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Force fields for simulating intrinsically disordered proteins: Assessing conformational sampling and structural dynamics

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Intrinsically disordered proteins (IDPs) lack a defined three-dimensional (3-D) structure but play crucial roles in biological pathways. They exist as conformational ensembles and experimental methods struggle to capture their dynamic nature, making molecular dynamics (MD) simulations a valuable tool. However, force field accuracy and sampling algorithms limit simulation fidelity. Most protein force fields are too stable to accurately model unstructured proteins like IDPs. Empirical force fields-based computer simulations are increasingly used to study the biophysics of disordered proteins, with the choice of force field significantly influencing simulation outcomes for studying the conformational ensemble of IDPs. This study evaluates three AMBER force fields (ff99SBildn-TIP3P, ff99SB-TIP3P, and ff19SB-OPC) for simulating an IDP (Histatin 5) and a partially folded protein (Trp-cage). Extensive MD simulations compared the structural dynamics and conformational sampling across all the force fields for these two systems. The results show ff99SBildn-TIP3P as the most balanced force field, efficiently sampling ordered and disordered regions in these proteins. We evaluated the performance of the force fields with enhanced sampling metrices including RMSD, RMSF, Rg and SASA. Our results reveal ff99SBildn-TIP3P model better samples the disorder regions in Histatin 5 than the other force fields. This study highlights the importance of understanding force field strengths and limitations for IDP simulations. By selecting suitable force fields, researchers can better simulate the IDPs and understand their complex behavior.

Keywords: Conformational ensemble, Disordered regions, Molecular dynamics simulations, Unstructured proteins, Water models

Intrinsically disordered proteins (IDPs) are a class of proteins that lack a fixed three-dimensional (3-D) structure under physiological conditions in contrast to the common paradigm that proteins fold into a single native form to perform its function[1,2]. Recent studies have revealed that IDPs are abundant in the human proteome, with a significant number of proteins containing disordered regions[3]. The results from human proteomes suggest that there are 35-50% of proteins with more than 40 consecutive disordered residues[4,5]. IDPs are proteins that can form different conformations depending on the environment and their binding partners[6]. IDPs or intrinsically disordered protein regions (IDRs) are characterized by lack of specific tertiary structure and unable to fold spontaneously into globular 3-D structures without partner binding[7,8]. Furthermore, IDPs have been found to be included in many biological processes, such as regulation, recognition, cell cycle control and signaling[3,9,10].

Experimental tools like small-angle X-ray scattering (SAXS)[11], nuclear magnetic resonance (NMR)[12] and Forster resonance energy transfer (FRET) spectroscopy[11] are commonly used to study IDP conformational ensembles. However, the data obtained from these experiments represents an average over the diverse, interconverting conformational states of IDPs[13].Since, IDPs cannot spontaneously fold into stable tertiary structure without binding to their partners[4,6], the key experimental method for exploring the dynamics conformation of IDPs is NMR spectroscopy[13]. In addition, Molecular Dynamics (MD) simulations can be used to reveal the structural continuum of IDPs[14], from tightly folded single domains and multidomain proteins with flexible or disordered regions, to disordered molten globules, highly extended, and heterogeneous unstructured states[15]. Recently, MD simulations have been employed to predict disordered regions in IDPs by analysing the dihedral angles of proteins[16]. This method capitalizes on the ability of MD simulations to capture dynamic fluctuations,

