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Understanding the conformational dynamics of an intrinsically disordered protein bound to a small molecule

Babli Sharma[#] and Venkata Satish Kumar Mattaparthi[#,*]

Molecular Modelling and Simulation Laboratory, Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur-784 028, Assam, India

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Intrinsically disordered proteins (IDPs) have been implicated in a wide range of human diseases. Due to their intrinsic conformational variability, IDPs are generally not effective for conventional structure-based drug design. Recently, ZZW-115 has been identified as a promising inhibitor of NUPR1 (an IDP whose overexpression is involved in various cancers), offering a potential lead for the development of novel cancer therapies. Understanding the conformational dynamics is crucial for comprehending the inhibitory action of ZZW-115, revealing the binding modes and dynamic interactions between ZZW-115 and NUPR1. Here, we present a computational study employing atomic-level Molecular dynamics (MD) simulations coupled with Umbrella sampling (US) to investigate the binding of ZZW-115 to NUPR1, elucidating the conformational changes and dynamics involved in the interaction. The Potential of Mean Force (PMF) plot showed a minimum value of 9Å at NUPR1-ZZW-115 separation with a dissociation energy of 2 kcal/mol. From Root Mean Square Deviation (RMSD) and secondary structure analysis, the conformational dynamics of NUPR1 was found to be varied as a function of its centre of mass (CoM) distance from ZZW-115. The NUPR1-ZZW-115 complex with the lowest potential energy extracted from the PMF plot was used to study the conformational dynamics of NUPR1 in unbound and complex form. The study reveals specific residues and binding pockets in NUPR1's disordered region that bind to ZZW-115, providing valuable insights into its inhibitory mechanism, enabling the development of effective drug design for therapeutic purposes.

Keywords: Molecular dynamics simulations, Umbrella sampling, Potential of mean force, Binding pocket

Intrinsically disordered proteins (IDPs) lack stable secondary or tertiary structures in certain regions or throughout their entire sequence due to their rapid inter-converting nature[1,2]. IDPs serve as hubs in interaction networks, performing various functions in cell-signalling routes and regulation, making them frequently involved in significant diseases[3]. Since IDPs are prevalent in a variety of diseases, including cancer and neurological disorders[4], they have emerged as attractive targets for drugs[4]. They have very high flexibility, hence do not maintain a stable conformation[5] and prediction of their disordered regions is an ongoing area of development[6]. IDPs are frequently involved in regulatory and signaling processes and their functions include cell cycle control[7], transcriptional regulation[8], replication, differentiation and RNA processing which are mediated by protein-protein or protein and other biomolecule interactions[9]. Since interactions between small molecules and IDPs can greatly affect IDP functions, understanding these interactions is essential for drug discovery[10]. Despite their significant potential for therapeutic intervention, IDPs have long been viewed as challenging and considered undruggable because of their lack of stable long-lived binding pockets for small molecules[11]. Currently, only a small number of instances have been identified where small molecules has been seen to interact with IDPs[12], and this condition is further complicated by the limitations of current experimental techniques, which often lack the sensitivity to detect these subtle binding events[13,14].

Here, we have employed molecular dynamics (MD) simulations[15] to gain an atomic-level insights into the binding mechanism of a small molecule (ZZW-115)[16] to an IDP (NUPR1)[17], specifically focusing on how this interaction influences the conformational dynamics of NUPR1.

*Correspondence:
[#]Equally contributed
Phone: +91-3712-275443, +91-8811806866 (Mob)
Fax: -91-3712-267005/267006
E-mail: mvenkatasatishkumar@gmail.com, venkata@tezu.ernet.in
Suppl. Data available on respective page of NOPR

NUPR1, or nuclear protein 1, is an 82-residue-long, basic IDP[17] activated in the exocrine pancreas in response to pancreatitis-induced cellular injury. NUPR1 is crucial for cell-cycle regulation and has been observed to be over-expressed in almost all cancer tissues under various stress conditions. Furthermore, it plays a crucial role in various protein cascades, including those that regulate apoptosis. Nupr1 genetic inactivation inhibits pancreatic cancer growth[18], hepatocarcinoma[19], osteosarcoma[20] and multiple myeloma[20]. NUPR1 could be a potential target for developing cancer therapies by preventing its protein-protein interactions[21], but its dynamic nature presents challenges for drug discovery.

An antipsychotic drug called trifluoperazine has been shown to interact with NUPR1and inhibit the growth of tumors dependent on NUPR1[18]. In various studies, ZZW-115, a novel trifluoperazine derivative, has shown improved binding and potent effects on pancreatic cancer cells, offering promising prospects for therapeutic targeting NUPR1[16]. NUPR1 is a crucial molecule in cancer research, involved in DNA repair, transcription regulation, cell cycle death, and metabolic activity of cancer cells over the past two decades[17-19]. The drug targets various cancers and shows early therapeutic potential by targeting NUPR1 in cancer treatment. Understanding ZZW-115 and NUPR1 interactions at an atomic levelis crucial for drug design. This study uses MD simulations to explore these interactions, focusing on binding dynamics and conformational flexibility.

We present a computational study that employs atomic-level molecular dynamics (MD) simulations coupled with Umbrella sampling (US) to investigate the binding of small molecule ZZW-115 to NUPR1. Our simulations elucidate the conformational changes and dynamics involved in the interaction, revealing the structural basis of their complex formation. We equilibrated the NUPR1-ZZW-115 complex structure and conducted a 10 ns production run, during which we measured the centre of mass (CoM) distance between NUPR1 and ZZW-115. We then employed a Potential of Mean Force (PMF) study to reveal the dynamic energetic landscape of binding and unbinding events, providing insights into the conformational ensembles defining this crucial interaction. Our results show that NUPR1 undergoes localized conformational changes upon binding to ZZW-115, suggesting the presence of active sites within NUPR1. This study provides valuable atomic-level insights into the impact of small molecule on the conformational dynamics of an IDP by identifying specific regions exhibiting characteristic changes. It sheds light on the molecular mechanisms of IDP-small molecule interactions, aiding in understanding protein function and regulation, and revealing key residues in small molecule-IDP interactions which may help in the rational design of IDP-targeted therapeutics.

