Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Molecular simulations integrated with experiments for probing the interaction dynamics and binding mechanisms of intrinsically disordered proteins

Catherine Ghosh[1,2], Suhani Nagpal[1,2,3] and Victor Muñoz[1,2]

Abstract

Intrinsically disordered proteins (IDPs) exploit their plasticity to deploy a rich panoply of soft interactions and binding phenomena. Advances in tailoring molecular simulations for IDPs combined with experimental cross-validation offer an atomistic view of the mechanisms that control IDP binding, function, and dysfunction. The emerging theme is that unbound IDPs autonomously form transient local structures and self-interactions that determine their binding behavior. Recent results have shed light on whether and how IDPs fold, stay disordered or drive condensation upon binding; how they achieve binding specificity and select among competing partners. The disorder-binding paradigm is now being proactively used by researchers to target IDPs for rational drug design and engineer molecular responsive elements for biosensing applications.

Addresses

[1] NSF-CREST Center for Cellular and Biomolecular Machines (CCBM), University of California at Merced, Merced, 95343 CA, USA
[2] Department of Bioengineering, University of California at Merced, Merced, 95343 CA, USA
[3] OpenEye, Cadence Molecular Sciences, Boston, 02114 MA, USA

Corresponding author: Muñoz, Victor (vmunoz3@ucmerced.edu)
(Ghosh C.)
(Muñoz V.)

Current Opinion in Structural Biology 2024, **84**:102756

This review comes from a themed issue on **Folding and Binding (2024)**

Edited by **H. Jane Dyson** and **Peter E. Wright**

For complete overview of the section, please refer the article collection - Folding and Binding (2024)

Available online 19 December 2023

https://doi.org/10.1016/j.sbi.2023.102756

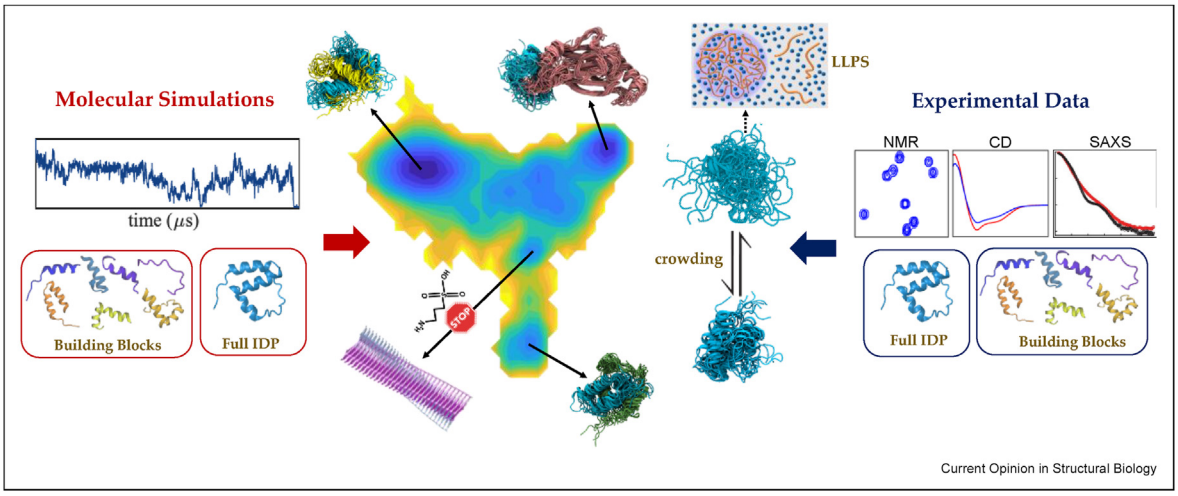
Introduction

Over one third of the eukaryotic proteome is constituted of intrinsically disordered proteins (IDPs) or regions (IDRs), which are flexible and highly dynamic in their functional states [1]. IDPs are key players in high level cellular processes that involve complex molecular coordination, such as signaling, gene expression, and transport [1,2]. Disordered proteins are also abundant in viruses, providing an essential economy of scale by fulfilling roles in viral infection, assembly, and proliferation [3]. IDP dysfunction is linked to many neurodegenerative pathological conditions [4] and cancer [5].

The myriad functions performed by IDPs rely on their inherent plasticity to bind and recruit partners. IDPs often fold upon binding, but can also form highly disordered (fuzzy) complexes, or morph to accommodate diverse binding partners [6]. Moreover, IDP dynamic interactions appear to drive the formation of biomolecular condensates [7]. The highly dynamic nature of IDP folding and binding has usually required the use of hybrid approaches that combine computational modeling and simulations with experimental analysis. Efforts have generally focused on characterizing IDP ensembles structurally [8] or folding upon binding reactions [9]. More recently, interest has broadened towards investigating the conformational and binding dynamics of IDPs. The switch to dynamics was facilitated by advances in multiscale simulation methods tailored to IDPs and their binding processes, as well as by modular approaches designed to dissect the conformational biases and energetics of IDP ensembles.

