Computational approaches to understanding the biological behaviour of intrinsically disordered proteins

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Intrinsically disordered proteins (IDPs) represent a class of proteins that lack a persistent folded conformation and exist as dynamic ensembles in their native state. Inherent lack of a well-defined structure and remarkable structural plasticity have facilitated their functioning in a wide range of crucial cellular processes such as signalling transduction and cell cycle regulation as well as responsible for their aberrant toxic amyloidogenic conformations implicated in a wide range of neurodegenerative diseases, cancer, etc. Their ubiquitous presence in nature, role in biological function and diseases have spurred interest in the biophysical and conformational characterization of IDPs. Conventional methods of structure determination are less feasible owing to structural and spatiotemporal heterogeneity of IDPs, which demand the development of novel biophysical methods as well as rigorous computational techniques for their characterization. In this review, we provide a brief overview of the widely used computational techniques probing the rugged conformational energy landscape of IDPs, their kinetics of structural transitions and molecular interactions key to their functions. Advances in the development of calibrated computational approaches for statistical representation of highly dynamic structural ensemble of IDPs are provided with examples. Challenges in modelling this unique class of proteins as well as the existing and futuristic avenues are also discussed.

Keywords: Chaperones, free-energy, intrinsically disordered proteins, molecular dynamics, Monte Carlo method.

Introduction

PROTEINS, complex biological polymers composed of various combinations of less than two dozen naturally occurring amino acids, fulfil myriad functions in biology. For over a hundred years, functional integrity of proteins has been associated with their ability to fold rapidly into unique, three-dimensional structures[1]. Interestingly, newer perspectives developed over the last few decades establish the existence of a class of polypeptides known as intrinsically disordered proteins (IDPs), whose biological roles are fundamentally associated to their ability to adopt multiple conformations at different physiological conditions[2–4]. In addition, many proteins also have intrinsically disordered segments or regions (IDRs) that are key to their functions. These IDPs or IDRs are highly abundant in nature and involved in a plethora of biological activities. The inherent structural plasticity of IDPs confers them with unique functional modalities in cellular processes such as cell–cycle regulation, gene expression, protein–protein interactions, etc. Several evidences have recognized structural disorders in proteins that act as chaperones[5–7]. A well-characterized example of such a protein is the heat shock protein Hsp-33 that functions as a chaperone under oxidative stress[5]. Transitions of this protein between ordered and disordered conformations are key to its functioning as a chaperone. Promiscuous binding nature of IDPs makes them well suited as hubs in protein interaction networks, especially those involved in cell signalling, as exemplified by the tumour suppressor protein, p53 (ref. 8). On the flip side, these characteristics also render IDPs more prone to aggregation and associated diseases. Formation of insoluble and intractable aggregates of IDPs highlights their implications in human diseases. Examples of such IDPs are amyloid beta (Aβ) and α-synuclein involved in neurodegenerative diseases[9] (Alzheimer's and Parkinson's diseases respectively), p53 and HPV associated with cancer[10], etc. In the remaining text, the term 'IDP' will be used to denote both intrinsically disordered proteins and regions.

Owing to their structural complexities and subtle dependence on solvent and thermodynamic conditions, probing the mechanistic origins of IDP function can pose greater challenges than the corresponding studies of well-defined folded proteins. In recent years, however, large strides have been made in biophysical experimental techniques for probing IDP structure and function, most notably in solution[11,12] and solid-state NMR[13], small-angle X-ray scattering[14,15], fluorescence microscopy[16,17], cryo-EM[18], fluorescence correlation spectroscopy[19,20] and

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some single-molecule techniques[21,22]. In Table 1, we have listed representative studies that use at least one of these methods to characterize physico-chemical characteristics of key IDPs. It is important to note, however, that experiments are essentially a 'top down' approach to understand IDP behaviour, and often provide only limited insights on the specific nature of interactions and the resultant physical forces underlying their observed thermodynamic, kinetic, structural and thereby functional behaviour. In this regard, it has been realized that theoretical models and advanced molecular computations can provide an alternative 'bottoms-up' view, and thereby provide a powerful repertoire of complementary methods to probe the physical underpinnings of IDP structure and function[23,24].

Conformational energy landscapes of IDPs describing their thermodynamic free energy as a function of one or more collective variables are typically more rugged in comparison to the 'funnel-shaped' landscapes adopted by landscapes of folded proteins (see Figure 1). Thus, the conformational ensembles of IDPs are characterized by several thermodynamically equivalent low energy states. These states are often separated by relatively small energy barriers and therefore the landscapes are sometimes referred to as 'glassy'[25]. However, infrequently, thermodynamically equivalent states may be separated by barriers that exceed thermal levels by more than an order of magnitude; such situations may 'trap' specific states, and may trigger the onset of systemic malfunction or disease in biological organisms[26,27]. Specially designed computational studies are often necessary to understand the thermodynamic and kinetic origins of such 'traps' and to strategize plausible physico-chemical means to lower the barriers and repopulate biologically advantageous conformations[28–30].

In this article, we attempt to provide an overview of popular computational techniques used for studying IDP conformations, their energy landscapes, kinetics of their transformations and their modulation by the surrounding environment as well as by nanomaterials and small molecules. The underpinnings of each method are touched upon briefly, and the reader is referred to other sources for more details[31–34]. Wherever possible, complementarity with experimental observations has been highlighted. Future challenges and the way forward have been discussed. Though not exhaustive, we hope to provide the reader a useful insight into the exciting world of IDPs, and possibly motivate theory and computational biophysicists to invest their interest.

Table 1. Representative experimental techniques used for characterizing IDPs; the list provided is non-exhaustive

Experimental technique	Reference
NMR spectroscopy	124–128
Small angle X-ray scattering	129–134
Single molecule spectroscopy	135–141
Fluorescence techniques	142–144
Atomic force microscopy	145, 146
Cryo-electron microscopy	147–149
X-ray diffraction	150, 151

Computational techniques

Molecular dynamics simulations

With the rapid evolution and advancement of technology, computational methods are proving indispensable in understanding the physics and chemistry of complex biomolecular systems. Of the myriad of computational techniques, molecular dynamics (MD) simulations is widely used to gain molecular-level insights into structural, dynamical and thermodynamic properties of macromolecular systems. The rapidly increasing computational power over the years has allowed harnessing MD simulations to delve into the intricacies of biological processes such as protein folding and misfolding, protein denaturation, protein aggregation, membrane dynamics,

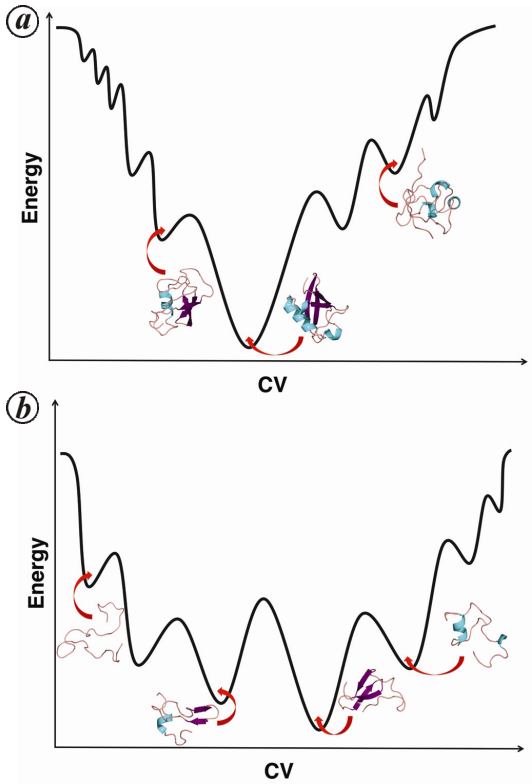

Figure 1. Schematic descriptions of conformational energy landscapes of (***a***) folded and (***b***) intrinsically disordered proteins (IDPs), as function of representative collective variables (CV). Folded proteins have funnel-shaped landscapes with a unique global minimum corresponding to the natively folded state. IDPs have a more rugged landscape characterized by multiple equivalent minima separated by barriers.

protein–protein interactions, proteins–nucleic acid interactions, influence of solvent environments, enzymatic processes, effects of biomimetic crowding, etc.[35–50]. This method is also, capable of complementing experimental measurements over fairly wide time and length scales.