Materials and Methods

Molecular docking and the preparation of initial structures

Protein preparation

The initial 3D structure of an IDP, nuclear protein 1 (NUPR1) was downloaded from AlphaFold[22] which served as the receptor protein molecule for our molecular docking studies.

Small molecule preparation

The chemical structure of the small molecule ZZW-115 was obtained from the PubChem online database (PubChem CID: 25010688) in SDF format and subsequently converted to pdb format, providing a precise representation of its atomic coordinates. This served as an input for our further analyses.

Preparation of the complex

NUPR1, retrieved from AlphaFold was then docked to the small molecule (ZZW-115) using HDOCK[23], an online docking server. The schematic representation of preparation of complex from is shown in (Fig. 1). The structure was further parameterized and prepared for simulations using the

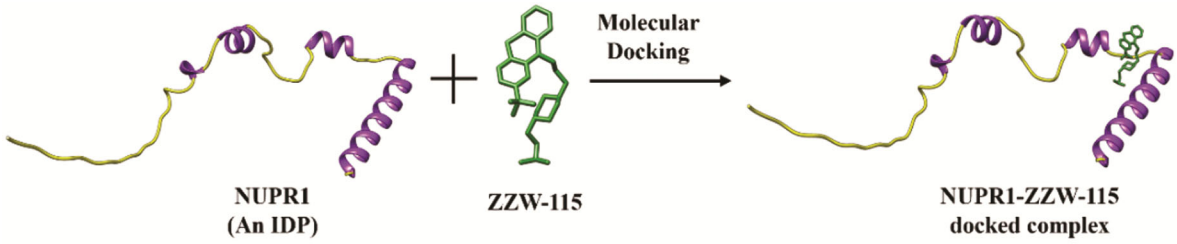

Fig. 1 — Schematic representation showing the formation of docked complex from NUPR1 and ZZW-115

Antechamber protocol in xLEaP. This involves assigning accurate partial charges using the bcc charge model, generating a force field modification file (frcmod) to tailor the molecular mechanics parameters and setting up the complex system in both explicit and implicit solvation environments. Next, we generated the topology and coordinate files for each system as well as for the complex. We then performed Molecular Dynamics (MD) simulations on the complex system using an explicit solvation environment, allowing for a detailed exploration of the molecular interactions and dynamics.

Identification of interacting residues and Binding pockets

We have identified the interacting residues of NUPR1 that directly engage with ZZW-115 using the LigPlot tool[24] and the binding pockets using CASTp web server[25], to confirm that the molecule docks precisely into any of the predicted binding pocket of NUPR1. These interacting residues, spanning hydrophobic, hydrophilic, electrostatic, and van der Waals interactions play a crucial role in understanding the binding mechanism and affinity between NUPR1 and ZZW-115.

Molecular dynamics (MD) simulations

NUPR1-ZZW-115 complex system was prepared for the simulation using the AMBER force field[26] in the Leap module of the AMBER software package. Recent studies have shown that structural ensembles of IDPs strongly depend on their force field[27]. In this study, we have carried out MD simulations using the ff99SBildn force field as it accurately represents molecular interactions and energy environment. The proteins were solvated in a cubic periodic box using the explicit TIP3P (transferable intermolecular potential with 3 points) water model[28]. Counter ions were added to neutralize the systems, and strong van der Waals were removed by minimizing energy. The MD simulations utilized a methodology starting with heating dynamics and progressing to density, equilibration, and production dynamics, with energy-minimized systems being used for the remaining phases. Protein systems were heated from 0 to 300 K under constant volume (NVT) conditions before the density method and adjusted for 1 ns at constant pressure (NPT) settings. After equilibrating the complex structure, a 10 ns production run was conducted. Following this the centre of mass (CoM) distance between NUPR1 and ZZW-115 in the complex structure (with cut off zone residues 53-70)was measured and the value was found to be 8Å. Next, the obtained structure served as the initial structure for PMF study.

Potential of mean force (PMF) study

The weighted histogram analysis method (WHAM) coupled with Umbrella Sampling (US) simulations was used to determine the PMF of the NUPR1-ZZW-115 complex[29,30]. To elucidate the atomistic details of the binding and unbinding mechanism of the NUPR1-ZZW-115 complex, we performed US simulations of the NUPR1-ZZW-115 binding process and employed the phase space analysis. This simulation provided a detailed understanding of the binding event that governs the association of NUPR1 and ZZW-115.This involved performing MD simulations across a series of windows along a defined reaction coordinate, with biasing potentials added to the Hamiltonian to constrain the system within specific phase space regions. Simulations were conducted for a specific duration at each window to generate biased probability distributions or histograms. The WHAM was then used to determine optimal free energy constants by combining the simulations. The resulting PMF profile quantifies the free energy profile of the system along the reaction coordinate, providing valuable insights into the binding process and dissociation pathway[31]. To examine the extent of association between NUPR1 and ZZW-115 in the formation of the complex, we determined the PMF by systematically varying the center of mass (CoM) distance between NUPR1 and ZZW-115. To generate the initial configurations for the US MD simulation windows, we employed CoM distance-constrained MD simulations of the NUPR1-ZZW-115 complex which allowed us to sample a range of configurations with varying distances between the CoMs of NUPR1 and ZZW-115, spanning from 3 to 20 Å. Due to the 10 Å water buffer distance from the solute, we anticipated that the complex structure might extend beyond the solvation box at larger separations (greater than 15 Å). To address this, we resolvated the complex using TIP3P water model, ensuring a sufficient solvent buffer surrounding the complex. Additionally, we neutralized the system with counterions for each US simulation window, maintaining a neutral charge state. Before the US simulation, the periodic boundary conditions and equilibration of the complex system are assessed.

For each US simulation window, we performed 5 ns of MD simulation, employing harmonic potentials to constrain the CoM distance between NUPR1 and ZZW-115 to the desired values.

Table 1 — Distance-based initial configurations of the NUPR1-ZZW-115 complex

Complex	Starting distance between NUPR1 and ZZW-115 (Å)	Distance sampled (Å)	Duration (ns) for umbrella sampling; each window size being 5 ns
NUPR1-ZZW-115	8	3 to 20	90

Visualization of the 3-D structure of the complex was performed using UCSF Chimera[32]. To standardize the PMF data, we employed the centering and standard deviation method, focusing on the region of large separation distances between NUPR1 and ZZW-115 in the complex. We conducted RMSD, SASA and DSSP analysis for NUPR1 across all windows, including both increasing and decreasing reaction coordinates. The PMF profile was computed using WHAM, enabling the calculation of the free energy landscape of the complex. The simulation details, including starting distances and total durations for the US windows between the CoMs of NUPR1 and ZZW-115, are summarized in (Table 1).

