Communications in Computational Chemistry
Commun. Comput. Chem., Vol. 7, No. 4, 350–360
doi: 10.4208/cicc.2025.217.02
Regular Article

Combining All-Atom Molecular Dynamics Simulation and NMR to Analyze Conformational Ensemble of Intrinsically Disordered Proteins

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Received on 5 August 2025; Accepted on 2 November 2025

Abstract: Intrinsically disordered proteins (IDPs) lack stable tertiary structures and instead populate dynamic conformational ensembles, presenting unique challenges for structural characterization. In this review, we discuss the synergistic integration of all-atom molecular dynamics (MD) simulations and nuclear magnetic resonance (NMR) spectroscopy to elucidate the structural and dynamic properties of IDPs. NMR spectroscopy provides ensemble-averaged, site-specific structural and dynamic information, though its inherently sparse data limits resolution. Conversely, MD simulations yield atomically detailed trajectories but are constrained by sampling limitations and potential force field inaccuracies. Integrating both methods, using NMR data as restraints or reweighting criteria for MD simulations, improves accuracy and provides a more complete understanding of IDP behavior. Recent advancements include statistical reweighting techniques and AI-assisted methods to enhance sampling efficiency and ensemble construction. Despite progress, challenges remain in force field accuracy and seamless data integration. Future work will focus on improving force fields, developing more dynamic data integration methods, and leveraging AI for more efficient and accurate ensemble generation.

Key words: intrinsically disordered protein, molecular dynamics simulation, NMR, structure ensemble.

1. Introduction

Intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs) defy the classical structure-function paradigm by lacking a stable tertiary structure under physiological conditions [1-5]. Instead of adopting a single, well-defined conformation, IDPs exist as dynamic ensembles of rapidly interconverting structures [6,7]. This inherent flexibility enables them to engage in diverse biological functions, including cellular signaling [8-10], transcription regulation [9,11], and molecular recognition [12], and closely associated with a range of diseases, such as neurodegenerative disorders, cancer, and cardiovascular diseases [13-15].

A central concept in the study of IDPs is that of the conformational ensemble, which provides a statistical description of all structural conformations [5,16]. Unlike folded proteins, whose structures can often be captured by single high-resolution models, IDPs require a more comprehensive depiction that accounts for their conformational heterogeneity, structural plasticity, and dynamic fluctuations [17-21]. Capturing these ensembles with sufficient accuracy is critical for understanding their biophysical behavior and functional mechanisms [22-26].

Due to their high flexibility, characterizing the conformational ensembles of IDPs remains an immense challenge [27]. Their transient and heterogeneous nature renders many conventional structural biology techniques insufficient [28]. High-resolution methods such as X-ray crystallography or cryo-electron microscopy often fail to resolve disordered regions due to their inherent flexibility [29]. In contrast, nuclear magnetic resonance (NMR) spectroscopy [30] has emerged as a powerful and complementary tool for probing IDP ensembles at atomic resolution. NMR spectroscopy is uniquely well-suited for studying IDPs, as it provides ensemble-averaged, site-specific information

on local structure and dynamics under physiological conditions. Parameters such as chemical shifts [31], residual dipolar couplings (RDCs) [32], nuclear Overhauser effects (NOEs) [33], paramagnetic relaxation enhancements (PREs), and spin relaxation rates [34] offer rich insights into both secondary structure propensities and backbone dynamics. However, NMR data are inherently sparse and indirect, requiring interpretation through structural modeling or computational support [35].

On the other hand, all-atom molecular dynamics (MD) simulations can provide atomistic trajectories of protein motions, thereby offering a detailed picture of conformational sampling and dynamics across a wide range of time scales [36-38]. However, due to the limited sampling efficiency and force field accuracy, MD simulations may deviate from physical reality or fail to capture functionally relevant states [35]. Given their respective strengths and limitations, integrating all-atom MD simulations with NMR data has become increasingly essential for constructing accurate and experimentally validated models of IDP ensembles [39,40]. By using NMR observables as restraints, validation metrics, or reweighting criteria, simulations can be refined to reflect true biophysical behaviors, while MD offers atomistic context and dynamical interpretation to otherwise averaged experimental data [41]. This synergistic approach holds great promise for unraveling the complexity of IDPs. Here, we present a review of the recent advances in the integration of MD simulations and NMR spectroscopy for characterizing the conformational ensembles of IDPs.