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enabling the identification of flexible and disordered regions in IDPs that are often associated with specific biological functions. Force field plays a key role in applications of MD simulations[17]. Protein force fields are mathematical representations of the potential energy functions and associated parameters that govern the interactions between atoms within amino acids and between polypeptides and water[18]. These force fields capture both bonded and non-bonded interactions, enabling the simulation of complex biomolecular systems at the atomic level[17]. However, some previous studies show that most protein force fields could not reproduce the flexible conformers of IDPs due to their original intended applications of folded proteins[19].Other researchers also reached similar conclusions regarding the use of existing force fields for sampling IDPs. Due to the heterogeneous nature of IDPs, MD simulations are widely used to study the conformational ensembles and dynamic properties[18,19]. Hence, MD simulations offer a computational approach to explore the diverse conformational ensemble and dynamics of IDPs at an atomistic level[20]. However, the accuracy of MD simulations depends heavily on the choice of force field, which governs the interatomic interactions within the system. Several force fields, such as CHARMM[21], AMBER[19], and OPLS-AA[22], are commonly employed in MD simulations, each with its own parameters and functional forms. Understanding how different force fields influence the behavior of IDPs is essential for interpreting simulation results and drawing meaningful conclusions about their biological function[23]. Traditional force fields are primarily parameterized for folded proteins and may not accurately represent the energetics of disordered regions in IDPs[24]. Creating force field parameters specifically designed to capture the distinctive structural characteristics and dynamic behaviour of IDPs is crucial for enhancing the accuracy of MD simulations in investigating these complex proteins[23-25]. Different force fields may lead to distinct conformational ensembles for IDPs, affecting the interpretation of simulation results and predictions of structural properties such as RMSD, RMSF, Rg and solvent accessibility[24]. IDPs often interact with partner molecules, such as other proteins, nucleic acids, or small molecules, to perform their biological functions. The choice of force field may affect the accuracy of predicting the binding affinity, specificity, and binding modes of IDPs with their partners. In the recent past, many studies have been focussed in addressing suitable force fields to sample IDPs and its interactions[23]. Force field parameters optimized for folded proteins may not transfer well to IDPs due to their unique sequence composition and structural properties. Developing transferable force field parameters that accurately capture the behaviour of both folded and disordered regions in proteins is an important challenge in the field of computational biophysics[24,26].

Here, we conducted MD simulations on two proteins, Histatin 5 (an IDP)[27] and Trp-cage (a partially folded protein)[28], using three widely employed AMBER force fields- ff99SBildn-TIP3P, ff99SB-TIP3P, and ff19SB-OPC water model. We systematically compared the structural dynamics of the two proteins using the three force fields by assessing their ability to reproduce experimentally observed characteristics. Our results reveal that ff99SBildn force field with TIP3P water model generates diverse range of conformations and picks up the ordered and disordered regions in these proteins quite well. This study may provide valuable insights into the strengths and limitations of commonly used force fields for simulating IDPs, highlighting the importance of force field selection in capturing the complex behaviour of IDPs.

Materials and Methods

Initial structure preparation

The proteins considered in this study are Histatin 5 (an IDP) and Trp-cage (partially folded protein). Since the initial structure of Histatin 5 is not available in the RCSB PDB[29], we generated its initial structure using I-TASSER[30] by submitting its amino acid sequence in FASTA format. The initial 3D structure of Trp-cage was obtained directly from the RCSB PDB (PDB ID: 1L2Y).

Selection of force fields for simulations

We have selected recently developed AMBER force fields (ff99SB, ff99SBildn with TIP3P water model, and ff19SB with OPC water model) to assess their performance in simulating IDPs. These force fields were chosen to evaluate their ability to capture the distinctive characteristics of IDPs, such as their flexibility, dynamics, and conformational heterogeneity.

Capping of N-terminal and C-terminal of the proteins

We have used xleap, a graphical user interface available with the AmberTools software suite to cap the N-terminal with an acetyl group (ACE) and C-terminal with an amide group (NME) of the proteins before MD

simulations to prevent artifacts and maintain structural integrity, ensuring accurate and reliable results.

Input File preparation for MD simulation

After capping the terminals, we generated topology and coordinate files from the PDB structures using xleap in AMBER. We then performed MD simulations[31] with explicit solvation allowing for a thorough investigation of protein-solvent interactions and system dynamics[32,33].