MD simulations are used to study macroscopic behaviour by means of numerical integration of classical equations of motion of a microscopic many-body system. It involves sampling of the phase space of a system in terms of position and velocities of the constituting particles. Temporal evolution of these quantities often referred to as the 'trajectory' of the system, can be processed to obtain macroscopic properties using principles of statistical mechanics. The positional coordinates and velocities of the particles are derived from Newton's second law of motion, wherein the force acting on a particle at a given time point is equal to the product of its mass and acceleration. The force on each particle i, is derived as the negative gradient of the potential, U_i, imposed on it by the rest of the system. The interaction potentials are described empirically. The potential function, commonly known as 'force fields', is a parametric description of interaction energy as a function of inter-atomic distance, and is used to obtain the force acting on each particle at every time point. The numerical integration of forces then provide velocities and positions, and thereby a description of phase space of the system[31–32].

It is now increasingly becoming possible to use all-atom unbiased MD simulations to explore simple biological macromolecules in microseconds time regime[51–53], or further[54]. It has been extensively used to study the highly polymorphic IDPs and thereby complement biophysical characterizations and help overcome the limitations of several experimental observations. Edward O'Brien *et al.* used all-atom MD simulations to study the thermodynamics of lactam congener, Aβ_{1-40} [D23–K28] in order to interpret the origins of the enhanced rate of fibril formation observed in experiments[55]. They simulated the wild type (WT) Aβ dimer, a monomer, with lactam bridge (Aβ_{10-35}-lactam [D23–K28]) and monomer and dimers with harmonically constrained D23-K28 salt bridge (Aβ_{10-35} [D23–K28]), to understand the origin of enhanced rate of fibril formation. Their results suggested that the reduction in entropy of lactam congener with constrained salt bridge [D23–K28] as well as enthalpic effects, contribute to the reduction in free energy barrier to nucleation and growth of Aβ_{1-40}-lactam [D23–K28] fibril as compared to WT. Zhu *et al.*[56] probed the mechanism of dimerization as well as structural features of the most stable dimers of the full-length Aβ_{1-42} peptide using classical MD simulations. Their simulations reaffirmed structural features of dimers as observed in experiments as well as identified several key structural properties of peptides. Extensive all-atom MD simulations were used to identify and understand the molecular determinants of relative propensities of IDPs to aggregate[57]. The results showed that the overall protein hydrophobicity, a property defined by the hydration-free energy of protein, predominates the aggregation propensities of proteins in aqueous environment and had a remarkable correlation with experimentally observed aggregation propensities. However, owing to multiple minima, sometimes separated by high kinetic barriers, unbiased MD techniques can fall short of the sampling requirements in IDPs. Hence, several enhanced MD protocols have been designed to enable crossing the energetic barriers and explore a large conformational space within finite computational times.

Replica exchange molecular dynamics (REMD)[33] is a parallel tempering method that samples multiple replicas of a system concurrently over a range of different temperatures. Coordinates of the replicas are periodically exchanged between ensembles, and the exchanges are governed by Metropolis criterion that is defined as

$$P(\text{exchange}) = \exp(-\beta\Delta E); \quad \Delta E \geq 0, \tag{1}$$

$$= 1, \quad \Delta E < 0, \tag{2}$$

where $\beta = 1/k_B T$, k_B is the Boltzmann constant and T is the temperature. Rapid exchange of replicas helps overcome free energy barriers in the conformational landscape. This technique has been widely used as an explorative tool to characterize the rugged energy landscapes of IDPs[58–66]. Sgourakis *et al.*[67] used REMD based methods to distinguish the conformational ensembles of two Aβ variants (Aβ_{1-40} and Aβ_{1-42}) which are key players in the pathogenesis of Alzheimer's disease (AD). Enhanced sampling of the conformational space of Aβ peptides revealed that the structural ensemble of Aβ_{1-42} is far more diverse than Aβ_{1-40}. They also identified structured regions primarily in the C-terminus of Aβ_{1-42} that may be responsible for higher propensity of this peptide to form amyloid. Another study exploited REMD to provide insights into the equilibrium structure of Aβ_{1-40} dimer by sampling the various transient peptide conformations and modes of organization to form the dimer[58]. Das *et al.*[66], using extensive atomistic REMD, studied the protective cross-interaction of experimentally revealed A2T variant of Aβ_{1-42} monomer and the wild type (WT). On comparison with WT homo-dimer, they found an overall weakening of a set of transient, intrachain contacts formed between the central and C-terminal hydrophobic residues of the heterodimer. Importantly, the A2T N-terminus, particularly residue F4, was observed to undergo hydrophobic burial owing to its persistent tertiary and quaternary interactions with hydrophobic residues of the central and C-terminus. They further remarked that the atypical behaviour of N-terminus in A2T heterodimer might have consequences in the aggregation of peptide, which was consistent with experiments. However, this method has its own drawbacks: REMD cannot substantially enhance sampling for large biomolecular systems,

which require a huge number of replicas to bridge the temperature range sampling the ensemble. Further, this method is not useful if high temperature does not facilitate the conformational transition of interest. In addition, temporal properties cannot be calculated since this method introduces discontinuity during swapping of replicas.

Accelerated molecular dynamics (AMD)[34] is an enhanced sampling method that introduces suitable biases into potential energy function in order to enable barrier crossings. A boost energy, E_b, and an acceleration parameter, α, is applied to the original potential, $V(r)$, of the system of reference such that the system evolves in a modified potential, $V^*(r)$, which can be written as

$$V^*(r) = V(r), \qquad V(r) \geq E_b, \tag{3}$$

$$V^*(r) = V(r) + \Delta V(r), \qquad V(r) < E_b, \tag{4}$$

where the 'bias potential' $\Delta V(r)$ is obtained as

$$\Delta V(r) = \frac{[E_b - V(r)]^2}{E_b - V(r) + \alpha}. \tag{5}$$

The introduction of bias potential preserves the landscape of the underlying original potential energy wells while allowing the wells to be sufficiently and accurately sampled.

AMD has been used effectively to investigate dynamical transitions in the natively unstructured Tau protein, whose misfolded and aggregated forms form neurofibrillary tangles implicated in AD and other neurodegenerative diseases[68]. In this study, AMD, in agreement with experimental data, revealed strong structural propensity of four homologous sequences in repeat domains to form turns and highlighted the potential of this specific conformational transition as an inhibitory mechanism against pathological transformation. Our previous studies have implemented AMD to sample the conformational space of full-length Aβ peptide, which has been further used to understand the correlations with the structure and dynamics of surrounding water molecules[69–70].

The probability of finding a molecular system in one state or another depends on the free-energy difference between two states. According to the principles of statistical mechanics, free energy difference between two states can be computed from the averages of ensembles of atomic-level configurations of the system, which can be generated from MD or MC simulation strategies. Free-energy calculations are quite useful for calculating biophysical properties of biomolecules such as protein–protein and protein-ligand binding free affinities, partition coefficients, etc. One of the techniques used to calculate free-energy difference between two states of a molecular system separated by an energy barrier is umbrella sampling[71], where multiple biased simulations are performed by applying restraints to a chosen set of configurations. These configurations are chosen such that they sample a region of a specific reaction coordinate. However, free-energy is only obtained as a function of the chosen reaction coordinate. The use of another reaction coordinate to link the end states may lead to a different free-energy difference, indicating the dependence of this method on the chosen reaction coordinate[72]. Lenkul and Bevan employed umbrella sampling simulations to study the thermodynamics of Aβ peptide dissociation from the core of a model protofibril at physiological temperature[73]. Another known method for free energy calculations is Adaptive Biasing Force (ABF)[74]. ABF is a method of thermodynamic integration in which the mean force along a chosen reaction coordinate, σ, is used to estimate the energy barriers between two states. The gradient of the free energy is then obtained from the average force F_σ as

$$\frac{dA(\sigma)}{d\sigma} = -\langle F_\sigma \rangle_\sigma. \tag{6}$$

In our previous work dealing with the study of Aβ self-association on a single-walled carbon nanotube (SWCNT), we implemented ABF method to evaluate the growth potential of Aβ oligomers immobilized on the surface of the nanotube. Although the intrinsic propensity of Aβ to self-assemble is highly impaired by adsorption on nano-surface, the oligomeric units showed high degrees of surface immobilization. Free-energy calculations revealed that though immobilized oligomers are capable of growth, there is a shift in monomer–oligomer equilibrium as compared to free states.