2. NMR characterization of IDP structure and dynamics

NMR spectroscopy yields a range of experimental observables that allow researchers to explore the structural and dynamical properties of biomolecules [42]. Crucially, the technique captures ensemble-averaged signals weighted by the population of interconverting conformational states under equilibrium [43].

2.1 Chemical shifts

Chemical shifts are fundamental parameters in NMR spectroscopy, describing the position of an NMR signal relative to a reference [44,45]. As highly sensitive reporters of molecular structure, chemical shifts have been extensively utilized in protein structure prediction, enabling accurate and efficient determination of structures based solely on chemical shift data. [45-49]. In recent years, chemical shifts have become standard tools for modelling the secondary and tertiary structures of IDPs, providing critical insights into their conformational ensembles [43,50-54]. Different types of chemical shifts exhibit distinct yet complementary dependencies on backbone dihedral angles, thereby enabling residue-specific conformational mapping of IDPs [55].

Among various chemical shift-based observables, secondary chemical shifts (SCSs)—defined as the difference between the measured chemical shift and the corresponding random coil chemical shift (RCCS)—serve as the primary atomic-scale indicators of local secondary structure propensities [56,57]:

$$SCS_i^A = \delta_i^A - RCCS_i^A \quad (1)$$

where i denotes the residue position and A indicates the atom type. Depending on the nucleus observed and the conformational space sampled (e.g., α-helical or β-sheet regions of the Ramachandran plot), SCS values can be either positive or negative. This sensitivity makes them powerful indicators of transient secondary structural elements, even within highly dynamic or disordered regions [55].

2.2 Nuclear overhauser effect (NOE)

The Nuclear Overhauser Effect (NOE) provides inter-atomic distance information, typically between hydrogen atoms within approximately 5 Å of each other [58]. NOEs arise from cross-relaxation processes between spatially close nuclear spins and are widely used as key constraints in the determination of 3D structures of biomolecules, especially proteins and nucleic acids [59-61].

Notably, in studies of IDPs, NOEs are often weak or sparse due to rapidly conformational averaging and lack of persistent tertiary contacts [62,63]. However, weak NOE signals can still provide valuable insight into local compaction, residue structure, or preferred interactions within dynamic ensembles. By scrutinizing the patterns of NOE cross-peaks, transient helices, β-strands, and other fleeting structural motifs can be identified within IDPs. Moreover, NOEs reveal long-range contacts—such as intramolecular hydrogen bonds and hydrophobic interactions—thereby mapping the sparse yet functionally relevant tertiary networks that persist in the disordered ensemble [64,65].

2.3 Paramagnetic relaxation enhancement (PRE)

PRE provides long-range distance information, by leveraging the influence of an unpaired electron—typically introduced via a paramagnetic probe—on the relaxation rates of the nuclear spins [66,67]. PRE is a useful tool for detecting long-range distance restraints, up to approximately 40 Å [68,69]. This technique is particularly valuable for investigating protein structure, dynamics, and interactions, as it can reveal sparsely populated conformational states and transient structural changes that are often inaccessible by other methods [70,71].

Because PREs are sensitive to a much wider distance range than NOEs, they are particularly valuable for detecting transient, long-range contacts, including those that exist in low-population conformational states [69,72,73]. For IDPs, PRE provides unique insight into transient tertiary interactions and compaction levels that are often invisible to other NMR observables such as chemical shifts or NOEs [74-76].