The emerging theme from recent results is that IDPs autonomously form transient local structures and tertiary contacts in response to subtle energetic biases encoded in their sequences [10–12]. These hard to pin down energetic biases control the intermolecular interactions and binding mechanisms of IDPs, and hold the keys to understand IDP function and dysfunction. In this review we discuss the most recent research topics, technical advances, and results pertaining IDP interaction dynamics and their roles in binding. We cover recent advances in the IDP simulation toolkit and discuss new insights in IDP function, binding mechanisms, and condensate formation that have come from integrating enhanced molecular simulations with experimental validation, as shown graphically in Figure 1. We end with a brief overview of recent technological efforts to use IDPs as therapeutic targets or scaffolds for biosensors.

Figure 1



Schematic of hybrid methods for studying IDP conformational and interaction dynamics, and how they are enabling to understand the mechanisms of IDP function and dysfunction. The general approach integrates advanced molecular simulations (left) with detailed experimental validation through a combination of structural and biophysical techniques (right). The studies can be done on the full IDP and/or on series of overlapping IDP fragments using a modular construction ansatz. The simulations provide high-resolution, even atomistic, descriptions of the IDP conformational and binding landscapes. To obtain the landscape, the simulated trajectories are projected onto one or several order parameters of interest. The landscape shown here was obtained from 36 μs of MD simulations for NCBD (from Ref. [11]). The cartesian coordinates for the molecule were transformed onto feature vectors that were subsequently processed using time-lagged independent component analysis (TICA) [53], as a means to find a projection from the maximization of the autocorrelation function that captures the slowest kinetic modes. The landscape permits to calculate the global ensemble features of the IDP and/or its fragments, which are then cross-validated or refined with the experimental data for compliance. Validated simulations are then used to identify any sub-ensembles and transient interactions that lead to binding to different partners, biomolecular condensation, or aberrant aggregation (center area). The detailed understanding of IDP binding mechanisms that emerges from these approaches can be used to effectively turn IDPs into therapeutic targets, or engineer IDP-based molecular responsive elements for biotechnological applications.

Simulation methods for IDP dynamics

Recent years have seen significant developments in addressing the intrinsic challenges of simulating IDP processes. One such challenge was the refinement of molecular mechanics force-fields, originally parameterized with 3D structures, to make them IDP friendly. Ongoing efforts in this direction have been recently discussed elsewhere [13,14]. A still useful alternative are coarse grained molecular models, which compensate the lack of atomistic detail with higher tunability to experimental data. In this regard, a recent performance analysis actually concluded that it could be advantageous to use coarse models for certain IDP simulations because they tend to reproduce the experimental data more closely [15].

A second challenge comes from the analysis and interpretation of the simulated trajectories. The analysis usually involves identifying structural targets *a priori*, such as the 3D structure of a folded state or bound complex, to define a suitable progress variable for projecting the trajectories. This protocol is difficult for IDPs since obvious targets are often not available. Some strides have been made in this direction recently. A reaction-coordinate-independent energy landscape approach has been developed specifically to analyze IDP simulations [16]. Other authors developed a topology-based method for extracting conformational patterns, critical contacts, and timescales from simulations that do not visit defined structures [17].

Atomistic simulations of IDPs also require increased sampling given the high degree of solvation and broad conformational landscapes of these systems. Ongoing efforts in enhanced sampling for molecular dynamics (MD) of IDPs are reviewed in Ref. [18]. Of note are advances in temperature-based replica exchange, or parallel tempering protocols. These methods tend to artificially elevate local energy barriers for simulations in water solutions, severely impacting sampling for IDPs. New hybrid tempering protocols have been developed that accelerate water dynamics in replica exchange simulations of IDPs without ensemble reweighting, and seem to scale well for large IDPs [19]. A similar approach introduces a solute tempering protocol that restricts the replica exchange process to only a few degrees of freedom of interest [20].

An exciting prospect is the application of machine learning for generating IDP ensembles with reduced computational resources. Generative autoencoders that learn from short MD simulations have been developed,

which produce ensembles comparable with those generated from extensive simulations [21]. This method has then been further improved by incorporating an additional inference layer that enhances sampling of IDP conformational landscapes [22].

Simulations of the extremely large multi-molecular systems involved in biomolecular condensate formation are being pursued using multiscale simulations. In these methods, coarse-grained simulations are used first to generate an equilibrated phase-separated supramolecular configuration that is then used as starting point for all-atom simulations [23]. Such approach was used to investigate how intermolecular contacts between IDPs induce liquid-liquid phase separation [24]. An alternative are atomistic MD simulations performed at high concentrations, but of just small IDR fragments, chosen with the idea of still capturing the most significant intermolecular interactions [25].