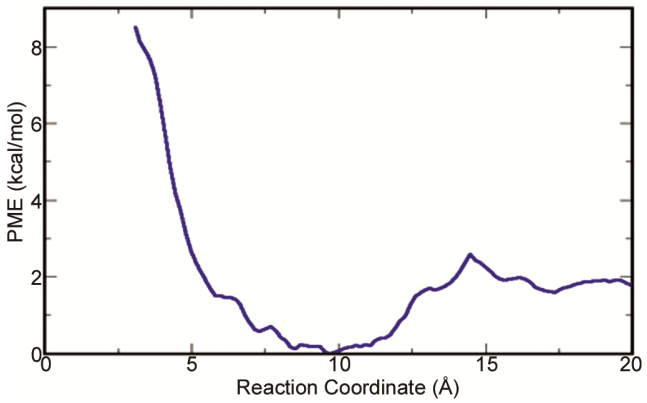

Fig. 2 — Potential of Mean Force (PMF) as a function of reaction coordinate for the association and dissociation of the NUPR1-ZZW-115 complex

MD simulation of the minimum PMF structure

We isolated the structure with the lowest potential energy, corresponding to the minimum PMF, from the ensemble of simulated NUPR1-ZZW-115 complex structures. The NUPR1-ZZW-115 complex structure corresponding to the minimum PMF was used for a 200 ns production run in two different states- NUPR1 in complex with ZZW-115 and in its apo form by removing the small molecule, ZZW-115, to analyze key structural features of NUPR1.To elucidate the structural dynamics of NUPR1 in both unbound and bound states, we analyzed the MD simulation trajectories using the AMBER Tools PTRAJ and CPPTRAJ modules[33]. The root mean square deviation (RMSD) of NUPR1 was calculated with respect to the energy-minimized PMF structure as a reference. Additionally, we evaluated the protein's flexibility through RMSF calculations and assessed the impact of simulation on the stability of the NUPR1-ZZW-115 complex by analyzing the Rg and SASA. These analyses offer significant insights into the conformational changes, flexibility, and stability of NUPR1 in the presence and absence of ZZW-115.

Results and Discussion

PMF profile of the NUPR1-ZZW-115 complex

To examine the degree of association between NUPR1 and ZZW-115 in the formation of the complex, we employed a PMF study that combined MD simulations with the US approach[31,34]. This method allowed us to calculate the free energy profile of the binding process, providing a quantitative measure of the association between NUPR1 and ZZW-115.

The PMF profile of the NUPR1-ZZW-115 complex in water at room temperature, plotted as a function of the reaction coordinate, defined as the distance between the centers of mass (CoM) of NUPR1 and ZZW-115 is shown is (Fig. 2). The PMF profile reveals a minimum PMF at a separation distance of 9 Å, corresponding to a dissociation energy of 2 kcal/mol. We observed that the interaction between NUPR1 and ZZW-115 ceases to exist when the distance of separation between them exceeds 16 Å. When the interchain distance reduces beyond 9 Å, a repulsive force arises between the two molecules, leading to an increase in PMF. We conducted US simulations for 5 ns each window to ensure proper sampling and convergence, verifying PMF convergence after each nanosecond of simulation (Suppl. Fig. 1). The method for examining the convergence of PMF was well-established[35].

Analysis of Conformational Dynamics of NUPR1 as a Function of its CoM Distance from ZZW-115

The snapshots of the NUPR1-ZZW-115 complex have been shown in (Fig. 3) at various separation distances, captured at different windows along the reaction coordinate. These snapshots were generated using UCSF Chimera v.1.17.3[32] and provide a visual representation of the complex's conformational changes as a function of its CoM distance between

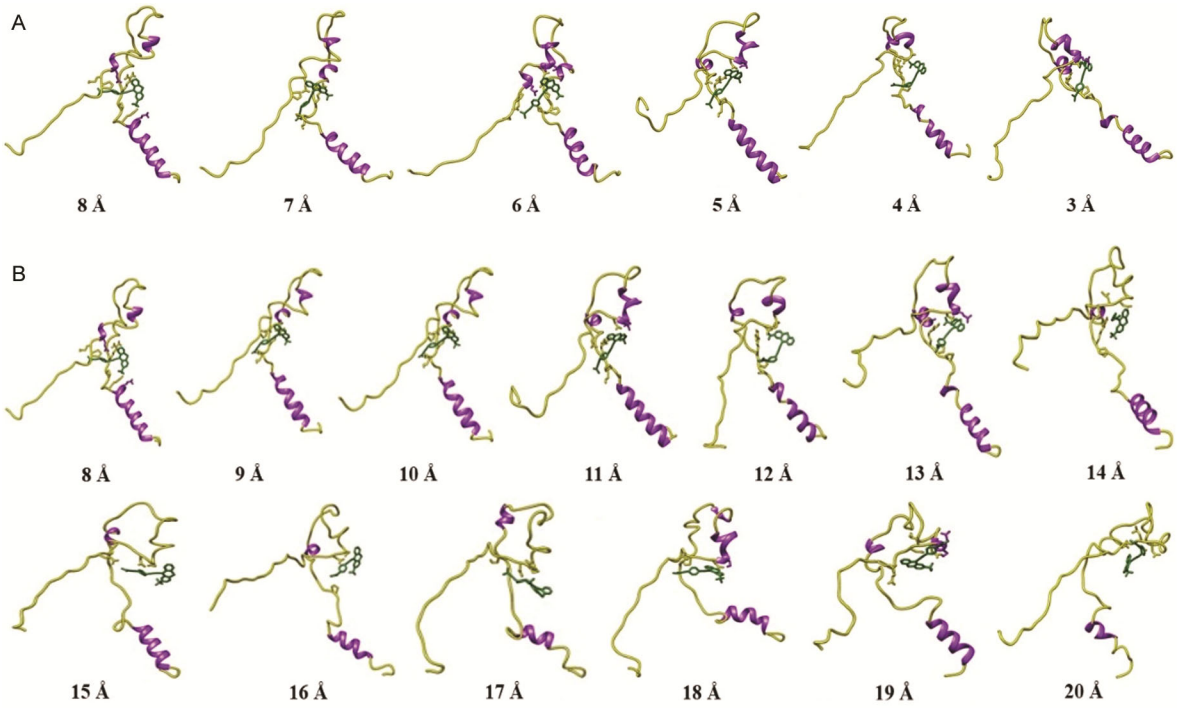

Fig. 3 — Snapshots of NUPR1-ZZW-115 complex structures at discrete distance of separation (in Å) from ZZW-115 (A) when the interchain distance is reduced from 8Å to 3Å (B) when the interchain distance is increased from 8Å to 20Å