Set up for MD Simulations

MD simulations of Histatin 5 and Trp-cage were performed using the AMBER force fields in the Leap module of AMBER[34]. The first step in performing MD simulations of IDPs is to select a force field that accurately represents the molecular interactions and energy environment[35]. This requires inclusion of dihedral angle potentials, which describe the energy associated with changes in dihedral angles, in the force field parameters. The choice of force field is crucial for accurately capturing the structural ensembles of IDPs[36]. While no single force field is ideal for IDP studies, we have utilized the three commonly used AMBER force fields (ff99SB, ff99SBildn with TIP3P water model and ff19SB with OPC water model)to sample the complex behavior of IDPs. To comprehensively assess the impact of these force fields on the simulations of the selected IDPs, we performed MD simulations on both proteins sequentially using each of the three force fields. This approach allowed us to compare the results obtained with different force fields, providing valuable insights into their strengths and limitations for IDP studies. Proteins were solvated in a cubic periodic box using the explicit TIP3P water model[37]. Counter ions were introduced to neutralize the system. Strong van der Waals interactions were eliminated through energy minimization. The conventional MD methodology involved heating the systems from 0 to 300 K, equilibrating them under NVT conditions, 1 ns NPT simulation at 300 K and 1 atm and later performing energy-minimized simulations for production dynamics[38-40]. Throughout the simulation, several key parameters including density, temperature, potential energy, kinetic energy, and total energy for both proteins were closely monitored to ensure the accuracy of the NPT algorithm (Suppl. Figs. 1-6).

Trajectory analysis was performed using PTRAJ and CPPTRAJ modules of AmberTools[41]. Structural visualization was done using UCSF Chimera[42]. RMSD, RMSF and Radius of gyration (Rg) were calculated using Amber tools, and graphs were generated with xmgrace plotting tools.

Results and Discussion

Root Mean Square Deviation analysis

Root Mean Square Deviation (RMSD) is a metric used in MD simulations to measure the average distance between atoms in two superimposed protein structures[43]. Analysis of the RMSD plots (Fig. 1A (i and ii)) reveals distinct differences in the conformational sampling of Histatin 5 and Trp-cage using various force fields. For Histatin 5 (Fig. 1A (i)), the ff99SBildn force field exhibits higher RMSD values which are the salient characteristics of IDPs, indicating its ability to effectively sample the disordered configurations. This suggests that ff99SBildn is well-suited for capturing protein flexibility and dynamics in disordered regions. In contrast, the ff19SB-OPC and ff99SB force field shows minimal changes in RMSD values, indicating its limited ability to sample the diverse structures that are inherent in IDPs. Notably, for the partially folded protein like Trp-cage (Fig. 1A (ii)), all three force fields perform equally well, suggesting that ff99SBildn is not only effective for IDPs but also suitable for partially folded proteins.

Root Mean Square Fluctuation analysis

Root Mean Square Fluctuation (RMSF) analysis measures protein atom or residue mobility by calculating the square root of the average squared displacement over a simulation trajectory. For Histatin 5, as depicted in (Fig. 1B (i)), the ff99SBildn force field excels in sampling disordered conformations, encompassing a wider conformational space and exhibiting greater flexibility. This suggests that ff99SBildn effectively captures the dynamic nature of IDPs. In contrast, the ff19SB-OPC and ff99SB force field yields the lower RMSF values for Histatin 5, indicating its limited ability to fully capture the conformational flexibility inherent in disordered proteins.

When it comes to simulating a partially folded protein like Trp-cage as shown in (Fig. 1B (ii)), the ff99SBildn force field shows lower RMSF values indicate a more accurate capture of the dynamic nature of folded proteins. Notably, the other two force fields, ff99SB and ff19SB-OPC, also produce comparable results, suggesting that all three force fields perform equally well for partially folded proteins.

