighting the link between stress and IDPs both in plants and animals.

C. Mechanisms underlying IDP interactions

In light of the incredible functional repertoire of the IDPs, understanding how they interact with partners in spite of (the perceived) lack of structure is of significant interest. It is now evident that some IDPs can undergo transitions from disorder to order upon binding to their cognate targets, a phenomenon referred to as "coupled folding and binding" (Dyson and Wright, 2002; Oldfield *et al.*, 2005; Mohan *et al.*, 2006; Cheng *et al.*, 2007; Vacic *et al.*, 2007; and Oldfield *et al.*, 2008). Two models have been advanced supporting this concept. While the "induced fit" mechanism postulates that folding occurs after association of the IDP with the target, the "conformational selection" mechanism suggests that all potential conformations of the ensemble exist *a priori* and the ligand then "selects" the most favored prefolded state from this preexisting pool (Boehr *et al.*, 2009). However, in studies on the interaction between pKID/KIX and KIX/Myb (Arai *et al.*, 2015) and the C-terminal domain (CTD) of the measles virus nucleoprotein (Wang *et al.*, 2013), some combination of both these mechanisms may also be applicable, suggesting that the exact binding mechanism is determined by the intrinsic secondary structure propensities of the IDPs (Wright and Dyson, 2015).

Coupled folding and binding is a complex process involving at least two steps—binding to the partner and folding of the IDP (Wright and Dyson, 1999; Uversky, 2002; Wright and Dyson, 2005; Tompa and Fuxreiter, 2008; Tompa, 2011; Kiefhaber *et al.*, 2012; Habchi *et al.*, 2014; and Gianni *et al.*, 2016). However, it is important to recognize that mechanistically, there are distinct differences between the classical spontaneous folding of globular proteins and binding-induced folding of IDPs. More specifically, while globular proteins fold via a robust mechanism consolidated by the presence of a loosely formed yet specific nucleus (Fersht, 1995 and Itzhaki *et al.*, 1995), IDPs appear to fold by heterogeneous nucleation via an overall mechanism, that is, induced by interaction with the partner (Rogers *et al.*, 2014a; Rogers *et al.*, 2014b; Toto and Gianni, 2016; Toto *et al.*, 2016; and Bonetti *et al.*, 2018). A recent study on protein folding employing molecular dynamics simulations with all-atom force fields, with folding pathways interpreted in terms of soliton structures, examined the presence of systematic dynamical patterns of self-organization that may govern protein folding. Simulations were performed on the conformational transformations of three different proteins, namely, the ordered region of the oncoprotein MYC, amylin, and indolicidin (IDPs with different length and binding dynamics). Interestingly, the authors observed the emergence of soliton-mediated secondary motifs only in the case of IDPs suggesting that, indeed, the folding mechanisms in IDP folds are different, and that soliton-like quasi-ordered conformations may serve

as an important intermediate stage in this process (Ilieva *et al.*, 2016). Consistent with this observation, a previous study (Austin *et al.*, 2009) on the protein myoglobin showed that there is no long-lived Davydov soliton, at least in this highly ordered protein. Similarly, a theoretical study on intrinsic localized modes (ILMs), which are members of the large soliton family (Nicolaï *et al.*, 2015), found that the probability of ILMs playing a significant functional role in the flexible regions of the proteins and in proteins in a non-native state is significantly higher than in folded proteins/regions lending further credence to the idea that soliton-mediated structural events may be prevalent in IDPs. Furthermore, in model systems, it was demonstrated that structural disorder facilitates transmission of solitons (Kartashov *et al.*, 2011).

Thus, it follows that the mechanisms of disorder-to-order induced folding in IDPs could be intrinsically different from the mechanisms seen in globular proteins. However, there are important similarities. For example, the cooperative nature of the reaction underlying disorder-to-order transitions in IDPs is comparable to that of ordered proteins. However, their folding pathways are strikingly more malleable because of the heterogeneous nature inherent in their folding nuclei (Toto *et al.*, 2020). Furthermore, the timescale that governs the conformational dynamics is an important factor in the binding mode for IDPs (Choi *et al.*, 2019). In the induced fit model, rapid conformational dynamics play an important role. In many cases, the energy from order transitions is coupled to the recognition event. On the other hand, the disorder persists even in the bound state in some IDPs (Borgia *et al.*, 2018 and Tsytlonok *et al.*, 2019). If the conformational dynamics are slow, then the binding mode is limited to conformational selection. In this case, interactions can only occur in the presence of the binding-competent configuration.

Another model is the "extended conformational selection," which is a repertoire of selection and adjustment processes (Csermely *et al.*, 2010). Here, the contribution of induced fit that constitutes a subset of this repertoire is affected by the bonds, which stabilize the interaction and the differences between the partners. Per this model, segments, or regions of the proteins with dynamics distinct from the rest of the molecule referred to as "discrete breathers," can impact conformational transitions and the propagation of allosteric signals that occur along with the binding processes.

Aside from the scenarios described above, some IDPs do not appear to assume any discernable structure even when bound to a cognate ligand. For example, regions of caldesmon (Permyakov *et al.*, 2003 and Permyakov *et al.*, 2015), anhydrin (Chakrabortee *et al.*, 2010), c-Myc (Andresen *et al.*, 2012), prostate-associated Gene 4 (PAGE4) (He *et al.*, 2015), and the transcription factors (TFs) Sp1 and TAF4 (Hibino and Hoshino, 2020) remain largely disordered even while interacting with their cognate partners. Such interactions are described as "fuzzy complexes" (Tompa and Fuxreiter, 2008) suggesting yet another molecular mechanism underlying IDP interactions (Choi *et al.*, 2011 and Latysheva *et al.*, 2015). Therefore, IDPs can form tight complexes in the absence of any ordered structure (Borgia *et al.*, 2018) while retaining long-range flexibility and highly dynamic character (Mittag *et al.*, 2010 and Borgia *et al.*, 2018). Thus, the conformational equilibria present in even the bound states facilitate pleiotropic functions of IDPs (Tompa *et al.*, 2005) underscoring their importance in regulatory processes (Berlow *et al.*, 2015).

D. IDP interactions involve specific motifs

Interaction of IDPs with binding partners involves short sequence motifs referred to as short linear motifs (SLiMs) and molecular recognition features (MoRFs) as well as low-complexity sequences (Mohan *et al.*, 2006; Davey *et al.*, 2012; and van der Lee *et al.*, 2014). Frequently, two or more of such motifs are found in the same IDP underscoring multivalent interactions (Davey *et al.*, 2012; van der Lee *et al.*, 2014; Van Roey *et al.*, 2014; Krystkowiak and Davey, 2017; and Bhowmick *et al.*, 2015) and increasing the overall avidity of the interaction by exploring conformational ensembles that are recognized by distinct binding partners (Fung *et al.*, 2018 and Uversky *et al.*, 2008). Thus, the same binding region can bind to several different partners with very similar affinities (Oldfield *et al.*, 2008).

Interactions of IDPs that involve SLiMs often involve contributions from the flanking regions and/or such interactions are contextual. These contributions are typically electrostatic acting through either highly negatively charged proteins, for example Rb binding proteins (Palopoli *et al.*, 2018), or positively charged proteins such as the PCNA binding PIP-box (Prestel *et al.*, 2019). However, some flanking regions have also been observed to be hydrophobic (Alanen *et al.*, 2011). Furthermore, the structure and dynamics of the flanking regions can contribute to competition, cooperativity, and allosteric regulation (Berlow *et al.*, 2017) in addition to ensuring proper orientation and the velocity with which interactions occur (Fuxreiter *et al.*, 2007). Nonetheless, the underlying thermodynamics and the exact structural requirements of such interactions are not fully understood (Bugge *et al.*, 2020).

E. Conformational dynamics and conformational noise

In addition to the energetics of binding reactions, conformational dynamics also enables IDPs to control and regulate their hydrodynamic volume and spacing. For example, conformational exchange allows IDPs to explore a large volume while seeking binding partners appropriately dubbed as the "fly-casting" model (Shoemaker *et al.*, 2000; Hoffman *et al.*, 2006; and Metskas and Rhoades, 2015). Similarly, the entropic clock model demonstrates how the degree of extension of an IDP linker region between a pore and its blocking domain modulates timing of an ion channel (Podlaha and Zhang, 2003). Finally, the entropic bristle model revealed how IDPs regulate protein interactions by exploring large search space before populating an appropriate conformation (Hoh, 1998). Together, these unique aspects of IDPs underscore how the timescale and the range of conformational sampling within the ensemble modulates their structural properties.

In addition to affecting structural properties of the IDPs, conformational dynamics also results in noise referred to as "conformational noise" (Mahmoudabadi *et al.*, 2013; Kulkarni and Kulkarni, 2019; and Kulkarni, 2020) that it is distinct from transcriptional noise (Eldar and Elowitz, 2010 and Hansen *et al.*, 2018). In biology, noise is defined as the random variability in biomolecular quantities. Such variation arises even in the absence of any genetic contribution, and as a result, even cells that are isogenic can exhibit significant stochastic fluctuations in protein levels that are leveraged to facilitate probabilistic bet-hedging decisions (Jolly *et al.*, 2018 and Hansen and Weinberger, 2019). Indeed, transcriptional noise that arises due to stochasticity in gene expression is well documented (Raj and van Oudenaarden, 2009 and

Hansen *et al.*, 2018), and isogenic cells in a population are observed to switch states (phenotypes) and behave differently in response to the same stimulus (Brock *et al.*, 2009 and Huang, 2009). Indeed, noise-driven phenotypic switching is now acknowledged to play an important role in development, stress response, disease pathological, and evolution (Mahmoudabadi *et al.*, 2013). Furthermore, stochasticity in phenotypic switching has been reported to modulate differentiation (Eldar and Elowitz, 2010 and Simon *et al.*, 2018), stem cell reprogramming (MacArthur *et al.*, 2008; Yamanaka, 2009; Wakao *et al.*, 2013; Chung *et al.*, 2014; Lin *et al.*, 2018a; Lin *et al.*, 2018b; and Raina *et al.*, 2021), and the conversion of cancer cells to cancer stem-like cells (Gupta *et al.*, 2011 and Sehl *et al.*, 2015).

Aside from transcription, noise also significantly affects information transduced in cellular PINs (Ladbury and Arold, 2012), especially noise contributed by random protein interactions (Kuwahara and Gao, 2013) due to promiscuous interactions (Kontogeorgaki *et al.*, 2017 and Azpeitia *et al.*, 2020). Consistent with this argument, most transcription factors (Liu *et al.*, 2006; Niklas *et al.*, 2015; Strzyz, 2018; and Brodsky *et al.*, 2020) and hub proteins in cellular PINs are IDPs (Haynes *et al.*, 2006; Dosztányi *et al.*, 2006; Gsponer and Babu, 2009; and Patil *et al.*, 2010). Therefore, in response to extrinsic or intrinsic perturbations, IDPs can unmask latent interactions to cause phenotypic switching (Mahmoudabadi *et al.*, 2013 and Kulkarni and Kulkarni, 2019). IDP conformational noise is implied as noise due to the random variability in sampling ensemble. Further, although interconversions of IDP conformations are in fast exchange, they are typically modified by post-translational modifications such as phosphorylation, which can result in variant conformational ensembles to have significantly longer half-lives (Kulkarni and Kulkarni, 2019).

Though conformational switching and even fold switching are well documented in folded/metamorphic proteins (Kulkarni *et al.*, 2018), some IDPs can switch between discrete conformational ensembles even while remaining disordered in both states (Choi *et al.*, 2011 and Choi *et al.*, 2019). While such transitions possess many common features, several IDPs stochastically switch between distinct states within the entire conformational space or display dynamics on slow timescales. Therefore, an in-depth understanding of the conformational dynamics beyond just the minimum energy states that characterize the ensemble both in terms of the landscape, that is, accessible, and the timescales is necessary to gain more insight into IDP structure and function.

F. Characterizing IDPs

Experimental characterization of IDPs, especially large proteins or regions within proteins, remains a challenge. X-ray crystallography and cryo-EM, which recover high-resolution images of proteins in their crystalline and frozen states, respectively, and provide a static view, are not well suited (Kaptein and Wagner, 2019). However, techniques, such as nuclear magnetic resonance (NMR), small-angle x-ray scattering (SAXS), single-molecule Förster resonance energy transfer (FRET), dynamic light scattering (DLS), and two-focus fluorescence correlation spectroscopy (2f-FCS), atomic force microscopy (AFM), circular dichroism (CD), fluorescence, Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy, and mass spectrometry (MS), are adapt at identifying the conformational transitions sampled by IDPs (Kjaergaard *et al.*, 2010a; Kjaergaard *et al.*, 2010b; Jurneczko

et al., 2012; Camilloni *et al.*, 2012; Bernadó and Svergun, 2012; Jensen *et al.*, 2013; Sterckx *et al.*, 2014; Borgia *et al.*, 2016; Khan *et al.*, 2017; Cordeiro *et al.*, 2017; Gomes and Gradinaru, 2017; LeBlanc *et al.*, 2018; Dyson and Wright, 2019; Chan-Yao-Chong *et al.*, 2019; Bax and Clore, 2019; Metskas and Rhoades, 2020; Casuso *et al.*, 2020; and Dyson and Wright, 2021) since they perform measurements of protein molecules as they fluctuate in their "natural" environment. On the other hand, the measurements provided by the above techniques are of limited resolution. Therefore, they are inadequate to discern the distribution of the multiple distinct IDP conformational ensembles. Molecular simulations that can complement and even validate the experimental observations, have emerged as increasingly important tools to elucidate IDP conformational ensembles (Bhattacharya and Lin, 2019; Hsu *et al.*, 2020; Dokholyan, 2020; Zhao *et al.*, 2020; and Wang, 2021). Nonetheless, visualizing their energy landscape presents a formidable challenge.

In this review, using specific examples of IDPs, we highlight recent advances in molecular dynamics simulations and in energy landscape visualization techniques that have shed new light on their conformational dynamics and its functional implications at a systems level. In addition, we also discuss the emerging role of IDPs as therapeutic targets that, until recently, were regarded as "undruggable." Thus, a deeper understanding of the IDPs can not only provide new insight on cellular decision making with wider implications in biology and medicine but may also help to refine and extend the structure/function paradigm beyond Anfinsen's postulate.

II. ENERGY LANDSCAPE

A. Molecular dynamics simulations (MD)

The energy landscape theory has provided a general conceptual framework to understand the folding and functional properties of proteins (Frauenfelder *et al.*, 1991; Onuchic and Wolynes, 2004; and Thirumalai *et al.*, 2010). Based on the principle of minimal frustration (Bryngelson and Wolynes, 1987 and Ferreiro *et al.*, 2018) and grounded on statistical mechanics principles, this approach led to the description of the protein folding funnel (Leopold *et al.*, 1992 and Bryngelson *et al.*, 1995), which has brought a comprehensive understanding of biomolecular processes, bridging theory and experiments (Hills and Brooks, 2009; Onuchic *et al.*, 1997; and Chung *et al.*, 2009). Indeed, these techniques not only aid the study of protein folding but also help elucidate the functional dynamics, which can involve large-scale conformational changes (Takada *et al.*, 2015), motor-like energy transfer, and assembly (Hirokawa *et al.*, 2009).

The general goal of this approach has been to describe the dynamics and thermodynamics of biological molecules in the context of funnel-like landscapes, which takes into account the interplay of topological, energetic, and entropic aspects (Koga and Takada, 2001). Despite the fact that protein folding and functional dynamics are intrinsically multidimensional, the energy landscape approach accurately describes the kinetic and thermodynamic properties in terms of a few key quantities that are used as reaction coordinates. The computational description is highly simplified when there are reference conformations, such as native or functional state, which are used as reaction coordinates and correlated with measurable experimental variables.

The analysis of MD trajectories seeks to capture properties of a system, such as the dominant kinetics and structural features of the

transition state ensembles, as a function of few-dimensional reaction coordinates. Beyond the straightforward structure-based coordinates, such as the fraction of native contacts and the root mean square distance (RMSD) from reference structures, there are alternative strategies for inferring suitable reaction coordinates to describe the energy landscape. For instance, transition-path analysis can be used to find the coordinates that best portray the underlying free-energy barrier (Best and Hummer, 2016). On the other hand, time-correlation analysis (Noé and Clementi, 2017) allows defining classes of collective variables (CVs) associated with the slowest motions. A common limitation of these techniques is that they require, in general, *a priori* definition of coordinates, which can be time-consuming and computationally expensive. Moreover, applying putative coordinates may hide the richness of the dynamics.

Other approaches for representing the landscape involve determining a connectivity map between long-lived states, which can be inferred by Markov state models (MSMs) (Chodera and Noé, 2014; Zimmerman *et al.*, 2017; and Jacobs and Shakhnovich, 2018). Local minima can be individually addressed and go beyond one-dimensional representation (Wales, 2010), and the visualization of distances between local minima in a hierarchical representation is also an appealing way to probe the energy landscape (Wales, 2018). The above methods suit well to investigate funnel-like landscapes with well-defined energy basins. However, IDPs are far more challenging systems due to the high disorder, shallow energy minima, and lack of reference structures.

B. Energy landscape visualization method (ELViM)

A novel approach called the energy landscape visualization method (ELViM) (Oliveira *et al.*, 2014 and Oliveira *et al.*, 2019) that relies upon a multidimensional scaling (MDS) method to examine IDP structure appears quite promising. This method is a reaction coordinate-free method, and it is based on pairwise distances between all structures of the ensemble (Ragonnet-Cronin *et al.*, 2013). Using a local structural similarity metric (Hardin *et al.*, 2000), one can survey and triangulate a high-dimensional conformational phase space and project the ensembles to two optimal dimensions but, at the same, preserve the local proximities. Thus, ELViM permits an intuitive visual analysis of the energy landscape. Moreover, different ensembles can be mapped into a single phase-space, which allows comparisons of ensembles investigated under different physical and chemical conditions.

Using the ELViM method, the authors focused on prostate-associated Gene 4 (PAGE4) in which three different phosphorylated versions of the PAGE4 protein were analyzed [wild type (WT)-PAGE4, HIPK1-PAGE4, and CLK2-PAGE4] (Oliveira *et al.*, 2021). In the ELViM 2D projection, each conformation is represented by a point, which can be fully examined, calculating any desirable variable, such as radius of gyration or specific distances between residues [Fig. 1(a)]. Moreover, ensembles can be analyzed separately, from which the density of states and free energies can be estimated [Fig. 1(b)]. In another study, the same group leveraged ELViM to amyloid-β (Aβ) monomer variants, all IPDs, to discern their propensities for fiber formation (Sanches *et al.*, 2022). Figure 2 shows the ELViM projection of different ensembles of amyloid-β monomers, all projected in the same 2D effective phase space. Each conformation of the dataset can be examined individually, and any chosen variable can
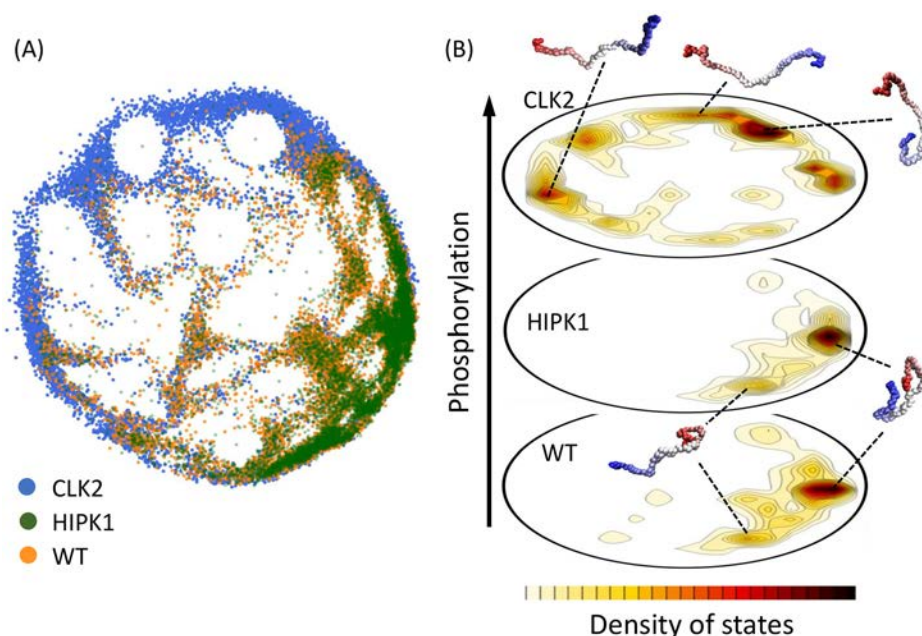
FIG. 1. The different PAGE4 ensembles represented in a single conformational phase space using the energy landscape visualization method. (a) Each conformation displayed by a point in the effective phase space. (b) Contour plots showing the density of states of the wild type (WT) and phosphorylated PAGE4. Each free energy valley is characterized by specific conformations that entail particular binding affinities. For WT-PAGE4, through a fly-casting mechanism, the C-terminal region is extended, facilitating its interaction with partner protiens. In the case of HIPK1-PAGE4, the lower free energy of the compact state decreases the affinity for c-Jun, while the extended conformations of CLK2-PAGE4 due to hyperphosporylation inhibit this interaction.

be tracked and colored accordingly. As an example, Fig. 2(b) shows all conformations of the amyloid-β monomers colored according to their radius of gyration (R_g), and examples of the conformations from different regions of the phase space are also shown. Therefore, one would expect that if such projection makes sense, meaningful coordinates would be "well behaved" and vary continuously throughout the 2D representation, which is shown by R_g in Fig. 2(b). Considered together, this MDS strategy appears to provide an insightful representation of IDP energy landscape.
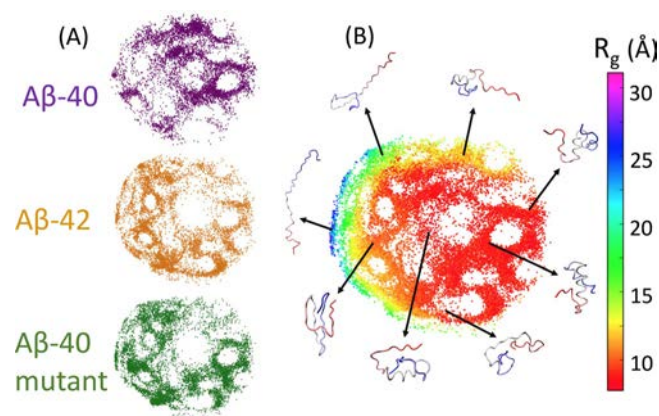

FIG. 2. Conformational phase space of the simulated amyloid-β (Ab) structures. (a) Distinct Ab ensembles present in the projection with the Ab-40 (purple), Ab-42 (beige), and the Ab-40 mutants (green). (b) Each point represents a conformation, which is colored as function of radius of gyration. Typical conformation examples of each region are displayed around the projection.