NUPR1 and ZZW-115. It has been observed that when the distance between NUPR1 and ZZW-115 decreases, we noticed subtle transitions in secondary structure from one to another. But significant secondary structure transitions have been noticed while the distance between the NUPR1 and ZZW-115 increases.

RMSD Analysis for NUPR1 as a Function of its CoM Distance from ZZW-115

The Root Mean Square Deviation (RMSD) is a widely used metric that evaluates protein stability during simulations by quantifying the average distance between simulated and reference protein atoms. This assesses the structural similarity or deviation of a molecule or biomolecule over time, providing valuable insights into conformational changes[36]. Specifically, RMSD measures the average distance between atoms in a reference structure and their corresponding counterparts in simulated structures, enabling the evaluation of structural deviations and stability. This study uses RMSD analysis to examine the structural variations of NUPR1 with respect to its CoM distance from ZZW-115. The structural stability of NUPR1 in the NUPR1-ZZW-115 complex has been verified by means of the RMSD analysis carried out during the US simulation. The RMSD analysis of NUPR1 in the complex, showcasing the structural deviations that occur as the interchain distance between NUPR1 and ZZW-115 is reduced from an optimal distance of 8 Å to 3 Å has been shown in (Fig. 4A). Conversely, (Fig. 4B) illustrates the RMSD analysis for the NUPR1 molecule in the complex when the interchain distance is increased from the optimal distance of 8Å to 20Å, providing insights into the structural changes.

As shown in Figure 4A, NUPR1 exhibits minimal conformational changes when its distance from ZZW-115 is reduced beyond the optimal distance. An initial increase in RMSD is observed which is followed by a plateau or decrease in RMSD values, suggesting that NUPR1 adopts a more ordered conformation to facilitate binding, thereby stabilizing the local structure. When NUPR1 is moved away from its optimal distance (Fig 4B), it displays an initial increase in RMSD, likely due to the loss of structural constraints and continues to rise as the NUPR1 reverts to its more disordered conformation, characterized by potential loss of structural features and increased flexibility. ZZW-115 binding to NUPR1 involves a localized interaction, affecting a specific binding pocket within the NUPR1.The interaction between NUPR1 and ZZW-115 showed minimal

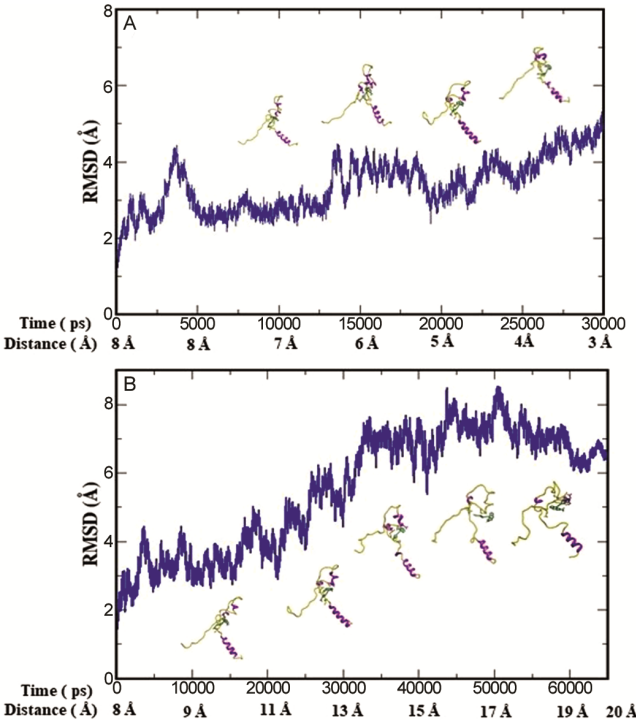

Fig. 4 — RMSD analysis for NUPR1 when the distance of separation between NUPR1 and ZZW-115 (A) decreased from 8 to 3 Å and (B) increased from 8 to 20 Å

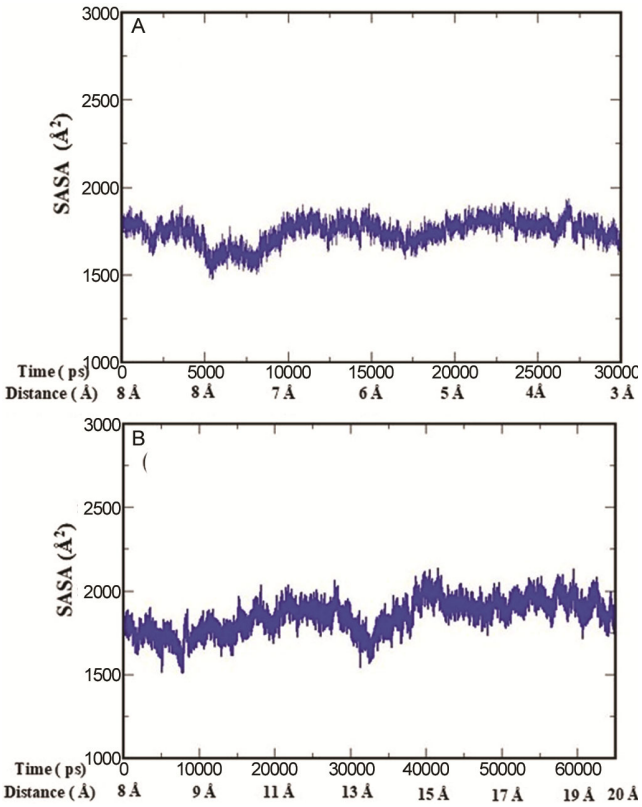

Fig. 5 — SASA analysis for NUPR1 when the distance of separation between NUPR1 and ZZW-115 (A) decreased from 8 to 3 Å and (B) increased from 8 to 20 Å

conformational changes across the entire NUPR1 unlike when an IDP binds to a protein, that involves multiple binding sites along the IDP, leading to an extended binding interface, resulting in partial or complete folding and hence can induce a significant conformational change in the IDP.