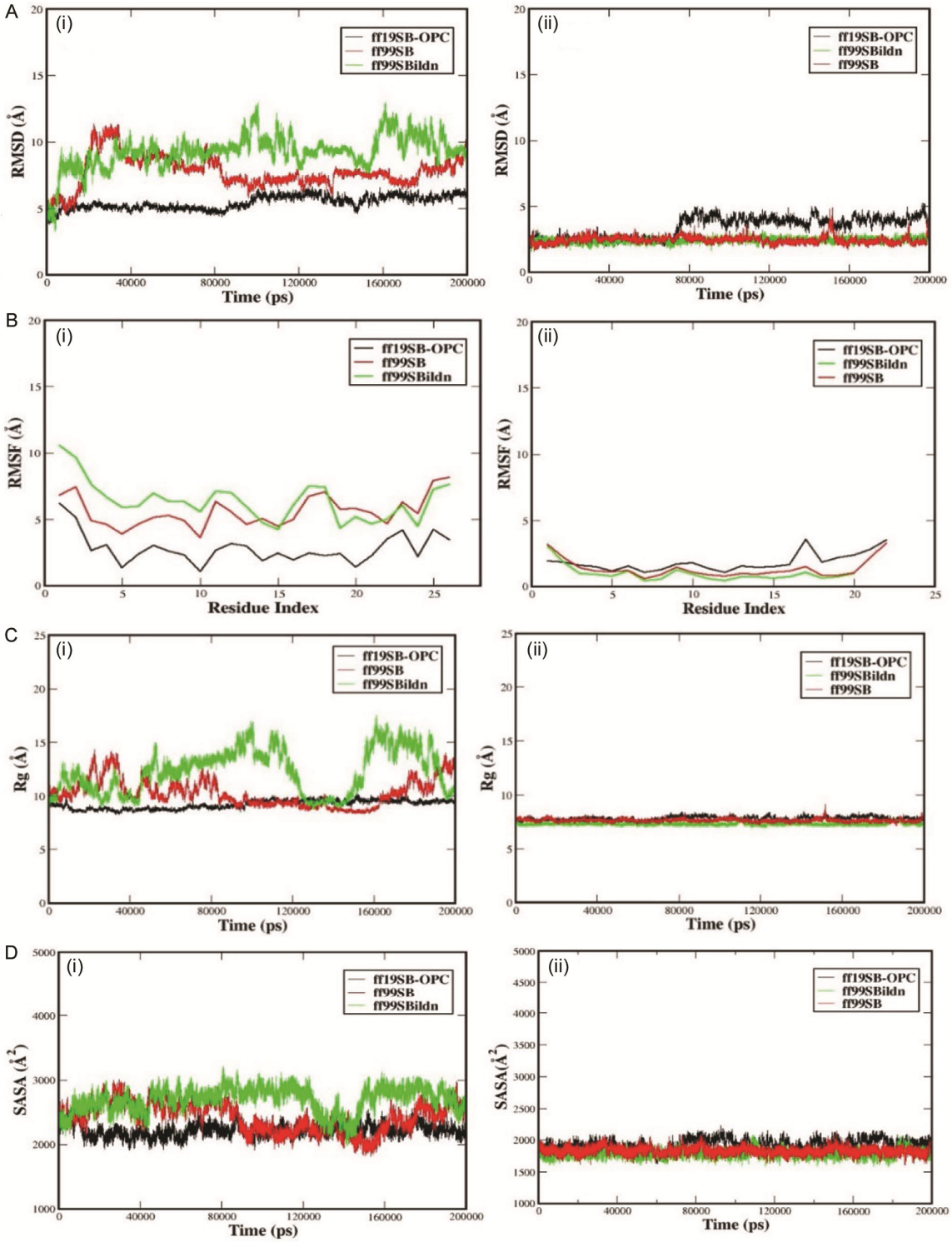

Fig. 1 — RMSD plots, RMSF plots, Rg plots, and SASA plots for the three different force fields showing for (i) Histatin 5; and (ii) Trp-cage

Radius of gyration analysis

The radius of gyration (Rg) is a measure of a protein's overall compactness, indicating the distribution of its atomic positions around its centre of mass[44]. As can be seen in (Fig. 1C (i)), the ff99SBildn force field exhibits a greater variation in Rg values, effectively capturing the diverse conformational ensembles of IDPs. This indicates that ff99SBildn

samples the broad range of conformations adopted by IDPs, making it a suitable choice for simulating proteins that are intrinsically disordered.

The Rg analysis for Trp-cage (Fig. 1C (ii)) shows that all three force fields — ff99SBildn, ff99SB and ff19SB-OPCyield nearly identical results for simulating folded proteins. This similarity in Rg values suggests that each of these force fields is equally capable of capturing the overall size and compactness of the protein's conformational ensemble.

SASA analysis

SASA (Solvent Accessible Surface Area) analysis is a method used to quantify the exposure of protein surfaces to the solvent. As shown in (Fig. 1D (i and ii)), the ff99SBildn force field excels in accurately capturing surface exposure across both ordered and disordered regions of the proteins. It performs particularly well in representing the dynamic changes in surface accessibility during transitions between these states, making it the most suitable force field for simulating IDPs that undergo such transitions. On the other hand, the ff19SB-OPC and ff99SB force fields exhibits limited ability to accurately capture the dynamic behavior of IDPs, struggle to adequately represent the flexible regions located at the surface of the proteins.