2.4 Residual dipolar couplings (RDCs)

Residue Dipolar Couplings (RDCs) are NMR observables that provide long-range internuclear orientational information [77-81]. They offer insights into the time- and ensemble-averaged orientation of bond vectors, thereby complementing high-resolution structural and dynamic studies of proteins and other biomolecules [80,82-84].RDCs are particularly valuable for refining global protein fold and domain orientation [85-88], studying conformational equilibria and dynamics [89-91], and investigating protein-protein and protein-ligand interactions [92,93]. As for IDPs, RDCs report on angular rather than distance constraints, can significantly enhance the accuracy and precision of NMR-based structure-determination, especially in cases where conventional data is sparse [94-97].

2.5 Spin-relaxation

NMR spin-relaxation analysis is a highly informative and widely used technique for probing protein dynamics [98-102]. By measuring longitudinal relaxation rates (R_1), transverse relaxation rates (R_2) and heteronuclear NOE, it could sensitively captures picosecond-to-nanosecond (ps-ns) timescale motions [44,101,103-105]. These parameters enable the construction of residue-specific dynamic profiles, offering detailed insights into the flexibility and functional dynamics of proteins [106,107]. For IDPs, spin relaxation measurements are particularly useful for identifying residual secondary structure [108-110], compaction [111,112], or heterogeneous local motions [109,113,114], even in the absence of stable tertiary structure.

In summary, different NMR parameters provide complementary insights into the spatial and temporal information of protein structure and dynamics. Chemical shifts and NOEs are primarily used for high-resolution local structure determination, while RDCs and PREs extend structural analysis to longer-range and global features. Relaxation measurements offer quantitative information on molecular motions across a wide range of timescales. Together, these techniques enable a comprehensive, site-specific understanding of biomolecular behavior in solution. However, each method has its own limitations in terms of resolution, interpretability, and experimental practicality.

While NMR spectroscopy provides rich, residue-specific information on both the structure and dynamics of proteins across a broad range of timescales, its resolution is often limited by the sparsity of experimentally accessible observables and the need for interpretative models. Moreover, for highly flexible systems such as IDPs, the complexity of conformational ensembles can render NMR data difficult to interpret in isolation.

To bridge these gaps, MD simulations have emerged as a powerful computational complement. By providing atomistic trajectories of protein motions, MD allows researchers to explore conformational landscapes with temporal and spatial continuity that is challenging to achieve experimentally. The following section discusses the unique contributions of MD simulations to biomolecular studies, as well as the inherent challenges and limitations that accompany their application.

3. Strategies for combining MD and NMR to decipher IDP conformational ensembles

MD simulations offer a unique and powerful means to study biomolecular systems at atomic resolution, complementing experimental techniques such as NMR spectroscopy [39,41,115]. One of the major advantages of MD is its ability to provide time-resolved, atomistic trajectories that reveal how protein structures fluctuate, fold, or interact with their environment over time. These simulations allow for a direct visualization of molecular motions that are often inaccessible or only indirectly inferred from experimental data, thereby offering a dynamic perspective to structural ensembles [116]. As illustrated in Figure 1, The approaches for MD-NMR integration could be divided into four types: post-processing validation, statistical reweighting, experimentally restrained MD and newly emerged experiment-guided AI methods.