It is important to keep in mind that a large number of MD simulation work on IDPs are carried out using fully atomistic force fields, where every atom is parametrized separately. However, this requires the integration of motion for each atom which inherently makes the sampling of phase space computationally expensive. While atomistic simulations are indispensable for eliciting detailed structural features of IDPs and their aggregates, it can be advantageous, in specific situations, to use a coarse-grained description of the force field. A coarse grained potential is essentially a description of interactions where the degrees of freedom of individual atoms are not considered independently, but a chemical group is described as a spherical mass with an overall charge. Coarse-grained simulations use longer time steps and are capable of sampling systems of much larger dimensions than atomistic force fields, and are therefore useful in investigating higher ordered growth in IDP aggregation[75–82]. A recent work by Kurcinski *et al.*[83] utilized a coarse-grained approach to characterize the various modes of complex formation and interactions of the IDP, phosphorylated kinase-inducible domain (pKID) and its interacting domain KIX. Sieradzan *et al.*[84] combined coarse graining and REMD sampling to investigate the process of

complex assembly and inter-protein interactions of a homotetrameric $\beta\beta\alpha$ (BBAT1) protein. They found that the association into a tetramer precedes the folding of individual protein chains, facilitating the folding process and stabilization of the complex. Coarse graining has also shown promising results in modelling intermediates of aggregation, following different aggregation pathways as well as exploring long-time scales of the aggregation process that cannot be captured via atomistic simulations. Using coarse-grained simulations of peptide self-assembly, Ranganathan *et al.*[85] presented a physical rationale for self-assembly of peptides/proteins into ordered aggregates and supramolecular structures as well as diversity in the self-assembled structures. By varying the inherent physical properties like polymer flexibility and interaction strengths, they identified and elucidated properties such as aggregate diversity and polymorphism associated with biological self-assemblies. As a general rule, coarse graining smoothens out the ruggedness of the underlying energy landscapes and may lead to inaccurate calculations of the thermodynamic properties of the system, and therefore must be used judiciously vis-à-vis pertinent biophysical questions.

Monte Carlo methods

Monte Carlo (MC) method, frequently used for sampling biomolecular systems, work by exploring the energy landscape by probing random conformations in the phase space. Briefly, a MC simulation commences from an initial configuration followed by a random move that generates a new configuration. This move is accepted or rejected based on an 'acceptance criterion' that ensures that the conformations are sampled from a statistical mechanical ensemble distribution with a correct weight. An important condition to be followed in MC simulations is, that an ergodic scheme must be used which means that any state of the system must be accessible from any other state in the conformational space in a finite number of MC moves. On accumulation of a large number of conformations from a stochastic simulation, statistical mechanical principles are used to calculate thermodynamic properties of the system. However, as MC does not involve solving Newton's equations of motion, no dynamical information can be obtained from MC simulation. Since it lacks an objective definition of time, no temporal evolution of a property can be calculated for a system. Detailed descriptions of MC techniques are found in several reports over the last few decades[86,87]. We, would like point out that MC simulations of proteins in explicit water are not efficient as stochastic moves causing drastic changes in the internal coordinates of the protein without simultaneously moving the solvent molecules, result in a high rejection probability due to possible steric hindrances[86]. Thus, MC methods are more popular in combination with coarse grained descriptions of potential functions.

Kinetic approaches

Despite rapid advances, there is a large possibility that the conformational sampling of complex biomolecules may remain incomplete by even the most efficient MD and MC methods. This is especially true of those IDPs whose conformational changes and aggregation involve crossing several large kinetic barriers. In this direction, several researchers have attempted to develop phenomenological models designed to reproduce the experimentally observed rates of conformational transformation and aggregation. An early mathematical model proposed by Lomakin *et al.* posited that amyloid growth must require a nucleation facilitated by Aβ micelles, followed by irreversible fibrillar elongation[88]. Adopting the nucleation-polymerization hypothesis, Pallitto and Murphy incorporated experimental data from dynamic light scattering, fluorescence, size exclusion chromatography as well as cytotoxicity assays to develop kinetic master equations yielding the rates of filament initiation, elongation and fibrillar growth[89]. The concentration dependence of kinetics of the nucleated polymerization process in amyloidogenesis, especially under supercritical concentrations, were further modelled by Powers and Powers[90]. A more recent two-state model of IDP kinetics embodies a key pathogenic structural transition in amyloid aggregation from a coil-like state to a β-sheet rich state. By employing distinct rates of polymerization and depolymerization based on the conformational state of the peptide, this model explains experimentally observed phenomena of amyloid self-assembly such as concentration-dependence of growth velocities, fibril length heterogeneity and intermittent nature of fibril growth[91].

Another quantitative approach for obtaining the rates of conformational transitions in IDPs and amyloidogenic proteins, harnesses the energy landscape methods pioneered by Wales *et al.*[92]. These methods first produce a database of stationary points for the system using global optimization techniques, and then employ discrete path sampling methods to connect minima and transition states in the energy landscape[93–96]. The repertoire of these methods has been developed over the last decade to overcome the limitations of thermodynamic sampling methods such as MD and MC simulations. A combination of these methods have been used to describe the kinetics of assembly process of the shortest known amyloid sequences[97], as well as key fragments of prion protein and Aβ peptide[98]. Interestingly, these methods have also yielded the most stable oligomeric size and conformations of full-length Aβ peptide in its toxic, membrane-spanning state[99].

Challenges and opportunities

Owing to the lack of global free-energy 'native' states and the ruggedness in conformational landscapes, studies

of IDPs present a more greater challenge than corresponding studies involving folded and functional proteins. The realization that a 'bottoms-up' view of underpinning interactions and the structural, thermodynamic, kinetic and assembly aspects of IDP behaviour can be obtained from theoretical and computational methods, has opened up a plethora of possibilities in IDP research. Herein, a brief overview of some of the key but persistent challenges and new opportunities is presented.

Sampling accuracy

Current generation force field parameters developed for amino acids that are used in most MD, and in some MC and kinetic methods, are benchmarked against experiments via structural data available for natively folded proteins[100,101]. Thus, an underlying assumption in their usage in IDP studies is, that the parameters are equally effective in sampling the conformational space of proteins prone to rapid fluctuations. However, given the sparse amount of ensemble information for IDPs that are available experimentally, benchmarking the sampling accuracy is challenging. Yet, some progress in weighing the relative accuracy of various force fields has been made, particularly for the archetypal Aβ, in conjunction with data from solution of NMR studies[102].