C. Parallel tempering (PT)

IDPs exist in shallow rugged free energy landscapes with multiple conformational populations that are in dynamic equilibrium with each other. As such, it is difficult to structurally resolve them at high-resolution with experimental techniques. Of late, in order to elucidate the structural and dynamical features of IDPs at higher resolution, molecular simulation is being routinely used in conjunction with low-resolution ensemble-averaged data (Lindorff-Larsen *et al.*, 2012; Bonomi *et al.*, 2017; Best, 2017; Peterson *et al.*, 2017; Bottaro and Lindorff-Larsen, 2018; Kasahara *et al.*, 2019; Gomes *et al.*, 2020; and Kassem *et al.*, 2021) from experiments such as SAXS (Henriques *et al.*, 2015; Hub, 2018; Hermann and Hub, 2019; Chan-Yao-Chong *et al.*, 2019; Ahmed *et al.*, 2021; and Kassem *et al.*, 2021), NMR (Fawzi *et al.*, 2008; Robustelli *et al.*, 2010; Fisette *et al.*, 2012; Fu and Vendruscolo, 2015; Salvi *et al.*, 2016; Papaleo *et al.*, 2018; Chan-Yao-Chong *et al.*, 2019; Heller *et al.*, 2020; and Kassem *et al.*, 2021), FRET (LeBlanc *et al.*, 2018 and Lerner *et al.*, 2021), and cryo-electron microscopy (Bonomi and Vendruscolo, 2019 and Nierzwicki and Palermo, 2021). Despite the many advances, extracting experimentally consistent ensemble for an IDP remains highly challenging. This, in large part, is due to the presence of diverse conformational states in an ensemble that render the experimental data noisy, sparse, and/or ambiguous. On the other hand, the molecular simulations typically sample only a tiny phase space of an IDP ensemble despite the underlying free energy landscape being shallow in nature. The presence of significant entropic barriers between different population clusters is an often-overlooked aspect of IDPs sampling and the primary reason for samples not reproducing the ensemble and thermodynamic averages of experiments. Also, modeling extremely fuzzy IDPs with very low hydrophobicity and high net charge is yet another example of entropically stabilized systems that are not sampled properly and need to be addressed post haste due to emerging roles of such IDPs in

interactions, both at single molecule recognition level (Jephthah et al., 2019; Schuler et al., 2020; and Sottini et al., 2020) and in assemblies (Li et al., 2001 and Rauscher and Pomès, 2017).

The determination of experimentally consistent ensemble data from simulation mandates adequate sampling, which is generally achieved in advanced sampling approaches by either applying structural restraints using collective variables or by reweighting the obtained conformations to arrive at the Boltzmann weighted populations (Cavalli et al., 2013; Rangan et al., 2018; and Köfinger et al., 2019). Parallel tempering (PT) sampling is an attractive alternative since it can be used effectively without any reweighing and restraining and without the need to have a low-dimensional collective variable (CV) to define the ensemble states. Moreover, instances when sampling outcomes are not commensurate with experimental data, PT can be coupled seamlessly with other CV-based restraining methods or reweighted appropriately to solve the problems of interest (Do et al., 2014; Zerze et al., 2015; Awasthi and Nair, 2017; and Liu et al., 2020). In the classical version of this method (Sugita and Okamoto, 1999) called temperature replica exchange MD (TREMD), multiple replicas are simulated simultaneously at a series of low and high temperatures and neighboring replicas are stochastically swapped at regular intervals based on criteria that honor detailed balance. These random walk swaps allow the broader phase space explored at the high temperature replicas to be accessed by the low temperature replicas, thereby generating an unbiased Boltzmann-weighted ensemble of conformations at a given temperature. The acceptance probability of swapping depends on the extent of potential energy overlap between the adjacent replicas and as the system size increases, more and more numbers of replicas are required for effective sampling (Baumketner and Shea, 2007; Wang et al., 2013; Zerze et al., 2015; and Jain et al., 2021). This problem is particularly exacerbated when simulating large proteins in explicit solvent where the bulk of solvent molecules contributes majorly toward the poor overlap. For instance, about 96 replicas were needed to sample a 20-residues long disordered N-tail of measles envelop virus protein in solvent, and 180 replicas were needed for effectively sampling a 63-residues long alpha-synuclein (Wang et al., 2013 and Baumketner and Shea, 2007).

Several variants of PT have evolved in recent years to alleviate the huge computational expenses of classical TREMD. One of the popular approaches is the replica exchange with solute tempering/scaling (REST/REST2 and gREST), where the Hamiltonian is designed in such a way that it effectively heats up the solute while keeping the solvent at room temperature (Liu et al., 2005; Wang et al., 2011; and Kamiya and Sugita, 2018). This transformation drastically reduces the required number of replicas as the exchange probability now depends only on the solute degrees of freedom and forgoes the calculations from the expensive solvent self-interactions. This method has found tremendous applications in sampling IDPs (Musiani et al., 2013; Brown et al., 2014; Smith et al., 2016; Peng et al., 2017; Shrestha et al., 2019; Liu and Chen, 2019; and Shrestha et al., 2021) and also on studies related to IDPs binding to their cognate partners (Miller et al., 2014; Smith et al., 2019; Khayat et al., 2020; Noda et al., 2020; Zhao et al., 2021; and Gopal et al., 2021).

The success of REST2, the evolved version of REST, is highly dependent on the choice of forcefield. In most of the cases, the combined application of REST2 with a99SB-disp forcefield (Robustelli et al., 2018) recapitulated almost all the experimental measurements

(Liu and Chen, 2019 and Shrestha et al., 2021). For instance, in p53-TAD, the REST2-a99SB-disp duo generated ensemble captures multiple local and long-range structural properties, including chain dimension, residual secondary structures, and transient long-range contacts in consistent with measurements from NMR, smFRET, and TR-FRET experiments. Whereas with other state-of-the-art forcefields including Charmm36m (Huang et al., 2017) and Amberff99SB-ILDN/ TIP4PD (Lindorff-Larsen et al., 2010 and Piana et al., 2015), the REST2 either suffered with inadequate convergence or over-compaction issue (Liu and Chen, 2019). REST2 was also used in studying the coupled folding induced binding in c-Myb/KIX (Gopal et al., 2021) and Bcl-XL/PUMA (Liu et al., 2017) complexes. In these simulations, the REST2-a99SB-disp combination provided high-precision accurate structural properties when compared to circular dichroism and secondary chemical shifts. However, the predicted nuclear Overhauser effect (NOE)-like distances show significant violations from the NMR derived values particularly at the interface residues. This indicates the formidable challenge of sampling tertiary packing of IDP segments likely originated from inadequate sampling and convergence in these simulations (Smith et al., 2016 and Liu et al., 2017).

In addition to poor convergence and inadequate sampling in some cases, the REST2 also suffers with poor mixing of replicas between the high and low temperature regimes in complex proteins (Huang et al., 2007 and Smith et al., 2016), where friction between the protein and solvent determines the rate of conformational transition, and most existing methods suffer in overcoming the solvent-imposed (entropy driven) free-energy bottlenecks. This aspect is amply highlighted in a recent study where REST2-derived conformations at high and low temperatures are shown trapped in local temperature basins with nominal exchange taking place between them (Appadurai et al., 2021). In this work, the entropic lock problem is solved by enabling differential tempering of both the solute and solvent in the Hamiltonian (Fig. 3). This scheme, called as replica exchange with hybrid tempering (REHT), specifically allows faster decay of water reorientation dynamics at non-base replica that, in turn, facilitates the faster and converged conformational sampling at the base replica (Appadurai et al., 2021). REHT is able to reproduce SAXS and Chemical Shift data for a range of proteins with a variety of free-energy landscape (folded, metamorphic as well as IDPs) without any need to restrain or reweight the ensemble. The details of REHT simulation setup and scripts for generating the input files are available at the github repository, https://doi.org/10.5281/zenodo.4361714.

Across these different sampling methods and forcefields, it is clear that the correct modeling of protein–solvent interactions is critical for generating ensembles to a level of precision sufficient to draw physical conclusions. At this point, it is important to point out again how sampling and forcefield are very interconnected and need to be addressed simultaneously. Here, we would like to mention how the popular IDP forcefield Charmm36m, which does not work well with REST2, seems to generate excellent results with REHT. REHT has its origin from REST2, and it solves an old problem arising out of "cold solvent" effects in REST2 while keeping the computational requirements tractable. There is a substantially shortened round trip of replicas in REHT with the same forcefield, and this is due to the way it treats the water self-interaction (in non-base replicas). This is, in some sense, equivalent to the ideas in work with optimized forcefields of water interactions for better sampling (Best et al., 2014; Piana et al.,
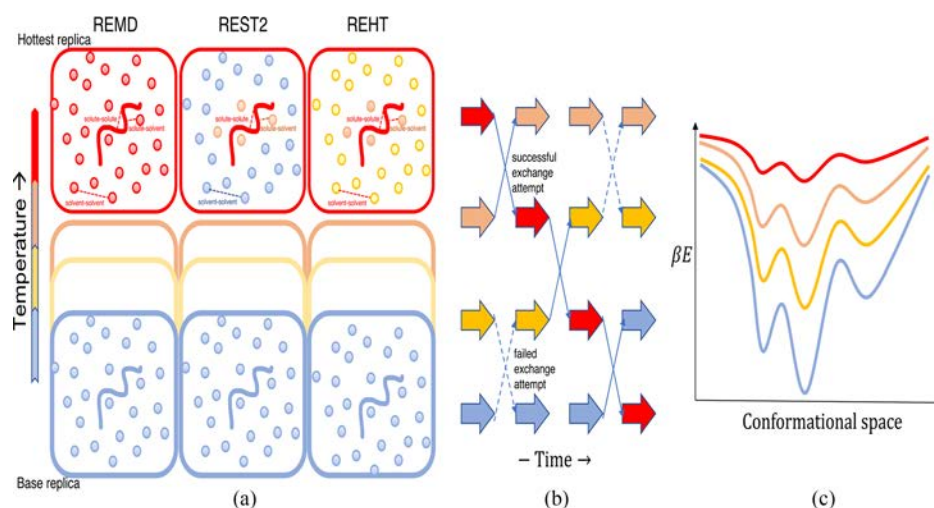
FIG. 3. Schematic diagram of different parallel tempering simulations. (a) In parallel tempering simulations, a series of low and high temperature replicas are simulated. The replicas in the conventional REMD differ by increasing bath temperatures across the ladder. Thus, the probability of accepting the exchange between adjacent replicas depends on the difference in the complete Hamiltonian of the system, including solute–solute, solute–solvent, and solvent–solvent contributions, which results in poor scaling in large systems. REST2 scales the energy function in a particle-wise manner, such that the solute is effectively heated up while keeping the solvent cold. Thus, the exchange acceptance probability depends on the energy difference in solute–solute interactions mainly and solute–solvent interactions subtly. The imbalance between hot solute and cold solvent causes entropic trap. REHT optimally heats the solute as well as the surrounding solvent by associating the replicas to different bath temperatures in addition to scaling down the potential function. Note that the base replica in all the cases is unbiased and has the same forcefield parameters and temperature conditions. (b) Stochastic swapping of replicas at regular intervals facilitates the equilibrium sampling at the base replica. (c) Schematic of energy landscape illustrating accessibility of broader conformational space facilitated by the high temperature replica.

2015; and Robustelli et al., 2018). Entropic barrier is more acute in larger IDPs (>100 residues long), and REHT has opened the door to extract the experimentally commensurate conformational ensemble at atomic resolutions for very long IDPs.

III. CONFORMATIONAL DYNAMICS AND PHENOTYPIC SWITCHING

A. IDP dynamics and stochasticity

Conformational noise can have important implications in cellular behavior. Our current understanding of noise in biological systems is largely focused on stochasticity arising due to the low copy number of biomolecules (Balázsi et al., 2011). Stochasticity can be seen at various regulatory levels: signaling cascades, transcription (DNA binding/unbinding), translation (microRNA-mRNA binding/unbinding), chromatin organization (Guillemin and Stumpf, 2021), eventually impacting cellular decision-making, and enabling phenotypic heterogeneity. Similar implications of stochasticity can also arise due to conformational noise. With IDPs acting as hubs in PINs, the interaction strength between members of the PIN and even the connections between them becomes a dynamical variable instead of fixed parameters in the "static" regulatory networks we often imagine. Thus, conformational noise can drive time-varying PIN(s) and/or regulatory network(s) where the interactions among the nodes change dynamically over time (Fig. 4). Such traits can amplify any preexisting cell-to-cell variability, despite identical genotype, facilitating non-genetic heterogeneity. Thus, it becomes important to delineate the impact of conformational noise and IDPs on phenotypic heterogeneity and plasticity (ability of cells to reversibly switch to a different cell-state, often

as an emergent property of underlying network dynamics) (Hari et al., 2020).

Various molecules involved in cell-state transitions and cellular transformation are known to be IDPs. For instance, various oncogenes and tumor-suppressor genes have IDRs. Similarly, transcription factors (TFs) such as ZEB1, SNAII, and OVOL1 and OVOL2 that are involved in phenotypic plasticity during cancer metastasis and therapy resistance have been shown to be IDPs (Mooney et al., 2016). These
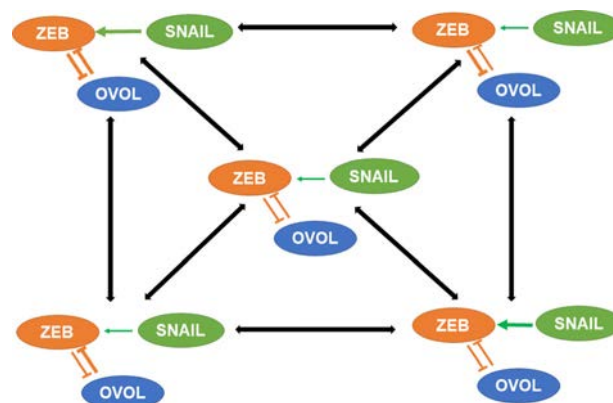
FIG. 4. Dynamic protein interaction networks. A "static" network consisting of IDPs (center) can be altered in terms of relative strengths of interactions across the nodes, in the presence of conformational noise. Orange hammer shows inhibition; green arrows show activation. Thicker lines show stronger activation or inhibition than weaker ones. These "dynamic" networks can interchange among themselves as well due to changes in conformational structure of IDPs/IDPRs involved.

TFs are the master regulators of epithelial-mesenchymal transition (EMT) and its reverse mesenchymal-epithelial transition (MET)—cellular processes, which enable cancer cells to alter their adhesion, migration, and invasion traits dynamically during different steps of the metastatic cascade. Even, the crucial drug targets for prostate and breast cancer, respectively—androgen receptor (AR) and estrogen receptor (ER)—contain IDRs (Myung *et al.*, 2013 and Peng *et al.*, 2019). EMT/MET can also impact drug resistance in cells by influencing the levels and/or activity of ER and AR (Graham *et al.*, 2010; Anose and Sanders, 2011; and Sahoo *et al.*, 2021) and *vice versa*. This crosstalk can lead to a dynamic PIN between these key nodes and can impact cancer cell fitness dynamics.

PAGE4, yet another IDP implicated in PCa, can show various conformations (He *et al.*, 2015). It can be phosphorylated at two residues (S9 and T51) by the kinase HIPK1; phosphorylation of PAGE4 enables its interactions with AP-1 transcription factor complex (Mooney *et al.*, 2014). PAGE4 can also be phosphorylated by another kinase CLK2, and these two phosphorylated versions of PAGE4 (HIPK1-PAGE4 and CLK2-PAGE) have opposing functions due to their different conformational dynamics. HIPK1-PAGE4 has a compact conformational ensemble that can bind AP-1 and potentiate c-Jun, but CLK2-PAGE4 has a reduced affinity for AP-1 due to its random coil-like structure (Kulkarni *et al.*, 2017 and Lin *et al.*, 2018a).

Because c-Jun potentiation can indirectly enhance CLK2 levels through AR, a negative feedback loop is formed, which can lead to oscillations [Fig. 5(a)] in the levels of AR and those of differently phosphorylated versions of PAGE4 (Kulkarni *et al.*, 2017). Such oscillations can generate non-genetic heterogeneity in a clonal prostate cancer cell population and also manifest in dynamic levels of AR in individual cells, impacting their therapeutic sensitivity.

B. Non-genetic heterogeneity due to conformational noise

Upon investigating the coupled dynamics of this negative feedback loop with that of EMT, a wider repertoire of cellular behavior can be realized. A core EMT circuit comprised of a mutually inhibitory loop between ZEB1 and microRNA family miR-200, driven by SNAI1, can lead to three distinct interconverting cell-states: epithelial (E; high miR-200 and low ZEB1), mesenchymal (M; low miR-200 and high ZEB1), and hybrid E/M (medium miR-200 and medium ZEB1) (Jolly *et al.*, 2017). Also, ZEB1 and AR can inhibit each other (Singh *et al.*, 2021). On coupling EMT and PAGE4/AR circuits, we see that these oscillations of PAGE4 circuit can convert to bistable behavior. Depending on the interaction strength between AR and ZEB, this coupled circuit can show both oscillations (sustained or damped) and
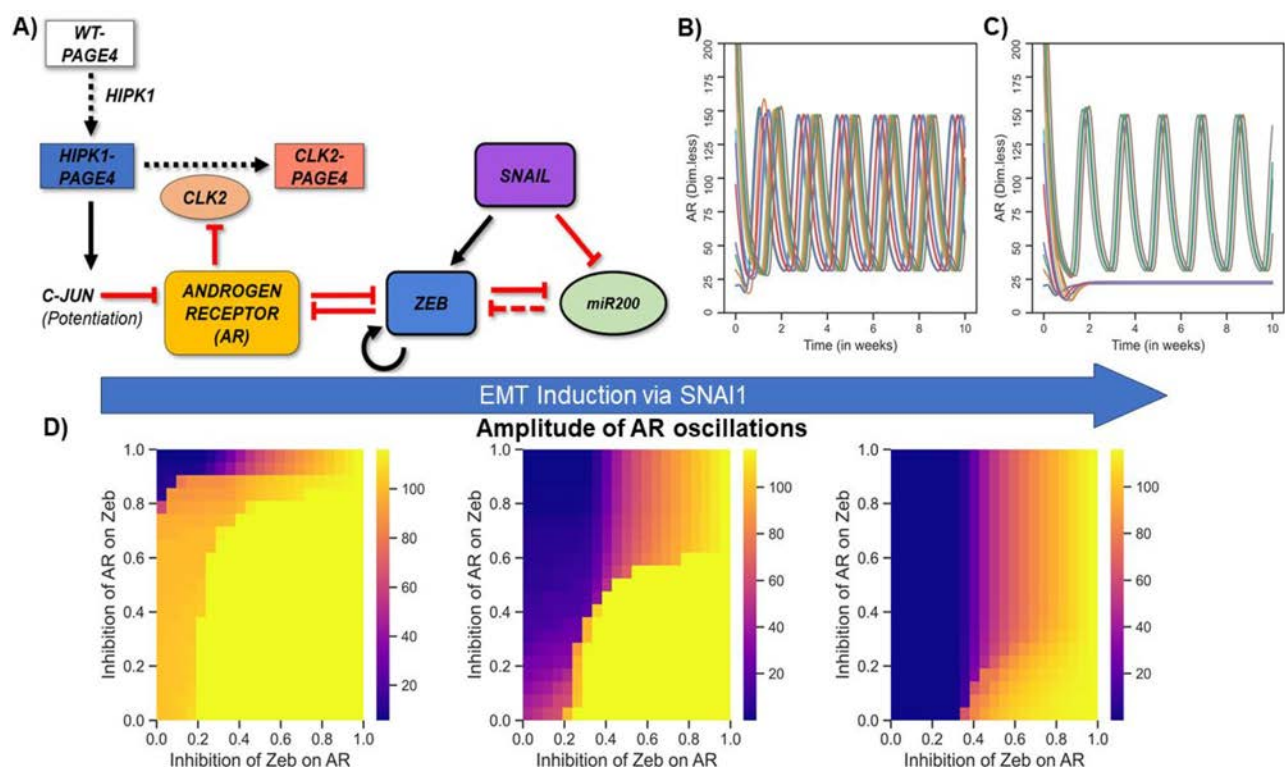
FIG. 5. Coupled dynamics of EMT and PAGE4 circuits containing IDPs. (a) Coupled network of EMT and PAGE4 circuit. Solid red hammer heads correspond to transcriptional inhibition, and dotted red hammer heads correspond to post transcriptional inhibition due to micro-RNA interaction. Solid black arrows correspond to transcriptional activation, and dotted arrows stand for phosphorylation. (b) and (c) Dynamics of AR: AR levels over time for two different values of interaction strength with ZEB. (d) Induction of EMT via SNAI1 leads to oscillations converting to bistability. Phase plot between interaction strengths of AR and Zeb, as EMT is induced via SNAI1, Zeb inhibits AR more strongly and leads to oscillations converting to bistability. [Adapted from Singh *et al.*, Entropy (Basel) **23**(3), 288 (2021). Copyright 2021 MDPI].

multistability—both of which are different examples of non-genetic heterogeneity in cancer cell populations [Fig. 5(b)].

Such non-genetic heterogeneity can often subvert the efficacy of therapeutic treatments. Because the interaction strength between members of these dynamic PINs can change, some cells may exhibit oscillatory dynamics for AR, while others may enable bistability (enabling cells to spontaneously switch from AR-high to AR-low state and *vice versa*). This diverse arsenal of cellular dynamics makes it difficult to design targeted therapies aimed to kill these cancer cells, thus aggravating disease progression in many patients.

IV. INTRINSICALLY DISORDERED REGIONS IN CELLULAR FUNCTIONING AND MALFUNCTIONING: CLASSIC CASE OF KIRSTEN RAT SARCOMA VIRUS (KRAS)

In addition to IDPs, proteins containing IDRs are abundantly found in modulating cellular functioning (Romero *et al.*, 2006 and Oldfield and Dunker, 2014). Most IDR regions are involved in membrane-associated activities and cell signaling (Buljan *et al.*, 2013; Wright and Dyson, 2015; Nussinov *et al.*, 2018; and Cornish *et al.*, 2020). The Ras superfamily of small GTPases represents a classic example where such signaling proteins act like binary molecular switches that regulate cell growth, proliferation, and differentiation (Colicelli, 2004 and Cox and Der, 2010). The switching function of Ras regulates an inactive GDP-bound off-state and active guanosine-5′-triphosphate (GTP)-bound on-state. Hyperactivation of RAS signaling is often triggered by direct mutations leading to Ras-induced cancer development (Biankin *et al.*, 2012 and Wood *et al.*, 2016).

A. Dynamics of disordered regions influence catalytic activity of KRAS: Insights from experimental data

Amongst RAS isoforms, KRAS is the most frequently mutated oncogene found in human cancers (Pleasance *et al.*, 2010 and Prior *et al.*, 2012). Only in the activated GTP-bound state, KRAS can associate with its effector protein like RAF-kinases, PI3K, and RalGDS to activate them (Pantsar, 2019 and Nussinov *et al.*, 2019). The activation of KRAS, however, depends on the guanosine exchange factor (GEF) that helps to replace GDP with GTP when the cellular concentration of GTP is higher [Fig. 6(a)]. On the other hand, despite having low intrinsic GTPase activity, the inactivation of KRAS is often induced by GTPase activating proteins (GAP) that catalyze GTP hydrolysis to GDP (Milburn *et al.*, 1990 and Bos *et al.*, 2007). For their catalytic function, all RAS isoforms have a very similar catalytic domain (residue: 1–166), including the N-terminal residues. This catalytic domain contains highly disordered functionally critical switch regions (switch 1: residues 25–40 and switch 2: residues 57–75). In particular, the positively charged hypervariable region (HVR), (residues 167–179) in the C terminus, and the flexible switch regions of small GTPases have drawn much attention of the recent investigations on the effects of such IDRs on the regulation and modulation of signaling output compared to its wild type (WT) and oncogenic variants (Gorfe, 2010; Abraham *et al.*, 2010; and Hunter *et al.*, 2014). The HVR mainly mediates membrane association [Fig. 6(b)]. A recent paramagnetic relaxation enhancement (PRE) NMR study has provided mechanistic insight into membrane-dependent RAS dimerization and the implications of the HVR region with membrane association (Lee *et al.*, 2020). In both the monomeric and the dimeric states of KRAS4B, the basic poly-lysine stretch in the C-terminal HVR through electrostatic

interactions helps to anchor the anionic lipid head groups of the membrane [Fig. 6(b)]. Although RAS dimerization has been proposed as an essential step in the cascade of RAS signaling, the oligomerization state of KRAS remains elusive. It has been proposed that its membrane association occurs only in the monomeric state (Chung *et al.*, 2018); however, other suggestions include dimers, trimers, and even oligomers (Muratcioglu *et al.*, 2015; Sarkar-Banerjee *et al.*, 2017; and Barklis *et al.*, 2019).