This suggests that the structure of NUPR1 is minimally influenced by its interaction with ZZW-115, with dissociation causing a reversion to its disordered state. Hence, small molecule binding, in contrast to protein binding, rarely causes the IDP to fold extensively. The conformational change is restricted to the region adjacent to the binding site.

SASA Analysis forNUPR1 as a Function of its CoM Distance from ZZW-115

The analysis of Solvent Accessible Surface Area (SASA) of NUPR1 reveals variations in surface exposure based on its distance from ZZW-115. This analysis provides insights into the dynamics of NUPR1's solvent-exposed regions, which may be involved in binding, protein-protein interactions, or post-translational modifications. By examining the SASA profiles at varying CoM distances, we can identify potential binding sites, conformational changes, and allosteric effects that modulate the IDP's interactions with ZZW-115.However, as shown in (Fig. 5), the SASA profile does not exhibit significant changes when ZZW-115 is moved away as well as approaches the NUPR1. This suggests that the region is neither buried nor exposed at the periphery/surface of NUPR1, indicating a relatively stable solvent accessibility.

DSSP Analysis of NUPR1 as a Function of its CoM Distance from ZZW-115

The Dictionary of Secondary Structure of Proteins (DSSP)[37] algorithm was employed to analyze changes in NUPR1's secondary structural elements, providing insights into the protein's conformational dynamics. The secondary structural transitions in NUPR1 as the distance between NUPR1 and ZZW-115 is decreased from 8 Å to 3 Å as shown in (Fig. 6A), while the transitions when the distance is increased from 8Å to 20 Å as presented in (Fig. 6B), highlights the dynamic changes in NUPR1's secondary structure in response to varying distances between the NUPR1 and ZZW-115.

The secondary structure of NUPR1 undergoes significant changes with respect to varying distances

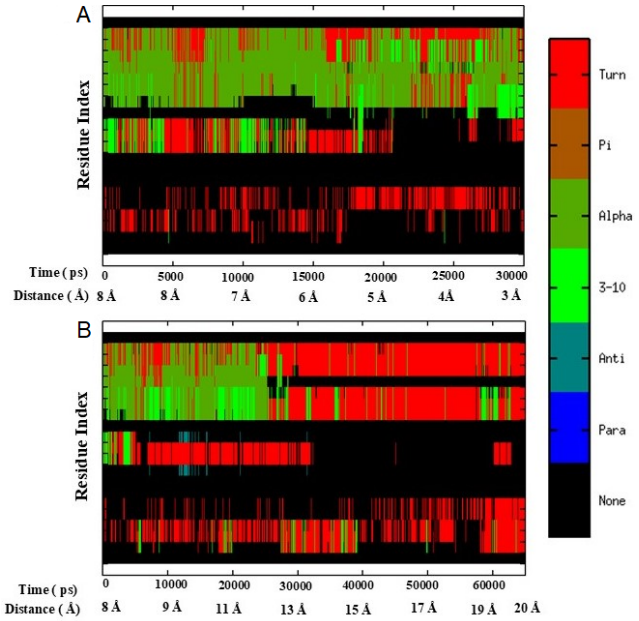

Fig. 6 — Secondary structure analysis for NUPR1 when the distance of separation between NUPR1 and ZZW-115 (A) decreased from 8 to 3 Å and (B) increased from 8 to 20 Å

from ZZW-115. Notably, the 3_{10}-helix content of NUPR1 remains stable as the distance between NUPR1 and ZZW-115 decreases (Fig. 6A). In contrast, increasing the distance leads to a conformational shift from 3_{10}-helix to turns (Fig. 6B), resulting in a significant increase in turn content relative to helix. These findings suggest that NUPR1 exhibits dynamic conformational rearrangements in response to changes in its interaction with ZZW-115.

Salient Structural Features of the Minimum PMF Structure of NUPR1 in bound to ZZW-115 and in unbound state

The most stable conformation of the NUPR1-ZZW-115 complex was identified by determining the minimum PMF structure through PMF analysis. To further investigate the conformational dynamics of NUPR1, we performed 200 ns MD simulations in two different states. The bound state where the NUPR1-ZZW-115 complex was simulated with ZZW-115 bound to NUPR1, allowing us to examine the interactions and conformational changes in the presence of the small molecule ZZW-115. The unbound structure of NUPR1 was obtained by removing ZZW-115 from the minimum PMF complex structure using Chimera and also subjected to a detailed MD simulation for 200 ns. This is done to observe and analyze the intrinsic structural dynamics and conformational flexibility of NUPR1 in its unbound state. To ensure the accuracy of the NPT (constant Number of particles, Pressure, and Temperature) simulation algorithm used in this study, several key parameters were monitored over the course of the simulation. These parameters include density, temperature, potential energy, kinetic energy, and total energy for both the NUPR1-ZZW-115 complex and NUPR1 in its apo (unbound) form. (Suppl. Figs 2 & 3). During the simulation, various structural parameters were monitored to gain insights into the behavior of bound and unbound NUPR1. The CPPTRAJ module of AMBER software package was used to perform all the preliminary analyses which includes, RMSD to track the stability and overall conformational changes, RMSF to identify regions of high flexibility and intrinsic disorder and Rg and SASA analysis.

The conformational dynamics of NUPR1 in its unbound and bound form has been shown at different intervals of the simulation period in (Figs 7 & 8), respectively, highlighting the structural rearrangements that occur in the absence and presence of its binding partner, ZZW-115.

The RMSD analysis revealed a significant difference in the structural stability of NUPR1 between its unbound and bound states. The unbound state of NUPR1 (Fig. 7A) exhibited a high RMSD value, which settled around 25 Å, indicating substantial conformational fluctuations. In contrast, the bound state of NUPR1 (Fig. 8A) showed a reduction in RMSD, suggesting a more stable and rigid structure with fewer fluctuations compared to its unbound state. This observation implies that the binding of ZZW-115 has a little stabilizing effect on the NUPR1 protein, leading to a defined and less dynamic conformation than the unbound state.