Conformational biases and dynamics

A fundamental question in the IDP field is to what extent the conformations that play functional roles, i.e. form upon binding, are primed in the conformational ensemble of free IDPs. IDP ensembles are generated using experimental data, often from NMR and small-angle X-ray scattering, as restraints to filter/bias computer generated conformations [8], and are generally deposited in the open-access repository Protein Ensemble Database (PED). The development of enhanced MD sampling methods has been pivotal for obtaining increasingly realistic IDP ensembles that reproduce experimental data well without reweighting [12,26–29]. Importantly, regardless of which IDP and what particular simulation method or experimental validation were used, these MD-produced IDP ensembles consistently contain local conformational preferences and transient tertiary interactions that are not directly apparent in the experimental data [12,26–29]. A word of caution is needed, however, because the experimental data only provide ensemble-averaged information, and hence the sampling-refinement procedures could underestimate the actual ensemble broadness. One possible way around this issue has been proposed in which the NMR chemical shifts of the IDP are interpreted probabilistically by comparing them against conformational distributions derived from a database of structure-chemical shift pairs [30].

Parallel efforts aimed to develop approaches for detecting the conformational biases of IDPs in both simulations and experiments. A modular construction ansatz can be invoked to split the IDP into basic building blocks and their combinations and thus detect and map subtle conformational biases in the relative behaviors of overlapping fragments [11]. The modular ansatz was first used to enhance computational sampling [31]. But it has proven most powerful when the IDP fragments are concertedly analyzed by simulations and experiments, as recently demonstrated on the partially disordered protein NCBD [11]. A modular ansatz was also used to determine local contributions to the global features of IDP ensembles [29]. Similar information can also be extracted from the effects on the IDP ensemble dynamics of local perturbations, such as point mutations [10] and/or posttranslational modifications [32].

Overall, recent results strongly indicate that IDP ensembles, as disordered as they might appear in bulk experiments, contain networks of fleeting local structures and tertiary interactions that render specific conformational biases. While highly dynamic, these interactions could certainly drive the specific responses of IDPs to binding partners and other functional cues.

Interactions, ordering transitions, and binding mechanisms

Folding upon binding reactions have become finally accessible to full atomistic simulations, whether using short parallel cascade runs to induce association and dissociation cycles [33], or through long unbiased MD simulations [34]. Two recent such studies have looked at short IDRs that form single helical structures upon binding to folded partners via MD simulations validated with experimental data [33,34]. In both cases, the IDP first bound to the partner from a partially helical subensemble, and then rearranged while in complex to consolidate a final α-helix structure. These results thus point to a general two-step mechanism of conformational selection followed by induced-fit. There was, however, a suggestive size difference. All the partially helical conformations in the bound ensemble of an 11-residue IDR could directly rearrange to form the 2.5 turn helix structure [33]. However, the bound ensemble of a twice longer IDR incorporated helical conformations that needed to unfold before consolidating the final 5-turn, bound straight helix, or unbind to start over, which resulted on slower pathways [34].

On the other hand, a recent NMR study of an IDP that folds into a 3-helix bundle upon binding to DNA discovered that the same globular structure forms transiently in the unbound IDP as an excited state [35]. The authors interpreted these results as indicative of binding via a strict conformational selection mechanism, which they termed dynamic lock-and-key [35]. Interestingly, this exact scenario was investigated previously via coarse-grained simulations of the folding upon binding to either DNA [36] or a folded protein [37] of unstable, autonomously-folding proteins. The computational studies showed that whether the IDP binds exclusively by conformational selection, or adds induced-fit pathways, is determined by its self-folding mechanism. Particularly, when the self-folding

mechanism was two-state the protein always used conformational selection, whereas a downhill folding mechanism led to the addition of induced-fit pathways [36,37].

IDPs can also form fuzzy complexes, which occur when highly disordered IDPs engage in delocalized charge-charge interactions with their partners [38,39]. Recent work combined simulations and experiments to investigate the inconspicuous source of binding specificity in fuzzy complexes. One study discovered that the disordered E-cadherin tail diffuses across the entire surface of its folded binding partner β-catenin in sub-msecs, and identified a few persistent intermolecular contacts as the determinants of binding specificity [40]. A similar study of the fuzzy complex formed between the IDP 4.1G-CTD and an IDR from NuMA also identified critical hydrophobic interactions between specific regions of the full IDP that act as molecular recognition "hot spots" for binding the IDR [41].

IDP ordering transitions are also expected to be highly sensitive to environmental cues. Recent studies have shown that the compactness, conformational dynamics, and ability to make intermolecular interactions of IDPs are strongly modulated by crowding [42,43] and monovalent salts [44]. The degree of IDP compaction has also been identified as an important factor in forming fuzzy complexes [41]. In contrast, IDP-mediated liquid-liquid phase separation (LLPS) and biomolecular condensation are facilitated by expanded, highly dynamic IDP ensembles [7].