Dynamical behavior of the flexible switch regions was first obtained via NMR spectroscopy where switch-I is found in two different conformations: open and closed states (Spoerner *et al.*, 2001). The closed state is essentially found when it is bound to other effector proteins. Specific mutations in the switch regions, such as D33E in the switch-I and A59G in the switch-II regions, have the potential to lock the conformation in its open form when one considers only the catalytic G-domain (Lu *et al.*, 2018). However, for the full-length RAS the equilibrium shifts toward the closed state. While such mutated open conformations are stated as an inactive GTP-bound state, these mutants show similar affinity to the RAS binding effector protein, RAF, when compared to the WT KRAS. This possibly occurs as the overall structure eventually moves toward the closed conformation leaving the open state presumably as an intermediate functional conformation where the disordered dynamics of switch regions still helps to maintain the association and affinity toward other effector proteins. NMR data also showed that the equilibrium-shift toward the open state is attainable if one perturbs Y32 position by replacing it with other amino acids in the dynamic switch-I region (Spoerner *et al.*, 2010). As dynamic switch-I is in the immediate close region of the catalytic cavity, this region is identical in all RAS isomers.

The allosteric behavior of RAS has been well-studied previously (Buhrman *et al.*, 2010 and Buhrman *et al.*, 2011) to elucidate its allosteric function. In WT RAS-GTP, an allosteric switch is found to promote disorder to order transition of switch-II through a network of H-bonding interactions connecting the allosteric site to switch-II involving key residues crucial for catalysis. These studies revealed that an "on" state of the allosteric switch may enhance the hydrolysis rate in a GAP-independent pathway with the signal being turned off. Again, when the allosteric switch is in the "off" state, GTP-hydrolysis is deprived and signaling remains on. While RAS and its effector RAF are crucial driver proteins to control the RAS/RAF/MEK/ERK (extracellular signal-regulated kinase 1) signaling pathway, several oncogenic mutations including Gly12 and Gln61 are found to impair the GTPase activity of RAS and are abundantly found in human cancer (Prior *et al.*, 2012). Thus, the allosteric mechanism helped to explain how such oncogenic mutations could affect the catalytic process.

B. Advancement of drug development to target oncogenic mutations of KRAS and drug resistance

Until recently, KRAS was considered undruggable. AMG510 was one of the first KRAS (G12C) inhibitors that was efficacious against KRAS G12C tumors (AMG510, 2019 and Canon *et al.*, 2019). Soon after, MRTX849 was found to be a potent mutant selective covalent inhibitor of KRAS G12C. MRTX849 is highly efficacious in tumor regression in KRAS G12C mutant cell lines, patient-derived xenograft models from multiple tumor types, and in lung and colon cancer patients.
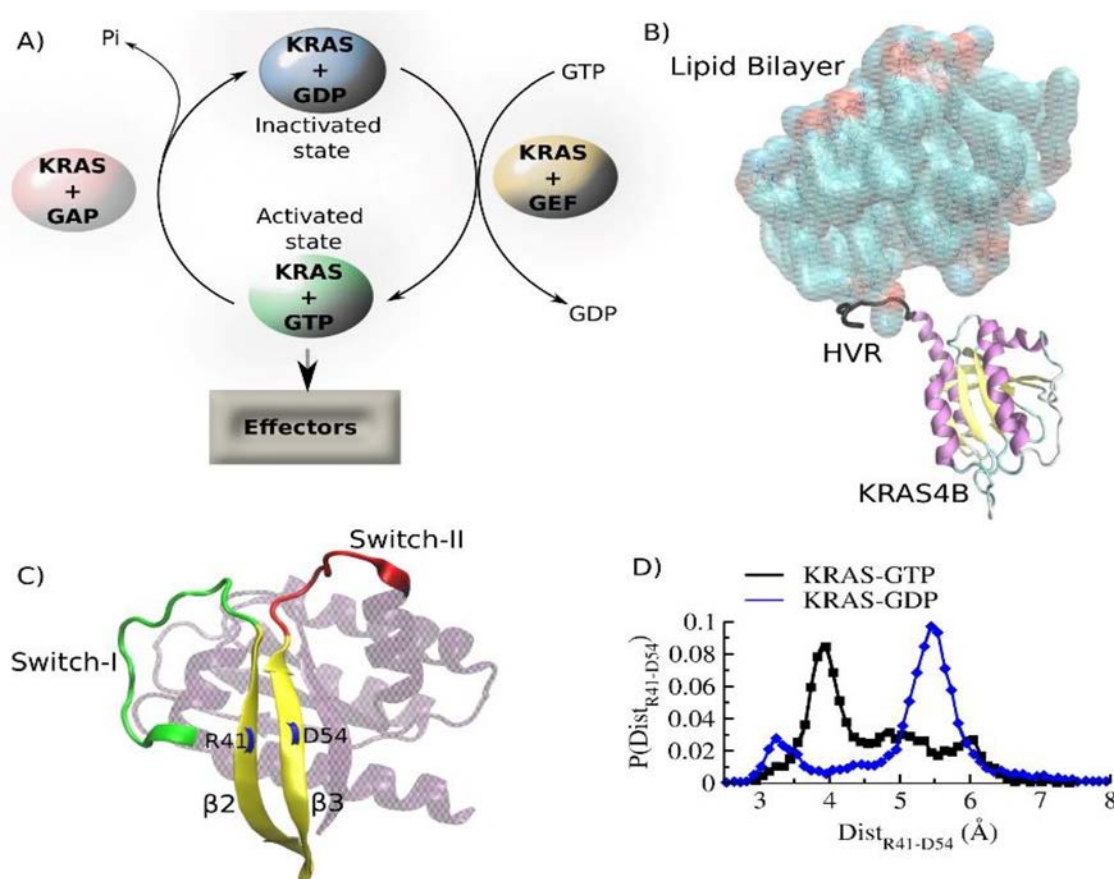
FIG. 6. The structure/function cycle of KRAS. (a) Activation/deactivation cycle of KRAS GTPase. GDP/GTP exchange in this cycle is mediated by two other proteins: guanine nucleotide-exchange factors (GEFs) and GTPase activating proteins (GAPs). While GEFs catalyze the exchange from GDP to GTP, GAPs enhance the rate of exchange from GTP to GDP. (b) NMR-driven structure of KRAS4B-GTP on a lipid bilayer (pdb id:6W4E) (Lee *et al.*, 2020). The positively charged intrinsically disordered hypervariable region (HVR) is shown in gray to highlight its mode of association with the lipid membrane. (c) Three-dimensional structure of inactive GDP-bound human KRAS highlighting the dynamic switch regions: Switch I (green) and Switch II (red) (pdb id: 4OBE) (Hunter *et al.*, 2014). These two switch regions are connected via two parallel β strands: β2 and β3. (d) The switch dynamics and their correlation with β2-β3 fluctuation are compared in GDP and GTP-bound states. The dynamics are assessed by quantifying the distance between two residues R41 (located in β2) and D54 (located in β3). The distance distribution indicates enhanced conformation fluctuation of the switches in the GDP-bound state. [Adapted from Vatansever *et al.*, Sci. Rep. **6**, 37012 (2016). Copyright 2016 Author(s), licensed under a Creative Commons Attribution 4.0 License.]

Mutations in KRAS are frequently observed in lung, pancreatic, and colorectal cancers. Lung adenocarcinoma has the highest percentage of KRAS mutations, and the most frequent mutations include substitution of glycine 12 with either cysteine, valine, aspartic acid, alanine, serine, or asparagine. Each of these substitutions leads to conformational changes in the KRAS molecule, which impinge on its biophysical property. For example, Moghadamchargari *et al.* (2019) reported that KRAS has intrinsic GTPase activity, that is, also involved in the conversion of the KRAS-GTP active form to KRAS-GDP inactive form, and this activity is higher in the native (WT), G12C, or G12D KRAS mutants, but lower in the G12A, G12V, G12S, and G12R mutants. Similarly, based on the affinity for Ras binding domain (RBD), KRAS can be grouped into a high affinity group (WT, G12A, and G12C) and a low affinity group (G12V, G12R, and G12D) (Hunter *et al.*, 2015). Since KRAS lacks a groove or pocket except the GTP binding domain for the small molecules to bind, GTP analogs were used to compete against the cellular GTP for the GTP binding pockets of KRAS but that approach did not work (Noonan *et al.*, 1991). The other option to target KRAS signaling was by targeting the upstream and downstream signaling pathways. Thus, various inhibitors of the RAF-MEK-ERK and AKT serine/threonine kinase 1-mTOR pathways were developed that were able to suppress the growth of KRAS driven tumor. Unfortunately, the activation of overly complex network of positive and negative feedback loops associated with KRAS signaling reduced the efficacy of these drugs and, eventually, caused tumor relapse. Therefore, a more directed approach was tried to target the KRAS molecules and block its activation.

The proposal was to block the KRAS function by developing the covalent inhibitors, which could covalently interact with KRAS and block its conversion from KRAS-GDP (inactive sate) to KRAS-GTP (active state). In the initial study, the small molecules were designed to covalently interact with the thiol group of cysteine (G12C) residue and lock KRAS in GDP-bound state. The inhibitors like SML-10–70-1 appeared selective for the KRAS G12C compared to the WT, inhibited

activation of AKT and ERK, and increased accumulation of KRAS-GDP (Lim *et al.*, 2014 and Hunter *et al.*, 2014). However, its ability to inhibit tumor expressing KRAS G12S mutants raised questions about its specificity. Other inhibitors such as vinyl sulfonamide and acrylamide analogs were developed by either changing the positions or altering the electrophilic group for efficient interaction with KRAS G12C. Compound 12 was developed, which could interact with the new allosteric pocket and change the preference of KRAS G12C for GDP compared to GTP (Ostrem *et al.*, 2013). The compound was selective for KRAS G12C but had poor pharmacological properties. Compound 12 was further modified and developed to ARS853, which had 600-fold more affinity for KRAS G12C and locked it in the inactive GDP bound state. However, the compound had lower metabolic stability in the plasma and poor oral bioavailability in mice, which restricted its use for *in vivo* studies.

Janes *et al.* (2018) reported a new covalent inhibitor ARS1620, which was based on the structure of ARS-853 by scaffold optimization. X-ray crystallography studies showed the binding on the ARS1620 to the allosteric pocket region located beneath the switch II loop of KRAS-GDP. It is a biochemically stable and orally bioavailable compound shown to inhibit KRAS G12C activity *in vitro* and *in vivo*, but it has suboptimal potency owing to small volume of pocket being occupied (Canon *et al.*, 2019). The crystallographic structure of ARS1620-KRAS G12C revealed a hydrogen bonding between the ARS1620 and histidine 95 residue. Canon *et al.* (2019) reported that this histidine residue could flip up and reveal a hidden groove, which could be targeted by covalent inhibitors leading to the discovery of the KRAS inhibitor AMG510 (Canon *et al.*, 2019). ARS1620 and AMG510 have structural similarity but enhanced the interaction of AMG510 with the H95 groove, which increases its potency by ten-fold compared to ARS1620.

MRTX849 also binds to cysteine 12 residue irreversibly and locks it in an inactive GDP-bound state inhibiting the KRAS driven downstream signaling pathways. It is highly selective against the KRAS G12C, and *in vivo* data demonstrate that it is effective against several solid tumors, including lung, pancreas, and colon. In *in vitro* studies, the drug was also shown to be effective against cell lines that have co-mutations in genes like P53, STK11, KEAP1, HER, or CDKN2A (Hallin *et al.*, 2020).

C. Investigations of conformational ensembles and disordered dynamics of KRAS by computer simulation methods

Several microsecond simulations have been performed for different RAS isoforms (HRAS, NRAS, and KRAS) to sample their conformational ensembles and understand the conformational dynamics in their GDP- and GTP-bound states (Kapoor and Travesset, 2015 and Prakash and Gorfe, 2013). These simulations capture the high flexibility of the switch regions, and the range of flexibility differs in different RAS isoforms. In wild-type KRAS, dynamics of switch regions are observed to influence the closure of two immediate parallel β-strands located between switch-I and switch-II regions [Fig. 6(c)]. The differential switch dynamics in GDP- and GTP-bound states are reflected when the distance closure between these two parallel β-strands (β2 and β3) is measured [Fig. 6(d)]. The distance distribution between β2 and β3 indicates that GTP-binding increases KRAS stiffness by restraining the switch dynamics, which possibly helps enable its

GTPase activity (Vatansever *et al.*, 2016). However, recent NMR analysis of GDP-bound G12V-HRAS and G12V-HRAS (GMPPNP, a stable GTP analog) obtained a different result, which shows that the latter is more flexible (Chen *et al.*, 2021). Investigations on WT-HRAS, WT-KRAS, and other RAS mutants are consistent with that recent NMR analysis (Kraulis *et al.*, 1994; Araki *et al.*, 2011; O'Connor and Kovrigin, 2008; Vo *et al.*, 2013; Fetics *et al.*, 2015; Matsumoto *et al.*, 2016; and Yin *et al.*, 2017). Moreover, HRAS (GMPPNP) is more susceptible to proteolytic cleavage by an engineered subtilisin protease than the GDP form. Protease recognition occurs specifically at the Switch II YSAM site, with cleavage right after the methionine. This region is in the alpha2 helix in the GDP form but is more disordered in the GTP form, thus making it more accessible to proteolysis (Chen *et al.*, 2021). Early MD simulations showed that these intrinsically flexible switch regions belong to an evolutionarily conserved nucleotide-binding lobe-1 (residue 1–86), which has an isoform-specific communication pathway with C-terminal lobe-2 (residue 87–171) (Gorfe *et al.*, 2008).

Apart from classical MD, accelerated MD and targeted MD approaches have also been employed to probe the large timescale and extensive length-scale conformational dynamics that are associated with GDP and GTP binding processes (Milburn *et al.*, 1990; Diaz *et al.*, 1997; and Grant *et al.*, 2009). It was proposed that the conformation selection and the population shift mechanisms might play an important role where allosteric interference is also associated in such ligand-binding phenomena. Allosteric regulations have an immense role in post-translation modifications (PTMs) of such signaling proteins (Nussinov *et al.*, 2012 and Ahearn *et al.*, 2018). PTMs that occur away from the functional site yet propagating through conformational and dynamical changes are called allosteric PTMs, while PTM events taking place at the functional site via direct recognition are often known as orthosteric PTMs (Nussinov *et al.*, 2012 and Clausen *et al.*, 2015). However, a theoretical dynamic energy landscape combining equilibrium fluctuation concepts has been proposed to explain such dynamic conformational changes of the substrate regulated by allosteric event (Kar *et al.*, 2010). Such a concept also shows promises with the allosteric drugs that allow modulation of signal and responses in comparison to targeted drug binding at active sites.

The full-length KRAS including the IDR dynamics of HVR regions in solution along with their oncogenic mutations was also investigated using large microsecond simulation data (Chavan *et al.*, 2015; Jang *et al.*, 2016; Sayyed-Ahmad *et al.*, 2017; and Pantsar *et al.*, 2018). The orientational dynamics of KRAS has also been studied including the membrane, and the results correlated well with the experimental findings (Li and Buck, 2017). Apart from early membrane-associated simulation with KRAS displaying distinct rotational conformations, a recent microsecond long membrane-associated simulation of G12V KRAS shows three unique conformations (Prakash *et al.*, 2019). These conformations are also found in the case of G12D and Q61H mutants but in a different population (Prakash and Gorfe, 2019). Coarse-grained simulation approaches have also been adopted to model clusters of inactive or lipid anchored RAS embedded in a phase-separating lipid mixture of DPPC, cholesterol (CHOL), and DLiPC (Janosi *et al.*, 2012). The lipid mixture was found to segregate between CHOL/DPPC containing the ordered domain and DLiPC containing disordered domain to form a raft and

non-raft like domains, respectively, indicating how asymmetric RAS-binding induces bilayer deformation.

Among different computational and theoretical studies, most investigations have focused on the G-domain. Conformational dynamics comparing WT KRAS and its different oncogenic mutants, namely, G12C, G12D, G12V, G13D, and Q61H, have been studied using microsecond long simulations, and differences were assessed using a residual contact probability network (Lu *et al.*, 2016 and Vatansever *et al.*, 2020). Simulation studies have been performed on all KRAS G12 missense mutants, and analyses were made using Markov state models (MSMs). MSMs highlight seven metastable conformational ensembles indicating different dynamic states and conformational plasticity of the flexible switch regions. MSMs also help evaluate the transition probabilities of those conformational ensembles (Husic and Pande, 2018). Comparing different oncogenic mutated KRAS variants, it appears that the dynamical shift in KRAS results in an allosteric manner, and that a mutation can rewire the crosstalk between the switch regions maneuvering the switch flexibility. However, current understanding is still not adequate to discern the driving force behind such allosteric communication and mutation-induced re-wiring mechanism, which is required for targeted inhibition of mutated KRAS.

MD simulations have also rationalized our understanding of how KRAS interacts with its effector proteins to instigate their activation process. To study the KRAS induced P13Kα activation mechanism, KRas4B and its interaction with the Ras binding domain (RBD) of PI3Kα in solution were investigated through extensive atomistic simulation of 10 μs. This study suggests that Ras recruitment shifts conformational ensemble of PI3Kα in such a way that it is likely to determine the recruitment and restriction of the PI3Kα population at the membrane (Zhang *et al.*, 2019). Recent computational modeling also provided mechanistic insight into how farnesylated/methylated KRAS4B interacts with calmodulin (CaM). Due to multiple interaction modes, various conformational ensembles of the KRas4B-CaM complex have been distinguished, effectively helping to activate PI3Kα/AKT signaling by recruiting PI3Kα to the plasma membrane (Jang *et al.*, 2019).

V. RATIONAL DRUG DISCOVERY TARGETING IDPs

As discussed above, IDPs constitute a significant portion of the human proteome, and their involvement in multiple diseases has been well documented (Uversky *et al.*, 2008 and Babu *et al.*, 2011). The pathological role of IDPs is related to their altered PTMs and their expression and lifetime in the cell since they can rewire PINs, leading to the activation of latent pathways (Babu, 2016 and Salgia and Kulkarni, 2018). Aggregation of IDPs such as the tau-protein is associated with neurodegenerative diseases. Moreover, dysregulated splicing in certain cancers such as chronic lymphocytic leukemia and colorectal carcinoma can produce novel spliced proteins that behave as IDPs (Sciarrillo *et al.*, 2020 and Romero *et al.*, 2006). These proteins are, therefore, considered promising yet challenging drug targets. Currently, some of the major obstacles involved in rational drug discovery targeting IDPs are (i) the identification of structurally stable druggable pockets (Ruan *et al.*, 2019; Joshi and Vendruscolo, 2015; and Cheng *et al.*, 2006), (ii) weak affinity of binders (Metallo, 2010), and (iii) lack of selectivity to the target (Metallo, 2010).

A. Computer aided drug discovery

Computer aided drug discovery relies on well-defined protein structures with druggable pockets that are deep, with a fair number of hydrophobic patches to facilitate partitioning of drug molecules from solvent (Volkamer *et al.*, 2012), although exceptions to these rules exist (Nisius *et al.*, 2012; and Zheng *et al.*, 2013). It is conceivable that IDPs, due to their inherent flexibility, may not fit into the above paradigm. Many IDPs show significantly higher fraction of hydrophilic residues in the sequence compared to folded proteins, implying that hydrophobic pockets that can bind drugs may be rare among IDPs. Yet, the possibility of stable hydrophobic pockets has been shown in certain IDPs such as the nuclear protein 1 (NUPR1) (Neira *et al.*, 2017). In other cases, such as the oncogenic transcription factor c-Myc, small molecules have been shown to bind to the disordered regions of the protein (Follis *et al.*, 2008 and Hammoudeh *et al.*, 2009). In the case of NUPR1, the binding compounds targeted the part of the protein sequence with lower flexibility than the rest of the protein. In a more recent study, a compound (epigallocatechin gallate or EGCG) was reported to bind to the disordered N terminal domain (NTD) of P53, which disrupted its interaction with the ligase MDM2 and stabilized P53 for enhanced antitumor activities (Zhao *et al.*, 2021). SAXS and NMR experiments showed that EGCG introduced subtle conformational changes to the P53 NTD, leading to a more compact conformational ensemble. The NMR and enhanced replica exchange MD simulations further revealed that EGCG interacts with the NTD through many dynamic contacts, as opposed to a few stable ones. Such reports regarding the interaction of small molecules with IDPs or IDRs are becoming increasingly frequent (Santofimia-Castaño *et al.*, 2020), while only a few years back, disordered proteins such as transcription factors were considered undruggable (Henley and Koehler, 2021).

In addition to small molecules, several alternative strategies have been promising in targeting IDPs. One such approach involves the use of peptide aptamers, which are short peptide sequences as part of a loop within a protein scaffold. Aptamers can be designed through a directed evolutionary process in live cells, where the aptamer sequences that result in the best desired phenotypes can be selected over several rounds of optimization. Since the aptamer sequences are constrained within a protein loop, they suffer less entropic loss upon binding compared to free peptides and, therefore, can achieve high binding affinities. Using yeast-based screening assays and in-cell NMR, the peptide aptamers were designed to bind to the disordered region of the ubiquitin-like protein Pup with nanomolar affinity (Cobbert *et al.*, 2015). These three aptamers were shown to interact with a disordered segment in Pup that folds into an alpha-helix upon binding to the partner Mpa. Despite targeting the same protein and roughly similar regions, the three aptamers led to different functional effects, underscoring the complexity in targeting IDPs for functional inhibition.

In computational drug discovery, IDPs as drug targets offer unprecedented opportunities, but with significant challenges. Some likely improvizations necessary for developing *in silico* pipelines for designing IDP binders are (i) methods to account for entropy loss upon binding of drugs, (ii) machine learning approaches, (iii) consideration of folding upon binding of IDPs to partner proteins, and (iv) application of MD, enhanced MD and NMR generated protein ensembles in the binder screening, among others. Recently, a virtual

screening method was reported that utilized the mechanism of disorder-to-order transition in IDPs to screen for inhibitors (Na *et al.*, 2020). The method involves generating short (20 AA) peptide conformations from the IDP region that undergoes order-to-disorder transition and docking small molecule libraries to these peptide conformations. Using a discriminatory score that combines peptide-compound interactions with peptide structural stability, the authors successfully identified the known inhibitor for the proto-oncogene Myc from among thousands of negatives.

B. *In silico* strategy to design peptide sequences

In contrast to the disorder-to-order mechanism, where a specific segment of the IDP interacts with the partner protein, many IDPs remain disordered upon binding to the partner (Freiberger *et al.*, 2021). In such cases, multiple residues in the disordered segment typically form transient contacts with a focused region in the partner protein, which is normally folded. This mechanism is referred to as the many-to-one mode of interaction. Such interactions can be targeted for inhibitor design, if the interaction hotspots in the partner protein cavity are known and the IDP conformations that interact with the partner protein can be resolved using NMR, MD, or other approaches. Recently, utilizing the above-described principle, Bhattacharya and co-workers designed an inhibitory peptide for the carbohydrate binding protein galectin-3 (Bhattacharya *et al.*, 2021). Galectin-3 consists of a disordered N terminal domain (NTD) that interacts with a folded C terminal domain (CTD). By combining accelerated MD simulations of full-length galectin-3 with available chemical shift perturbations, the ensemble of NTD that interacts with the CTD was determined. This ensemble was used to derive peptide scaffolds, and a hierarchical *in silico* strategy was used to design peptide sequences that were predicted to disrupt the NTD–CTD interaction. The sensitivity of this approach was demonstrated where one out of only three tested peptides was found to be a positive hit. Such approaches can be easily applied to other IDPs, which bind to folded partners, for which experimental structural data in the form of NMR or SAXS are available. Moreover, the inhibitory peptide sequences and their bound structures can be used to construct pharmacophores for searching large libraries of small molecules for potential lead compounds.