IDP interactions on surfaces

In the last few decades, growth of nanotechnology has encouraged several new ventures in drug development. Protein surface interactions have been capturing increasing attention owing to their ubiquitous occurrence in biological processes and a wide range of applications in bioengineering and nanotechnology[30,103–105]. Understanding protein-surface interactions is key to the development of new strategies in the field of nanomedicine, biomaterial sciences and nanobiotechnology. The physicochemical properties of the surfaces and the nature of their interactions may have an altering effect on the structural stability and activities of biomolecules[30,103,104,106,107]. Computational modelling and simulations can help to unravel the mechanisms of protein surface binding, the determinants of binding specificity as well as thermodynamics adsorption. Molecular level information obtained from computational studies has the potential to leverage experimental efforts in drug development. Harnessing the power of theoretical methods such as atomistic MD simulations as well as quantum mechanical (QM) calculations, we elucidated the interactions of Aβ monomer on the surface of a SWCNT as well as provided insights into the effects of surface geometry on the interactions with biomolecules[30,108]. MD simulations have been extensively used to study the binding and interactions of IDPs with surfaces of various biomimetic membrane models as well as membrane permeation phenomenon. A recent study employed MD simulations combined with experimental techniques to study the role of cholesterol in the aggregation of islet amyloid polypeptide (IAPP), an IDP linked to type-2 diabetes mellitus (T2DM), and the related membrane disruption[109]. It demonstrated that the presence of cholesterol has a modulatory effect on the IAPP-evoked membrane disruption.

It is evident that the interaction of IDPs with surfaces has a complex dependence on their physical and chemical characteristics. Consequently, IDP adsorption as well as its potential toxic effects may, in principle, be modulated via properties of the interaction surfaces. Thus, further computational research on IDP-surface interactions in conjunction with suitable experiments, should have high significance in terms of understanding IDP behaviour on biological surfaces such as cellular membranes, as well in controlling aspects such as toxicity and function.

Devices and detection

Computational approaches have also been utilized in the proof-of-concept design of biomolecular sensors. The bottoms-up approach of computational methods to understand the atomistic-level interactions in biomolecules is capable of contributing immensely to the process of designing high-performance biomaterials for applications in biosensors with diagnostic applications or point-of-care assessment. Surface immobilized biomolecular probes or lab-on-a-chip devices are revolutionizing many areas of biomedical research such as genomics, proteomics, immunology and pathology. The adsorption of the biomolecule to the surface plays a key role in these applications and hence needs to be engineered to build a successful device. For example, the orientation of ligand molecules, which have specific binding sites that need to be accessible to the target molecules, is crucial for the proper functioning of a biosensor. MD simulations have proved their merit by providing atomic level information in biomolecular adsorption studies[110,111]. Such insights could be applied to control and manipulate the orientation of biomolecules in experiments that can maximize the efficiency of biosensors. Our recent work has demonstrated the detectability of Aβ oligomeric states adsorbed to SWCNT in an ionic solution upon application of optimal electric currents[108]. The results encourage the development of carbon nanomaterial based electrical sensors for the detection of small Aβ oligomers.

The use of fluorescent dyes such as Congo red and Thioflavin T (ThT) for characterizing amyloid aggregates to diagnose amyloid diseases and for the development of therapeutics is well established[112]. From a biomedical standpoint, it is essential to obtain a molecular level understanding of the binding events in order to design better dyes for diagnosis. Theoretical studies have been

used to delve deeper into the various interactions and binding modes of the routinely used fluorescent dye, ThT, to protofibrillar models of Aβ[113,114].

Drug design

Since the identification of IDPs more than two decades ago and the essential roles that they play in a broad range of critical cellular functions, 'unstructural biology' has paved its way into the mainstream of molecular and cell biology[115–117]. Additionally, IDPs are implicated in a number of human diseases such as cancer, neurodegenerative and cardiovascular disorders[8]. Given the prevalence of IDPs in human diseases, they are attractive therapeutic targets; however, rational drug designing for IDPs is still in its infancy. With the steadily improving knowledge of structural and dynamical behaviour of IDPs characterized by experiments as well as computational methods, IDPs are now considered 'druggable'[118], and hence drug development is a new frontier being explored in this domain. In this regard, it is crucial to study and establish the mechanisms of molecular recognition between small molecules and IDPs that can guide rational drug design endeavours. Conformational sampling is being increasingly exploited to gain insights into the structural diversity and dynamical aspects of IDPs as well as mechanisms of interactions with other proteins[119,120]. The feasibility of small molecule inhibition of IDPs is demonstrated in the case of oncoprotein c-Myc, which is a potential cancer drug target[121]. Extensive REMD simulation studies have helped to build the conformational ensemble of c-Myc and explore its interactions and binding mechanism with an experimentally reported small molecule inhibitor[122,123]. Such computational investigations have important implications in rational ligand design efforts targeting IDPs, and can be used for *in-silico* designs of therapeutics aimed at several debilitating diseases.

Conclusions

The requirement of disorder in some proteins as a cornerstone of their biological function is beginning to emerge as a new paradigm. This realization has fuelled vast experimental research with several biophysical and biochemical methods. Yet, experimental limitations in capturing the complex details of conformational fluctuations can hinder the understanding of the functional behaviour of this subclass of protein molecules generally referred to as IDPs. Fortunately, advanced computations, which are based on modelling the interactions at the atomic level, emerge as powerful complementary techniques in this area of research. These methods can be useful in directly capturing the conformational energy landscapes, and thereby unearthing thermodynamic and kinetic properties underlying the complex IDP behaviour in realistic environments. While several challenges remain, particularly in terms of accuracy and scales of thermodynamic sampling in appropriate media, it is encouraging to note how rapid advances in these methods have been harnessed appropriately to gain major insights into the structural, self-assembly and functional properties of IDPs. Further, computational methods are being increasingly used to predict as well as propose ways to alter key properties of IDPs, paving way for several applications ranging from drug discovery to sensor design. In conjunction with appropriate experimental data, advanced computational methods therefore provide unprecedented possibilities in the scope of IDP research. The aim of this article has been to provide a short but meaningful glimpse of this exciting field, and it is hoped that it will motivate computational biophysicists to pursue the key challenges that emerge in this field.

1. Fischer, E., Influence of configuration on the action of enzymes. *Ber. Dtsch. Chem. Ges.*,1894, **27**, 2985–2993.
2. Wright, P. E. and Dyson, H. J., Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. *J. Mol. Biol.*, 1999, **293**(2), 321–331.
3. Tompa, P., Intrinsically disordered proteins: a 10-year recap. *Trends Biochem. Sci.*, 2012, **37**(12), 509–516.
4. Dyson, H. J., Making sense of intrinsically disordered proteins. *Biophys. J.*, 2016, **110**(5), 1013–1016.
5. Tompa, P. and Csermely, P., The role of structural disorder in the function of RNA and protein chaperones. *FASEB J.*, 2004, **18**(11), 1169–1175.
6. Kovacs, D. and Tompa, P., Diverse functional manifestations of intrinsic structural disorder in molecular chaperones. *Biochem. Soc. Trans.*, 2012, **40**(5), 963–968.
7. Foit, L., George, J. S., Zhang, B. W., Brooks, C. L. and Bardwell, J. C. A., Chaperone activation by unfolding. *Proc. Natl. Acad. Sci. USA*, 2013, **110**(14), E1254–E1262.
8. 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*, 2008, **9**(S1), 1–20.
9. Knowles, T. P. J., Vendruscolo, M. and Dobson, C. M., The amyloid state and its association with protein misfolding diseases. *Nat. Rev. Mol. Cell. Biol.*, 2014, **15**(6), 384–396.
10. 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.*, 2002, **323**(3), 573–584.
11. Bussell, R. and Eliezer, D., Residual structure and dynamics in Parkinson's disease-associated mutants of α-synuclein. *J. Biol. Chem.*, 2001, **276**(49), 45996–46003.
12. Eliezer, D., Barré, P., Kobaslija, M., Chan, D., Li, X. and Heend, L., Residual structure in the repeat domain of tau: echoes of microtubule binding and paired helical filament formation. *Biochemistry*, 2005, **44**(3), 1026–1036.
13. Havlin, R. H. and Tycko, R., Probing site-specific conformational distributions in protein folding with solid-state NMR. *Proc. Natl. Acad. Sci. USA*, 2005, **102**(9), 3284–3289.
14. Mylonas, E., Hascher, A., Bernadó, P., Blackledge, M., Mandelkow, E. and Svergun, D. I., Domain conformation of tau protein studied by solution small-angle X-ray scattering. *Biochemistry*, 2008, **47**(39), 10345–10353.
15. Bernado, P. and Svergun, D. I., Structural analysis of intrinsically disordered proteins by small-angle X-ray scattering. *Mol. BioSyst. I*, 2012, **8**(1), 151–167.

