Helix-coil transition and conformational deformity in Aβ42-monomer: a case study using the Zn$^{2+}$ cation

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1. Introduction

The aggregation of amyloid beta peptides and its progression is regarded as the primary cause for Alzheimer’s disease (AD) (Hartmann et al., 1997; Kayed et al., 2003). AD is one of the most common causes of Dementia which imparts a considerable decline in the cognitive processes of an individual (Dominy, 2019; Selkoe & Hardy, 2016). Amyloid-β peptides and their aggregation is deemed as an important problem for the NMR and molecular dynamics (MD) studies (Lemkul & Bevan, 2010). Owing to their hitherto disorderedness they are classified as intrinsically disordered proteins or IDPs (Dunker et al., 2001). The self-aggregation of the amyloid-β is triggered as a result of the amyloid precursor protein (APP) being sequentially cleaved by the β- and γ-secretase. The AD is characterized by the deposition of amyloid fibrils which are densely packed and aggregated peptide oligomers of the 42 (Aβ42) and 40 (Aβ40) residue monomers. It has been found that the 42 residue monomer is more prone toward aggregation and hence more toxic to the brain, and also the dominant species in the fibrils that are deposited in the brain. There is a high prevalence of metal ions (Fe$^{2+}$, Zn$^{2+}$, Cu$^{2+}$) in the fibrils that are found in the dementia affected parts in the brain (Barnham & Bush, 2014; Lovell et al., 1998; Miller et al., 2006). Many studies have reported the interaction of the metal ions with the Aβ monomer and fibrils. These metal ions are the potential hidden actors in the interaction of the APP and the fibril oligomers. Previous studies have found that the H6, H13 and H14 regions are the binding junctions at the N-terminal region to accommodate the Zn$^{2+}$ ion (Alies et al., 2016; Danielsson et al., 2007; Kozin et al., 2001; Xu et al., 2013; Zirah et al., 2006). Structural models of these metal bound peptides are important to understand the fibrillation kinetics and the structural changes involved. Experiments (such as FRET, dynamic light scattering, mass spectrometry, atomic force microscopy) at times prove to be important in predicting the structural changes of the oligomers involved in the fibrillation (Bitan et al., 2003; Innocenti et al., 2010; Nag et al., 2011; Sitkiewicz et al., 2014). But with the advent of highly effective sampling methods along with the increase of available computational resources, classical MD simulations have provided deep insights on the fibril aggregation mechanism (Asadbegi & Shamloo, 2021; Li et al., 2007; Miller et al., 2010; Nasica-Labouze et al., 2015). In

ABSTRACT

The metal ions (like Fe$^{2+}$, Zn$^{2+}$, Cu$^{2+}$) are known to influence the amyloid beta (Aβ) aggregation. In this study, we have examined the conformational and dynamical changes during the coordination of Aβ-monomer with the Zn$^{2+}$ ion using all-atom molecular dynamics (MD) simulations using explicit solvent models. We have probed the unfolding of the full-length Aβ42 monomer both inclusive and exclusive of the Zn$^{2+}$ cation, with 1:1 ratio of the peptide and the Zn$^{2+}$ cation. The inclusion of the Zn$^{2+}$ cation shows differential intra-peptide interactions which has been probed using various analyses. The Helix – Coil transition of the wild type Aβ42 monomer is studied using the steered molecular dynamics simulations by taking the end-to-end Cα distance across the peptide. This gives an idea of the unequal intra – peptide and peptide – water interactions being found across the length of the Aβ monomer. The transition of an α-helix dominated wild-type (WT) Aβ structure to the unfolded coil structure gives significant evidence of the intra-peptide hydrogen bonding shifts in the presence of the Zn$^{2+}$ cation. This accounts for the structural and the dynamical variations that take place in the Aβ monomer in the presence of the Zn$^{2+}$ cation to mimic the conditions/environment at the onset of fibrillation.

Abbreviations: Aβ: Amyloid beta; WT: wild type; RDF: Radial Distribution Function; REMD: Replica Exchange Molecular Dynamics; CHC: Central Hydrophobic Core; FRET: Fluorescence resonance energy transfer; H-bond: Hydrogen bond; PMF: Potential of mean force; MD: molecular dynamics; DSSP: Define Secondary Structure of Proteins; WHAM: Weighted Histogram Analysis Method

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many cases short fragments of K16 to E22 or G33 to A42 is modeled to study the aggregation mechanism of the Aβ monomer (Dasari & Mallik, 2020; Pal & Paul, 2020). In this study, we have taken the entire Aβ monomer with and without Zn$^{2+}$ and estimated the potential of mean force (PMF) for the unfolding of the entire peptide from α-helix to coil. Keten and coworkers have analyzed the mechanical unfolding of a series of alpha-helical and beta-helical proteins through a number of steered molecular dynamics (SMD) simulations using a variety of pull-rates (Ackbarow et al., 2007; DeBenedictis & Keten, 2019).