The purpose of a therapeutic agent is to modulate the biological function of its target. Since IDPs are multi-functional proteins, targeting IDPs requires the consideration not only of the thermodynamic and structural aspects of the drug binding, but also of the specific function, that is, being targeted. Examples include targeting a transcription factor for disrupting DNA binding vs preventing phosphorylation via interaction with kinases or inhibiting the formation of liquid–liquid separated granules. A single IDP can carry out each of these functions, through different structural regions. Targeting such specific functions not only improves the selectivity of the therapeutic agent but also requires deeper structural understanding of the mechanisms governing IDPs. Rational designing of agents targeting an IDP and its specific function should, therefore, begin with the accurate determination of the segment of the protein sequence, that is, responsible for the functional effect. Next, a variety of methods can be applied to search for binders, which can include both *in silico* methods and directed evolution to find aptamers. Another promising avenue is to search for small molecule fragments (molecular weight < 200 Da) that bind to the region of interest using high throughput screening methods such as

differential scanning fluorimetry, solution small angle x ray scattering, and isothermal titration calorimetry (Murray and Rees, 2009). The most promising fragments can then be linked together through appropriate linkers to develop drug molecules with high affinity. Such approaches have been proposed as viable avenues for targeting IDP related pathologies, such as aggregation (Joshi *et al.*, 2016).

VI. CONCLUSIONS AND FUTURE DIRECTIONS

Since their discovery >20 years ago (see Dyson and Wright, 2019 and Uversky and Kulkarni, 2021, for historical accounts), there has been an explosion in the IDP field. Almost 10 000 papers dedicated to IDPs were published by the end of 2021 with more than 350 000 citations (Fig. 7). These statistics confirm the increased attention that the IDPs have attracted and will undoubtedly continue to do so in the years to come. The challenges they pose have led to new thinking such as IDPs and dynamical systems theory (Uversky, 2014 and Kulkarni, 2020) and new technical advances such as mass spectrometry technologies for protein structure analysis, "footprinting" studies, and cryo-electron microscopy (Nwanochie and Uversky, 2019 and Chance *et al.*, 2020). Concomitant with these developments, we have also seen significant advances in computational methods such as new developments in force field strategies (Huang and MacKerell, 2018; Masetti *et al.*, 2020; Hsu *et al.*, 2020; Ahmed *et al.*, 2020; Mu *et al.*, 2021;
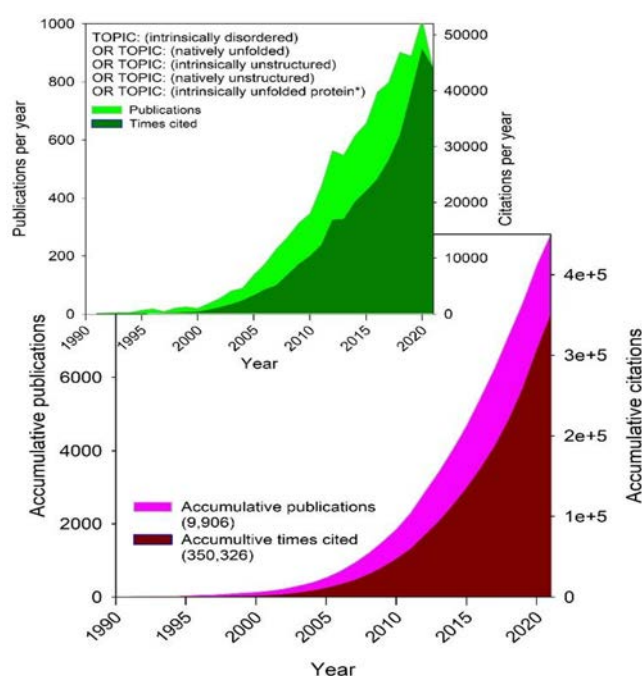

FIG. 7. An explosion in the protein intrinsic disorder literature. The plots represent the time-courses of the increase in the number of publications dealing with the intrinsic disorder and the number of papers citing those publications (inset). Plot shows total publications per year and accumulative number of publications. Inset shows the data for the sum of times cited per year and accumulative sum of times cited. Data for these plots were retrieved from Web of Science on November 17, 2021 using the following search criteria: TOPIC: (intrinsically disordered) OR TOPIC: (natively unfolded) OR TOPIC: (intrinsically unstructured) OR TOPIC: (natively unstructured) OR TOPIC: (intrinsically unfolded protein).

Wang, 2021; and Gopal *et al.*, 2021) and physics-based computational and theoretical approaches (Shea *et al.*, 2021 and Sieradzan *et al.*, 2021).

Aside from the biological functions discussed here, IDPs are important constituents of proteinaceous membrane-less organelles (PMLOs). PMLOs are formed by liquid–liquid phase separation when a polypeptide coalesces into a dense phase in an aqueous solution (Uversky, 2021). PMLOs play important roles in myriad cellular processes from responding to stress to transcriptional regulation of gene expression. Furthermore, it is also postulated that PMLOs very likely played a critical role in prebiotic evolution of the predecessor of the first universal common ancestor (Kulkarni and Uversky, 2018b). We suspect that these aspects of the IDPs would be intensely investigated going forward. Finally, since several proteins associated with drug-resistance in cancer and prion proteins associated with neurodegenerative disease are IDPs (Kulkarni and Uversky, 2019 and Salahuddin *et al.*, 2021), a deeper understanding of IDPs can help better understand their role in phenotypic switching and adaptive evolution via non-genetic, protein-based mechanisms (Kulkarni, 2020).

To further inspire work on IDPs, we put forth the Janus challenge (Kulkarni and Uversky, 2018b). We believe that meeting this challenge is likely to lead to technological advances with important biomedical applications. Finally, IDPs, for example, c-Myc and KRAS, that were once considered as "undruggable" are emerging as favorite therapeutic targets. Thus, it is very likely that IDPs including many transcription factors (Tsafou *et al.*, 2018), will be targeted for therapeutic development. The availability of several dedicated databases to the community that house a wealth of information related to IDPs (Hatos *et al.*, 2020; Lazar *et al.*, 2021; Piovesan *et al.*, 2021; and Quaglia *et al.*, 2021) as well as powerful tools to analyze big data that are designed using machine learning and artificial intelligence (Katuwawala *et al.*, 2019; Ramanathan *et al.*, 2021; Lindorff-Larsen and Kragelund, 2021; and Strodel, 2021) should help realize the full potential of IDPs.

Although IDPs are incorrectly perceived to lack structure and, hence, presumed to defy Anfinsen's dogma, IDPs are not random coils but exist as conformational ensembles. However, IDP ensembles have conformational preferences. Therefore, IDPs do have "structure" (or a set of interconverting structures), albeit subtle perhaps, at the limit of Anfinsen's dogma. In fact, being dynamical multifunctional systems, IDPs represent a logical extension to the Anfinsen's dogma, since different members of their conformational ensembles might have different functions.

ACKNOWLEDGMENTS

AUTHOR DECLARATIONS

Conflict of Interest

The authors declare no conflict of interest.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Abraham, S. J., Muhamed, I., Nolet, R., Yeung, F., and Gaponenko, V., "Expression, purification, and characterization of soluble K-Ras4B for structural analysis," Protein Expression Purif. **73**(2), 125–131 (2010).

Adamski, W., Salvi, N., Maurin, D., Magnat, J., Milles, S., Jensen, M. R., Abyzov, A., Moreau, C. J., and Blackledge, M., "A unified description of intrinsically disordered protein dynamics under physiological conditions using NMR spectroscopy," J. Am. Chem. Soc. **141**(44), 17817–17829 (2019).

Ahearn, I., Zhou, M., and Philips, M. R., "Posttranslational modifications of RAS proteins," Cold Spring Harbor Perspect. Med. **8**(11), a031484 (2018).

Ahmed, M. C., Crehuet, R., and Lindorff-Larsen, K., "Computing, analyzing, and comparing the radius of gyration and hydrodynamic radius in conformational ensembles of intrinsically disordered proteins," Methods Mol. Biol. **2141**, 429–445 (2020).

Ahmed, M. C., Skaanning, L. K., Jussupow, A., Newcombe, E. A., Kragelund, B. B., Camilloni, C., Langkilde, A. E., and Lindorff-Larsen, K., "Refinement of α-Synuclein ensembles against SAXS data: Comparison of force fields and methods," Front. Mol. Biosci. **8**, 654333 (2021).

Alanen, H. I., Raykhel, I. B., Luukas, M. J., Salo, K. E., and Ruddock, L. W., "Beyond KDEL: The role of positions 5 and 6 in determining ER localization," J. Mol. Biol. **409**(3), 291–297 (2011).

AMG 510, "AMG 510 first to inhibit 'Undruggable' KRAS," Cancer Discovery **9**(8), 988–989 (2019).

Andresen, C., Helander, S., Lemak, A., Farès, C., Csizmok, V., Carlsson, J., Penn, L. Z., Forman-Kay, J. D., Arrowsmith, C. H., Lundström, P., and Sunnerhagen, M., "Transient structure and dynamics in the disordered c-Myc transactivation domain affect Bin1 binding," Nucl. Acids Res. **40**(13), 6353–6366 (2012).

Anfinsen, C. B., "Principles that govern the folding of protein chains," Science **181**(4096), 223–230 (1973).

Anfinsen, C. B., Haber, E., Sela, M., and White, F. H., Jr.,"The kinetics of formation of native ribonuclease during oxidation of the reduced polypeptide chain," Proc. Natl. Acad. Sci. U.S.A. **47**(9), 1309–1314 (1961).

Anose, B. M., and Sanders, M. M., "Androgen receptor regulates transcription of the ZEB1 transcription factor," Int. J. Endocrinol. **2011**, 903918.

Appadurai, R., Nagesh, J., and Srivastava, A., "High resolution ensemble description of metamorphic and intrinsically disordered proteins using an efficient hybrid parallel tempering scheme," Nat. Commun. **12**(1), 958 (2021).

Arai, M., Sugase, K., Dyson, H. J., and Wright, P. E., "Conformational propensities of intrinsically disordered proteins influence the mechanism of binding and folding," Proc. Natl. Acad. Sci. U.S.A. **112**(31), 9614–9619 (2015).

Araki, M., Shima, F., Yoshikawa, Y., Muraoka, S., Ijiri, Y., Nagahara, Y., Shirono, T., Kataoka, T., and Tamura, A., "Solution structure of the state 1 conformer of GTP-bound H-Ras protein and distinct dynamic properties between the state 1 and state 2 conformers," J. Biol. Chem. **286**(45), 39644–39653 (2011).

Austin, R. H., Xie, A., Fu, D., Warren, W. W., Redlich, B., and van der Meer, L., "Tilting after Dutch windmills: Probably no long-lived Davydov solitons in proteins," J. Biol. Phys. **35**(1), 91–101 (2009).

Awasthi, S., and Nair, N. N., "Exploring high dimensional free energy landscapes: Temperature accelerated sliced sampling," J. Chem. Phys. **146**, 094108 (2017).

Azpeitia, E., Balanzario, E. P., and Wagner, A., "Signaling pathways have an inherent need for noise to acquire information," BMC Bioinf. **21**(1), 462 (2020).

Babu, M. M., "The contribution of intrinsically disordered regions to protein function, cellular complexity, and human disease," Biochem. Soc. Trans. **44**(5), 1185–1200 (2016).

Babu, M. M., van der Lee, R., de Groot, N. S., and Gsponer, J., "Intrinsically disordered proteins: Regulation and disease," Curr. Opin. Struct. Biol. **21**(3), 432–440 (2011).

Balázsi, G., van Oudenaarden, A., and Collins, J. J., "Cellular decision making and biological noise: From microbes to mammals," Cell **144**(6), 910–925 (2011).

Balcerowicz, M., "A new order through disorder: Intrinsically disordered proteins reshape the cytoskeleton under drought stress," Plant Physiol. **183**(2), 425–426 (2020).

Barabási, A. L., "Scale-free networks: A decade and beyond," Science **325**(5939), 412–413 (2009).

Barabasi, A. L., and Albert, R., "Emergence of scaling in random networks," Science **286**(5439), 509–512 (1999).

Barabási, A. L., and Oltvai, Z. N., "Network biology: Understanding the cell's functional organization," Nat. Rev. Genet. **5**(2), 101–113 (2004).

Barklis, E., Stephen, A. G., Staubus, A. O., Barklis, R. L., and Alfadhli, A., "Organization of farnesylated, carboxymethylated KRAS4B on membranes," J. Mol. Biol. **431**(19), 3706–3717 (2019).

Baul, U., Chakraborty, D., Mugnai, M. L., Straub, J. E., and Thirumalai, D., "Sequence effects on size, shape, and structural heterogeneity in intrinsically disordered proteins," J. Phys. Chem. B **123**(16), 3462–3474 (2019).

Baumketner, A., and Shea, J. E., "The structure of the alzheimer amyloid beta 10–35 peptide probed through replica-exchange molecular dynamics simulations in explicit solvent," J. Mol. Biol. **366**(1), 275–285 (2007).

Bax, A., and Clore, G. M., "Protein NMR: Boundless opportunities," J. Magn. Reson. **306**, 187–191 (2019).

Berlow, R. B., Dyson, H. J., and Wright, P. E., "Functional advantages of dynamic protein disorder," FEBS Lett. **589**(19 Pt A), 2433–2440 (2015).

Berlow, R. B., Dyson, H. J., and Wright, P. E., "Hypersensitive termination of the hypoxic response by a disordered protein switch," Nature **543**(7645), 447–451 (2017).

Berlow, R. B., Dyson, H. J., and Wright, P. E., "Expanding the paradigm: Intrinsically disordered proteins and allosteric regulation," J. Mol. Biol. **430**(16), 2309–2320 (2018).

Bernadó, P., and Svergun, D. I., "Structural analysis of intrinsically disordered proteins by small-angle X-ray scattering," Mol. Biosyst. **8**(1), 151–167 (2012).

Best, R. B., "Computational and theoretical advances in studies of intrinsically disordered proteins," Curr. Opin. Struct. Biol. **42**, 147–154 (2017).

Best, R. B., and Hummer, G., "Microscopic interpretation of folding ϕ-values using the transition path ensemble," Proc. Natl. Acad. Sci. U.S.A. **113**(12), 3263–3268 (2016).

Best, R. B., Zheng, W., and Mittal, J., "Balanced protein-water interactions improve properties of disordered proteins and non-specific protein association," J. Chem. Theory Comput. **10**(11), 5113–5124 (2014).

Bhattacharya, S., and Lin, X., "Recent advances in computational protocols addressing intrinsically disordered proteins," Biomolecules **9**(4), 146 (2019).

Bhattacharya, S., Zhang, M., Hu, W., Qi, T., and Heisterkamp, N., "Targeting interactions between the Galectin-3 intrinsically disordered and structured domains based on long time-scale accelerated molecular dynamics," arXiv:10.1101/2021.09.27.461147 (2021).

Bhowmick, P., Guharoy, M., and Tompa, P., "Bioinformatics approaches for predicting disordered protein motifs," Adv. Exp. Med. Biol. **870**, 291–318 (2015).

Biankin, A. V., Waddell, N., Kassahn, K. S., Gingras, M. C., Muthuswamy, L. B., Johns, A. L., Miller, D. K., Wilson, P. J., Patch, A. M., Wu, J., Chang, D. K., Cowley, M. J., Gardiner, B. B., Song, S., Harliwong, I., Idrisoglu, S., Nourse, C., Nourbakhsh, E., Manning, S., Wani, S., Gongora, M., Pajic, M., Scarlett, C. J., Gill, A. J., Pinho, A. V., Rooman, I., Anderson, M., Holmes, O., Leonard, C., Taylor, D., Wood, S., Xu, Q., Nones, K., Fink, J. L., Christ, A., Bruxner, T., Cloonan, N., Kolle, G., Newell, F., Pinese, M., Mead, R. S., Humphris, J. L., Kaplan, W., Jones, M. D., Colvin, E. K., Nagrial, A. M., Humphrey, E. S., Chou, A., Chin, V. T., Chantrill, L. A., Mawson, A., Samra, J. S., Kench, J. G., Lovell, J. A., Daly, R. J., Merrett, N. D., Toon, C., Epari, K., Nguyen, N. Q., Barbour, A., Zeps, N., Kakkar, N., Zhao, F., Wu, Y. Q., Wang, M., Muzny, D. M., Fisher, W. E., Brunicardi, F. C., Hodges, S. E., Reid, J. G., Drummond, J., Chang, K., Han, Y., Lewis, L. R., Dinh, H., Buhay, C. J., Beck, T., Timms, L., Sam, M., Begley, K., Brown, A., Pai, D., Panchal, A., Buchner, N., De Borja, R., Denroche, R. E., Yung, C. K., Serra, S., Onetto, N., Mukhopadhyay, D., Tsao, M. S., Shaw, P. A., Petersen, G. M., Gallinger, S., Hruban, R. H., Maitra, A., Iacobuzio-Donahue, C. A., Schulick, R. D., Wolfgang, C. L., Morgan, R. A., Lawlor, R. T., Capelli, P., Corbo, V., Scardoni, M., Tortora, G., Tempero, M. A., Mann, K. M., Jenkins, N. A., Perez-Mancera, P. A., Adams, D. J., Largaespada, D. A., Wessels, L. F., Rust, A. G., Stein, L. D., Tuveson, D. A., Copeland, N. G., Musgrove, E. A., Scarpa, A., Eshleman, J. R., Hudson, T. J., Sutherland, R. L., Wheeler, D. A., Pearson, J. V., McPherson, J. D., Gibbs, R. A., Grimmond, S. M, and Australian Pancreatic Cancer Genome Initiative, "Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes," Nature **491**(7424), 399–405 (2012).

Boehr, D. D., Nussinov, R., and Wright, P. E., "The role of dynamic conformational ensembles in biomolecular recognition," Nat. Chem. Biol. **5**(11), 789–796 (2009).

Bonetti, D., Troilo, F., Brunori, M., Longhi, S., and Gianni, S., "How robust is the mechanism of folding-upon-binding for an intrinsically disordered protein?," Biophys. J. **114**(8), 1889–1894 (2018).

Bonomi, M., Heller, G. T., Camilloni, C., and Vendruscolo, M., "Principles of protein structural ensemble determination," Curr. Opin. Struct. Biol. **42**, 106–116 (2017).

Bonomi, M., and Vendruscolo, M., "Determination of protein structural ensembles using cryo-electron microscopy," Curr. Opin. Struct. Biol. **56**, 37–45 (2019).

Boothby, T. C., Tapia, H., Brozena, A. H., Piszkiewicz, S., Smith, A. E., Giovannini, I., Rebecchi, L., Pielak, G. J., Koshland, D., and Goldstein, B., "Tardigrades use intrinsically disordered proteins to survive desiccation," Mol. Cell **65**(6), 975–984 (2017).

Borgia, A., Borgia, M. B., Bugge, K., Kissling, V. M., Heidarsson, P. O., Fernandes, C. B., Sottini, A., Soranno, A., Buholzer, K. J., Nettels, D., Kragelund, B. B., Best, R. B., and Schuler, B., "Extreme disorder in an ultrahigh-affinity protein complex," Nature **555**(7694), 61–66 (2018).

Borgia, A., Zheng, W., Buholzer, K., Borgia, M. B., Schüler, A., Hofmann, H., Soranno, A., Nettels, D., Gast, K., Grishaev, A., Best, R. B., and Schuler, B., "Consistent view of polypeptide chain expansion in chemical denaturants from multiple experimental methods," J. Am. Chem. Soc. **138**(36), 11714–11726 (2016).

Bos, J. L., Rehmann, H., and Wittinghofer, A., "GEFs and GAPs: Critical elements in the control of small G proteins," Cell **129**(5), 865–877 (2007).

Bottaro, S., and Lindorff-Larsen, K., "Biophysical experiments and biomolecular simulations: A perfect match?," Science **361**(6400), 355–360 (2018).

Brocca, S., Grandori, R., Longhi, S., and Uversky, V., "Liquid-liquid phase separation by intrinsically disordered protein regions of viruses: Roles in viral life cycle and control of virus-host interactions," Int. J. Mol. Sci. **21**(23), 9045 (2020).

Brock, A., Chang, H., and Huang, S., "Non-genetic heterogeneity—A mutation-independent driving force for the somatic evolution of tumours," Nat. Rev. Genet. **10**(5), 336–342 (2009).

Brodsky, S., Mittelman, J. T., Chapal, K., Kumar, M., Carmi, D. K., and Barkai, N., "Intrinsically disordered regions direct transcription factor *in vivo* binding specificity," Mol. Cell **79**(3), 459–471 (2020).

Brown, A. H., Rodger, P. M., Evans, J. S., and Walsh, T. R., "Equilibrium conformational ensemble of the intrinsically disordered peptide n16N: Linking subdomain structures and function in nacre," Biomacromolecules **15**(12), 4467–4479 (2014).

Bryngelson, J. D., and Wolynes, P. G., "Spin glasses and the statistical mechanics of protein folding," Proc. Natl. Acad. Sci. U.S.A. **84**(21), 7524–7528 (1987).

Bryngelson, J. D., Onuchic, J. N., Socci, N. D., and Wolynes, P. G., "Funnels, pathways, and the energy landscape of protein folding: A synthesis," Proteins **21**(3), 167–195 (1995).

Bugge, K., Brakti, I., Fernandes, C. B., Dreier, J. E., Lundsgaard, J. E., Olsen, J. G., Skriver, K., and Kragelund, B. B., "Interactions by disorder—A matter of context," Front. Mol. Biosci. **7**, 110 (2020).

Buhrman, G., Holzapfel, G., Fetics, S., and Mattos, C., "Allosteric modulation of Ras positions Q61 for a direct role in catalysis," Proc. Natl. Acad. Sci. U.S.A. **107**(11), 4931–4936 (2010).

Buhrman, G., Kumar, V. S., Cirit, M., Haugh, J. M., and Mattos, C., "Allosteric modulation of Ras-GTP is linked to signal transduction through RAF kinase," J. Biol. Chem. **286**(5), 3323–3331 (2011).

Buljan, M., Chalancon, G., Dunker, A. K., Bateman, A., Balaji, S., Fuxreiter, M., and Babu, M. M., "Alternative splicing of intrinsically disordered regions and rewiring of protein interactions," Curr. Opin. Struct. Biol. **23**(3), 443–450 (2013).

Burger, V. M., Nolasco, D. O., and Stultz, C. M., "Expanding the range of protein function at the far end of the order-structure continuum," J. Biol. Chem. **291**(13), 6706–6713 (2016).

Buske, P. J., Mittal, A., Pappu, R. V., and Levin, P. A., "An intrinsically disordered linker plays a critical role in bacterial cell division," Semin. Cell Dev. Biol. **37**, 3–10 (2015).

Camilloni, C., De Simone, A., Vranken, W. F., and Vendruscolo, M., "Determination of secondary structure populations in disordered states of proteins using nuclear magnetic resonance chemical shifts," Biochemistry **51**(11), 2224–2231 (2012).

Canon, J., Rex, K., Saiki, A. Y., Mohr, C., Cooke, K., Bagal, D., Gaida, K., Holt, T., Knutson, C. G., Koppada, N., Lanman, B. A., Werner, J., Rapaport, A. S., San Miguel, T., Ortiz, R., Osgood, T., Sun, J. R., Zhu, X., McCarter, J. D., Volak, L. P., Houk, B. E., Fakih, M. G., O'Neil, B. H., Price, T. J., Falchook, G. S., Desai, J., Kuo, J., Govindan, R., Hong, D. S., Ouyang, W., Henary, H., Arvedson, T., Cee, V. J., and Lipford, J. R., "The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity," Nature **575**(7781), 217–223 (2019).