16. Maji, S. K. *et al.*, Functional amyloids as natural storage of peptide hormones in pituitary secretory granules. *Science*, 2009, **325**(5938), 328.
17. Singh, P. K. and Maji, S. K., Amyloid-like fibril formation by tachykinin neuropeptides and its relevance to amyloid β-protein aggregation and toxicity. *Cell Biochem. Biophys.*, 2012, **64**(1), 29–44.
18. Schmidt, M., Rohou, A., Lasker, K., Yadav, J. K., Schiene-Fischer, C., Fändrich, M. and Grigorieff, N., Peptide dimer structure in an Aβ(1–42) fibril visualized with cryo-EM. *Proc. Natl. Acad. Sci. USA*, 2015, **112**(38), 11858–11863.
19. Bhowmik, D., MacLaughlin, C. M., Chandrakesan, M., Ramesh, P., Venkatramani, R., Walker, G. C. and Maiti, S., pH changes the aggregation propensity of amyloid-[small beta] without altering the monomer conformation. *Phys. Chem. Chem. Phys.*, 2014, **16**(3), 885–889.
20. Chandrakesan, M. *et al.*, Steric crowding of the turn region alters the tertiary fold of amyloid-β18–35 and makes it soluble. *J. Biol. Chem.*, 2015, **290**(50), 30099–30107.
21. Elbaum-Garfinkle, S. and Rhoades, E., Identification of an aggregation-prone structure of tau. *J. Am. Chem. Soc.*, 2012, **134**(40), 16607–16613.
22. Arya, S. and Mukhopadhyay, S., Ordered water within the collapsed globules of an amyloidogenic intrinsically disordered protein. *J. Phys. Chem. B*, 2014, **118**(31), 9191–9198.
23. 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.*, 2015, **11**(6), 2776–2782.
24. Ithuralde, R. E., Roitberg, A. E. and Turjanski, A. G., Structured and unstructured binding of an intrinsically disordered protein as revealed by atomistic simulations. *J. Am. Chem. Soc.*, **138**(28), 8742–8751.
25. Vitalis, A., Wang, X. and Pappu, R. V., Quantitative characterization of intrinsic disorder in polyglutamine: insights from analysis based on polymer theories. *Biophys. J.*, 2007, **93**(6), 1923–1937.
26. Jahn, T. R. and Radford, S. E., The Yin and Yang of protein folding. *FEBS J.*, 2005, **272**(23), 5962–5970.
27. Hartl, F. U., Bracher, A. and Hayer-Hartl, M., Molecular chaperones in protein folding and proteostasis. *Nature*, 2011, **475**(7356), 324–332.
28. Wales, D. J. and Bogdan, T. V., Potential energy and free energy landscapes. *J. Phys. Chem. B*, 2006, **110**(42), 20765–20776.
29. Raval, A., Piana, S., Eastwood, M. P. and Shaw, D. E., Assessment of the utility of contact-based restraints in accelerating the prediction of protein structure using molecular dynamics simulations. *Protein Sci.*, 2016, **25**(1), 19–29.
30. Jana, A. K. and Sengupta, N., Adsorption mechanism and collapse propensities of the full-length, monomeric Aβ1–42 on the surface of a single-walled carbon nanotube: a molecular dynamics simulation study. *Biophys. J.*, 2012, **102**(8), 1889–1896.
31. Allen, M. P. and Tildesley, D. J., *Computer Simulation of Liquids*, Oxford University Press, 1989.
32. Frenkel, D. and Smit, B., *Understanding Molecular Simulations: From Algorithms to Applications*, Academic, San Diego, 1996.
33. Sugita, Y. and Okamoto, Y., Replica-exchange molecular dynamics method for protein folding. *Chem. Phys. Lett.*, 1999, **314**(1–2), 141–151.
34. Hamelberg, D., Mongan, J. and McCammon, J. A., Accelerated molecular dynamics: a promising and efficient simulation method for biomolecules. *J. Chem. Phys.*, 2004, **120**(24), 11919–11929.
35. Paschek, D., Nymeyer, H. and García, A. E., Replica exchange simulation of reversible folding/unfolding of the Trp-cage miniprotein in explicit solvent: On the structure and possible role of internal water. *J. Struct. Biol.*, 2007, **157**(3), 524–533.
36. Tran, H. T., Mao, A. and Pappu, R. V., Role of backbone−solvent interactions in determining conformational equilibria of intrinsically disordered proteins. *J. Am. Chem. Soc.*, 2008, **130**(23), 7380–7392.
37. Reddy, G., Straub, J. E. and Thirumalai, D., Dynamics of locking of peptides onto growing amyloid fibrils. *Proc. Natl. Acad. Sci. USA*, 2009, **106**(29), 11948–11953.
38. Canchi, D. R., Paschek, D. and García, A. E., Equilibrium study of protein denaturation by urea. *J. Am. Chem. Soc.*, 2010, **132**(7), 2338–2344.
39. Reddy, G., Straub, J. E. and Thirumalai, D., Dry amyloid fibril assembly in a yeast prion peptide is mediated by long-lived structures containing water wires. *Proc. Natl. Acad. Sci. USA*, 2010, **107**(50), 21459–21464.
40. O'Brien, E. P., Straub, J. E., Brooks, B. R. and Thirumalai, D., Influence of nanoparticle size and shape on oligomer formation of an amyloidogenic peptide. *J. Phys. Chem. Lett.*, 2011, **2**(10), 1171–1177.
41. Chong, S. H. and Ham, S., Impact of chemical heterogeneity on protein self-assembly in water. *Proc. Natl. Acad. Sci. USA*, 2012, **109**(20), 7636–7641.
42. Thirumalai, D., Reddy, G. and Straub, J. E., Role of water in protein aggregation and amyloid polymorphism. *Acc. Chem. Res.*, 2012, **45**(1), 83–92.
43. Reddy, G., Liu, Z. and Thirumalai, D., Denaturant-dependent folding of GFP. *Proc. Natl. Acad. Sci. USA*, 2012, **109**(44), 17832–17838.
44. Das, R. K. and Pappu, R. V., Conformations of intrinsically disordered proteins are influenced by linear sequence distributions of oppositely charged residues. *Proc. Natl. Acad. Sci. USA*, 2013, **110**(33), 13392–13397.
45. Thirumalai, D., Liu, Z., O'Brien, E. P. and Reddy, G., Protein folding: from theory to practice. *Curr. Opin. Struct. Biol.*, 2013, **23**(1), 22–29.
46. English, C. A. and Garcia, A. E., Folding and unfolding thermodynamics of the TC10b Trp-cage miniprotein. *Phys. Chem. Chem. Phys.*, 2014, **16**(7), 2748–2757.
47. Barz, B., Olubiyi, O. O. and Strodel, B., Early amyloid [small beta]-protein aggregation precedes conformational change. *Chem. Commun.*, 2014, **50**(40), 5373–5375.
48. Han, W. and Schulten, K., Fibril elongation by Aβ17–42: kinetic network analysis of hybrid-resolution molecular dynamics simulations. *J. Am. Chem. Soc.*, 2014, **136**(35), 12450–12460.
49. Baram, M., Atsmon-Raz, Y., Ma, B., Nussinov, R. and Miller, Y., Amylin-A[small beta] oligomers at atomic resolution using molecular dynamics simulations: a link between Type 2 diabetes and Alzheimer's disease. *Phys. Chem. Chem. Phys.*, 2016, **18**(4), 2330–2338.
50. Muttathukattil, A. N. and Reddy, G., Osmolyte effects on the growth of amyloid fibrils. *J. Phys. Chem. B*, 2016, **120**(42), 10979–10989.
51. Duan, Y. and Kollman, P. A., Pathways to a protein folding intermediate observed in a 1-microsecond simulation in aqueous solution. *Science*, 1998, **282**(5389), 740–744.
52. Freddolino, P. L., Liu, F., Gruebele, M. and Schulten, K., Ten-microsecond molecular dynamics simulation of a fast-folding WW domain. *Biophys. J.*, 2008, **94**(10), L75–L77.
53. Lindorff-Larsen, K., Maragakis, P., Piana, S. and Shaw, D. E., Picosecond to millisecond structural dynamics in human ubiquitin. *J. Phys. Chem. B*, 2016, **120**(33), 8313–8320.
54. Shaw, D. E. *et al.*, Anton, a special-purpose machine for molecular dynamics simulation. *Commun. ACM, Commun. ACM*, **51**(7), 91–97.
55. O'Brien, E. P., Okamoto, Y., Straub, J. E., Brooks, B. R. and Thirumalai, D., Thermodynamic perspective on the Dock–Lock growth mechanism of amyloid fibrils. *J. Phys. Chem. B*, 2009, **113**(43), 14421–14430.