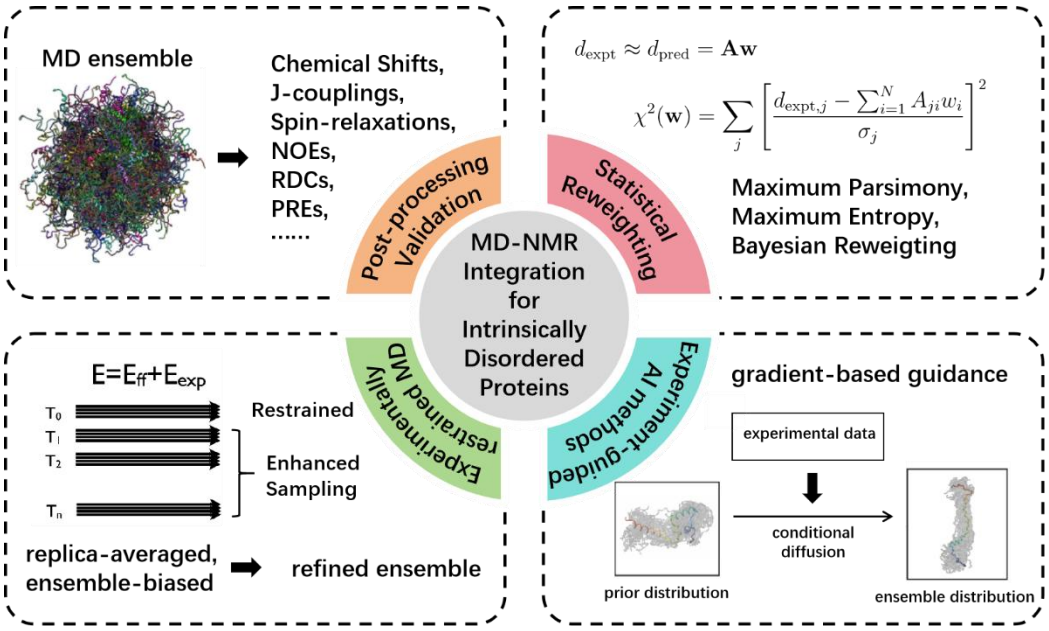

Figure 1. The integration methods for MD simulations and NMR experiments.

3.1 Post-processing validation

To assess the accuracy of MD simulations in reproducing experimental observations, researchers often compare computed observables with experimental NMR data. A widely used method is post-processing validation, in which NMR observables, such as chemical shifts, residual dipolar couplings (RDCs), and paramagnetic relaxation enhancements (PREs), are calculated from MD-generated trajectories [40,117-123]. By comparing these computed values with experimental measurements, researchers can evaluate the reliability of the simulations or the underlying force fields [124-128]. For IDPs, which sample a diverse ensemble of

transient conformations, well-converged MD simulations can generate structural ensembles that accurately reflect the conformational heterogeneity observed in experimental NMR data [41,129].

These methods provide an important consistency check between simulation and experiment, but their effectiveness is fundamentally limited by the initial quality of the MD-generated ensemble and the accuracy of the models used to predict NMR observables. For example, conventional AMBER99 or AMBER99SB-ILDN force fields have been reported to over-stabilize α-helices in α-synuclein [27] or Aβ peptides [130], deviating from experimental NMR and SAXS observations. To overcome these limitations, two key directions are typically pursued: improving conformational sampling [131] and enhancing the accuracy of models that predict experimental observables from structure. Recent force fields, such as CHARMM36m [132], AMBER ff03ws [133], and a99SB-disp [134], have made significant improvement in addressing these issues by better balancing protein-water interactions and adjusting torsional potentials. However, even with improved force fields, the validation against NMR data remains crucial, as subtle inaccuracies in the forward models for calculating NMR parameters or insufficient sampling can still lead to misinterpretations of IDP conformational landscapes.

3.2 Statistical reweighting

In parallel, statistical reweighting methods have been widely applied to refine MD ensembles and improve their agreement with experimental data [135]. Since a conformational ensemble could be defined by a set of structures and there correspongding populations (i.e., relative weights), methods such as the Maximum Entropy principle [136-138], Bayesian inference [138,139], and Maximum Parsimony approaches [140] are used to adjust the weights of conformations to refine the ensembles that better match experimental observations. Maximum Entropy methods maximize uncertainty under experimental constraints, often producing a highly heterogeneous ensemble with many low-weight conformations. While this approach requires minimal changes to the initial model, the resulting ensemble can be difficult to visualize [140]. Maximum Parsimony method, in contrast, assumes the experimental data can be explained by a sparse subset of key conformations. This results in a simple, interpretable set of structures but may inadequate for modeling the the complexity of IDPs. Bayesian methods offer a more rigorous framework by treating weights as variables and inferring the probability distribution over all possible ensembles [141].