Casuso, I., Redondo-Morata, L., and Rico, F., "Biological physics by high-speed atomic force microscopy," Philos. Trans. R. Soc. A **378**(2186), 20190604 (2020).

Cavalli, A., Camilloni, C., and Vendruscolo, M., "Molecular dynamics simulations with replica-averaged structural restraints generate structural ensembles according to the maximum entropy principle," J. Chem. Phys. **138**(9), 094112 (2013).

Chakrabortee, S., Byers, J. S., Jones, S., Garcia, D. M., Bhullar, B., Chang, A., She, R., Lee, L., Fremin, B., Lindquist, S., and Jarosz, D. F., "Intrinsically disordered proteins drive emergence and inheritance of biological traits," Cell **167**(2), 369–381 (2016).

Chakrabortee, S., Meersman, F., Kaminski Schierle, G. S., Bertoncini, C. W., McGee, B., Kaminski, C. F., and Tunnacliffe, A., "Catalytic and chaperone-like functions in an intrinsically disordered protein associated with desiccation tolerance," Proc. Natl. Acad. Sci. U.S.A. **107**(37), 16084–16089 (2010).

Chance, M. R., Farquhar, E. R., Yang, S., Lodowski, D. T., and Kiselar, J., "Protein footprinting: Auxiliary engine to power the structural biology revolution," J. Mol. Biol. **432**(9), 2973–2984 (2020).

Chan-Yao-Chong, M., Durand, D., and Ha-Duong, T., "Molecular dynamics simulations combined with nuclear magnetic resonance and/or small-angle x-ray scattering data for characterizing intrinsically disordered protein conformational ensembles," J. Chem. Inf. Model. **59**(5), 1743–1758 (2019).

Chavan, T. S., Jang, H., Khavrutskii, L., Abraham, S. J., Banerjee, A., Freed, B. C., Johannessen, L., Tarasov, S. G., Gaponenko, V., Nussinov, R., and Tarasova, N. I., "High-affinity interaction of the K-Ras4B hypervariable region with the Ras active site," Biophys. J. **109**(12), 2602–2613 (2015).

Chen, Y., Toth, E. A., Ruan, B., Choi, E. J., Simmerman, R., Chen, Y., He, Y., Wang, R., Godoy-Ruiz, R., King, H., Custer, G., Travis Gallagher, D., Rozak, D. A., Solomon, M., Muro, S., Weber, D. J., Orban, J., Fuerst, T. R., and Bryan, P. N., "Engineering subtilisin proteases that specifically degrade active RAS," Commun. Biol. **4**(1), 299 (2021).

Cheng, Y., LeGall, T., Oldfield, C. J., Mueller, J. P., Van, Y. Y., Romero, P., Cortese, M. S., Uversky, V. N., and Dunker, A. K., "Rational drug design via intrinsically disordered protein," Trends Biotechnol. **24**(10), 435–442 (2006).

Cheng, Y., Oldfield, C. J., Meng, J., Romero, P., Uversky, V. N., and Dunker, A. K., "Mining alpha-helix-forming molecular recognition features with cross species sequence alignments," Biochemistry **46**(47), 13468–13477 (2007).

Chodera, J. D., and Noé, F., "Markov state models of biomolecular conformational dynamics," Curr. Opin. Struct. Biol. **25**, 135–144 (2014).

Choi, J. H., Laurent, A. H., Hilser, V. J., and Ostermeier, M., "Design of protein switches based on an ensemble model of allostery," Nat. Commun. **6**, 6968 (2015).

Choi, U. B., McCann, J. J., Weninger, K. R., and Bowen, M. E., "Beyond the random coil: Stochastic conformational switching in intrinsically disordered proteins," Structure **19**(4), 566–576 (2011).

Choi, U. B., Sanabria, H., Smirnova, T., Bowen, M. E., and Weninger, K. R., "Spontaneous switching among conformational ensembles in intrinsically disordered proteins," Biomolecules **9**(3), 114 (2019).

Chung, K. M., Kolling, F. W. IV, Gajdosik, M. D., Burger, S., Russell, A. C., and Nelson, C. E., "Single cell analysis reveals the stochastic phase of reprogramming to pluripotency is an ordered probabilistic process," PLoS One **9**(4), e95304 (2014).

Chung, J. K., Lee, Y. K., Denson, J. P., Gillette, W. K., Alvarez, S., Stephen, A. G., and Groves, J. T., "K-Ras4B remains monomeric on membranes over a wide range of surface densities and lipid compositions," Biophys. J. **114**(1), 137–145 (2018).

Chung, H. S., Louis, J. M., and Eaton, W. A., "Experimental determination of upper bound for transition path times in protein folding from single-molecule photon-by-photon trajectories," Proc. Natl. Acad. Sci. U.S.A. **106**(29), 11837–11844 (2009).

Clausen, R., Ma, B., Nussinov, R., and Shehu, A., "Mapping the conformation space of wildtype and mutant H-Ras with a memetic, cellular, and multiscale evolutionary algorithm," PLoS Comput. Biol. **11**(9), e1004470 (2015).

Cobbert, J. D., DeMott, C., Majumder, S., Smith, E. A., Reverdatto, S., Burz, D. S., McDonough, K. A., and Shekhtman, A., "Caught in action: Selecting peptide aptamers against intrinsically disordered proteins in live cells," Sci. Rep. **5**, 9402 (2015).

Colicelli, J., "Human RAS superfamily proteins and related GTPases," Sci. STKE **2004**(250), RE13.

Cordeiro, T. N., Herranz-Trillo, F., Urbanek, A., Estaña, A., Cortés, J., Sibille, N., and Bernadó, P., "Structural characterization of highly flexible proteins by small-angle scattering," Adv. Exp. Med. Biol. **1009**, 107–129 (2017).

Cornish, J., Chamberlain, S. G., Owen, D., and Mott, H. R., "Intrinsically disordered proteins and membranes: A marriage of convenience for cell signalling?," Biochem. Soc. Trans. **48**(6), 2669–2689 (2020).

Covarrubias, A. A., Cuevas-Velazquez, C. L., Romero-Pérez, P. S., Rendón-Luna, D. F., and Chater, C. C. C., "Structural disorder in plant proteins: Where plasticity meets sessility," Cell. Mol. Life Sci. **74**(17), 3119–3147 (2017).

Cox, A. D., and Der, C. J., "Ras history: The saga continues," Small GTPases **1**(1), 2–27 (2010).

Csermely, P., Kunsic, N., Mendik, P., Kerestély, M., Faragó, T., Veres, D. V., and Tompa, P., "Learning of signaling networks: Molecular mechanisms," Trends Biochem. Sci. **45**(4), 284–294 (2020).

Csermely, P., Palotai, R., and Nussinov, R., "Induced fit, conformational selection and independent dynamic segments: An extended view of binding events," Trends Biochem. Sci. **35**(10), 539–546 (2010).

Das, P., Matysiak, S., and Mittal, J., "Looking at the disordered proteins through the computational microscope," ACS Cent. Sci. **4**(5), 534–542 (2018).

Davey, N. E., Van Roey, K., Weatheritt, R. J., Toedt, G., Uyar, B., Altenberg, B., Budd, A., Diella, F., Dinkel, H., and Gibson, T. J., "Attributes of short linear motifs," Mol. Biosyst. **8**(1), 268–281 (2012).

DeForte, S., and Uversky, V. N., "Order, disorder, and everything in between," Molecules **21**(8), 1090 (2016).

Deiana, A., Forcelloni, S., Porrello, A., and Giansanti, A., "Intrinsically disordered proteins and structured proteins with intrinsically disordered regions have different functional roles in the cell," PLoS One **14**(8), e0217889 (2019).

Diaz, J. F., Wroblowski, B., Schlitter, J., and Engelborghs, Y., "Calculation of pathways for the conformational transition between the GTP- and GDP-bound states of the Ha-ras-p21 protein: Calculations with explicit solvent simulations and comparison with calculations in vacuum," Proteins **28**(3), 434–451 (1997).

Di Domenico, T., Walsh, I., and Tosatto, S. C., "Analysis and consensus of currently available intrinsic protein disorder annotation sources in the MobiDB database," BMC Bioinf. **14**(Suppl 7), S3 (2013).

Diernfellner, A. C. R., and Brunner, M., "Phosphorylation timers in the neurospora crassa circadian clock," J. Mol. Biol. **432**(12), 3449–3465 (2020).

Do, T. N., Choy, W. Y., and Karttunen, M., "Accelerating the conformational sampling of intrinsically disordered proteins," J. Chem. Theory Comput. **10**(11), 5081–5094 (2014).

Dokholyan, N. V., "Experimentally-driven protein structure modeling," J. Proteomics **220**, 103777 (2020).

Dong, P., Fan, Y., Sun, J., Lv, M., Yi, M., Tan, X., and Liu, S., "A dynamic interaction process between KaiA and KaiC is critical to the cyanobacterial circadian oscillator," Sci. Rep. **6**, 25129 (2016).

Dosztányi, Z., Chen, J., Dunker, A. K., Simon, I., and Tompa, P., "Disorder and sequence repeats in hub proteins and their implications for network evolution," J. Proteome Res. **5**(11), 2985–2995 (2006).

Dunker, A. K., Babu, M. M., Barbar, E., Blackledge, M., Bondos, S. E., Dosztányi, Z., Dyson, H. J., Forman-Kay, J., Fuxreiter, M., Gsponer, J., Han, K. H., Jones, D. T., Longhi, S., Metallo, S. J., Nishikawa, K., Nussinov, R., Obradovic, Z., Pappu, R. V., Rost, B., Selenko, P., Subramaniam, V., Sussman, J. L., Tompa, P., and Uversky, V. N., "What's in a name? Why these proteins are intrinsically disordered," Intrinsically Disord. Proteins **1**(1), e24157 (2013).

Dunker, A. K., Brown, C. J., Lawson, J. D., Iakoucheva, L. M., and Obradović, Z., "Intrinsic disorder and protein function," Biochemistry **41**(21), 6573–6582 (2002).

Dunker, A. K., Cortese, M. S., Romero, P., Iakoucheva, L. M., and Uversky, V. N., "Flexible nets: The roles of intrinsic disorder in protein interaction networks," FEBS J. **272**(20), 5129–5148 (2005).

Dunker, A. K., Lawson, J. D., Brown, C. J., Williams, R. M., Romero, P., Oh, J. S., Oldfield, C. J., Campen, A. M., Ratliff, C. M., Hipps, K. W., Ausio, J., Nissen, M. S., Reeves, R., Kang, C., Kissinger, C. R., Bailey, R. W., Griswold, M. D., Chiu, W., Garner, E. C., and Obradovic, Z., "Intrinsically disordered protein," J. Mol. Graphics Modell. **19**(1), 26–59 (2001).

Dunker, A. K., Obradovic, Z., Romero, P., Garner, E. C., and Brown, C. J., "Intrinsic protein disorder in complete genomes," Genome Inf. **11**, 161–171 (2000).

Dyson, H. J., "Expanding the proteome: Disordered and alternatively folded proteins," Q. Rev. Biophys. **44**(4), 467–518 (2011).

Dyson, H. J., and Wright, P. E., "Coupling of folding and binding for unstructured proteins," Curr. Opin. Struct. Biol. **12**(1), 54–60 (2002).

Dyson, H. J., and Wright, P. E., "Intrinsically unstructured proteins and their functions," Nat. Rev. Mol. Cell. Biol. **6**(3), 197–208 (2005).

Dyson, H. J., and Wright, P. E., "Perspective: The essential role of NMR in the discovery and characterization of intrinsically disordered proteins," J. Biomol. NMR **73**(12), 651–659 (2019).

Dyson, H. J., and Wright, P. E., "NMR illuminates intrinsic disorder," Curr. Opin. Struct. Biol. **70**, 44–52 (2021).

Edwards, Y. J., Lobley, A. E., Pentony, M. M., and Jones, D. T., "Insights into the regulation of intrinsically disordered proteins in the human proteome by analyzing sequence and gene expression data," Genome Biol. **10**(5), R50 (2009).

Eldar, A., and Elowitz, M. B., "Functional roles for noise in genetic circuits," Nature **467**(7312), 167–173 (2010).

Fawzi, N. L., Phillips, A. H., Ruscio, J. Z., Doucleff, M., Wemmer, D. E., and Head-Gordon, T., "Structure and dynamics of the Abeta(21–30) peptide from the interplay of NMR experiments and molecular simulations," J. Am. Chem. Soc. **130**(19), 6145–6158 (2008).

Ferreon, A. C., Ferreon, J. C., Wright, P. E., and Deniz, A. A., "Modulation of allostery by protein intrinsic disorder," Nature **498**(7454), 390–394 (2013).

Ferreiro, D. U., Komives, E. A., and Wolynes, P. G., "Frustration, function and folding," Curr. Opin. Struct. Biol. **48**, 68–73 (2018).

Fersht, A. R., "Optimization of rates of protein folding: The nucleation-condensation mechanism and its implications," Proc. Natl. Acad. Sci. U.S.A. **92**(24), 10869–10873 (1995).

Fetics, S. K., Guterres, H., Kearney, B. M., Buhrman, G., Ma, B., Nussinov, R., and Mattos, C., "Allosteric effects of the oncogenic RasQ61L mutant on Raf-RBD," Structure **23**(3), 505–516 (2015).

Fisette, O., Lagüe, P., Gagné, S., and Morin, S., "Synergistic applications of MD and NMR for the study of biological systems," J. Biomed. Biotechnol. **2012**, 254208.

Follis, A. V., Hammoudeh, D. I., Wang, H., Prochownik, E. V., and Metallo, S. J., "Structural rationale for the coupled binding and unfolding of the c-Myc oncoprotein by small molecules," Chem. Biol. **15**(11), 1149–1155 (2008).

Fonin, A. V., Darling, A. L., Kuznetsova, I. M., Turoverov, K. K., and Uversky, V. N., "Multi-functionality of proteins involved in GPCR and G protein signaling: Making sense of structure-function continuum with intrinsic disorder-based proteoforms," Cell. Mol. Life Sci. **76**(22), 4461–4492 (2019).

Frauenfelder, H., Sligar, S. G., and Wolynes, P. G., "The energy landscapes and motions of proteins," Science **254**(5038), 1598–1603 (1991).

Freiberger, M. I., Wolynes, P. G., Ferreiro, D. U., and Fuxreiter, M., "Frustration in fuzzy protein complexes leads to interaction versatility," J. Phys. Chem. B **125**(10), 2513–2520 (2021).

Fu, B., and Vendruscolo, M., "Structure and dynamics of intrinsically disordered proteins," Adv. Exp. Med. Biol. **870**, 35–48 (2015).

Fung, H. Y. J., Birol, M., and Rhoades, E., "IDPs in macromolecular complexes: The roles of multivalent interactions in diverse assemblies," Curr. Opin. Struct. Biol. **49**, 36–43 (2018).

Fusco, G., and Gianni, S., "Function, regulation, and dysfunction of intrinsically disordered proteins," Life (Basel) **11**(2), 140 (2021).

Fuxreiter, M., Tompa, P., and Simon, I., "Local structural disorder imparts plasticity on linear motifs," Bioinformatics **23**(8), 950–956 (2007).

Galea, C. A., Wang, Y., Sivakolundu, S. G., and Kriwacki, R. W., "Regulation of cell division by intrinsically unstructured proteins: Intrinsic flexibility, modularity, and signaling conduits," Biochemistry **47**(29), 7598–7609 (2008).

Garcia-Pino, A., Balasubramanian, S., Wyns, L., Gazit, E., De Greve, H., Magnuson, R. D., Charlier, D., van Nuland, N. A., and Loris, R., "Allostery and intrinsic disorder mediate transcription regulation by conditional cooperativity," Cell **142**(1), 101–111 (2010).

Garcia-Pino, A., De Gieter, S., Talavera, A., De Greve, H., Efremov, R. G., and Loris, R., "An intrinsically disordered entropic switch determines allostery in Phd-Doc regulation," Nat. Chem. Biol. **12**(7), 490–496 (2016).

Gomes, G. N., and Gradinaru, C. C., "Insights into the conformations and dynamics of intrinsically disordered proteins using single-molecule fluorescence," Biochim. Biophys. Acta, Proteins Proteomics **1865**(11 Pt B), 1696–1706 (2017).

Gomes, G. W., Krzeminski, M., Namini, A., Martin, E. W., Mittag, T., Head-Gordon, T., Forman-Kay, J. D., and Gradinaru, C. C., "Conformational ensembles of an intrinsically disordered protein consistent with NMR, SAXS, and single-molecule FRET," J. Am. Chem. Soc. **142**(37), 15697–15710 (2020).

Gopal, S. M., Wingbermühle, S., Schnatwinkel, J., Juber, S., Herrmann, C., and Schäfer, L. V., "Conformational preferences of an intrinsically disordered protein domain: A case study for modern force fields," J. Phys. Chem. B **125**(1), 24–35 (2021).

Gorfe, A. A., "Mechanisms of allostery and membrane attachment in Ras GTPases: Implications for anti-cancer drug discovery," Curr. Med. Chem. **17**(1), 1–9 (2010).

Gorfe, A. A., Grant, B. J., and McCammon, J. A., "Mapping the nucleotide and isoform-dependent structural and dynamical features of Ras proteins," Structure **16**(6), 885–896 (2008).

Graham, T. R., Yacoub, R., Taliaferro-Smith, L., Osunkoya, A. O., Odero-Marah, V. A., Liu, T., Kimbro, K. S., Sharma, D., and O'Regan, R. M., "Reciprocal regulation of ZEB1 and AR in triple negative breast cancer cells," Breast Cancer Res. Treat. **123**(1), 139–147 (2010).

Grant, B. J., Gorfe, A. A., and McCammon, J. A., "Ras conformational switching: Simulating nucleotide-dependent conformational transitions with accelerated molecular dynamics," PLoS Comput. Biol. **5**(3), e1000325 (2009).

Gsponer, J., and Babu, M. M., "The rules of disorder or why disorder rules," Prog. Biophys. Mol. Biol. **99**(2–3), 94–103 (2009).

Gsponer, J., Futschik, M. E., Teichmann, S. A., and Babu, M. M., "Tight regulation of unstructured proteins: From transcript synthesis to protein degradation," Science **322**(5906), 1365–1368 (2008).

Guillemin, A., and Stumpf, M. P. H., "Noise and the molecular processes underlying cell fate decision-making," Phys. Biol. **18**(1), 011002 (2021).

Gunasekaran, K., Ma, B., and Nussinov, R., "Is allostery an intrinsic property of all dynamic proteins?," Proteins **57**(3), 433–443 (2004).

Gupta, P. B., Fillmore, C. M., Jiang, G., Shapira, S. D., Tao, K., Kuperwasser, C., and Lander, E. S., "Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells," Cell **146**(4), 633–644 (2011).

Habchi, J., Tompa, P., Longhi, S., and Uversky, V. N., "Introducing protein intrinsic disorder," Chem. Rev. **114**(13), 6561–6588 (2014).

Hallin, J., Engstrom, L. D., Hargis, L., Calinisan, A., Aranda, R., Briere, D. M., Sudhakar, N., Bowcut, V., Baer, B. R., Ballard, J. A., Burkard, M. R., Fell, J. B., Fischer, J. P., Vigers, G. P., Xue, Y., Gatto, S., Fernandez-Banet, J., Pavlicek, A., Velastagui, K., Chao, R. C., Barton, J., Pierobon, M., Baldelli, E., Patricoin, E. F.

III, Cassidy, D. P., Marx, M. A., Rybkin, I. I., Johnson, M. L., Ou, S. I., Lito, P., Papadopoulos, K. P., Jänne, P. A., Olson, P., and Christensen, J. G., "The KRASG12C inhibitor MRTX849 provides insight toward therapeutic susceptibility of KRAS-mutant cancers in mouse models and patients," Cancer Discovery **10**(1), 54–71 (2020).

Hammoudeh, D. I., Follis, A. V., Prochownik, E. V., and Metallo, S. J., "Multiple independent binding sites for small-molecule inhibitors on the oncoprotein c-Myc," J. Am. Chem. Soc. **131**(21), 7390–7401 (2009).

Hansen, M. M. K., and Weinberger, L. S., "Post-transcriptional noise control," Bioessays **41**(7), e1900044 (2019).

Hansen, M. M. K., Desai, R. V., Simpson, M. L., and Weinberger, L. S., "Cytoplasmic amplification of transcriptional noise generates substantial cell-to-cell variability," Cell Syst. **7**(4), 384–397 (2018).

Hardin, C., Eastwood, M. P., Luthey-Schulten, Z., and Wolynes, P. G., "Associative memory hamiltonians for structure prediction without homology: Alpha-helical proteins," Proc. Natl. Acad. Sci. U.S.A. **97**(26), 14235–14240 (2000).

Hari, K., Sabuwala, B., Subramani, B. V., La Porta, C. A. M., Zapperi, S., Font-Clos, F., and Jolly, M. K., "Identifying inhibitors of epithelial-mesenchymal plasticity using a network topology-based approach," NPJ Syst. Biol. Appl. **6**(1), 15 (2020).

Hatos, A., Hajdu-Soltész, B., Monzon, A. M., Palopoli, N., Álvarez, L., Aykac-Fas, B., Bassot, C., Benítez, G. I., Bevilacqua, M., Chasapi, A., Chemes, L., Davey, N. E., Davidović, R., Dunker, A. K., Elofsson, A., Gobeill, J., Foutel, N. S. G., Sudha, G., Guharoy, M., Horvath, T., Iglesias, V., Kajava, A. V., Kovacs, O. P., Lamb, J., Lambrughi, M., Lazar, T., Leclercq, J. Y., Leonardi, E., Macedo-Ribeiro, S., Macossay-Castillo, M., Maiani, E., Manso, J. A., Marino-Buslje, C., Martínez-Pérez, E., Mészáros, B., Mičetić, I., Minervini, G., Murvai, N., Necci, M., Ouzounis, C. A., Pajkos, M., Paladin, L., Pancsa, R., Papaleo, E., Parisi, G., Pasche, E., Barbosa Pereira, P. J., Promponas, V. J., Pujols, J., Quaglia, F., Ruch, P., Salvatore, M., Schad, E., Szabo, B., Szaniszló, T., Tamana, S., Tantos, A., Veljkovic, N., Ventura, S., Vranken, W., Dosztányi, Z., Tompa, P., Tosatto, S. C. E., and Piovesan, D., "DisProt: Intrinsic protein disorder annotation in 2020," Nucl. Acids Res. **48**(D1), D269–D276 (2020).

Haynes, C., Oldfield, C. J., Ji, F., Klitgord, N., Cusick, M. E., Radivojac, P., Uversky, V. N., Vidal, M., and Iakoucheva, L. M., "Intrinsic disorder is a common feature of hub proteins from four eukaryotic interactomes," PLoS Comput. Biol. **2**(8), e100 (2006).

He, Y., Chen, Y., Mooney, S. M., Rajagopalan, K., Bhargava, A., Sacho, E., Weninger, K., Bryan, P. N., Kulkarni, P., and Orban, J., "Phosphorylation-induced conformational ensemble switching in an intrinsically disordered cancer/testis antigen," J. Biol. Chem. **290**(41), 25090–25102 (2015).

Hegyi, H., and Tompa, P., "Increased structural disorder of proteins encoded on human sex chromosomes," Mol. Biosyst. **8**(1), 229–236 (2012).