56. Zhu, X., Bora, R. P., Barman, A., Singh, R. and Prabhakar, R., Dimerization of the full-length Alzheimer amyloid β-peptide (Aβ42) in explicit aqueous solution: a molecular dynamics study. *J. Phys. Chem. B*, 2012, **116**(15), 4405–4416.
57. Chong, S. H. and Ham, S., Interaction with the surrounding water plays a key role in determining the aggregation propensity of proteins. *Angew. Chemie Inter. Ed*., 2014, **53**(15), 3961–3964.
58. Tarus, B., Tran, T. T., Nasica-Labouze, J., Sterpone, F., Nguyen, P. H. and Derreumaux, P., Structures of the Alzheimer's wild-type Aβ1–40 dimer from atomistic simulations. *J. Phys. Chem. B*, 2015, **119**(33), 10478–10487.
59. Dupuis, N. F., Wu, C., Shea, J.-E. and Bowers, M. T., Human islet amyloid polypeptide monomers form ordered β-hairpins: a possible direct amyloidogenic precursor. *J. Am. Chem. Soc.*, 2009, **131**(51), 18283–18292.
60. Sgourakis, N. G., Merced-Serrano, M., Boutsidis, C., Drineas, P., Du, Z., Wang, C. and Garcia, A. E., Atomic-level characterization of the ensemble of the Aβ(1–42) monomer in water using unbiased molecular dynamics simulations and spectral algorithms. *J. Mol. Biol.*, 2011, **405**(2), 570–583.
61. Wu, C. and Shea, J. E., Structural similarities and differences between amyloidogenic and non-amyloidogenic islet amyloid polypeptide (IAPP) sequences and implications for the dual physiological and pathological activities of these peptides. *PLoS Comput. Biol.*, 2013, **9**(8), e1003211.
62. Mittal, J., Yoo, T. H., Georgiou, G. and Truskett, T. M., Structural ensemble of an intrinsically disordered polypeptide. *J. Phys. Chem. B*, 2013, **117**(1), 118–124.
63. Miller, C., Zerze, G. H. and Mittal, J., Molecular simulations indicate marked differences in the structure of amylin mutants, correlated with known aggregation propensity. *J. Phys. Chem. B*, 2013, **117**(50), 16066–16075.
64. Qi, R., Luo, Y., Wei, G., Nussinov, R. and Ma, B., Aβ 'stretching-and-tacking' cross-seeding mechanism can trigger tau protein aggregation. *J. Phys. Chem. Lett.*, 2015, **6**(16), 3276–3282.
65. Zou, Y., Sun, Y., Zhu, Y., Ma, B., Nussinov, R. and Zhang, Q., Critical nucleus structure and aggregation mechanism of the C-terminal fragment of copper–zinc superoxide dismutase protein. *ACS Chem. Neurosci.*, 2016, **7**(3), 286–296.
66. Das, P., Chacko, A. R. and Belfort, G., Alzheimer's protective cross–interaction between wild-type and A2T variants alters Aβ42 dimer structure. *ACS Chem. Neurosci*., 2017, **8**(3), 606–618.
67. Sgourakis, N. G., Yan, Y., McCallum, S. A., Wang, C. and Garcia, A. E., The Alzheimer's peptides Aβ40 and 42 adopt distinct conformations in water: a combined MD/NMR study. *J. Mol. Biol*., 2007, **368**(5), 1448–1457.
68. Mukrasch, M. D. *et al.*, Highly populated turn conformations in natively unfolded tau protein identified from residual dipolar couplings and molecular simulation. *J. Am. Chem. Soc*., 2007, **129**(16), 5235–5243.
69. Jose, J. C., Khatua, P., Bansal, N., Sengupta, N. and Bandyopadhyay, S., Microscopic hydration properties of the Aβ1–42 peptide monomer and the globular protein ubiquitin: a comparative molecular dynamics study. *J. Phys. Chem. B*, 2014, **118**(40), 11591–11604.
70. Khatua, P., Jose, J. C., Sengupta, N. and Bandyopadhyay, S., Conformational features of the Aβ42 peptide monomer and its interaction with the surrounding solvent. *Phys. Chem. Chem. Phys*., 2016, **18**(43), 30144–30159.
71. Torrie, G. M. and Valleau, J. P., Nonphysical sampling distributions in Monte Carlo free-energy estimation: umbrella sampling. *J. Comput. Phys*., 1977, **23**(2), 187–199.
72. Fraternali, F. and Van Gunsteren, W. F., Conformational transitions of a dipeptide in water: effects of imposed pathways using umbrella sampling techniques. *Biopolymers*, 1994, **34**(3), 347–355.
73. Lemkul, J. A. and Bevan, D. R., Assessing the stability of Alzheimer's amyloid protofibrils using molecular dynamics. *J. Phys. Chem. B*, 2010, **114**(4), 1652–1660.
74. Darve, E., Rodríguez-Gómez, D. and Pohorille, A., Adaptive biasing force method for scalar and vector free energy calculations. *J. Chem. Phys*., 2008, **128**(14), 144120.
75. Pellarin, R. and Caflisch, A., Interpreting the aggregation kinetics of amyloid peptides. *J. Mol. Biol*., 2006, **360**(4), 882–892.
76. Hyeon, C. and Thirumalai, D., Capturing the essence of folding and functions of biomolecules using coarse-grained models. *Nature Commun*., 2011, **2**(487), 1–11.
77. Ruff, Kiersten M., Khan, Siddique, J., Pappu and Rohit, V., A coarse-grained model for polyglutamine aggregation modulated by amphipathic flanking sequences. *Biophys. J*., 2014, **107**(5), 1226–1235.
78. Šarić, A., Chebaro, Y. C., Knowles, T. P. J. and Frenkel, D., Crucial role of nonspecific interactions in amyloid nucleation. *Proc. Natl. Acad. Sci. USA*, 2014, **111**(50), 17869–17874.
79. Zhuravlev, P. I., Reddy, G., Straub, J. E. and Thirumalai, D., Propensity to form amyloid fibrils is encoded as excitations in the free energy landscape of monomeric proteins. *J. Mol. Biol*., 2014, **426**(14), 2653–2666.
80. Dominguez, L., Foster, L., Meredith, S. C., Straub, J. E. and Thirumalai, D., Structural heterogeneity in transmembrane amyloid precursor protein homodimer is a consequence of environmental selection. *J. Am. Chem. Soc*., 2014, **136**(27), 9619–9626.
81. Kmiecik, S., Gront, D., Kolinski, M., Wieteska, L., Dawid, A. E. and Kolinski, A., Coarse-grained protein models and their applications. *Chem. Rev*., 2016, **116**(14), 7898–7936.
82. Maity, H. and Reddy, G., Folding of protein L with implications for collapse in the denatured state ensemble. *J. Am. Chem. Soc*., 2016, **138**(8), 2609–2616.
83. Kurcinski, M., Kolinski, A. and Kmiecik, S., Mechanism of folding and binding of an intrinsically disordered protein as revealed by *ab initio* simulations. *J. Chem. Theory Comput*., 2014, **10**(6), 2224–2231.
84. Sieradzan, A. K., Liwo, A. and Hansmann, U. H. E., Folding and self-assembly of a small protein complex. *J. Chem. Theory Comput*., 2012, **8**(9), 3416–3422.
85. Ranganathan, S., Maji, S. K. and Padinhateeri, R., Defining a physical basis for diversity in protein self-assemblies using a minimal model. *J. Am. Chem. Soc*., 2016, **138**(42), 13911–13922.
86. Earl, D. J. and Deem, M. W., Monte Carlo simulations. In *Molecular Modeling of Proteins* (ed. Kukol, A.), Springer, 2008, pp. 25–36.
87. Harrison, R. L., Introduction to Monte Carlo simulation. *AIP Conf. Proc*., 2010, **1204**, 17–21.
88. Lomakin, A., Teplow, D. B., Kirschner, D. A. and Benedek, G. B., Kinetic theory of fibrillogenesis of amyloid β-protein. *Proc. Natl. Acad. Sci. USA*, 1997, **94**(15), 7942–7947.
89. Pallitto, M. M. and Murphy, R. M., A mathematical model of the kinetics of beta-amyloid fibril growth from the denatured state. *Biophys. J*., 2001, **81**(3), 1805–1822.
90. Powers, E. T. and Powers, D. L., The kinetics of nucleated polymerizations at high concentrations: amyloid fibril formation near and above the 'supercritical concentration'. *Biophys. J*., 2006, **91**(1), 122–132.
91. Ranganathan, S., Ghosh, D., Maji, S. K. and Padinhateeri, R., A minimal conformational switching-dependent model for amyloid self-assembly. *Sci. Rep*., 2016, **6**, 21103(1–14).
92. Wales, D. J., Doye, J. P. K., Miller, M. A., Mortenson, P. N. and Walsh, T. R., Energy landscapes: from clusters to biomolecules. In *Advances in Chemical Physics*, John Wiley, 2000, pp. 1–111.
93. Wales, D. J. and Doye, J. P. K., Global optimization by basin-hopping and the lowest energy structures of Lennard–Jones clusters containing up to 110 atoms. *J. Phys. Chem. A*, 1997, **101**(28), 5111–5116.