All three force fields-ff19SB-OPC, ff99SB, and ff99SBildn produce similar results for Trp-cage (as shown in Fig. 1D (ii)), indicating their ability to accurately simulate the surface behavior of proteins that maintain a stable, folded structure.

Analysis of the Conformational dynamics of Histatin 5 at different time intervals

The snapshots created using UCSF Chimera show intriguing insights into the conformational dynamics of Histatin 5. Using ff19SB-OPC force field, we noticed Histatin 5 to adopt a compact conformation, indicating a highly ordered and rigid structure with limited flexibility (Fig. 2A). The tendency of this force field to promote stability and rigidity could limit its ability to accurately capture the dynamic, disordered nature of IDPs.

Histatin 5 exhibits a moderate conformation, representing an intermediate state using ff99SB force field (Fig. 2B). The ff99SB force field appears to strike a balance between stability and flexibility, allowing Histatin 5 to maintain some ordered structure while still permitting a degree of dynamic behavior.

As can be seen from (Fig. 2C), Histatin 5 displays a highly extended and disordered conformation using

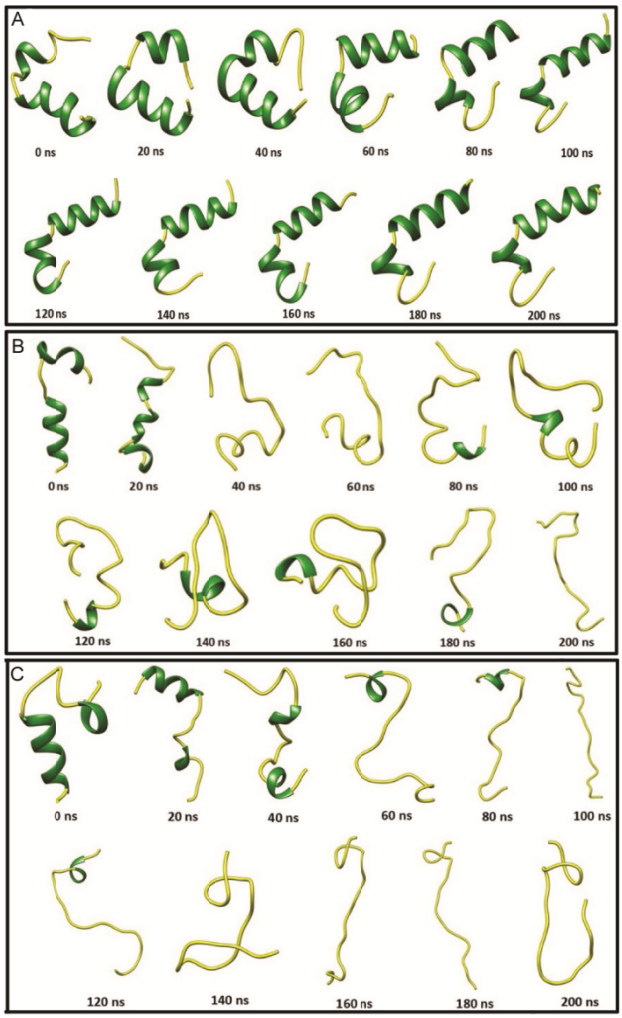

Fig. 2 — Snapshots of the conformers of Histatin 5 taken at different intervals of simulation time using force fields (A) ff19SB-OPC; (B) ff99SB-TIP3P; and (C) ff99SBildn-TIP3P

ff99SBildn force field. The protein adopts a more open, unfolded structure with increased flexibility, indicating that the ff99SBildn force field effectively captures the dynamic, disordered state. This force field allows Histatin 5 to sample the disordered regions, providing a more accurate representation of its intrinsically disordered nature. In some instances, the disordered regions in IDPs have been reported to undergo transition to ordered secondary structure in the presence of secondary structure inducers[45].