Heller, G. T., Aprile, F. A., Michaels, T. C. T., Limbocker, R., Perni, M., Ruggeri, F. S., Mannini, B., Löhr, T., Bonomi, M., Camilloni, C., Simone, D., Felli, A., Pierattelli, I. C., Knowles, R., Dobson, T. P. J., and Vendruscolo, C. M., "Small-molecule sequestration of amyloid-β as a drug discovery strategy for Alzheimer's disease," Sci. Adv. **6**(45), eabb5924 (2020).

Henley, M. J., and Koehler, A. N., "Advances in targeting 'undruggable' transcription factors with small molecules," Nat. Rev. Drug Discovery **20**(9), 669–688 (2021).

Henriques, J., Cragnell, C., and Skepö, M., "Molecular dynamics simulations of intrinsically disordered proteins: Force field evaluation and comparison with experiment," J. Chem. Theory Comput. **11**(7), 3420–3431 (2015).

Hermann, M. R., and Hub, J. S., "SAXS-restrained ensemble simulations of intrinsically disordered proteins with commitment to the principle of maximum entropy," J. Chem. Theory Comput. **15**(9), 5103–5115 (2019).

Hesgrove, C., and Boothby, T. C., "The biology of tardigrade disordered proteins in extreme stress tolerance," Cell Commun. Signaling **18**(1), 178 (2020).

Hibino, E., and Hoshino, M., "A novel mode of interaction between intrinsically disordered proteins," Biophys. Physicobiol. **17**, 86–93 (2020).

Hills, R. D., Jr., and Brooks, C. L. III, "Insights from coarse-grained Gō models for protein folding and dynamics," Int. J. Mol. Sci. **10**(3), 889–905 (2009).

Hirokawa, N., Noda, Y., Tanaka, Y., and Niwa, S., "Kinesin superfamily motor proteins and intracellular transport," Nat. Rev. Mol. Cell Biol. **10**(10), 682–696 (2009).

Hoffman, R. M., Blumenschein, T. M., and Sykes, B. D., "An interplay between protein disorder and structure confers the Ca^{2+} regulation of striated muscle," J. Mol. Biol. **361**(4), 625–633 (2006).

Hoh, J. H., "Functional protein domains from the thermally driven motion of polypeptide chains: A proposal," Proteins **32**(2), 223–228 (1998).

Hsu, C. C., Buehler, M. J., and Tarakanova, A., "The order-disorder continuum: Linking predictions of protein structure and disorder through molecular simulation," Sci. Rep. **10**(1), 2068 (2020).

Hu, G., Wu, Z., Uversky, V. N., and Kurgan, L., "Functional analysis of human hub proteins and their interactors involved in the intrinsic disorder-enriched interactions," Int. J. Mol. Sci. **18**(12), 2761 (2017).

Huang, S., "Non-genetic heterogeneity of cells in development: More than just noise," Development **136**(23), 3853–3862 (2009).

Huang, J., and MacKerell, A. D., Jr., "Force field development and simulations of intrinsically disordered proteins," Curr. Opin. Struct. Biol. **48**, 40–48 (2018).

Huang, J., Rauscher, S., Nawrocki, G., Ran, T., Feig, M., de Groot, B. L., Grubmüller, H., and MacKerell, A. D., Jr., "CHARMM36m: An improved force field for folded and intrinsically disordered proteins," Nat. Methods **14**(1), 71–73 (2017).

Huang, X., Hagen, M., Kim, B., Friesner, R. A., Zhou, R., and Berne, B. J., "Replica exchange with solute tempering: Efficiency in large scale systems," J. Phys. Chem. B **111**(19), 5405–5410 (2007).

Hub, J. S., "Interpreting solution X-ray scattering data using molecular simulations," Curr. Opin. Struct. Biol. **49**, 18–26 (2018).

Hunter, J. C., Gurbani, D., Ficarro, S. B., Carrasco, M. A., Lim, S. M., Choi, H. G., Xie, T., Marto, J. A., Chen, Z., Gray, N. S., and Westover, K. D., "In situ selectivity profiling and crystal structure of SML-8–73-1, an active site inhibitor of oncogenic K-Ras G12C," Proc. Natl. Acad. Sci. U.S.A. **111**(24), 8895–8900 (2014).

Hunter, J. C., Manandhar, A., Carrasco, M. A., Gurbani, D., Gondi, S., and Westover, K. D., "Biochemical and structural analysis of common cancer-associated KRAS mutations," Mol. Cancer Res. **13**(9), 1325–1335 (2015).

Hurley, J. M., Larrondo, L. F., Loros, J. J., and Dunlap, J. C., "Conserved RNA helicase FRH acts nonenzymatically to support the intrinsically disordered neurospora clock protein FRQ," Mol. Cell **52**(6), 832–843 (2013).

Husic, B. E., and Pande, V. S., "Markov state models: From an art to a science," J. Am. Chem. Soc. **140**(7), 2386–2396 (2018).

Iakoucheva, L. M., Brown, C. J., Lawson, J. D., Obradović, Z., and Dunker, A. K., "Intrinsic disorder in cell-signaling and cancer-associated proteins," J. Mol. Biol. **323**(3), 573–584 (2002).

Ilieva, N., Liu, J., Marinova, R., Petkov, P., Litov, L., He, J., and Niemi, A. J., "Are there folding pathways in the functional stages of intrinsically disordered proteins?," AIP Conf. Proc. **1773**, 110008 (2016).

Itzhaki, L. S., Otzen, D. E., and Fersht, A. R., "The structure of the transition state for folding of chymotrypsin inhibitor 2 analysed by protein engineering methods: Evidence for a nucleation-condensation mechanism for protein folding," J. Mol. Biol. **254**(2), 260–288 (1995).

Jacobs, W. M., and Shakhnovich, E. I., "Accurate protein-folding transition-path statistics from a simple free-energy landscape," J. Phys. Chem. B **122**(49), 11126–11136 (2018).

Jain, K., Ghribi, O., and Delhommelle, J., "Folding free-energy landscape of α-Synuclein (35–97) via replica exchange molecular dynamics," J. Chem. Inf. Modell. **61**(1), 432–443 (2021).

Janes, M. R., Zhang, J., Li, L. S., Hansen, R., Peters, U., Guo, X., Chen, Y., Babbar, A., Firdaus, S. J., Darjania, L., Feng, J., Chen, J. H., Li, S., Li, S., Long, Y. O., Thach, C., Liu, Y., Zarieh, A., Ely, T., Kucharski, J. M., Kessler, L. V., Wu, T., Yu, K., Wang, Y., Yao, Y., Deng, X., Zarrinkar, P., Brehmer, D., Dhanak, D., Lorenzi, M. V., Hu-Lowe, D., Patricelli, M. P., Ren, P., and Liu, Y., "Targeting KRAS mutant cancers with a covalent G12C-specific inhibitor," Cell **172**(3), 578–589 (2018).

Jang, H., Banerjee, A., Chavan, T. S., Lu, S., Zhang, J., Gaponenko, V., and Nussinov, R., "The higher level of complexity of K-Ras4B activation at the membrane," FASEB J. **30**(4), 1643–1655 (2016).

Jang, H., Banerjee, A., Marcus, K., Makowski, L., Mattos, C., Gaponenko, V., and Nussinov, R., "The structural basis of the farnesylated and methylated KRas4B interaction with calmodulin," Structure **27**(11), 1647–1659 (2019).

Janis, B., Belott, C., and Menze, M. A., "Role of intrinsic disorder in animal desiccation tolerance," Proteomics **18**(21–22), e1800067 (2018).

Janosi, L., Li, Z., Hancock, J. F., and Gorfe, A. A., "Organization, dynamics, and segregation of Ras nanoclusters in membrane domains," Proc. Natl. Acad. Sci. U.S.A. **109**(21), 8097–8102 (2012).

Jensen, M. R., Ruigrok, R. W., and Blackledge, M., "Describing intrinsically disordered proteins at atomic resolution by NMR," Curr. Opin. Struct. Biol. **23**(3), 426–435 (2013).

Jensen, M. R., Zweckstetter, M., Huang, J. R., and Blackledge, M., "Exploring free-energy landscapes of intrinsically disordered proteins at atomic resolution using NMR spectroscopy," Chem. Rev. **114**(13), 6632–6660 (2014).

Jephthah, S., Staby, L., Kragelund, B. B., and Skepö, M., "Temperature dependence of intrinsically disordered proteins in simulations: What are we missing?," J. Chem. Theory Comput. **15**(4), 2672–2683 (2019).

Jia, D., Jolly, M. K., Kulkarni, P., and Levine, H., "Phenotypic plasticity and cell fate decisions in cancer: Insights from dynamical systems theory," Cancers (Basel) **9**(7), 70 (2017).

Jolly, M. K., Kulkarni, P., Weninger, K., Orban, J., and Levine, H., "Phenotypic plasticity, bet-hedging, and androgen independence in prostate cancer: Role of non-genetic heterogeneity," Front. Oncol. **8**, 50 (2018).

Jolly, M. K., Tripathi, S. C., Somarelli, J. A., Hanash, S. M., and Levine, H., "Epithelial/mesenchymal plasticity: How have quantitative mathematical models helped improve our understanding?," Mol. Oncol. **11**(7), 739–754 (2017).

Joshi, P., and Vendruscolo, M., "Druggability of intrinsically disordered proteins," Adv. Exp. Med. Biol. **870**, 383–400 (2015).

Joshi, P., Chia, S., Habchi, J., Knowles, T. P., Dobson, C. M., and Vendruscolo, M., "A fragment-based method of creating small-molecule libraries to target the aggregation of intrinsically disordered proteins," ACS Comb. Sci. **18**(3), 144–153 (2016).

Jurneczko, E., Cruickshank, F., Porrini, M., Nikolova, P., Campuzano, I. D., Morris, M., and Barran, P. E., "Intrinsic disorder in proteins: A challenge for (un)structural biology met by ion mobility-mass spectrometry," Biochem. Soc. Trans. **40**(5), 1021–1026 (2012).

Kamiya, M., and Sugita, Y., "Flexible selection of the solute region in replica exchange with solute tempering: Application to protein-folding simulations," J. Chem. Phys. **149**(7), 072304 (2018).

Kapoor, A., and Travesset, A., "Differential dynamics of RAS isoforms in GDP- and GTP-bound states," Proteins **83**(6), 1091–1106 (2015).

Kaptein, R., and Wagner, G., "Integrative methods in structural biology," J. Biomol. NMR **73**(6–7), 261–263 (2019).

Kar, G., Keskin, O., Gursoy, A., and Nussinov, R., "Allostery and population shift in drug discovery," Curr. Opin. Pharmacol. **10**(6), 715–722 (2010).

Kartashov, Y. V., Vysloukh, V. A., and Torner, L., "Disorder-induced soliton transmission in nonlinear photonic lattices," Opt. Lett. **36**(4), 466–468 (2011).

Kasahara, K., Terazawa, H., Takahashi, T., and Higo, J., "Studies on molecular dynamics of intrinsically disordered proteins and their fuzzy complexes: A mini-review," Comput. Struct. Biotechnol. J. **17**, 712–720 (2019).

Kassem, N., Araya-Secchi, R., Bugge, K., Barclay, A., Steinocher, H., Khondker, A., Wang, Y., Lenard, A. J., Bürck, J., Sahin, C., Ulrich, A. S., Landreh, M., Pedersen, M. C., Rheinstädter, M. C., Pedersen, P. A., Lindorff-Larsen, K., Arleth, L., and Kragelund, B. B., "Order and disorder—An integrative structure of the full-length human growth hormone receptor," Sci. Adv. **7**(27), eabh3805 (2021).

Katuwawala, A., Ghadermarzi, S., and Kurgan, L., "Computational prediction of functions of intrinsically disordered regions," Prog. Mol. Biol. Transl. Sci. **166**, 341–369 (2019).

Kazakov, A. S., Markov, D. I., Gusev, N. B., and Levitsky, D. I., "Thermally induced structural changes of intrinsically disordered small heat shock protein Hsp22," Biophys. Chem. **145**(2–3), 79–85 (2009).

Kern, D., and Zuiderweg, E. R., "The role of dynamics in allosteric regulation," Curr. Opin. Struct. Biol. **13**(6), 748–757 (2003).

Khan, E., Mishra, S. K., and Kumar, A., "Emerging methods for structural analysis of protein aggregation," Protein Pept. Lett. **24**(4), 331–339 (2017).

Khayat, E., Klimov, D. K., and Smith, A. K., "Phosphorylation promotes $A\beta$25-35 peptide aggregation within the DMPC bilayer," ACS Chem. Neurosci. **11**(20), 3430–3441 (2020).

Kiefhaber, T., Bachmann, A., and Jensen, K. S., "Dynamics and mechanisms of coupled protein folding and binding reactions," Curr. Opin. Struct. Biol. **22**(1), 21–29 (2012).

Kjaergaard, M., Nørholm, A. B., Hendus-Altenburger, R., Pedersen, S. F., Poulsen, F. M., and Kragelund, B. B., "Temperature-dependent structural changes in intrinsically disordered proteins: Formation of alpha-helices or loss of polyproline II?," Protein Sci. **19**(8), 1555–1564 (2010b).

Kjaergaard, M., Teilum, K., and Poulsen, F. M., "Conformational selection in the molten globule state of the nuclear coactivator binding domain of CBP," Proc. Natl. Acad. Sci. U.S.A. **107**(28), 12535–12540 (2010a).

Köfinger, J., Stelzl, L. S., Reuter, K., Allande, C., Reichel, K., and Hummer, G., "Efficient ensemble refinement by reweighting," J. Chem. Theory Comput. **15**(5), 3390–3401 (2019).

Koga, N., and Takada, S., "Roles of native topology and chain-length scaling in protein folding: A simulation study with a go-like model," J. Mol. Biol. **313**(1), 171–180 (2001).

Kontogeorgaki, S., Sánchez-García, R. J., Ewing, R. M., Zygalakis, K. C., and MacArthur, B. D., "Noise-processing by signaling networks," Sci. Rep. **7**(1), 532 (2017).

Korneta, I., and Bujnicki, J. M., "Intrinsic disorder in the human spliceosomal proteome," PLoS Comput. Biol. **8**(8), e1002641 (2012).

Kraulis, P. J., Domaille, P. J., Campbell-Burk, S. L., Van Aken, T., and Laue, E. D., "Solution structure and dynamics of ras p21.GDP determined by heteronuclear three- and four-dimensional NMR spectroscopy," Biochemistry **33**(12), 3515–3531 (1994).

Krishnan, N., Koveal, D., Miller, D. H., Xue, B., Akshinthala, S. D., Kragelj, J., Jensen, M. R., Gauss, C. M., Page, R., Blackledge, M., Muthuswamy, S. K., Peti, W., and Tonks, N. K., "Targeting the disordered C terminus of PTP1B with an allosteric inhibitor," Nat. Chem. Biol. **10**(7), 558–566 (2014).

Krystkowiak, I., and Davey, N. E., "SLiMSearch: A framework for proteome-wide discovery and annotation of functional modules in intrinsically disordered regions," Nucl. Acids Res. **45**(W1), W464–W469 (2017).

Kulkarni, P., "Intrinsically disordered proteins: Insights from Poincaré, Waddington, and Lamarck," Biomolecules **10**(11), 1490 (2020).

Kulkarni, P., "The Boscombe Valley mystery: A lesson in the perils of dogmatism in science," J. Biosci. **46**, 59 (2021).

Kulkarni, V., and Kulkarni, P., "Intrinsically disordered proteins and phenotypic switching: Implications in cancer," Prog. Mol. Biol. Transl. Sci. **166**, 63–84 (2019).

Kulkarni, P., and Uversky, V. N., "Intrinsically disordered proteins: The dark horse of the dark proteome," Proteomics **18**(21–22), e1800061 (2018a).

Kulkarni, P., and Uversky, V. N., "Intrinsically disordered proteins and the Janus challenge," Biomolecules **8**(4), 179 (2018b).

Kulkarni, P., and Uversky, V. N., "Intrinsically disordered proteins in chronic diseases," Biomolecules **9**(4), 147 (2019).

Kulkarni, P., Jolly, M. K., Jia, D., Mooney, S. M., Bhargava, A., Kagohara, L. T., Chen, Y., Hao, P., He, Y., Veltri, R. W., Grishaev, A., Weninger, K., Levine, H., and Orban, J., "Phosphorylation-induced conformational dynamics in an intrinsically disordered protein and potential role in phenotypic heterogeneity," Proc. Natl. Acad. Sci. U.S.A. **114**(13), E2644–E2653 (2017).

Kulkarni, P., Nathan, A., Salgia, R., Rangarajan, A., and Jolly, M. K., "Phenotypic switching and prostate diseases: A model proposing a causal link between benign prostatic hyperplasia and prostate cancer," in *Phenotypic Switching: Implications in Biology and Medicine*, edited by H. Levine, M. K. Jolly, P. Kulkarni, and V. Nanjundiah (Academic Press, New York, 2020).

Kulkarni, P., Solomon, T. L., He, Y., Chen, Y., Bryan, P. N., and Orban, J., "Structural metamorphism and polymorphism in proteins on the brink of thermodynamic stability," Protein Sci. **27**(9), 1557–1567 (2018).

Kuwahara, H., and Gao, X., "Stochastic effects as a force to increase the complexity of signaling networks," Sci. Rep. **3**, 2297 (2013).

Ladbury, J. E., and Arold, S. T., "Noise in cellular signaling pathways: Causes and effects," Trends Biochem. Sci. **37**(5), 173–178 (2012).

Latysheva, N. S., Flock, T., Weatheritt, R. J., Chavali, S., and Babu, M. M., "How do disordered regions achieve comparable functions to structured domains?," Protein Sci. **24**(6), 909–922 (2015).

Lazar, T., Martínez-Pérez, E., Quaglia, F., Hatos, A., Chemes, L. B., Iserte, J. A., Méndez, N. A., Garrone, N. A., Saldaño, T. E., Marchetti, J., Rueda, A. J. V., Bernadó, P., Blackledge, M., Cordeiro, T. N., Fagerberg, E., Forman-Kay, J. D., Fornasari, M. S., Gibson, T. J., Gomes, G. W., Gradinaru, C. C., Head-Gordon, T., Jensen, M. R., Lemke, E. A., Longhi, S., Marino-Buslje, C., Minervini, G.,

Mittag, T., Monzon, A. M., Pappu, R. V., Parisi, G., Ricard-Blum, S., Ruff, K. M., Salladini, E., Skepö, M., Svergun, D., Vallet, S. D., Varadi, M., Tompa, P., Tosatto, S. C. E., and Piovesan, D., "PED in 2021: A major update of the protein ensemble database for intrinsically disordered proteins," Nucl. Acids Res. **49**(D1), D404–D411 (2021).

LeBlanc, S. J., Kulkarni, P., and Weninger, K. R., "Single molecule FRET: A powerful tool to study intrinsically disordered proteins," Biomolecules **8**(4), 140 (2018).

Lee, C. W., Ferreon, J. C., Ferreon, A. C., Arai, M., and Wright, P. E., "Graded enhancement of p53 binding to CREB-binding protein (CBP) by multisite phosphorylation," Proc. Natl. Acad. Sci. U.S.A. **107**(45), 19290–19295 (2010).

Lee, K. Y., Fang, Z., Enomoto, M., Gasmi-Seabrook, G., Zheng, L., Koide, S., Ikura, M., and Marshall, C. B., "Two distinct structures of membrane-associated homodimers of GTP- and GDP-bound KRAS4B revealed by paramagnetic relaxation enhancement," Angew. Chem. Int. Ed. Engl. **59**(27), 11037–11045 (2020).

Leopold, P. E., Montal, M., and Onuchic, J. N., "Protein folding funnels: A kinetic approach to the sequence-structure relationship," Proc. Natl. Acad. Sci. U.S.A. **89**(18), 8721–8725 (1992).

Lermyte, F., "Roles, characteristics, and analysis of intrinsically disordered proteins: A minireview," Life (Basel) **10**(12), 320 (2020).

Lerner, E., Barth, A., Hendrix, J., Ambrose, B., Birkedal, V., Blanchard, S. C., Börner, R., Sung Chung, H., Cordes, T., Craggs, T. D., Deniz, A. A., Diao, J., Fei, J., Gonzalez, R. L., Gopich, I. V., Ha, T., Hanke, C. A., Haran, G., Hatzakis, N. S., Hohng, S., Hong, S. C., Hugel, T., Ingargiola, A., Joo, C., Kapanidis, A. N., Kim, H. D., Laurence, T., Lee, N. K., Lee, T. H., Lemke, E. A., Margeat, E., Michaelis, J., Michalet, X., Myong, S., Nettels, D., Peulen, T. O., Ploetz, E., Razvag, Y., Robb, N. C., Schuler, B., Soleimaninejad, H., Tang, C., Vafabakhsh, R., Lamb, D. C., Seidel, C. A., and Weiss, S., "FRET-based dynamic structural biology: Challenges, perspectives and an appeal for open-science practices," Elife **10**, e60416 (2021).

Li, B., Alonso, D. O., and Daggett, V., "The molecular basis for the inverse temperature transition of elastin," J. Mol. Biol. **305**(3), 581–592 (2001).

Li, M., Cao, H., Lai, L., and Liu, Z., "Disordered linkers in multidomain allosteric proteins: Entropic effect to favor the open state or enhanced local concentration to favor the closed state?," Protein Sci. **27**(9), 1600–1610 (2018).

Li, Z. L., and Buck, M., "Computational modeling reveals that signaling lipids modulate the orientation of K-Ras4A at the membrane reflecting protein topology," Structure **25**(4), 679–689 (2017).

Li, J., White, J. T., Saavedra, H., Wrabl, J. O., Motlagh, H. N., Liu, K., Sowers, J., Schroer, T. A., Thompson, E. B., and Hilser, V. J., "Genetically tunable frustration controls allostery in an intrinsically disordered transcription factor," Elife **6**, e30688 (2017).

Lim, S. M., Westover, K. D., Ficarro, S. B., Harrison, R. A., Choi, H. G., Pacold, M. E., Carrasco, M., Hunter, J., Kim, N. D., Xie, T., Sim, T., Jänne, P. A., Meyerson, M., Marto, J. A., Engen, J. R., and Gray, N. S., "Therapeutic targeting of oncogenic K-Ras by a covalent catalytic site inhibitor," Angew. Chem. Int. Ed. Engl. **53**(1), 199–204 (2014).

Lin, X., Roy, S., Jolly, M. K., Bocci, F., Schafer, N. P., Tsai, M. Y., Chen, Y., He, Y., Grishaev, A., Weninger, K., Orban, J., Kulkarni, P., Rangarajan, G., Levine, H., and Onuchic, J. N., "PAGE4 and conformational switching: Insights from molecular dynamics simulations and implications for prostate cancer," J. Mol. Biol. **430**(16), 2422–2438 (2018a).

Lin, Y. T., Hufton, P. G., Lee, E. J., and Potoyan, D. A., "A stochastic and dynamical view of pluripotency in mouse embryonic stem cells," PLoS Comput. Biol. **14**(2), e1006000 (2018b).

Lindorff-Larsen, K., Piana, S., Palmo, K., Maragakis, P., Klepeis, J. L., Dror, R. O., and Shaw, D. E., "Improved side-chain torsion potentials for the Amber ff99SB protein force field," Proteins **78**(8), 1950–1958 (2010).

Lindorff-Larsen, K., Trbovic, N., Maragakis, P., Piana, S., and Shaw, D. E., "Structure and dynamics of an unfolded protein examined by molecular dynamics simulation," J. Am. Chem. Soc. **134**(8), 3787–3791 (2012).

Lindorff-Larsen, K., and Kragelund, B. B., "On the potential of machine learning to examine the relationship between sequence, structure, dynamics and function of intrinsically disordered proteins," J. Mol. Biol. **433**(20), 167196 (2021).