IDP function and disease

The coupling of a binding process to an IDP ordering transition provides a direct mechanism to control binding readiness and affinity through the inner dynamics of the IDP. Such systems can act as molecular logic gates, but the operation of that mechanism has been difficult to investigate, particularly for highly disordered IDPs-IDRs. Interestingly, recent results provide some examples that illustrate how such logic gates might actually operate. The discovery that free CytR transiently populates the same 3-helix bundle structure that it forms upon binding DNA [35] suggests a DNA recognition gate controlled by the metastability of the IDP's excited state. The observation of folding induced by multisite phosphorylation [32] demonstrates that classical kinase-dependent activation can be coupled to a folding gate with potential for producing analog outputs through the stepwise stabilization of the folded state by varying numbers of phosphorylated sites. The competition between conformational sub-ensembles in free NCBD exemplifies more complex gating mechanisms for the coordination of multiple binding partners [11]. An example of inhibitory gate, in which the binding of an IDP changes the target's conformation to block its binding to functional partners, has been recently proposed in the parasite *Toxoplasma gondii* as strategy to stop the transcription of immune response genes in the host [45].

The effects of disease-causing mutations on IDPs are starting to offer important clues on the connections between IDP dysfunction and the onset of cancer and neurodegenerative diseases. For instance, a recent study showed that cancer-related mutations in the IDR TAD-p53, which are known to disrupt the interactions of the tumor suppressor p53 with regulators without localizing on the known interaction interfaces, actually alter the disordered state of TAD-p53 [46]. Similarly, the conformational analysis of Alzheimer's related mutations in a tau protein region, containing the four microtubule binding repeats and the amyloid formation site, discovered that turn-like conformations consistent with a microtubule-binding function that occur in the wildtype switch to extended conformations akin to disease-associated tau fibrils in the mutants [28].

Biotechnological applications

The high-order regulatory functions of IDPs make them highly attractive therapeutic targets. IDP-based drug discovery has proven difficult because IDP disorder hampers the conventional structure-based computational drug design approaches. However, new computational protocols designed for multi-conformer targets are showing promising results. Peptide inhibitors against the disordered tail of histone H4 and the NET-resident histone H2A have been achieved [47]. Another IDP-based drug discovery effort identified two organic compounds that bind to TAD-p53 and restore the normal p53 function of cancer cells [48]. A small molecule that binds to the amyloid-β peptide and impedes its aggregation into β-amyloids has also been reported [49].

Finally, IDP-based design offers exciting opportunities to engineer proteins for biotechnological applications that need responsive molecular elements. This concept is proving particularly useful for the design of protein-based biosensors for monitoring signals of interest in living cells. For instance, the inherent sensitivity of IDP ensembles to the surrounding solution was exploited to develop a fluorescence biosensor for tracking intracellular changes in osmotic pressure [50] (Un)folding coupled to binding provides a general design principle to engineer signal transducers into any protein-IDP of interest and tune their responses. This idea was recently demonstrated by engineering conformational pH transducers into a naturally pH insensitive fast-folding protein [51]. The study demonstrated that an uncooperative ordering transition, as are generally expected for IDPs [52], facilitates the optimization of the transducer's response and operational range [51].

Author contributions

Catherine Ghosh Writing-Original Draft, Visualization **Suhani Nagpal** Investigation, Resources, **Victor Muñoz** Supervision, Conceptualization, Writing – Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Acknowledgements

This work was supported by the National Science Foundation [grant numbers MCB-2112710 and HRD-2112675] and the W.M. Keck Foundation.

References

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Bhowmick A, Brookes DH, Yost SR, Dyson HJ, Forman-Kay JD, Gunter D, Head-Gordon M, Hura GL, Pande VS, Wemmer DE: **Finding our way in the dark proteome**. *J Am Chem Soc* 2016, **138**:9730–9742.

2. Kulkarni P, Bhattacharya S, Achuthan S, Behal A, Jolly MK, Kotnala S, Mohanty A, Rangarajan G, Salgia R, Uversky V: **Intrinsically disordered proteins: critical components of the wetware**. *Chem Rev* 2022, **122**:6614–6633.

3. Dyson HJ: **Vital for viruses: intrinsically disordered proteins**. *J Mol Biol* 2023, **435**:167860.

4. Coskuner-Weber O, Mirzanli O, Uversky VN: **Intrinsically disordered proteins and proteins with intrinsically disordered regions in neurodegenerative diseases**. *Biophysical Reviews* 2022, **14**:679–707.

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

6. Tompa P, Schad E, Tantos A, Kalmar L: **Intrinsically disordered proteins: emerging interaction specialists**. *Curr Opin Struct Biol* 2015, **35**:49–59.

7. • Abyzov A, Blackledge M, Zweckstetter M: **Conformational dynamics of intrinsically disordered proteins regulate biomolecular condensate chemistry**. *Chem Rev* 2022, **122**:6719–6748.

This review covers emerging research on the mechanisms underlying the formation of biomolecular condensates and liquid-liquid phase separation mediated by IDP interactions, making connections with the biological functions of condensates and their aging in human diseases.