The Root Mean Square Fluctuation (RMSF) analysis was employed to investigate the dynamic behavior of individual amino acids in NUPR1 and local flexibility over the course of the simulation. The results reveal that NUPR1 exhibits reduced fluctuations when in complex with ZZW-115 (Fig. 8B), compared to its unbound state (Fig. 7B). This suggests that the binding of ZZW-115 to NUPR1 leads to a decrease in local deformability, indicating a relatively stable structure in the bound state.

The radius of gyration (Rg) analysis shows a notable difference in the overall compactness of NUPR1 between its bound and unbound states with ZZW-115. In the unbound state (Fig. 7C), NUPR1 exhibited a higher Rg value, indicating a more

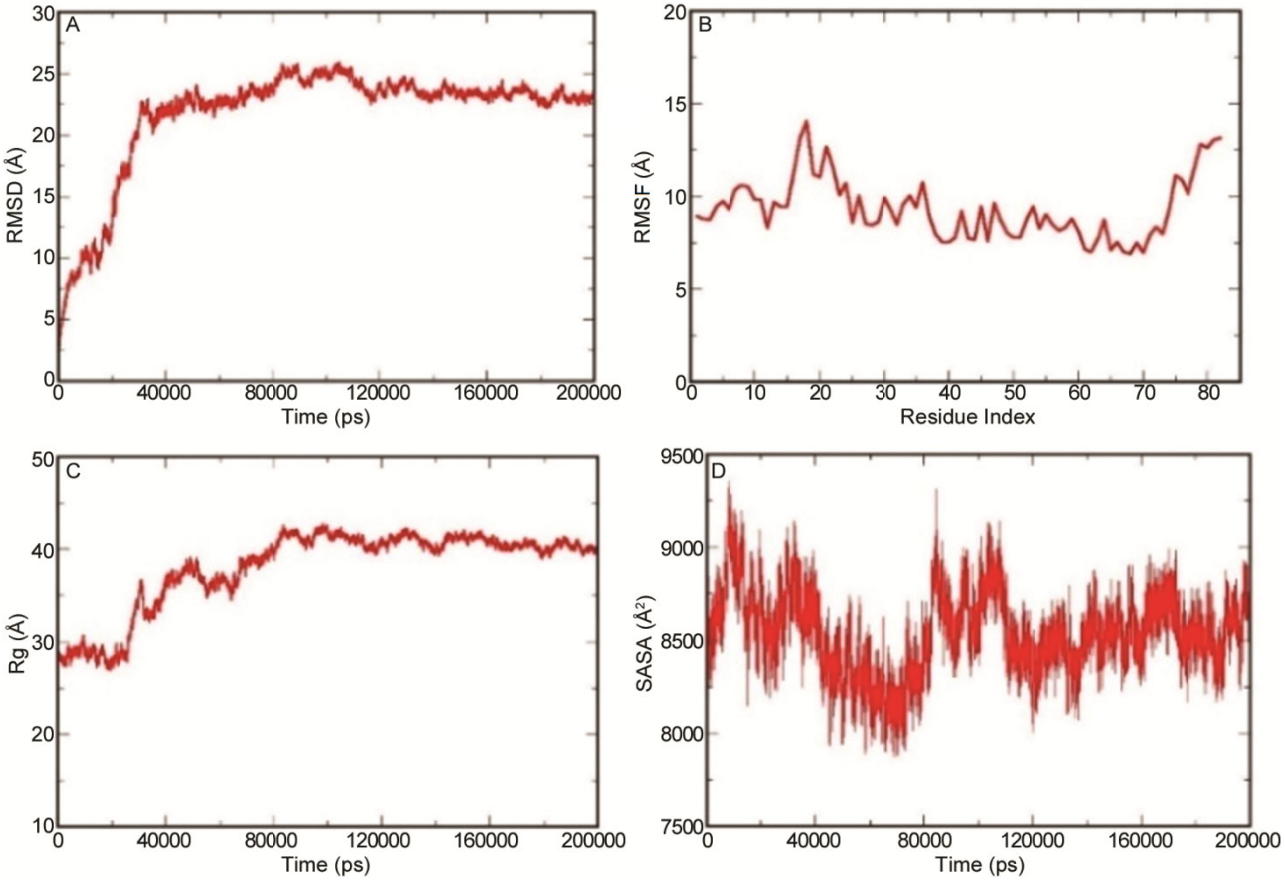

Fig. 7 — Conformational dynamics of NUPR1 in unbound state (A) RMSD (B) RMSF (C) Rg and (D) SASA analyses

extended and less compact structure. In contrast, the Rg value significantly decreased upon binding to ZZW-115 (Fig. 8C), suggesting a more compact conformation. This reduction in Rg value implies that the binding of ZZW-115 to NUPR1 induces a structural reorganization, leading to a more condensed and stable structure.

In the unbound state, NUPR1 exhibited a higher SASA value (Fig. 7D), indicating a larger portion of its surface was exposed to the solvent. However, upon binding to ZZW-115 (Fig. 8D), initially there was an increase in the SASA values but later it decreases, suggesting a reduction in solvent accessibility. This decrease in SASA implies that the binding of ZZW-115 to NUPR1 leads to a shielding of certain regions of the NUPR1's surface or a conformational twist in the NUPR1 protein, as evident in the snapshots.

Conformational changes of NUPR1in unbound and bound state observed during different intervals of simulation period

The snapshots taken at different intervals throughout the simulation period as depicted in (Fig. 9), reveal a progressive loss of standard secondary structure in NUPR1, indicating a transition to a more disordered conformation. Furthermore, in the absence of ZZW-115 (apo state), NUPR1 adopts an unfolded conformation, characterized by an opening of its structure. This unfolded conformation is distinct from the more compact, bound conformation observed in the presence of ZZW-115 (Fig. 10). This observation suggests that the binding partner plays a stabilizing role in maintaining NUPR1's structural integrity, and its absence leads to a reversion to a more intrinsically disordered state.