Computation tools such as ENSEMBLE [142] and ASTEROIDS [143] have been developed for IDPs ensemble selections. ENSEMBLE focuses on selecting a minimal subset of conformers from a large pool, while ASTEROIDS employs genetic algorithm to construct probabilistic ensembles.

3.3 Experimentally restrained MD

In some cases, methods based on statistical reweighting could significantly improve the accuracy of structural ensembles for IDPs [139,144]. However, it will still be influenced by the initial ensemble and the experimental conditions [145]. To more tightly couple simulations with experiments, experimentally restrained MD, often referred to as ensemble-restrained MD, has been developed [146-148]. For example, chemical shifts and NOEs could be used to refine protein's secondary structural elements formation since its sensitivity to local structure [148,149]; RDC restraints could be applied to correct the global orientations [150-152]. In this approach, experimental NMR data are incorporated as restraints or biasing potentials during the simulation, guiding the conformational sampling towards ensembles that satisfy the experimental constraints. This technique improves the relevance of MD-generated ensembles and can reveal minor populations or transient states that are difficult to capture otherwise [153,154].

When applied to IDPs, however, these approaches will face more difficulties. Since most experimental observables for IDPs are inherently ensemble-averaged, the use of direct single-structure restraints can lead to over-constraining. To overcome this, replica-averaged strategies have been introduced, in which multiple replicas are simulated in parallel and experimental observables are back-calculated and averaged across replicas before being restrained to match the data, either through bias potentials [155] or Bayesian inference [156]. For example, Replica Averaged Metadynamics [157] has shown good agreement with experimental data for a 13-residue peptide. However, the requirement of conducting simulations across multiple replicas and temperatures, along the need with real-time adjustments, significantly increases computational costs.

To mitigate such limitations, alternative ensemble-based approaches have been developed. Ensemble-Biased Metadynamics (EBMetaD) [158,159], for example, applies an adaptive bias potential based on a prior probability distribution from experiments. This enables a single MD trajectory to yield maximal-entropy ensembles consistent with one or more experimental observables, without the need for multiple replicas, thereby offering improved computational efficiency. It has been successfully applied to T4 lysozyme, reproduced three Double Eletron-Electron Resonance distance distributions concurrently within a few tens of nanoseconds of MD simulations [158].

More broadly, the principle of maximum entropy is widely employed to reconstruct IDP ensembles that are both maximally unbiased and consistent with experimental data. This approach ensures that no artificial constraints are introduced beyond those supported by the data. Furthermore, the refined ensemble was found to be minimally influenced by the applied force field [160].

3.4 Experiment-guided AI Methods

More recently, artificial intelligence (AI) - assisted methods have become important tools for studying intrinsically disordered proteins [161-165]. Unlike traditional MD simulations, which can be limited by the need for extensive sampling, AI-driven approaches can bridge the gap between arbitrary starting structures and physically consistent conformations by learning statistical distributions from physics-informed priors and experimental observables. Specifically, recent AI-driven approaches use generative frameworks, such as variational autoencoders [166,167] and diffusion models [167,168], that map high-dimensional conformational ensemble onto compact latent representations. These latent spaces enable far more efficient exploration of the vast conformational landscape of IDPs while retaining key structural and physical constraints.

However, without appropriate guidance, these samplers may generate ensembles that deviate substantially from the Boltzmann distribution [169]. To address this, models can incorporate experimental restraints such as chemical shifts, NOEs, or PREs,

thereby biasing the sampling toward more realistic ensembles. For instance, tools like DynamICE [170] employs a generative recurrent neural network to predict residue-by-residue torsion angles (ϕ, ψ, ω, χ), and dynamically refine torsional distributions based on NMR observables through Bayesian reward scheme. However, its performance is constrained by two main limitations: (i) dependence on a pre-generated conformational pool, which reduces generalizability and necessitates retraining for each new system; and (ii) use of internal-coordinate representations for conformers, which hampers the straightforward incorporation of distance-dependent restraints (e.g., NOEs or PREs).