8. • Thomasen FE, Lindorff-Larsen K: **Conformational ensembles of intrinsically disordered proteins and flexible multidomain proteins**. *Biochem Soc Trans* 2022, **50**:541–554.

This review provides, among several other things, an excellent discussion of the current methods for producing IDP conformational ensembles combining computational modeling and experiments for validation.

9. • Malagrinò F, Diop A, Pagano L, Nardella C, Toto A, Gianni S: **Unveiling induced folding of intrinsically disordered proteins–Protein engineering, frustration and emerging themes**. *Curr Opin Struct Biol* 2022, **72**:153–160.

Detailed update on the recent methods, results, and biological implications of folding upon binding processes with an emphasis on experimental approaches.

10. Zou J, Simmerling C, Raleigh DP: **Dissecting the energetics of intrinsically disordered proteins via a hybrid experimental and computational approach**. *J Phys Chem B* 2019, **123**:10394–10402.

11. •• Luong TD, Nagpal S, Sadqi M, Muñoz V: **A modular approach to map out the conformational landscapes of unbound intrinsically disordered proteins**. *Proc Natl Acad Sci USA* 2022, **119**, e2113572119.

This article presents proof of concept implementation of a modular approach for dissecting the energetic biases existing on unbound IDP ensembles. The authors demonstrate that comparative analysis of overlapping fragments can detect and crosscheck partial uncooperative ordering transitions, quantify their energetics, and map out their localization in the sequence. When applied to the partially disordered protein NCBD this approach revealed a highly dynamic ensemble that results from a tug of war between conflicting sets of interactions, each preparing NCBD to bind to one of its different partners. This result suggests an underlying rheostatic mechanism for the coordination of binding events among multiple partners.

12. •• Yu L, Brüschweiler R: **Quantitative prediction of ensemble dynamics, shapes and contact propensities of intrinsically disordered proteins**. *PLoS Comput Biol* 2022, **18**, e1010036.

This article shows that the combination of replica exchange sampling with microsecond-long atomistic MD simulations can generate, for several IDPs and without reweighting, conformational ensembles that are highly consistent with NMR experimental data that report both on global and local ensemble properties. The authors report that a significant fraction of the individual MD trajectories show the formation of transient secondary structures and interresidue interactions that are not apparent in the experimental data, suggesting that there is much more to IDP ensembles that meets the eye.

13. Mu J, Liu H, Zhang J, Luo R, Chen H-F: **Recent force field strategies for intrinsically disordered proteins**. *J Chem Inf Model* 2021, **61**:1037–1047.

14. Rahman MU, Rehman AU, Liu H, Chen H-F: **Comparison and evaluation of force fields for intrinsically disordered proteins**. *J Chem Inf Model* 2020, **60**:4912–4923.

15. • Fagerberg E, Skepö M: **Comparative performance of computer simulation models of intrinsically disordered proteins at different levels of coarse-graining**. *J Chem Inf Model* 2023, **63**:4079–4087.

The authors evaluate the performance of three models with different levels of coarse-graining in generating ensembles for several IDPs, and find that the least-coarse model shows in fact the poorest agreement with experimental data. These results challenge the intuition that, other conditions being equal, a more sophisticated model is generally superior.

16. •• Oliveira Junior AB, Lin X, Kulkarni P, Onuchic JN, Roy S, Leite VB: **Exploring energy landscapes of intrinsically disordered proteins: insights into functional mechanisms**. *J Chem Theor Comput* 2021, **17**:3178–3187.

This article introduces an energy landscape-based method for the analysis of molecular simulations that does not require the definition of a reaction coordinate *a priori*, which they term energy landscape visualization method (ELViM). The authors demonstrate the potential of the method by exploring the energy landscape of an IDP with a highly frustrated energy landscape.

17. •• Scalvini B, Sheikhhassani V, van de Brug N, Heling LWHJ, Schmit JD, Mashaghi A: **Circuit topology approach for the comparative analysis of intrinsically disordered proteins**. *J Chem Inf Model* 2023, **63**:2586–2602.

This article introduces a low-dimensional representation of protein conformation in the topology space. Such description enables the analysis of the conformational dynamics of IDPs in simulations in terms of topological similarity, and hence without the need to pre-define structural targets for the projection.

18. Gong X, Zhang Y, Chen J: **Advanced sampling methods for multiscale simulation of disordered proteins and dynamic interactions**. *Biomolecules* 2021, **11**:1416.

19. •• Appadurai R, Nagesh J, Srivastava A: **High resolution ensemble description of metamorphic and intrinsically**

disordered proteins using an efficient hybrid parallel tempering scheme. *Nat Commun* 2021, **12**:958.
Here the authors introduce a new replica exchange hybrid tempering method (REHT) that minimizes energy differences in solvent self-interaction to aid the fast restructuring of the hydration shell during simulations. Using this protocol the authors generated conformational ensembles for the IDP Histatin-5 that were in close agreement with NMR, SAXS, and CD experiments without any reweighting.