Identification of interacting residues and Binding pockets

We have identified the key residues of the NUPR1 that are interacting with ZZW-115 in the formation of the NUPR1-ZZW-115 complex using LigPlot and binding pockets using CASTp (Fig. 11). The LigPlot analysis of the NUPR1-ZZW-115 complex reveals interacting residues such as Asn53, Asn55, Arg56, Gly61, Lys65, Thr68 and Asn72. The interactions may reveal valuable insights into the binding mechanism, which could aid in further research on

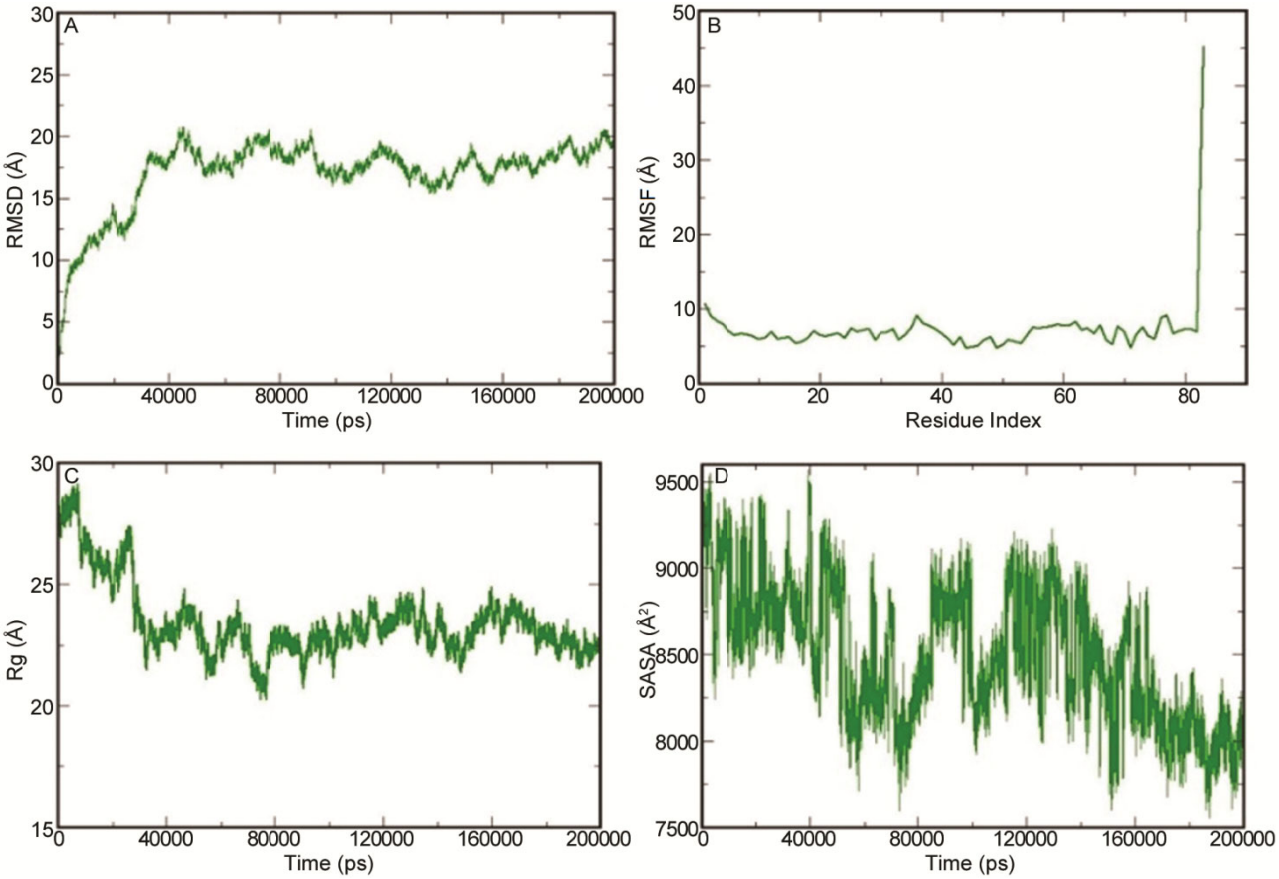

Fig. 8 — Conformational dynamics of NUPR1 in bound with ZZW-115 (A) RMSD (B) RMSF (C) Rg and (D) SASA analyses

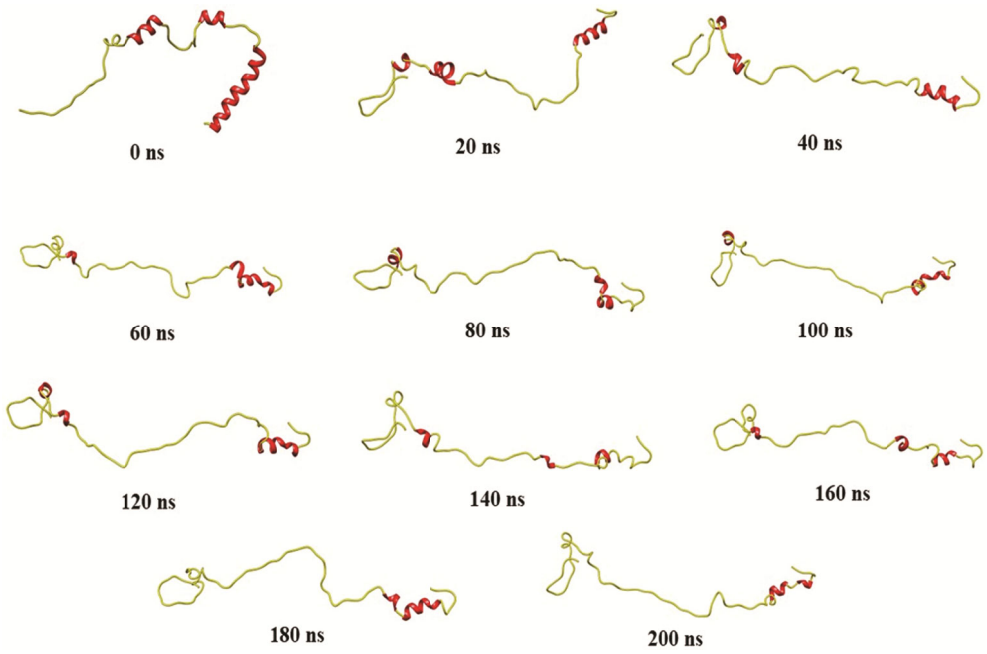

Fig. 9 — Snapshots of the conformers of NUPR1 taken at different intervals of simulation time in unbound (apo) state