In our study, we have pulled the monomer(Aβ-42) α-helix to the unfolded state to look at the mechanical robustness of the helix monomer. This also provides information of the energetics involved in the folding of the Aβ monomer with the addition of cations (Zn$^{2+}$, Cu$^{2+}$, Fe$^{2+}$ etc).

2. Methodology and simulation details

The initial structure for the MD simulations was taken from the PDB-id 1Z0Q. The N- and C-terminals are free amino (NH$_2$) and carboxyl (COO$^-$) groups and hence have a net charge of −3e. The Zinc ion was initially positioned nearby the Zn-binding residues (H6, H13 and H14). The proteins were solvated in a cubic box using the CHARMM-GUI (Brooks et al., 2009; Jo et al., 2008; Lee et al., 2016) interface using the parameters from CHARMM-36m (Huang et al., 2017) force field and TIP3P water model. The LINCS (Hess et al., 1997) algorithm was used to constrain the bonds involving hydrogen atoms. The particle mesh Ewald summation (Darden et al., 1993) was used to describe the long-range interactions with a cutoff of 1.2 nm. For the van der Waals forces, a cutoff of 1.2 nm was used. The temperature and pressure were controlled using the Nose-Hoover thermostat (Evans & Holian, 1985) and Parrinello-Rahman barostat (Parrinello & Rahman, 1981) respectively. Initially, the systems were energy minimized using the method of steepest descent. A timestep of 2.0 fs was used for the simulations. The velocities and coordinates were stored at an interval of 10.0 ps. The peptide was simulated with and without Zn$^{2+}$ ion, starting from four independent configurations. The initial configurations were obtained by simulating the peptide at four different temperatures (400K, 310K, 350K and 380K) and 1 atmosphere pressure for 10 ns. The simulations were then extended for another 1 μs in isothermal-isobaric (constant NPT) ensemble at 310K and 1 atmosphere pressure. These simulations are termed as sim1, sim2, sim3 and sim4. We have also carried out simulations on another configuration of the multiple configurations deposited in the pdb 1Z0Q, at 310K, which is referred to as sim5. The cumulative sampling of the equilibrium simulations add up to 9 μs. The details of the simulations are given in the Table 1.

The initial equilibration was carried out with the restraints applied on the protein molecules. All the systems were simulated using GROMACS MD engine (5.1.4 version) (Hess et al., 2008; Kutzner et al., 2007). The images were rendered using VMD software (Humphrey et al., 1996). To elucidate the changes in the secondary structure content, the DSSP utility in gmx was used.

Table 1. Details of the simulated systems.

| System                  | Number of Atoms | Box length (nm) |
|-------------------------|-----------------|-----------------|
| Aβ monomer              |                 |                 |
| sim1                    | 35,454          | 7.06            |
| sim2                    | 52,659          | 8.06            |
| sim3                    | 52,653          | 8.06            |
| sim4                    | 52,659          | 8.05            |
| sim5                    | 58,395          | 8.35            |
| Aβ monomer + Zn$^{2+}$  |                 |                 |
| sim1                    | 35,471          | 7.08            |
| sim2                    | 52,658          | 8.07            |
| sim3                    | 52,658          | 8.06            |
| sim4                    | 52,658          | 8.07            |

To study the helix to coil transition, we have stretched the Aβ peptide of 42 residues to a coil structure from the α-helix. The box dimensions were chosen in such a way to avoid interactions between the periodic images even when the peptide was fully stretched. The box dimensions were taken to be 31.5 nm × 8.4 nm × 8.4 nm, with the stretching direction being toward the x-axis. In the first steered MD simulation, the α-helix was pulled to the coil structure and the extent of the unfolding was scrutinized using DSSP. In all the pull simulation studies the pull rate and the force constant of the pull spring was 0.2 nm per nanosecond and $10^5$ kJ/mol/nm$^2$ respectively. The mdp files for the steered MD are given in the supplementary information. DSSP algorithm was used to analyze the extent of unfolding in the α-helix. A complete coil conformation in the DSSP plot points to the total unfolded state. The peptide was pulled to a completely unfolded state and the umbrella sampling was done taking successive configurations along the collective variable with the equilibration of 10 ns at each window. The spacing between the umbrella sampling was 0.102 nm for the alpha-helices to coil conversion. In this way, about 100 configurations across the reaction coordinate were generated to account for the entire length of the peptide.

The potential of mean force (PMF) was generated taking the pull profiles from all these configurations using the WHAM module of GROMACS. The equilibration at each window varied between 5 ns per window and 10 ns per window. The analysis was done using perl scripting, gmx modules and in-house scripts. A total of 3.1 μs of steered MD and umbrella sampling simulations have been performed. The histograms for the verification of the convergence of the PMF data are given as supplementary information (Figures S10–S12).