Liu, X., and Chen, J., "Residual structures and transient long-range interactions of p53 transactivation domain: Assessment of explicit solvent protein force fields," J. Chem. Theory Comput. **15**(8), 4708–4720 (2019).

Liu, N., Guo, Y., Ning, S. *et al.*, "Phosphorylation regulates the binding of intrinsically disordered proteins via a flexible conformation selection mechanism," Commun. Chem. **3**, 123 (2020).

Liu, X., Jia, Z., and Chen, J., "Enhanced sampling of intrinsic structural heterogeneity of the BH3-only protein binding interface of Bcl-xL," J. Phys. Chem. B **121**(39), 9160–9168 (2017).

Liu, P., Kim, B., Friesner, R. A., and Berne, B. J., "Replica exchange with solute tempering: A method for sampling biological systems in explicit water," Proc. Natl. Acad. Sci. U.S.A. **102**(39), 13749–13754 (2005).

Liu, J., Perumal, N. B., Oldfield, C. J., Su, E. W., Uversky, V. N., and Dunker, A. K., "Intrinsic disorder in transcription factors," Biochemistry **45**(22), 6873–6888 (2006).

Lu, J., Bera, A. K., Gondi, S., and Westover, K. D., "KRAS switch mutants D33E and A59G crystallize in the state 1 conformation," Biochemistry **57**(3), 324–333 (2018).

Lu, S., Jang, H., Nussinov, R., and Zhang, J., "The structural basis of oncogenic mutations G12, G13 and Q61 in small GTPase K-Ras4B," Sci. Rep. **6**, 21949 (2016).

MacArthur, B. D., Please, C. P., and Oreffo, R. O., "Stochasticity and the molecular mechanisms of induced pluripotency," PLoS One **3**(8), e3086 (2008).

Machida, K., Kono-Okada, A., Hongo, K., Mizobata, T., and Kawata, Y., "Hydrophilic residues 526 KNDAAD 531 in the flexible C-terminal region of the chaperonin GroEL are critical for substrate protein folding within the central cavity," J. Biol. Chem. **283**(11), 6886–6896 (2008).

Mahmoudabadi, G., Rajagopalan, K., Getzenberg, R. H., Hannenhalli, S., Rangarajan, G., and Kulkarni, P., "Intrinsically disordered proteins and conformational noise: Implications in cancer," Cell Cycle **12**(1), 26–31 (2013).

Marcotte, E. M., and Tschansky, M., "Disorder, promiscuity, and toxic partnerships," Cell **138**(1), 16–18 (2009).

Masetti, M., Bernetti, M., and Cavalli, A., "Enhanced molecular dynamics simulations of intrinsically disordered proteins," Methods Mol. Biol. **2141**, 391–411 (2020).

Matsumoto, S., Miyano, N., Baba, S., Liao, J., Kawamura, T., Tsuda, C., Takeda, A., Yamamoto, M., Kumasaka, T., Kataoka, T., and Shima, F., "Molecular mechanism for conformational dynamics of Ras·GTP elucidated from in-situ structural transition in crystal," Sci. Rep. **6**, 25931 (2016).

Metallo, S. J., "Intrinsically disordered proteins are potential drug targets," Curr. Opin. Chem. Biol. **14**(4), 481–488 (2010).

Metskas, L. A., and Rhoades, E., "Conformation and dynamics of the troponin I C-Terminal domain: Combining single-molecule and computational approaches for a disordered protein region," J. Am. Chem. Soc. **137**(37), 11962–11969 (2015).

Metskas, L. A., and Rhoades, E., "Single-molecule FRET of intrinsically disordered proteins," Annu. Rev. Phys. Chem. **71**, 391–414 (2020).

Midic, U., and Obradovic, Z., "Intrinsic disorder in putative protein sequences," Proteome Sci. **10**(Suppl 1), S19 (2012).

Midic, U., Oldfield, C. J., Dunker, A. K., Obradovic, Z., and Uversky, V. N., "Protein disorder in the human diseasome: Unfoldomics of human genetic diseases," BMC Genomics **10**(Suppl 1), S12 (2009).

Milburn, M. V., Tong, L., deVos, A. M., Brünger, A., Yamaizumi, Z., Nishimura, S., and Kim, S. H., "Molecular switch for signal transduction: Structural differences between active and inactive forms of protooncogenic ras proteins," Science **247**(4945), 939–945 (1990).

Miller, C. M., Brown, A. C., and Mittal, J., "Disorder in cholesterol-binding functionality of CRAC peptides: A molecular dynamics study," J. Phys. Chem. B **118**(46), 13169–13174 (2014).

Mitrea, D. M., Yoon, M. K., Ou, L., and Kriwacki, R. W., "Disorder-function relationships for the cell cycle regulatory proteins p21 and p27," Biol. Chem. **393**(4), 259–274 (2012).

Mittag, T., Kay, L. E., and Forman-Kay, J. D., "Protein dynamics and conformational disorder in molecular recognition," J. Mol. Recognit. **23**(2), 105–116 (2010).

Moghadamchargari, Z., Huddleston, J., Shirzadeh, M., Zheng, X., Clemmer, D. E., Raushel, F. M., Russell, D. H., and Laganowsky, A., "Intrinsic GTPase

activity of K-RAS monitored by native mass spectrometry," Biochemistry **58**(31), 3396–3405 (2019).

Mohan, A., Oldfield, C. J., Radivojac, P., Vacic, V., Cortese, M. S., Dunker, A. K., and Uversky, V. N., "Analysis of molecular recognition features (MoRFs)," J. Mol. Biol. **362**(5), 1043–1059 (2006).

Mooney, S. M., Jolly, M. K., Levine, H., and Kulkarni, P., "Phenotypic plasticity in prostate cancer: Role of intrinsically disordered proteins," Asian J. Androl. **18**(5), 704–710 (2016).

Mooney, S. M., Qiu, R., Kim, J. J., Sacho, E. J., Rajagopalan, K., Johng, D., Shiraishi, T., Kulkarni, P., and Weninger, K. R., "Cancer/testis antigen PAGE4, a regulator of c-Jun transactivation, is phosphorylated by homeodomain-interacting protein kinase 1, a component of the stress-response pathway," Biochemistry **53**(10), 1670–1679 (2014).

Motlagh, H. N., Li, J., Thompson, E. B., and Hilser, V. J., "Interplay between allostery and intrinsic disorder in an ensemble," Biochem. Soc. Trans. **40**(5), 975–980 (2012).

Motlagh, H. N., Wrabl, J. O., Li, J., and Hilser, V. J., "The ensemble nature of allostery," Nature **508**(7496), 331–339 (2014).

Mu, J., Liu, H., Zhang, J., Luo, R., and Chen, H. F., "Recent force field strategies for intrinsically disordered proteins," J. Chem. Inf. Model. **61**(3), 1037–1047 (2021).

Muratcioglu, S., Chavan, T. S., Freed, B. C., Jang, H., Khavrutskii, L., Freed, R. N., Dyba, M. A., Stefanisko, K., Tarasov, S. G., Gursoy, A., Keskin, O., Tarasova, N. I., Gaponenko, V., and Nussinov, R., "GTP-dependent K-Ras dimerization," Structure **23**(7), 1325–1335 (2015).

Murray, C. W., and Rees, D. C., "The rise of fragment-based drug discovery," Nat. Chem. **1**(3), 187–192 (2009).

Musiani, F., Ippoliti, E., Micheletti, C., Carloni, P., and Ciurli, S., "Conformational fluctuations of UreG, an intrinsically disordered enzyme," Biochemistry **52**(17), 2949–2954 (2013).

Myung, J. K., Banuelos, C. A., Fernandez, J. G., Mawji, N. R., Wang, J., Tien, A. H., Yang, Y. C., Tavakoli, I., Haile, S., Watt, K., McEwan, I. J., Plymate, S., Andersen, R. J., and Sadar, M. D., "An androgen receptor N-terminal domain antagonist for treating prostate cancer," J. Clin. Invest. **123**(7), 2948–2960 (2013).

Na, I., Choi, S., Son, S. H., Uversky, V. N., and Kim, C. G., "Drug discovery targeting the disorder-to-order transition regions through the conformational diversity mimicking and statistical analysis," Int. J. Mol. Sci. **21**(15), 5248 (2020).

Na, I., Redmon, D., Kopa, M., Qin, Y., Xue, B., and Uversky, V. N., "Ordered disorder of the astrocytic dystrophin-associated protein complex in the norm and pathology," PLoS One **8**(8), e73476 (2013).

Neira, J. L., Bintz, J., Arruebo, M., Rizzuti, B., Bonacci, T., Vega, S., Lanas, A., Velázquez-Campoy, A., Iovanna, J. L., and Abián, O., "Identification of a drug targeting an intrinsically disordered protein involved in pancreatic adenocarcinoma," Sci. Rep. **7**, 39732 (2017).

Nicolaï, A., Delarue, P., and Senet, P., "Intrinsic localized modes in proteins," Sci. Rep. **5**, 18128 (2015).

Nierzwicki, Ł., and Palermo, G., "Molecular dynamics to predict cryo-EM: Capturing transitions and short-lived conformational states of biomolecules," Front. Mol. Biosci. **8**, 641208 (2021).

Niklas, K. J., Bondos, S. E., Dunker, A. K., and Newman, S. A., "Rethinking gene regulatory networks in light of alternative splicing, intrinsically disordered protein domains, and post-translational modifications," Front. Cell Dev. Biol. **3**, 8 (2015).

Nisius, B., Sha, F., and Gohlke, H., "Structure-based computational analysis of protein binding sites for function and druggability prediction," J. Biotechnol. **159**(3), 123–134 (2012).

Noda, K., Tachi, Y., and Okamoto, Y., "Structural characteristics of monomeric Aβ42 on fibril in the early stage of secondary nucleation process," ACS Chem. Neurosci. **11**(19), 2989–2998 (2020).

Noé, F., and Clementi, C., "Collective variables for the study of long-time kinetics from molecular trajectories: Theory and methods," Curr. Opin. Struct. Biol. **43**, 141–147 (2017).

Noonan, T., Brown, N., Dudycz, L., and Wright, G., "Interaction of GTP derivatives with cellular and oncogenic ras-p21 proteins," J. Med. Chem. **34**(4), 1302–1307 (1991).

Nussinov, R., Tsai, C. J., and Jang, H., "Oncogenic Ras isoforms signaling specificity at the membrane," Cancer Res. **78**(3), 593–602 (2018).

Nussinov, R., Tsai, C. J., and Jang, H., "Does Ras activate Raf and PI3K allosterically?," Front. Oncol. **9**, 1231 (2019).

Nussinov, R., Tsai, C. J., Xin, F., and Radivojac, P., "Allosteric post-translational modification codes," Trends Biochem. Sci. **37**(10), 447–455 (2012).

Nwanochie, E., and Uversky, V. N., "Structure determination by single-particle cryo-electron microscopy: Only the sky (and intrinsic disorder) is the limit," Int. J. Mol. Sci. **20**(17), 4186 (2019).

O'Connor, C., and Kovrigin, E. L., "Global conformational dynamics in ras," Biochemistry **47**(39), 10244–10246 (2008).

Oldfield, C. J., Cheng, Y., Cortese, M. S., Romero, P., Uversky, V. N., and Dunker, A. K., "Coupled folding and binding with alpha-helix-forming molecular recognition elements," Biochemistry **44**(37), 12454–12470 (2005).

Oldfield, C. J., and Dunker, A. K., "Intrinsically disordered proteins and intrinsically disordered protein regions," Annu. Rev. Biochem. **83**, 553–584 (2014).

Oldfield, C. J., Meng, J., Yang, J. Y., Yang, M. Q., Uversky, V. N., and Dunker, A. K., "Flexible nets: Disorder and induced fit in the associations of p53 and 14-3-3 with their partners," BMC Genomics **9**(Suppl 1), S1 (2008).

Oliveira, A. B., Jr., Fatore, F. M., Paulovich, F. V., Oliveira, O. N., Jr., and Leite, V. B., "Visualization of protein folding funnels in lattice models," PLoS One **9**(7), e100861 (2014).

Oliveira, A. B., Jr., Lin, X., Kulkarni, P., Onuchic, J. N., Roy, S., and Leite, V. B. P., "Exploring energy landscapes of intrinsically disordered proteins: Insights into functional mechanisms," J. Chem. Theory Comput. **17**(5), 3178–3187 (2021).

Oliveira, A. B., Jr., Yang, H., Whitford, P. C., and Leite, V. B. P., "Distinguishing biomolecular pathways and metastable states," J. Chem. Theory Comput. **15**(11), 6482–6490 (2019).

Onuchic, J. N., and Wolynes, P. G., "Theory of protein folding," Curr. Opin. Struct. Biol. **14**(1), 70–75 (2004).

Onuchic, J. N., Luthey-Schulten, Z., and Wolynes, P. G., "Theory of protein folding: The energy landscape perspective," Annu. Rev. Phys. Chem. **48**, 545–600 (1997).

Ostrem, J. M., Peters, U., Sos, M. L., Wells, J. A., and Shokat, K. M., "K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions," Nature **503**(7477), 548–551 (2013).

Palopoli, N., González Foutel, N. S., Gibson, T. J., and Chemes, L. B., "Short linear motif core and flanking regions modulate retinoblastoma protein binding affinity and specificity," Protein Eng. Des. Sel. **31**(3), 69–77 (2018).

Pancsa, R., and Tompa, P., "Structural disorder in eukaryotes," PLoS One **7**(4), e34687 (2012).

Pantsar, T., "The current understanding of KRAS protein structure and dynamics," Comput. Struct. Biotechnol. J. **18**, 189–198 (2019).

Pantsar, T., Rissanen, S., Dauch, D., Laitinen, T., Vattulainen, I., and Poso, A., "Assessment of mutation probabilities of KRAS G12 missense mutants and their long-timescale dynamics by atomistic molecular simulations and Markov state modeling," PLoS Comput. Biol. **14**(9), e1006458 (2018).

Papaleo, E., Camilloni, C., Teilum, K., Vendruscolo, M., and Lindorff-Larsen, K., "Molecular dynamics ensemble refinement of the heterogeneous native state of NCBD using chemical shifts and NOEs," PeerJ **6**, e5125 (2018).

Patil, A., Kinoshita, K., and Nakamura, H., "Hub promiscuity in protein-protein interaction networks," Int. J. Mol. Sci. **11**(4), 1930–1943 (2010).

Pelham, J. F., Dunlap, J. C., and Hurley, J. M., "Intrinsic disorder is an essential characteristic of components in the conserved circadian circuit," Cell Commun. Signaling **18**(1), 181 (2020).

Pelham, J. F., Mosier, A. E., and Hurley, J. M., "Characterizing time-of-day conformational changes in the intrinsically disordered proteins of the circadian clock," Methods Enzymol. **611**, 503–529 (2018).

Peng, Z., Yan, J., Fan, X., Mizianty, M. J., Xue, B., Wang, K., Hu, G., Uversky, V. N., and Kurgan, L., "Exceptionally abundant exceptions: Comprehensive characterization of intrinsic disorder in all domains of life," Cell. Mol. Life Sci. **72**(1), 137–151 (2015).

Peng, E., Todorova, N., and Yarovsky, I., "Effects of forcefield and sampling method in all-atom simulations of inherently disordered proteins: Application to conformational preferences of human amylin," PLoS One **12**(10), e0186219 (2017).

Peng, Y., Cao, S., Kiselar, J., Xiao, X., Du, Z., Hsieh, A., Ko, S., Chen, Y., Agrawal, P., Zheng, W., Shi, W., Jiang, W., Yang, L., Chance, M. R., Surewicz, W. K., Buck, M., and Yang, S., "A metastable contact and structural disorder in the estrogen receptor transactivation domain," Structure **27**(2), 229–240 (2019).

Permyakov, S. E., Millett, I. S., Doniach, S., Permyakov, E. A., and Uversky, V. N., "Natively unfolded C-terminal domain of caldesmon remains substantially unstructured after the effective binding to calmodulin," Proteins **53**(4), 855–862 (2003).

Permyakov, S. E., Permyakov, E. A., and Uversky, V. N., "Intrinsically disordered caldesmon binds calmodulin via the 'buttons on a string' mechanism," PeerJ **3**, e1265 (2015).

Peterson, L. X., Roy, A., Christoffer, C., Terashi, G., and Kihara, D., "Modeling disordered protein interactions from biophysical principles," PLoS Comput. Biol. **13**(4), e1005485 (2017).

Piana, S., Donchev, A. G., Robustelli, P., and Shaw, D. E., "Water dispersion interactions strongly influence simulated structural properties of disordered protein states," J. Phys. Chem. B **119**(16), 5113–5123 (2015).

Piovesan, D., Necci, M., Escobedo, N., Monzon, A. M., Hatos, A., Mičetić, I., Quaglia, F., Paladin, L., Ramasamy, P., Dosztányi, Z., Vranken, W. F., Davey, N. E., Parisi, G., Fuxreiter, M., and Tosatto, S. C. E., "MobiDB: Intrinsically disordered proteins in 2021," Nucl. Acids Res. **49**(D1), D361–D367 (2021).

Pleasance, E. D., Cheetham, R. K., Stephens, P. J., McBride, D. J., Humphray, S. J., Greenman, C. D., Varela, I., Lin, M. L., Ordóñez, G. R., Bignell, G. R., Ye, K., Alipaz, J., Bauer, M. J., Beare, D., Butler, A., Carter, R. J., Chen, L., Cox, A. J., Edkins, S., Kokko-Gonzales, P. I., Gormley, N. A., Grocock, R. J., Haudenschild, C. D., Hims, M. M., James, T., Jia, M., Kingsbury, Z., Leroy, C., Marshall, J., Menzies, A., Mudie, L. J., Ning, Z., Royce, T., Schulz-Trieglaff, O. B., Spiridou, A., Stebbings, L. A., Szajkowski, L., Teague, J., Williamson, D., Chin, L., Ross, M. T., Campbell, P. J., Bentley, D. R., Futreal, P. A., and Stratton, M. R., "A comprehensive catalogue of somatic mutations from a human cancer genome," Nature **463**(7278), 191–196 (2010).

Podlaha, O., and Zhang, J., "Positive selection on protein-length in the evolution of a primate sperm ion channel," Proc. Natl. Acad. Sci. U.S.A. **100**(21), 12241–12246 (2003).

Pontius, B. W., "Close encounters: Why unstructured, polymeric domains can increase rates of specific macromolecular association," Trends Biochem. Sci. **18**(5), 181–186 (1993).

Potenza, E., Di Domenico, T., Walsh, I., and Tosatto, S. C., "MobiDB 2.0: An improved database of intrinsically disordered and mobile proteins," Nucl. Acids Res. **43**, D315–D320 (2015).

Prakash, P., and Gorfe, A. A., "Lessons from computer simulations of Ras proteins in solution and in membrane," Biochim. Biophys. Acta **1830**(11), 5211–5218 (2013).

Prakash, P., and Gorfe, A. A., "Probing the conformational and energy landscapes of KRAS membrane orientation," J. Phys. Chem. B **123**(41), 8644–8652 (2019).

Prakash, P., Litwin, D., Liang, H., Sarkar-Banerjee, S., Dolino, D., Zhou, Y., Hancock, J. F., Jayaraman, V., and Gorfe, A. A., "Dynamics of membrane-bound G12V-KRAS from simulations and single-molecule FRET in native nanodiscs," Biophys. J. **116**(2), 179–183 (2019).

Prestel, A., Wichmann, N., Martins, J. M., Marabini, R., Kassem, N., Broendum, S. S., Otterlei, M., Nielsen, O., Willemoës, M., Ploug, M., Boomsma, W., and Kragelund, B. B., "The PCNA interaction motifs revisited: Thinking outside the PIP-box," Cell. Mol. Life Sci. **76**(24), 4923–4943 (2019).

Prior, I. A., Lewis, P. D., and Mattos, C., "A comprehensive survey of Ras mutations in cancer," Cancer Res. **72**(10), 2457–2467 (2012).

Quaglia, F., Lazar, T., Hatos, A., Tompa, P., Piovesan, D., and Tosatto, S. C. E., "Exploring curated conformational ensembles of intrinsically disordered proteins in the protein ensemble database," Curr. Protoc. **1**(7), e192 (2021).

Rae, G. M., Uversky, V. N., David, K., and Wood, M., "DRM1 and DRM2 expression regulation: Potential role of splice variants in response to stress and environmental factors in Arabidopsis," Mol. Genet. Genomics **289**(3), 317–332 (2014).

Ragonnet-Cronin, M., Hodcroft, E., Hué, S., Fearnhill, E., Delpech, V., Brown, A. J., and Lycett, S., "Automated analysis of phylogenetic clusters," BMC Bioinf. **14**, 317 (2013).

Raina, K., Dey, C., Thool, M., Sudhagar, S., and Thummer, R. P., "An insight into the role of UTF1 in development, stem cells, and cancer," Stem Cell Rev. Rep. **17**(4), 1280–1293 (2021).

Raj, A., and van Oudenaarden, A., "Single-molecule approaches to stochastic gene expression," Annu. Rev. Biophys. **38**, 255–270 (2009).

Ramanathan, A., Ma, H., Parvatikar, A., and Chennubhotla, S. C., "Artificial intelligence techniques for integrative structural biology of intrinsically disordered proteins," Curr. Opin. Struct. Biol. **66**, 216–224 (2021).

Rangan, R., Bonomi, M., Heller, G. T., Cesari, A., Bussi, G., and Vendruscolo, M., "Determination of structural ensembles of proteins: Restraining vs reweighting," J. Chem. Theory Comput. **14**(12), 6632–6641 (2018).

Rangarajan, N., Kulkarni, P., and Hannenhalli, S., "Evolutionarily conserved network properties of intrinsically disordered proteins," PLoS One **10**(5), e0126729 (2015).

Rauscher, S., and Pomès, R., "The liquid structure of elastin," Elife **6**, e26526 (2017).

Rehman, A. U., Rahman, M. U., Arshad, T., and Chen, H. F., "Allosteric modulation of intrinsically disordered proteins," Adv. Exp. Med. Biol. **1163**, 335–357 (2019).

Robustelli, P., Kohlhoff, K., Cavalli, A., and Vendruscolo, M., "Using NMR chemical shifts as structural restraints in molecular dynamics simulations of proteins," Structure **18**(8), 923–933 (2010).

Robustelli, P., Piana, S., and Shaw, D. E., "Developing a molecular dynamics force field for both folded and disordered protein states," Proc. Natl. Acad. Sci. U.S.A. **115**(21), E4758–E4766 (2018).

Rogers, J. M., Oleinikovas, V., Shammas, S. L., Wong, C. T., De Sancho, D., Baker, C. M., and Clarke, J., "Interplay between partner and ligand facilitates the folding and binding of an intrinsically disordered protein," Proc. Natl. Acad. Sci. U.S.A. **111**(43), 15420–15425 (2014a).

Rogers, J. M., Wong, C. T., and Clarke, J., "Coupled folding and binding of the disordered protein PUMA does not require particular residual structure," J. Am. Chem. Soc. **136**(14), 5197–5200 (2014b).

Romero, P. R., Zaidi, S., Fang, Y. Y., Uversky, V. N., Radivojac, P., Oldfield, C. J., Cortese, M. S., Sickmeier, M., LeGall, T., Obradovic, Z., and Dunker, A. K., "Alternative splicing in concert with protein intrinsic disorder enables increased functional diversity in multicellular organisms," Proc. Natl. Acad. Sci. U.S.A. **103**(22), 8390–8395 (2006).

Ruan, H., Sun, Q., Zhang, W., Liu, Y., and Lai, L., "Targeting intrinsically disordered proteins at the edge of chaos," Drug Discovery Today **24**(1), 217–227 (2019).