20. • Zhang Y, Liu X, Chen J: **Re-balancing replica exchange with solute tempering for sampling dynamic protein conformations**. *J Chem Theor Comput* 2023, **19**:1602–1614.
This article describes a new replica exchange solute tempering protocol (REST3) that treats the solute–solvent van der Waals interactions as free parameters at each temperature to influence the conformational properties of the solute. The protocol enhances the efficiency of random walks in temperature space and permits to achieve convergence with reduced numbers of replicas.

21. • Gupta A, Dey S, Hicks A, Zhou H-X: **Artificial intelligence guided conformational mining of intrinsically disordered proteins**. *Commun Biol* 2022, **5**:610.
This article introduces the use of machine learning generative autoencoders that learn from short MD simulations to generate full IDP ensembles that compare remarkably well with those generated by extensive MD simulations.

22. •• Zhu J-J, Zhang N-J, Wei T, Chen H-F: **Enhancing conformational sampling for intrinsically disordered and ordered proteins by variational autoencoder**. *Int J Mol Sci* 2023, **24**:6896.
The authors introduce a modification of the machine learning methods for generating IDP ensembles based on variational generative autoencoders. Such variational generative autoencoders help enhance the coverage of the IDP conformational landscape, and produce IDP ensembles with closer agreement to experimental data than those generated by autoencoders.

23. • Shea J-E, Best RB, Mittal J: **Physics-based computational and theoretical approaches to intrinsically disordered proteins**. *Curr Opin Struct Biol* 2021, **67**:219–225.
Excellent review of the recent computational and theoretical approaches developed to study the structure and dynamics of IDP condensates and liquid-liquid phase separation.

24. Zheng W, Dignon GL, Jovic N, Xu X, Regy RM, Fawzi NL, Kim YC, Best RB, Mittal J: **Molecular details of protein condensates probed by microsecond long atomistic simulations**. *J Phys Chem B* 2020, **124**:11671–11679.

25. Paloni M, Bailly R, Ciandrini L, Barducci A: **Unraveling molecular interactions in liquid–liquid phase separation of disordered proteins by atomistic simulations**. *J Phys Chem B* 2020, **124**:9009–9016.

26. Salvi N, Abyzov A, Blackledge M: **Solvent-dependent segmental dynamics in intrinsically disordered proteins**. *Sci Adv* 2019, **5**, eaax2348.

27. Dey S, MacAinsh M, Zhou H-X: **Sequence-dependent backbone dynamics of intrinsically disordered proteins**. *J Chem Theor Comput* 2022, **18**:6310–6323.

28. •• Stelzl LS, Pietrek LM, Holla A, Oroz J, Sikora M, Köfinger Jr , Schuler B, Zweckstetter M, Hummer G: **Global structure of the intrinsically disordered protein tau emerges from its local structure**. *Jacs Au* 2022, **2**:673–686.
Here the authors use a new reweighted hierarchical chain growth (RHCG) algorithm to generate conformational ensembles for the tau protein region K18, which contains the four functional microtubule binding repeats and the amyloid nucleation site. They calculate ensembles for the wildtype protein and several pathogenic single-point mutations. What is particularly appealing in this study is that the conformational ensemble of tau K18 appears to change from populating functionally relevant turn-like conformations in the wildtype to form extended conformations in the mutants that are consistent with the conformation of the pathological tau fibrils associated with the onset of dementia in Alzheimer's disease.

29. Heesink G, Marseille MJ, Fakhree MAA, Driver MD, van Leijenhorst-Groener KA, Onck PR, Blum C: **Claessens MMAE: exploring intra- and inter-regional interactions in the IDP α-synuclein using smFRET and MD simulations**. *Biomacromolecules* 2023:10. 1021/acs.biomac.3c00404.

30. He Y, Nagpal S, Sadqi M, de Alba E, Muñoz V: **Glutton: a tool for generating structural ensembles of partly disordered proteins from chemical shifts**. *Bioinformatics* 2019, **35**:1234–1236.

31. Lindsay RJ, Mansbach RA, Gnanakaran S, Shen T: **Effects of pH on an IDP conformational ensemble explored by molecular dynamics simulation**. *Biophys Chem* 2021, **271**:106552.

32. Sieradzan AK, Korneev A, Begun A, Kachlishvili K, Scheraga HA, Molochkov A, Senet P, Niemi AJ, Maisuradze GG: **Investigation of phosphorylation-induced folding of an intrinsically disordered protein by coarse-grained molecular dynamics**. *J Chem Theor Comput* 2021, **17**:3203–3220.