94. Wales, D. J. and Scheraga, H. A., Global optimization of clusters, crystals, and biomolecules. *Science*, 1999, **285**(5432), 1368.
95. Wales, D. J., Some further applications of discrete path sampling to cluster isomerization. *Mol. Phys*., 2004, **102**(9–10), 891–908.
96. Wales, D. J., Discrete path sampling. *Mol. Phys*., 2002, **100**(20), 3285–3305.
97. Strodel, B. and Wales, D. J., Implicit solvent models and the energy landscape for aggregation of the amyloidogenic KFFE peptide. *J. Chem. Theory Comput*., 2008, **4**(4), 657–672.
98. Barz, B., Wales, D. J. and Strodel, B., A kinetic approach to the sequence–aggregation relationship in disease-related protein assembly. *J. Phys. Chem. B*, 2014, **118**(4), 1003–1011.
99. Strodel, B., Lee, J. W. L., Whittleston, C. S. and Wales, D. J., Transmembrane structures for Alzheimer's Aβ1–42 oligomers. *J. Am. Chem. Soc*., 2010, **132**(38), 13300–13312.
100. MacKerell, A. D., Protein force fields. In *Encyclopedia of Computational Chemistry*, John Wiley & Sons, Ltd, 2002.
101. Lindorff-Larsen, K., Maragakis, P., Piana, S., Eastwood, M. P., Dror, R. O. and Shaw, D. E., Systematic validation of protein force fields against experimental data. *PLOS ONE*, 2012, **7**(2), e32131.
102. Sgourakis, N. G., Merced-Serrano, M., Boutsidis, C., Drineas, P., Du, Z., Wang, C. and Garcia, A. E., Atomic-level characterization of the ensemble of the Aβ(1–42) monomer in water using unbiased molecular dynamics simulations and spectral algorithms. *J. Mol. Biol*., 2011, **405**(2), 570–583.
103. Jana, A. K., Jose, J. C. and Sengupta, N., Critical roles of key domains in complete adsorption of A[small beta] peptide on single-walled carbon nanotubes: insights with point mutations and MD simulations. *Phys. Chem. Chem. Phys*., 2013, **15**(3), 837–844.
104. Kim, S. and Klimov, D. K., Binding to the lipid monolayer induces conformational transition in Aβ monomer. *J. Mol. Model.*, 2013, **19**(2), 737–750.
105. Shemetov, A. A., Nabiev, I. and Sukhanova, A., Molecular interaction of proteins and peptides with nanoparticles. *ACS Nano*, 2012, **6**(6), 4585–4602.
106. Arce, F. T., Jang, H., Ramachandran, S., Landon, P. B., Nussinov, R. and Lal, R., Polymorphism of amyloid [small beta] peptide in different environments: implications for membrane insertion and pore formation. *Soft Matter*, 2011, **7**(11), 5267–5273.
107. Tofoleanu, F. and Buchete, N.-V., Alzheimer Aβ peptide interactions with lipid membranes: fibrils, oligomers and polymorphic amyloid channels. *Prion*, 2012, **6**(4), 339–345.
108. Jana, A. K. and Sengupta, N., A [small beta] self-association and adsorption on a hydrophobic nanosurface: competitive effects and the detection of small oligomers via electrical response. *Soft Matter*, 2015, **11**(2), 269–279.
109. Sciacca, Michele F. M., Lolicato, F., Di Mauro, G., Milardi, D., D'Urso, L., Satriano, C., Ramamoorthy, A. and La Rosa, C., The role of cholesterol in driving IAPP-membrane interactions. *Biophys. J*., 2016, **111**(1), 140–151.
110. Chen, Y., Cruz-Chu, E. R., Woodard, J., Gartia, M. R., Schulten, K. and Liu, L., Electrically induced conformational change of peptides on metallic nano-surfaces. *ACS Nano*, 2012, **6**(10), 8847–8856.
111. Liu, J., Liao, C. and Zhou, J., Multiscale simulations of protein G B1 adsorbed on charged self-assembled monolayers. *Langmuir*, 2013, **29**(36), 11366–11374.
112. Hawe, A., Sutter, M. and Jiskoot, W., Extrinsic fluorescent dyes as tools for protein characterization. *Pharm. Res*., 2008, **25**(7), 1487–1499.
113. Wu, C., Wang, Z., Lei, H., Duan, Y., Bowers, M. T. and Shea, J.-E., The binding of thioflavin-T and its neutral analog BTA-1 to protofibrils of the Alzheimer Aβ(16–22) peptide probed by molecular dynamics simulations. *J. Mol. Biol*., 2008, **384**(3), 718–729.
114. Rodriguez-Rodriguez, C., Rimola, A., Rodriguez-Santiago, L., Ugliengo, P., Alvarez-Larena, A., Gutierrez-de-Teran, H., Sodupe, M. and Gonzalez-Duarte, P., Crystal structure of thioflavin-T and its binding to amyloid fibrils: insights at the molecular level. *Chem. Commun*., 2010, **46**(7), 1156–1158.
115. Dyson, H. J. and Wright, P. E., Intrinsically unstructured proteins and their functions. *Nat. Rev. Mol. Cell Biol*., 2005, **6**(3), 197–208.
116. Uversky, V. N., Intrinsically disordered proteins from A to Z. *Int. J. Biochem. Cell Biol.*, 2011, **43**(8), 1090–1103.
117. Tompa, P., Unstructural biology coming of age. *Curr. Opin. Struct. Biol*., 2011, **21**(3), 419–425.
118. Zhang, Y., Cao, H. and Liu, Z., Binding cavities and druggability of intrinsically disordered proteins. *Prot. Sci*., 2015, **24**(5), 688–705.
119. Chen, J., Intrinsically disordered p53 extreme C-terminus binds to S100B($\beta\beta$) through 'fly-casting'. *J. Am. Chem. Soc*., 2009, **131**(6), 2088–2089.
120. Ganguly, D. and Chen, J., Atomistic details of the disordered states of KID and pKID. Implications in coupled binding and folding. *J. Am. Chem. Soc*., 2009, **131**(14), 5214–5223.
121. Soucek, L. *et al.*, Modelling Myc inhibition as a cancer therapy. *Nature*, 2008, **455**(7213), 679–683.
122. Michel, J. and Cuchillo, R., The impact of small molecule binding on the energy landscape of the intrinsically disordered protein C-Myc. *PLoS ONE*, 2012, **7**(7), e41070.
123. Jin, F., Yu, C., Lai, L. and Liu, Z., Ligand clouds around protein clouds: a scenario of ligand binding with intrinsically disordered proteins. *PLoS Comput. Biol.*, 2013, **9**(10), e1003249.
124. Lührs, T., Ritter, C., Adrian, M., Riek-Loher, D., Bohrmann, B., Döbeli, H., Schubert, D. and Riek, R., 3D structure of Alzheimer's amyloid-β(1–42) fibrils. *Proc. Natl. Acad. Sci. USA*, 2005, **102**(48), 17342–17347.
125. Shewmaker, F., Wickner, R. B. and Tycko, R., Amyloid of the prion domain of Sup35p has an in-register parallel β-sheet structure. *Proc. Natl. Acad. Sci. USA*, 2006, **103**(52), 19754–19759.
126. Sugase, K., Dyson, H. J. and Wright, P. E., Mechanism of coupled folding and binding of an intrinsically disordered protein. *Nature*, 2007, **447**(7147), 1021–1025.
127. Jensen, M. R., Salmon, L., Nodet, G. and Blackledge, M., Defining conformational ensembles of intrinsically disordered and partially folded proteins directly from chemical shifts. *J. Am. Chem. Soc*., 2010, **132**(4), 1270–1272.
128. Ozenne, V. *et al.*, Mapping the potential energy landscape of intrinsically disordered proteins at amino acid resolution. *J. Am. Chem. Soc*., 2012, **134**(36), 15138–15148.
129. Bernadó, P., Mylonas, E., Petoukhov, M. V., Blackledge, M. and Svergun, D. I., Structural characterization of flexible proteins using small-angle X-ray scattering. *J. Am. Chem. Soc*., 2007, **129**(17), 5656–5664.
130. Shell, S. S., Putnam, C. D. and Kolodner, R. D., The N terminus of *Saccharomyces cerevisiae* Msh6 is an unstructured tether to PCNA. *Mol. Cell*, 2007, **26**(4), 565–578.
131. Tidow, H. *et al*., Quaternary structures of tumor suppressor p53 and a specific p53–DNA complex. *Proc. Natl. Acad. Sci. USA*, 2007, **104**(30), 12324–12329.
132. Wells, M. *et al.*, Structure of tumor suppressor p53 and its intrinsically disordered *N*-terminal transactivation domain. *Proc. Natl. Acad. Sci. USA*, 2008, **105**(15), 5762–5767.
133. Giehm, L., Svergun, D. I., Otzen, D. E. and Vestergaard, B., Low-resolution structure of a vesicle disrupting α-synuclein oligomer that accumulates during fibrillation. *Proc. Natl. Acad. Sci. USA*, 2011, **108**(8), 3246–3251.
134. Bourhis, J.-M. *et al.*, The intrinsically disordered C-terminal domain of the measles virus nucleoprotein interacts with the C-terminal domain of the phosphoprotein via two distinct sites