Sahoo, S., Mishra, A., Kaur, H., Hari, K., Muralidharan, S., Mandal, S., and Jolly, M. K., "A mechanistic model captures the emergence and implications of non-genetic heterogeneity and reversible drug resistance in ER+ breast cancer cells," NAR Cancer **3**(3), zcab027 (2021).

Salahuddin, P., Fatima, M. T., Uversky, V. N., Khan, R. H., Islam, Z., and Furkan, M., "The role of amyloids in Alzheimer's and Parkinson's diseases," Int. J. Biol. Macromol. **190**, 44–55 (2021).

Salgia, R., and Kulkarni, P., "The genetic/non-genetic duality of drug 'resistance' in cancer," Trends Cancer **4**(2), 110–118 (2018).

Salvi, N., Abyzov, A., and Blackledge, M., "Multi-timescale dynamics in intrinsically disordered proteins from NMR relaxation and molecular simulation," J. Phys. Chem. Lett. **7**(13), 2483–2489 (2016).

Sanches, M. N., Knapp, K., Oliveira, Jr., A. B., Wolynes, P. G., Onuchic, J. N., and Leite, V. B. P., "Examining the ensembles of amyloid-β monomer variants and their propensities to form fibers using an energy landscape visualization method," J. Phys. Chem. B **126**(1), 93–99 (2022).

Santofimia-Castaño, P., Rizzuti, B., Xia, Y., Abian, O., Peng, L., Velázquez-Campoy, A., Neira, J. L., and Iovanna, J., "Targeting intrinsically disordered proteins involved in cancer," Cell. Mol. Life Sci. **77**(9), 1695–1707 (2020).

Sarkar-Banerjee, S., Sayyed-Ahmad, A., Prakash, P., Cho, K. J., Waxham, M. N., Hancock, J. F., and Gorfe, A. A., "Spatiotemporal analysis of K-Ras plasma membrane interactions reveals multiple high order homo-oligomeric complexes," J. Am. Chem. Soc. **139**(38), 13466–13475 (2017).

Sayyed-Ahmad, A., Prakash, P., and Gorfe, A. A., "Distinct dynamics and interaction patterns in H- and K-Ras oncogenic P-loop mutants," Proteins **85**(9), 1618–1632 (2017).

Schad, E., Tompa, P., and Hegyi, H., "The relationship between proteome size, structural disorder and organism complexity," Genome Biol. **12**(12), R120 (2011).

Schneider, R., Blackledge, M., and Jensen, M. R., "Elucidating binding mechanisms and dynamics of intrinsically disordered protein complexes using NMR spectroscopy," Curr. Opin. Struct. Biol. **54**, 10–18 (2019).

Schuler, B., Borgia, A., Borgia, M. B., Heidarsson, P. O., Holmstrom, E. D., Nettels, D., and Sottini, A., "Binding without folding—The biomolecular function of disordered polyelectrolyte complexes," Curr. Opin. Struct. Biol. **60**, 66–76 (2020).

Sciarrillo, R., Wojtuszkiewicz, A., Assaraf, Y. G., Jansen, G., Kaspers, G. J. L., Giovannetti, E., and Cloos, J., "The role of alternative splicing in cancer: From oncogenesis to drug resistance," Drug Resist. Update **53**, 100728 (2020).

Sehl, M. E., Shimada, M., Landeros, A., Lange, K., and Wicha, M. S., "Modeling of cancer stem cell state transitions predicts therapeutic response," PLoS One **10**(9), e0135797 (2015).

Shea, J. E., Best, R. B., and Mittal, J., "Physics-based computational and theoretical approaches to intrinsically disordered proteins," Curr. Opin. Struct. Biol. **67**, 219–225 (2021).

Shoemaker, B. A., Portman, J. J., and Wolynes, P. G., "Speeding molecular recognition by using the folding funnel: The fly-casting mechanism," Proc. Natl. Acad. Sci. U.S.A. **97**(16), 8868–8873 (2000).

Shrestha, U. R., Juneja, P., Zhang, Q., Gurumoorthy, V., Borreguero, J. M., Urban, V., Cheng, X., Pingali, S. V., Smith, J. C., O'Neill, H. M., and Petridis, L., "Generation of the configurational ensemble of an intrinsically disordered protein from unbiased molecular dynamics simulation," Proc. Natl. Acad. Sci. U.S.A. **116**(41), 20446–20452 (2019).

Shrestha, U. R., Smith, J. C., and Petridis, L., "Full structural ensembles of intrinsically disordered proteins from unbiased molecular dynamics simulations," Commun. Biol. **4**(1), 243 (2021).

Sieradzan, A. K., Korneev, A., Begun, A., Kachlishvili, K., Scheraga, H. A., Molochkov, A., Senet, P., Niemi, A. J., and Maisuradze, G. G., "Investigation of phosphorylation-induced folding of an intrinsically disordered protein by coarse-grained molecular dynamics," J. Chem. Theory Comput. **17**(5), 3203–3220 (2021).

Simon, C. S., Hadjantonakis, A. K., and Schröter, C., "Making lineage decisions with biological noise: Lessons from the early mouse embryo," Wiley Interdiscip. Rev. Dev. Biol. **7**(4), e319 (2018).

Singh, D., Bocci, F., Kulkarni, P., and Jolly, M. K., "Coupled feedback loops involving PAGE4, EMT and notch signaling can give rise to non-genetic heterogeneity in prostate cancer cells," Entropy (Basel) **23**(3), 288 (2021).

Smith, A. K., Khayat, E., Lockhart, C., and Klimov, D. K., "Do cholesterol and sphingomyelin change the mechanism of Aβ25-35 peptide binding to zwitterionic bilayer?," J. Chem. Inf. Model. **59**(12), 5207–5217 (2019).

Smith, A. K., Lockhart, C., and Klimov, D. K., "Does replica exchange with solute tempering efficiently sample Aβ peptide conformational ensembles?," J. Chem. Theory Comput. **12**(10), 5201–5214 (2016).

Sottini, A., Borgia, A., Borgia, M. B., Bugge, K., Nettels, D., Chowdhury, A., Heidarsson, P. O., Zosel, F., Best, R. B., Kragelund, B. B., and Schuler, B., "Polyelectrolyte interactions enable rapid association and dissociation in high-affinity disordered protein complexes," Nat. Commun. **11**(1), 5736 (2020).

Spoerner, M., Herrmann, C., Vetter, I. R., Kalbitzer, H. R., and Wittinghofer, A., "Dynamic properties of the Ras switch I region and its importance for binding to effectors," Proc. Natl. Acad. Sci. U.S.A. **98**(9), 4944–4949 (2001).

Spoerner, M., Hozsa, C., Poetzl, J. A., Reiss, K., Ganser, P., Geyer, M., and Kalbitzer, H. R., "Conformational states of human rat sarcoma (Ras) protein complexed with its natural ligand GTP and their role for effector interaction and GTP hydrolysis," J. Biol. Chem. **285**(51), 39768–39778 (2010).

Sterckx, Y. G., Volkov, A. N., Vranken, W. F., Kragelj, J., Jensen, M. R., Buts, L., Garcia-Pino, A., Jové, T., Van Melderen, L., Blackledge, M., van Nuland, N. A., and Loris, R., "Small-angle X-ray scattering- and nuclear magnetic resonance-derived conformational ensemble of the highly flexible antitoxin PaaA2," Structure **22**(6), 854–865 (2014).

Strodel, B., "Energy landscapes of protein aggregation and conformation switching in intrinsically disordered proteins," J. Mol. Biol. **433**(20), 167182 (2021).

Strzyz, P., "Concentrating on intrinsic disorder," Nat. Rev. Genet. **19**(9), 534 (2018).

Sudnitsyna, M. V., Mymrikov, E. V., Seit-Nebi, A. S., and Gusev, N. B., "The role of intrinsically disordered regions in the structure and functioning of small heat shock proteins," Curr. Protein Pept. Sci. **13**(1), 76–85 (2012).

Sugita, Y., and Okamoto, Y., "Replica-exchange molecular dynamics method for protein folding," Chem. Phys. Lett. **314**, 141–151 (1999).

Sun, X., Jones, W. T., Harvey, D., Edwards, P. J., Pascal, S. M., Kirk, C., Considine, T., Sheerin, D. J., Rakonjac, J., Oldfield, C. J., Xue, B., Dunker, A. K., and Uversky, V. N., "N-terminal domains of DELLA proteins are intrinsically unstructured in the absence of interaction with GID1/gibberellic acid receptors," J. Biol. Chem. **285**(15), 11557–11571 (2010).

Sun, X., Xue, B., Jones, W. T., Rikkerink, E., Dunker, A. K., and Uversky, V. N., "A functionally required unfoldome from the plant kingdom: Intrinsically disordered N-terminal domains of GRAS proteins are involved in molecular recognition during plant development," Plant Mol. Biol. **77**(3), 205–223 (2011).

Sun, X., Rikkerink, E. H., Jones, W. T., and Uversky, V. N., "Multifarious roles of intrinsic disorder in proteins illustrate its broad impact on plant biology," Plant Cell **25**(1), 38–55 (2013).

Sun, X., Greenwood, D. R., Templeton, M. D., Libich, D. S., McGhie, T. K., Xue, B., Yoon, M., Cui, W., Kirk, C. A., Jones, W. T., Uversky, V. N., and Rikkerink, E. H., "The intrinsically disordered structural platform of the plant defence hub protein RPM1-interacting protein 4 provides insights into its mode of action in the host-pathogen interface and evolution of the nitrate-induced domain protein family," FEBS J. **281**(17), 3955–3979 (2014).

Takada, S., Kanada, R., Tan, C., Terakawa, T., Li, W., and Kenzaki, H., "Modeling structural dynamics of biomolecular complexes by coarse-grained molecular simulations," Acc. Chem. Res. **48**(12), 3026–3035 (2015).

Teilum, K., Olsen, J. G., and Kragelund, B. B., "On the specificity of protein-protein interactions in the context of disorder," Biochem. J. **478**(11), 2035–2050 (2021).

Thirumalai, D., O'Brien, E. P., Morrison, G., and Hyeon, C., "Theoretical perspectives on protein folding," Annu. Rev. Biophys. **39**, 159–183 (2010).

Tompa, P., "Unstructural biology coming of age," Curr. Opin. Struct. Biol. **21**(3), 419–425 (2011).

Tompa, P., "Intrinsically disordered proteins: A 10-year recap," Trends Biochem. Sci. **37**(13), 509–516 (2012).

Tompa, P., and Fuxreiter, M., "Fuzzy complexes: Polymorphism and structural disorder in protein-protein interactions," Trends Biochem. Sci. **33**(1), 2–8 (2008).

Tompa, P., and Kovacs, D., "Intrinsically disordered chaperones in plants and animals," Biochem. Cell Biol. **88**(2), 167–174 (2010).

Tompa, P., Szász, C., and Buday, L., "Structural disorder throws new light on moonlighting," Trends Biochem. Sci. **30**(9), 484–489 (2005).

Toto, A., and Gianni, S., "Mutational analysis of the binding-induced folding reaction of the mixed-lineage leukemia protein to the KIX domain," Biochemistry **55**(28), 3957–3962 (2016).

Toto, A., Camilloni, C., Giri, R., Brunori, M., Vendruscolo, M., and Gianni, S., "Molecular recognition by templated folding of an intrinsically disordered protein," Sci. Rep. **6**, 21994 (2016).

Toto, A., Malagrinò, F., Visconti, L., Troilo, F., Pagano, L., Brunori, M., Jemth, P., and Gianni, S., "Templated folding of intrinsically disordered proteins," J. Biol. Chem. **295**(19), 6586–6593 (2020).

Tsafou, K., Tiwari, P. B., Forman-Kay, J. D., Metallo, S. J., and Toretsky, J. A., "Targeting intrinsically disordered transcription factors: Changing the paradigm," J. Mol. Biol. **430**(16), 2321–2341 (2018).

Tsai, C. J., and Nussinov, R., "A unified view of 'how allostery works'," PLoS Comput. Biol. **10**(2), e10033 (2014).

Tsytlonok, M., Sanabria, H., Wang, Y., Felekyan, S., Hemmen, K., Phillips, A. H., Yun, M. K., Waddell, M. B., Park, C. G., Vaithiyalingam, S., Iconaru, L., White, S. W., Tompa, P., Seidel, C. A. M., and Kriwacki, R., "Dynamic anticipation by Cdk2/cyclin A-bound p27 mediates signal integration in cell cycle regulation," Nat. Commun. **10**(1), 1676 (2019).

Uversky, V. N., "Natively unfolded proteins: A point where biology waits for physics," Protein Sci. **11**(4), 739–756 (2002).

Uversky, V. N., "The mysterious unfoldome: Structureless, underappreciated, yet vital part of any given proteome," J. Biomed. Biotechnol. **2010**, 568068.

Uversky, V. N., "Flexible nets of malleable guardians: Intrinsically disordered chaperones in neurodegenerative diseases," Chem. Rev. **111**(2), 1134–1166 (2011).

Uversky, V. N., "A decade and a half of protein intrinsic disorder: Biology still waits for physics," Protein Sci. **22**(6), 693–724 (2013a).

Uversky, V. N., "Unusual biophysics of intrinsically disordered proteins," Biochim. Biophys. Acta **1834**(5), 932–951 (2013b).

Uversky, V. N., "Wrecked regulation of intrinsically disordered proteins in diseases: Pathogenicity of deregulated regulators," Front. Mol. Biosci. **1**, 6 (2014).

Uversky, V. N., "Functional roles of transiently and intrinsically disordered regions within proteins," FEBS J. **282**(7), 1182–1189 (2015).

Uversky, V. N., "Dancing protein clouds: The strange biology and chaotic physics of intrinsically disordered proteins," J. Biol. Chem. **291**(13), 6681–6688 (2016a).

Uversky, V. N., "p53 proteoforms and intrinsic disorder: An illustration of the protein structure-function continuum concept," Int. J. Mol. Sci. **17**(11), 1874 (2016b).

Uversky, V. N., "Paradoxes and wonders of intrinsic disorder: Complexity of simplicity," Intrinsically Disord. Proteins **4**(1), e1135015 (2016c).

Uversky, V. N., "Intrinsically disordered proteins and their 'mysterious' (meta)-physics," Front. Phys. **7**, 1–18 (2019a).

Uversky, V. N., "Protein intrinsic disorder and structure-function continuum," Prog. Mol. Biol. Transl. Sci. **166**, 1–17 (2019b).

Uversky, V. N., "Per aspera ad chaos: A personal journey to the wonderland of intrinsic disorder," Biochem. J. **478**(15), 3015–3024 (2021).

Uversky, V. N., and Dunker, A. K., "Understanding protein non-folding," Biochim. Biophys. Acta **1804**(6), 1231–1264 (2010).

Uversky, V. N., and Kulkarni, P., "Intrinsically disordered proteins: Chronology of a discovery," Biophys. Chem. **279**, 106694 (2021).

Uversky, V. N., Oldfield, C. J., and Dunker, A. K., "Intrinsically disordered proteins in human diseases: Introducing the D2 concept," Annu. Rev. Biophys. **37**, 215–246 (2008).

Uversky, V. N., Oldfield, C. J., Midic, U., Xie, H., Xue, B., Vucetic, S., Iakoucheva, L. M., Obradovic, Z., and Dunker, A. K., "Unfoldomics of human diseases: Linking protein intrinsic disorder with diseases," BMC Genomics **10**(Suppl 1), S7 (2009).

Vacic, V., Oldfield, C. J., Mohan, A., Radivojac, P., Cortese, M. S., Uversky, V. N., and Dunker, A. K., "Characterization of molecular recognition features, MoRFs, and their binding partners," J. Proteome Res. **6**(6), 2351–2366 (2007).

van der Lee, R., Buljan, M., Lang, B., Weatheritt, R. J., Daughdrill, G. W., Dunker, A. K., Fuxreiter, M., Gough, J., Gsponer, J., Jones, D. T., Kim, P. M., Kriwacki, R. W., Oldfield, C. J., Pappu, R. V., Tompa, P., Uversky, V. N., Wright, P. E., and Babu, M. M., "Classification of intrinsically disordered regions and proteins," Chem. Rev. **114**(13), 6589–6631 (2014).

Van Roey, K., Dinkel, H., Weatheritt, R. J., Gibson, T. J., and Davey, N. E., "The switches.ELM resource: A compendium of conditional regulatory interaction interfaces," Sci. Signaling **6**(269), rs7 (2013).

Van Roey, K., Gibson, T. J., and Davey, N. E., "Motif switches: Decision-making in cell regulation," Curr. Opin. Struct. Biol. **22**(3), 378–385 (2012).

Van Roey, K., Uyar, B., Weatheritt, R. J., Dinkel, H., Seiler, M., Budd, A., Gibson, T. J., and Davey, N. E., "Short linear motifs: Ubiquitous and functionally diverse protein interaction modules directing cell regulation," Chem. Rev. **114**(13), 6733–6778 (2014).

Vatansever, S., Gümüş, Z. H., and Erman, B., "Intrinsic K-Ras dynamics: A novel molecular dynamics data analysis method shows causality between residue pair motions," Sci. Rep. **6**, 37012 (2016).

Vatansever, S., Erman, B., and Gümüş, Z. H., "Comparative effects of oncogenic mutations G12C, G12V, G13D, and Q61H on local conformations and dynamics of K-Ras," Comput. Struct. Biotechnol. J. **18**, 1000–1011 (2020).

Vavouri, T., Semple, J. I., Garcia-Verdugo, R., and Lehner, B., "Intrinsic protein disorder and interaction promiscuity are widely associated with dosage sensitivity," Cell **138**(1), 198–208 (2009).

Vila, J. A., "Metamorphic proteins in light of Anfinsen's dogma," J. Phys. Chem. Lett. **11**(13), 4998–4999 (2020).

Vo, U., Embrey, K. J., Breeze, A. L., and Golovanov, A. P., "^{1}H, ^{13}C and ^{15}N resonance assignment for the human K-Ras at physiological pH," Biomol. NMR Assign. **7**(2), 215–219 (2013).

Volkamer, A., Kuhn, D., Grombacher, T., Rippmann, F., and Rarey, M., "Combining global and local measures for structure-based druggability predictions," J. Chem. Inf. Model. **52**(2), 360–372 (2012).

Wakao, S., Kitada, M., and Dezawa, M., "The elite and stochastic model for iPS cell generation: Multilineage-differentiating stress enduring (muse) cells are readily reprogrammable into iPS cells," Cytometry, Part A **83**(1), 18–26 (2013).

Wales, D. J., "Energy landscapes: Some new horizons," Curr. Opin. Struct. Biol. **20**(1), 3–10 (2010).

Wales, D. J., "Exploring energy landscapes," Annu. Rev. Phys. Chem. **69**, 401–425 (2018).

Wang, W., "Recent advances in atomic molecular dynamics simulation of intrinsically disordered proteins," Phys. Chem. Chem. Phys. **23**(2), 777–784 (2021).

Wang, L., Friesner, R. A., and Berne, B. J., "Replica exchange with solute scaling: A more efficient version of replica exchange with solute tempering (REST2)," J. Phys. Chem. B **115**(30), 9431–9438 (2011).

Wang, Y., Chu, X., Longhi, S., Roche, P., Han, W., Wang, E., and Wang, J., "Multiscaled exploration of coupled folding and binding of an intrinsically disordered molecular recognition element in measles virus nucleoprotein," Proc. Natl. Acad. Sci. U.S.A. **110**(40), E3743–E3752 (2013).

Ward, J. J., Sodhi, J. S., McGuffin, L. J., Buxton, B. F., and Jones, D. T., "Prediction and functional analysis of native disorder in proteins from the three kingdoms of life," J. Mol. Biol. **337**(3), 635–645 (2004).

Webster, J. M., Darling, A. L., Uversky, V. N., and Blair, L. J., "Small heat shock proteins, big impact on protein aggregation in neurodegenerative disease," Front. Pharmacol. **10**, 1047 (2019).

Wood, K., Hensing, T., Malik, R., and Salgia, R., "Prognostic and predictive value in KRAS in non-small-cell lung cancer: A review," JAMA Oncol. **2**(6), 805–812 (2016).

Wright, P. E., and Dyson, H. J., "Intrinsically unstructured proteins: Re-assessing the protein structure-function paradigm," J. Mol. Biol. **293**(2), 321–331 (1999).

Wright, P. E., and Dyson, H. J., "Intrinsically disordered proteins in cellular signalling and regulation," Nat. Rev. Mol. Cell Biol. **16**(1), 18–29 (2015).

Xue, B., Dunker, A. K., and Uversky, V. N., "Orderly order in protein intrinsic disorder distribution: Disorder in 3500 proteomes from viruses and the three domains of life," J. Biomol. Struct. Dyn. **30**(2), 137–149 (2012).

Xue, B., Oldfield, C. J., Van, Y. Y., Dunker, A. K., and Uversky, V. N., "Protein intrinsic disorder and induced pluripotent stem cells," Mol. Biosyst. **8**(1), 134–150 (2012).

Xue, B., Williams, R. W., Oldfield, C. J., Dunker, A. K., and Uversky, V. N., "Archaic chaos: Intrinsically disordered proteins in Archaea," BMC Syst. Biol. **4**(Suppl 1), S1 (2010).

Yamanaka, S., "Elite and stochastic models for induced pluripotent stem cell generation," Nature **460**(7251), 49–52 (2009).

Yin, G., Kistler, S., George, S. D., Kuhlmann, N., Garvey, L., Huynh, M., Bagni, R. K., Lammers, M., Der, C. J., and Campbell, S. L., "A KRAS GTPase K104Q mutant retains downstream signaling by offsetting defects in regulation," J. Biol. Chem. **292**(11), 4446–4456 (2017).

Yoon, M. K., Mitrea, D. M., Ou, L., and Kriwacki, R. W., "Cell cycle regulation by the intrinsically disordered proteins p21 and p27," Biochem. Soc. Trans. **40**(5), 981–988 (2012).

Zerze, G. H., Miller, C. M., Granata, D., and Mittal, J., "Free energy surface of an intrinsically disordered protein: Comparison between temperature replica exchange molecular dynamics and bias-exchange metadynamics," J. Chem. Theory Comput. **11**(6), 2776–2782 (2015).

Zhang, L., Li, M., and Liu, Z., "A comprehensive ensemble model for comparing the allosteric effect of ordered and disordered proteins," PLoS Comput. Biol. **14**(12), e1006393 (2018).

Zhang, M., Jang, H., and Nussinov, R., "The structural basis for Ras activation of PI3Kα lipid kinase," Phys. Chem. Chem. Phys. **21**(22), 12021–12028 (2019).

Zhao, Y., Cortes-Huerto, R., Kremer, K., and Rudzinski, J. F., "Investigating the conformational ensembles of intrinsically disordered proteins with a simple physics-based model," J. Phys. Chem. B **124**(20), 4097–4113 (2020).

Zhao, J., Blayney, A., Liu, X., Gandy, L., Jin, W., Yan, L., Ha, J. H., Canning, A. J., Connelly, M., Yang, C., Liu, X., Xiao, Y., Cosgrove, M. S., Solmaz, S. R., Zhang, Y., Ban, D., Chen, J., Loh, S. N., and Wang, C., "EGCG binds intrinsically disordered N-terminal domain of p53 and disrupts p53-MDM2 interaction," Nat. Commun. **12**(1), 986 (2021).

Zheng, X., Gan, L., Wang, E., and Wang, J., "Pocket-based drug design: Exploring pocket space," AAPS J. **15**(1), 228–241 (2013).

Zimmerman, M. I., Hart, K. M., Sibbald, C. A., Frederick, T. E., Jimah, J. R., Knoverek, C. R., Tolia, N. H., and Bowman, G. R., "Prediction of new stabilizing mutations based on mechanistic insights from Markov state models," ACS Cent. Sci. **3**(12), 1311–1321 (2017).