33. • Tran DP, Kitao A: **Kinetic selection and relaxation of the intrinsically disordered region of a protein upon binding**. *J Chem Theor Comput* 2020, **16**:2835–2845.
Computational study of the mechanisms by which a short IDR segment from the transactivation domain of p53 (TAD-p53) binds to its folded protein partner MDM2 by performing parallel cascade MD simulations to simulate cycles of complex association and dissociation, jointly with standard MD and a Markov-state model. The simulations, which agreed well with the known complex structure and also with the experimentally determined binding free energy and rates, showed a two-step process in which TAD-p53 binds to MDM2 from a pre-formed partially helical sub-ensemble, and then rearranges while in the complex to form the final 2.5 turn α-helix structure.

34. • Robustelli P, Piana S, Shaw DE: **Mechanism of coupled folding-upon-binding of an intrinsically disordered protein**. *J Am Chem Soc* 2020, **142**:11092–11101.
The authors perform long timescale unbiased MD simulations of an IDP segment that forms a 5-turn α-helix upon binding to its folded protein partner. They use the recently developed a99SB-disp force-field for IDPs and observe over 70 binding and unbinding events, which allows extracting the folding upon binding mechanisms at atomistic detail, and benchmarking it with existing experimental data. The observed mechanism is one in which the IDR, which is significantly helical on its own, makes a few key intermolecular contacts with the partner leading to a structurally heterogeneous transition state ensemble from which the final α-helix consolidates. This scenario is generally consistent with a two-step mechanism of conformational selection followed by induced-folding. But there is an important twist, because the bound IDR conformations that are more likely to proceed to the final formation of the complex are the least helical ones; in contrast, the more helical conformations unbind more often. This presents an intriguing case in which more native-like structure in the unbound IDP results in slower pathways, suggesting that optimal folding upon binding requires a fine balance between conformational selection in the free IDP ensemble and induced-fit from specific intermolecular contacts.

35. •• Madhurima K, Nandi B, Munshi S, Naganathan AN, Sekhar A: **Functional regulation of an intrinsically disordered protein via a conformationally excited state**. *Sci Adv* 2023, **9**, eadh4591.
This article presents an experimental structural study of the conformational ensemble of the DNA binding domain of CytR, an IDP that folds into a 3-helix bundle upon binding to DNA. Using chemical exchange saturation transfer NMR experiments the authors discover that unbound CytR transiently forms as an excited state the same 3-helix bundle found in the complex with DNA. This result is particularly interesting because it provides a clear case in which the structure that the IDP forms upon binding, which in this case is actually globular, is fully encoded in the IDP sequence with the partner providing only binding energy to stabilize it. CytR thus provides an exciting opportunity to investigate the role of the self-folding mechanism, whether two-state or downhill, in controlling a functionally significant folding upon binding process.

36. Chu X, Muñoz V: **Roles of conformational disorder and downhill folding in modulating protein–DNA recognition**. *Phys Chem Chem Phys* 2017, **19**:28527–28539.

37. Sharma R, De Sancho D, Muñoz V: **Interplay between the folding mechanism and binding modes in folding coupled to binding processes**. *Phys Chem Chem Phys* 2017, **19**:28512–28516.

38. Borgia A, Borgia MB, Bugge K, Kissling VM, Heidarsson PO, Fernandes CB, Sottini A, Soranno A, Buholzer KJ, Nettels D: **Extreme disorder in an ultrahigh-affinity protein complex**. *Nature* 2018, **555**:61–66.

39. Holmstrom ED, Liu Z, Nettels D, Best RB, Schuler B: **Disordered RNA chaperones can enhance nucleic acid folding via local charge screening**. *Nat Commun* 2019, **10**:2453.

40. •• Wiggers F, Wohl S, Dubovetskyi A, Rosenblum G, Zheng W, Hofmann H: **Diffusion of a disordered protein on its folded ligand**. *Proc Natl Acad Sci USA* 2021, **118**, e2106690118.
This article presents a study of the conformational and interaction dynamics of the fuzzy complex formed between the disordered tail of E-cadherin and proto-oncogene β-catenin, a folded protein, using single-molecule FRET and simulations. The authors find that, in the complex, E-cadherin diffuses in less than 1 msec across the entire β-catenin surface. They also identify a few persistent contacts, which they argue provide the binding specificity whereas the affinity is boosted by the many other unspecific interactions that are dynamically formed in the complex.

41. •• Wang D, Wu S, Wang D, Song X, Yang M, Zhang W, Huang S, Weng J, Liu Z, Wang W: **The importance of the compact disordered state in the fuzzy interactions between intrinsically disordered proteins**. *Chem Sci* 2022, **13**:2363–2377.
The authors use MD simulations and experiments to investigate the mechanism behind the fuzzy complex formed by two disordered proteins, the C-terminal domain of 4.1G and a 26-residue region from NuMA. This study is interesting in that it involves two disordered proteins, but one is capable of folding into a globular structure whereas the other is an IDR like those often looked at as models for folding upon binding to a structured partner. The authors identify two features of the full IDP C-term-4.1 G that drive binding: the formation of tertiary interactions between two short structural motifs which act as binding recognition hot spots, and its overall degree of chain compaction.