and remains predominantly unfolded. *Prot. Sci*., 2005, **14**(8), 1975–1992.
135. Sengupta, P., Garai, K., Sahoo, B., Shi, Y., Callaway, D. J. E. and Maiti, S., The Amyloid β peptide (Aβ1–40) is thermodynamically soluble at physiological concentrations. *Biochemistry*, 2003, **42**(35), 10506–10513.
136. Mukhopadhyay, S., Krishnan, R., Lemke, E. A., Lindquist, S. and Deniz, A. A., A natively unfolded yeast prion monomer adopts an ensemble of collapsed and rapidly fluctuating structures. *Proc. Natl. Acad. Sci. USA*, 2007, **104**(8), 2649–2654.
137. Sahoo, B., Balaji, J., Nag, S., Kaushalya, S. K. and Maiti, S., Protein aggregation probed by two-photon fluorescence correlation spectroscopy of native tryptophan. *J. Chem. Phys*., 2008, **129**(7), 08B608.
138. Nag, S. *et al.*, Nature of the Amyloid-β monomer and the monomer–oligomer equilibrium. *J. Biol. Chem*., 2011, **286**(16), 13827–13833.
139. Hofmann, H., Soranno, A., Borgia, A., Gast, K., Nettels, D. and Schuler, B., Polymer scaling laws of unfolded and intrinsically disordered proteins quantified with single-molecule spectroscopy. *Proc. Natl. Acad. Sci. USA*, 2012, **109**(40), 16155–16160.
140. Soranno, A. *et al.*, Quantifying internal friction in unfolded and intrinsically disordered proteins with single-molecule spectroscopy. *Proc. Natl. Acad. Sci. USA*, 2012, **109**(44), 17800–17806.
141. Dalal, V., Arya, S., Bhattacharya, M. and Mukhopadhyay, S., Conformational switching and nanoscale assembly of human prion protein into polymorphic amyloids via structurally labile oligomers. *Biochemistry*, 2015, **54**(51), 7505–7513.
142. Maji, S. K., Amsden, J. J., Rothschild, K. J., Condron, M. M. and Teplow, D. B., Conformational dynamics of amyloid β-protein assembly probed using intrinsic fluorescence. *Biochemistry*, 2005, **44**(40), 13365–13376.
143. Ghosh, D. *et al.*, Structure based aggregation studies reveal the presence of helix-rich intermediate during α-synuclein aggregation. *Sci. Rep.,* 2015, **5**, 9228.
144. Sahay, S., Anoop, A., Krishnamoorthy, G. and Maji, S. K., Site-specific fluorescence dynamics of α-synuclein fibrils using time-resolved fluorescence studies: effect of familial Parkinson's disease-associated mutations. *Biochemistry*, 2014, **53**(5), 807–809.
145. Yu, J., Malkova, S. and Lyubchenko, Y. L., α-Synuclein misfolding: single molecule AFM force spectroscopy study. *J. Mol. Biol*., 2008, **384**(4), 992–1001.
146. Sandal, M. *et al.*, Conformational equilibria in monomeric α-synuclein at the single-molecule level. *PLoS Biol*., 2008, **6**(1), e6.
147. Jiménez, J. L., Nettleton, E. J., Bouchard, M., Robinson, C. V., Dobson, C. M. and Saibil, H. R., The protofilament structure of insulin amyloid fibrils. *Proc. Natl. Acad. Sci. USA*, 2002, **99**(14), 9196–9201.
148. Sumner Makin, O. and Serpell, L. C., Structural characterisation of islet amyloid polypeptide fibrils. *J. Mol. Biol*., 2004, **335**(5), 1279–1288.
149. Krotee, P. *et al.*, Atomic structures of fibrillar segments of hIAPP suggest tightly mated β-sheets are important for cytotoxicity. *eLife*, 2017, **6**, e19273.
150. Nelson, R., Sawaya, M. R., Balbirnie, M., Madsen, A. O., Riekel, C., Grothe, R. and Eisenberg, D., Structure of the cross-[beta] spine of amyloid-like fibrils. *Nature*, 2005, **435**(7043), 773–778.
151. Kishimoto, A., Hasegawa, K., Suzuki, H., Taguchi, H., Namba, K. and Yoshida, M., β-helix is a likely core structure of yeast prion Sup35 amyloid fibers. *Biochem. Biophys. Res. Commun*., 2004, **315**(3), 739–745.

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