Recently developed frameworks such as IDPForge [167] and ExEnDiff [169] integrate experimental data directly into the diffusion process, either by adding a correction term into the score function or by defining a back-calculator. These models operate in Cartesian coordinates and can be used to generate physically plausible starting conformations for brief MD simulations. Notably, ExEnDiff has been shown to reproduce ensembles consistent with those derived from long trajectories [169].

Compared to traditional MD-based sampling, these AI-enhanced methods offer improved sampling efficiency and accuracy in ensemble construction. As a result, they are becoming valuable complements to simulation and re-weighting strategies, helping to address the challenge of modeling heterogeneous and flexible protein states in a data-driven manner. For greater accuracy, multiple experimental datasets can be combined to guide sampling; doing so improves ensemble precision but requires careful balancing (for example via weighting or regularization) to avoid overfitting or conflicting restraints.

In conclusion, the integration of MD simulations with NMR data is a rapidly evolving field that leverages a range of computational strategies from direct validation to advanced statistical inference and AI-assisted modeling. These combined approaches are crucial for capturing the full complexity of biomolecular conformational landscapes and improving the reliability of structural and dynamic interpretations.

4. Challenges and perspectives

Despite significant advances in both experimental and computational methodologies, the accurate and comprehensive characterization of biomolecular dynamics—particularly for intrinsically disordered proteins (IDPs)—remains a formidable challenge. Looking ahead, further progress will hinge on continued improvements in force field accuracy, more seamless integration of simulation and experimental data, and the growing role of artificial intelligence in structural ensemble generation.

A major ongoing challenge lies in the refinement of force fields. Current force fields, while increasingly sophisticated, still exhibit limitations in accurately describing the delicate balance of interactions that govern disordered and flexible protein regions [171]. Small inaccuracies in torsional potentials, solvation models, or side chain interactions can lead to significant errors in predicted conformational ensembles [172]. The development of next-generation force fields—either empirically optimized against high-quality experimental data or derived through data-driven and machine-learning-based approaches [173-175]—will be crucial for improving the fidelity of MD simulations in representing real biomolecular behavior. Specifically, future efforts should prioritize the establishment of standardized community-wide benchmarks for force-field validation, such as large-scale comparisons against NMR chemical shifts, scalar couplings, and spin relaxation data for IDPs, complemented by cross-validation with SAXS and single-molecule FRET measurements. Another critical area for future work is tighter integration of simulation and experimental data. Although post hoc validation and ensemble re-weighting have proven effective, there remains a need for more dynamic, feedback-informed methods that steer simulations in real time using experimental observables. No single experimental technique could fully characterize a heterogeneous conformational ensemble, so it is important to combine diverse measurements, such as NMR observables (chemical shifts, RDCs, PREs, relaxation rates) together with complementary data from SAXS, FRET, or cryo-EM. Building reliable multimodal data-fusion methods underpinned by rigorous statistical and physical models will be essential to produce ensembles that are both experimentally consistent and physically meaningful.

Finally, the emergence of AI-assisted conformational sampling and modeling offers exciting new opportunities. Machine learning methods have already shown promise in accelerating sampling, improving structural prediction from sparse data, and learning complex mappings between structure and observables. As AI tools become more interpretable and physically grounded, their integration with traditional MD-NMR workflows could lead to transformative improvements in both accuracy and efficiency.

In conclusion, a comprehensive understanding of IDPs will depend on the integration of experimental NMR data and advanced computational modeling approaches. This offers a powerful framework for capturing the dynamic and heterogeneous nature of IDPs with greater accuracy and biological relevance of IDP conformational landscapes.

Acknowledgments

This work was supported by National Science Foundation of China (22073018, 22377015).

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