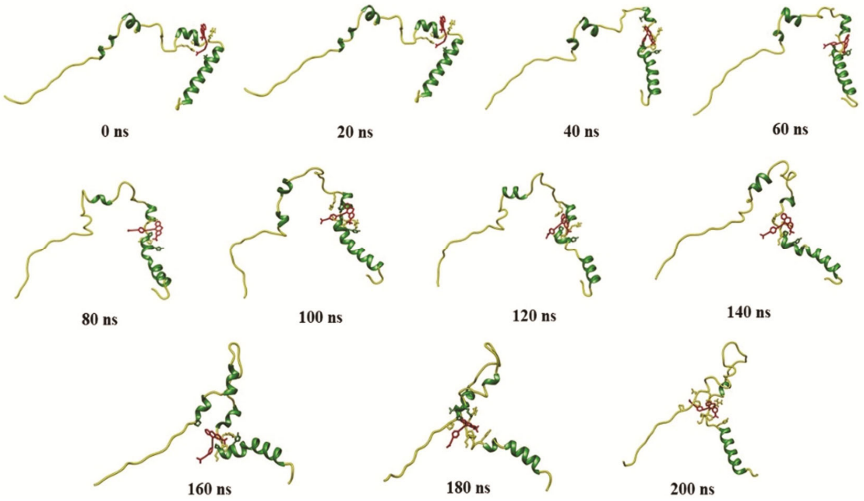

Fig. 10 — Snapshots of the conformers of NUPR1 taken at different intervals of simulation time in bound with ZZW-115

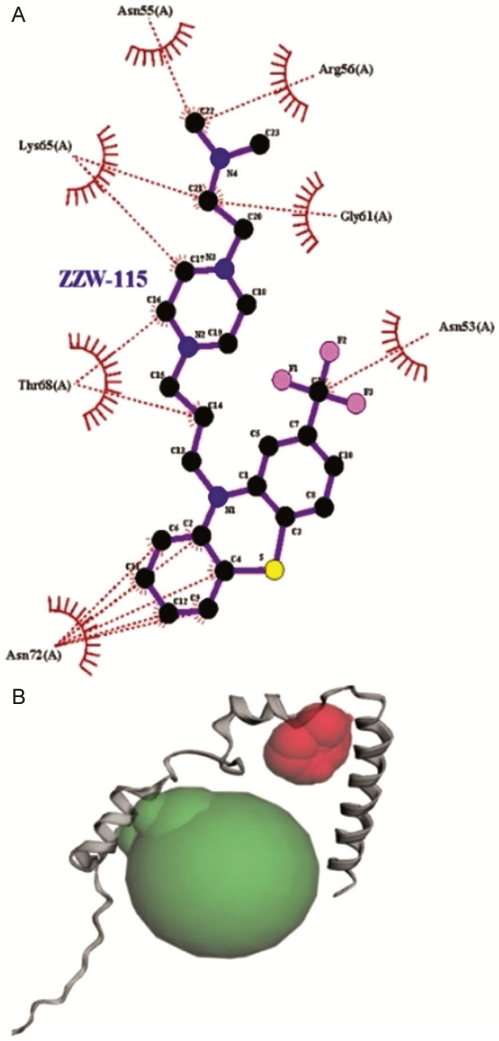

Fig. 11 — (A) Plot showing thekey residues of the NUPR1 that are interacting with ZZW-115 generated using LigPlotand (B) Binding pockets in NUPR1 predicted using CASTp online server (Pocket1: green; Pocket2: Red)

Table 2 — List of residues in the predicted binding pockets of NUPR1

CASTp	
Pocket 1	Pocket 2
24, 28, 31, 32, 35, 36, 34, 37, 82	45, 53, 54, 55, 56, 58, 61, 64, 65, 68

NUPR1 function and inhibitor design. We have further investigated the binding site of NUPR1 by analysing the residues that line its binding pocket using CASTp. The results of this analysis are summarized in (Table 2). Notably, CASTp predicted two potential binding pockets in NUPR1. Subsequent molecular docking simulations revealed that the small molecule (ZZW-115) binds to the second predicted pocket, suggesting a specific interaction between the ZZW-115 and these region of NUPR1.

Our findings align with previous research highlighting the importance of these residues in NUPR1-ZZW-115 interactions offering potential targets for designing more potent NUPR1 inhibitors. Notably, our results validate the notion that Thr68 is essential for binding, as mutations or modifications at this site significantly impair NUPR1's function[38,39].

Conclusion

This study provides an understanding on the conformational dynamics of an IDP (NUPR1) in the presence of a small molecule ZZW-115. Through MD simulations and US calculations, we have elucidated the binding and unbinding mechanisms of the NUPR1-ZZW-115 complex, revealing a minimum PMF value at a separation distance of 9 Å. Our results demonstrate that the distance between NUPR1 and ZZW-115 significantly influences the complex's

stability and secondary structural composition, underscoring the importance of considering conformational flexibility in IDP-small molecule interactions. This study reveals that the binding of ZZW-115 to NUPR1 is a localized event, specifically targeting a distinct binding pocket within the NUPR1, with minimal conformational changes across the entire IDP. This differs with the binding of IDPs to proteins, which often involves multiple binding sites and induces significant conformational changes. The localized nature of the NUPR1-ZZW-115 interaction highlights the unique characteristics and dynamics of small molecule binding to IDPs. The study identified key interacting residues serving as hot-spot regions for ZZW-115 binding to NUPR1's disordered region thereby enhancing the design of targeted therapeutic strategies against NUPR1.Hence, the binding of ZZW-115 to NUPR1 selectively targets a specific binding pocket and affects the conformational dynamics to a significant extent in the vicinity of the binding region. This study contributes to our understanding of IDP behavior and provides valuable insights for the design of drugs targeting IDP-small molecule interactions. These findings may contribute to the rational design of NUPR1 inhibitors and pave the way for developing effective therapeutic strategies against cancers involving NUPR1 dysregulation.

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Conflicts of interest

Both the authors declare no conflict of interest.

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