42. • Stringer MA, Cubuk J, Incicco JJ, Roy D, Hall KB, Stuchell-Brereton MD, Soranno A: **Excluded volume and weak interactions in crowded solutions modulate conformations and RNA binding of an intrinsically disordered tail**. *J Phys Chem B* 2023, **127**:5837–5849.
A thorough single-molecule Förster resonance energy transfer study of the effects of crowding on the compaction and partner interactions of an IDR from the SARS-CoV-2 nucleocapsid protein. The study offers insights into the interplay between crowding agents and the conformation and binding properties of disordered proteins.

43. • Guseva S, Schnapka V, Adamski W, Maurin D, Ruigrok RWH, Salvi N, Blackledge M: **Liquid–liquid phase separation modifies the dynamic properties of intrinsically disordered proteins**. *J Am Chem Soc* 2023, **145**:10548–10563.
An interesting NMR-simulations study of the changes in conformational dynamics that IDPs, in this case, experience when they form biomolecular condensates through LLPS. A major finding is that the self-interactions occurring in the dense liquid phase results in a slow down of all the conformational dynamic modes of the IDP, with also an increase in the relative amplitude of the larger scale chain-like motions.

44. • Maity H, Baidya L, Reddy G: **Salt-induced transitions in the conformational ensembles of intrinsically disordered proteins**. *J Phys Chem B* 2022, **126**:5959–5971.
This article introduces a simulation-experimental methodology tailored to study salt-mediated ordering transitions in IDP ensembles. The authors propose that such an approach can be effectively used to identify which type of salt will induce LLPS of an IDP of interest.

45. Huang Z, Liu H, Nix J, Xu R, Knoverek CR, Bowman GR, Amarasinghe GK, Sibley LD: **The intrinsically disordered protein TgIST from Toxoplasma gondii inhibits STAT1 signaling by blocking cofactor recruitment**. *Nat Commun* 2022, **13**:4047.

46. •• Schrag LG, Liu X, Thevarajan I, Prakash O, Zolkiewski M, Chen J: **Cancer-associated mutations perturb the disordered ensemble and interactions of the intrinsically disordered p53 transactivation domain**. *J Mol Biol* 2021, **433**:167048.
This article presents a computational-experimental study of how cancer-associated mutations affect the conformational ensemble and binding properties of the intrinsically disordered transactivation domain (TAD) of the tumor suppresor p53. The obtained evidence indicates that cancer-associated mutations known to disrupt the interactions between p53 and regulators do not affect the interaction interfaces between partners but modulate the conformational expansion and rigidity of the TAD ensemble. These results provide indirect, but appealing, evidence of a connection between IDP conformational dysfunction and the onset of cancer

47. Wichapong K, Silvestre-Roig C, Braster Q, Schumski A, Soehnlein O, Nicolaes GA: **Structure-based peptide design targeting intrinsically disordered proteins: novel histone H4 and H2A peptidic inhibitors**. *Comput Struct Biotechnol J* 2021, **19**:934–948.

48. •• Ruan H, Yu C, Niu X, Zhang W, Liu H, Chen L, Xiong R, Sun Q, Jin C, Liu Y: **Computational strategy for intrinsically disordered protein ligand design leads to the discovery of p53 transactivation domain I binding compounds that activate the p53 pathway**. *Chem Sci* 2021, **12**:3004–3016.
This article introduces a new hierarchical computational strategy for IDP drug virtual screening that allows to search for compounds that can bind to multiple conformations of the IDP. To demonstrate the approach the authors target TAD-p53 and identify two organic compounds that bind to TAD-p53 with high affinity and restore the normal function of p53 in cultured cancer cells.

49. Heller GT, Aprile FA, Michaels TC, Limbocker R, Perni M, Ruggeri FS, Mannini B, Löhr T, Bonomi M, Camilloni C: **Small-molecule sequestration of amyloid-β as a drug discovery strategy for Alzheimer's disease**. *Sci Adv* 2020, **6**, eabb5924.

50. Cuevas-Velazquez CL, Vellosillo T, Guadalupe K, Schmidt HB, Yu F, Moses D, Brophy JA, Cosio-Acosta D, Das A, Wang L: **Intrinsically disordered protein biosensor tracks the physical-chemical effects of osmotic stress on cells**. *Nat Commun* 2021, **12**:5438.

51. Nagpal S, Luong TD, Sadqi M, Muñoz V: **Downhill (Un) folding coupled to binding as a mechanism for engineering broadband protein conformational transducers**. *ACS Synth Biol* 2020, **9**:2427–2439.

52. Campos LA, Sadqi M, Muñoz V: **Lessons about protein folding and binding from archetypal folds**. *Acc Chem Res* 2020, **53**:2180–2188.

53. Pérez-Hernández G, Paul F, Giorgino T, De Fabritiis G, Noé F: **Identification of slow molecular order parameters for Markov model construction**. *J Chem Phys* 2013:139.