Analysis of the Conformational dynamics of Trp-cage at different time intervals

The snapshots reveal that the Trp-cage protein consistently exhibits an ordered conformation across all three force fields—ff19SB-OPC, ff99SBildn, and ff99SB (Fig. 3A-C). This consistency is expected since Trp-cage possesses a relatively stable and well-

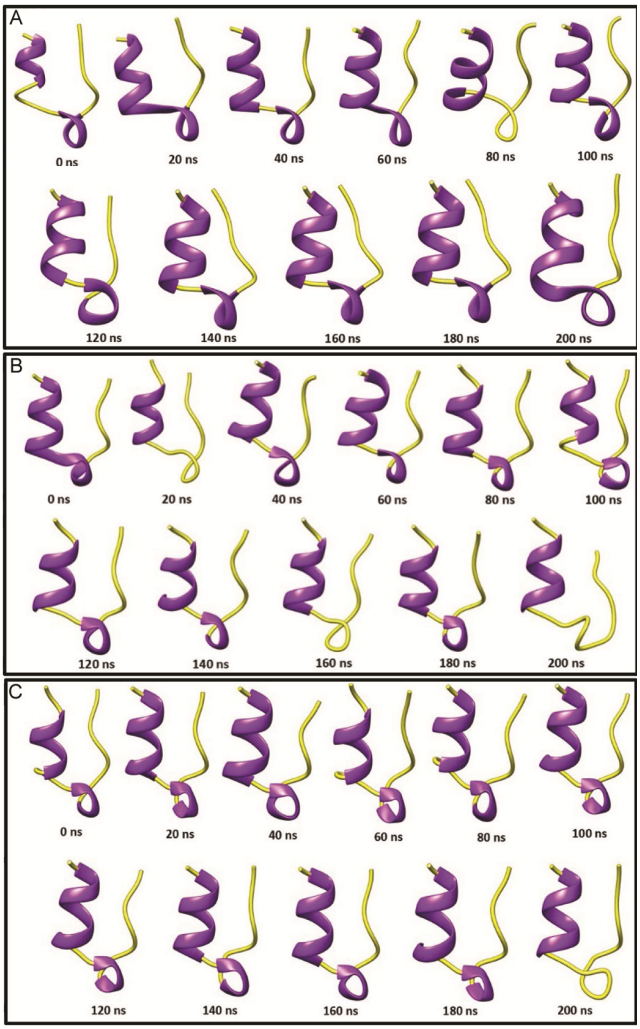

Fig. 3 — Snapshots of the conformers of Trp-cage taken at different intervals of simulation time using force fields (A) ff19SB-OPC; (B) ff99SB-TIP3P; and (C) ff99SBildn-TIP3P

defined structure, unlike the more flexible Histatin 5. All three force fields effectively sampled this ordered conformation, indicating their capability to accurately represent the protein's stable structure. Trp-cage has a strong hydrophobic core, which provides stability and helps to maintain its ordered conformation. The similar snapshots across force fields show that current force fields accurately capture the protein's structure, thereby providing reliance in their use for simulations for folded proteins.

Conclusion

In conclusion, our comprehensive study evaluated the performance of different AMBER force fields in simulating the conformational dynamics of an IDP and a partially folded protein. Our results show that the ff99SBildn force field, combined with the TIP3P water model, excels in sampling the diverse conformational ensemble characteristic of IDPs, as well as sampling the ordered structure of partially folded proteins. This demonstrates its ability to accurately represent both the dynamic and stable aspects of protein conformations. While the other two force fields (ff99SB and ff19SB-OPC) exhibit moderate performance in simulating the IDP dynamics, but all three force fields samples well for partially folded protein. Through a systematic comparison of Histatin 5 and Trp-cage structural dynamics, we assessed each force field's capability to accurately capture both ordered and disordered regions of the proteins. Hence, ff99SBildn-TIP3P excels in sampling the dynamic, flexible nature of IDPs while also accurately representing stable, ordered conformations when necessary. This study underscores the critical role of force field selection in MD simulations, particularly for complex systems like IDPs, where capturing both stability and disorder is essential. The insights gained from our analysis highlight the strengths and limitations of commonly used force fields, providing valuable guidance for future studies aiming to explore the intricate behaviors of IDPs.

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Conflicts of interest

All authors declare no conflict of interest.

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