To elucidate the Zn$^{2+}$ binding in the Aβ monomer, we have pulled the Zn$^{2+}$ away from the solvation shell of the Aβ monomer toward the y-direction using a harmonic force constant of $10^5$ kJmol$^{-1}$nm$^{-2}$. WHAM is used to extract the PMF from 30 individual windows being placed across the pulling coordinate. The spacing between the umbrella sampling windows was 0.08 nm. The pulling rate was 0.2 nm/ns with the collective variable being chosen as the distance between the residue E-22 and the Zn$^{2+}$ atom. E-22 was chosen to tether the Aβ monomer from the Zn$^{2+}$ cation as it is close to the center-of-mass of the monomer peptide. The WHAM profiles are extracted through the equilibration of
3. Results and discussion

3.1. Hydrophilicity/hydrophobicity

There have been many theoretical and experimental studies that demonstrate that the Zn$^{2+}$ binds in the N-terminal region with the amino acid residues H6, E11, H13 and H14. These studies conclude that the central hydrophobic core (CHC) plays a major role in the aggregation process leading to the oligomerization. It has been found (Asadbegi & Shamloo, 2021) that the presence of the Zn$^{2+}$ decreases the $\beta$-strand content. Another study has demonstrated the change in hydrophobicity of the whole length peptides upon Zn$^{2+}$ binding (Shi et al., 2014). The increased hydrophobicity in the increased interaction among the hydrophobic residues which facilitates aggregation (Cejas et al., 2008; Lin & Shell, 2010; Mu & Yu, 2014; Savelieff et al., 2013).

In this study, we have investigated the effect of Zn$^{2+}$ incorporation in the peptide in 1:1 ratio. The hydrophobicity is probed using the radial distribution function (RDF) of the water oxygen with the C-$\alpha$ of the protein shown in the Figure 1 which provides the relative probability distribution of the water oxygen around the protein. The results are averaged across all the trajectories with and without Zn$^{2+}$. The peptide zones 1, 2, 3 and 4 with and without the Zn$^{2+}$ show differential plots for their relative interactions with the water oxygen, which gives an idea about the relative hydrophobicity or hydrophilicity. The RDFs show differential interaction of various zones; zone 3 (hydrophilic patch between 22 and 29) shows greater affinity toward the water oxygen and the zone 2 (the hydrophobic core between 17 and 21) shows least preference toward water. The zone 1 (hydrophilic N-terminal) and zone 4 (hydrophobic C-terminal) show comparable affinity toward water in the presence and absence of Zn$^{2+}$. The Zn$^{2+}$ binding thus induces change in the hydrophobicity of various regions in the peptide confirming the previous reports.

3.2. Hydrogen bonding

The hydrogen bond is an attractive interaction between a hydrogen atom of a molecule or a molecular fragment $X$ – H in which $X$ is more electronegative than H, and an atom or a group of atoms in the same or a different molecule, in which there is evidence of bond formation. In a strong H bond, the H atom and the acceptor are separated by a distance less than 0.22 nm, and the angle made by the donor, H atom, and the acceptor is within the range 130 – 180°. The corresponding distance range and angular range are 0.2 – 0.3 nm and 90 – 180°, respectively, in a weak hydrogen bond (Desiraju & Steiner, 2001). Thus we have used the weak hydrogen bonding criterion to compare across the results as no strong H-bond is observed in our cases.

The pattern of H-bonding is analyzed across the two representative trajectories with and without Zn$^{2+}$ ion in Figure 2. The H-bonding profiles of the other trajectories are given in the supplementary information (Figures S3–S5). The average number of the intramolecular H bonds in the A$\beta$ monomer system, and the monomer + Zn$^{2+}$ system are 13.93 and 14.89 respectively being averaged out of all the trajectories. However, the number of peptide-water H-bonds show the opposite trend, with the peptide showing greater number of H-bonds in the absence of Zn$^{2+}$ ion as shown in Table 2.

The average number of peptide – water H-bonds in the solution decreases upon the addition of Zn$^{2+}$ cation, due to the increase in the number of intramolecular H-bonds. The increase in the intra-peptide hydrogen bonding is lesser than the decrease in the number of peptide-water hydrogen bonding due to the availability of lesser number of intra-peptide contacts for the hydrogen bond formation. The higher number of intra-peptide hydrogen bonds gives rise to the more favorable interactions within the peptide residues in the presence of the Zn$^{2+}$ ion.

Hence, in the solvent, the Zn$^{2+}$ cation induces more number of intra-peptide H-bonds facilitating the favorable intra-peptide interactions for maintaining the secondary structure, which comes at the cost of reduction in the number of peptide-water H-bonds. Thus Zn$^{2+}$ cation initiates the favorable H-bonded interactions within the peptide, by increasing the
favorable interactions to attain a particular secondary structure. The H-bonding profiles corresponding to other independent simulations are qualitatively similar, with the Zn\(^{2+}\) cation inducing slightly higher number of intra-peptide hydrogen bonds and reducing the peptide–water hydrogen bonding.

### 3.3. Contact maps

To explore the structural disparity of the Zn\(^{2+}\) bound peptide, we have studied the contact maps for the residue distances. The contact maps elucidate the presence of long-range interactions and the contact probabilities and are shown in Figure 3. The presence of Zn\(^{2+}\) cation induces the long range interactions between the residues, the most important among them being H13 – I32, F4 – N27, I32 – V40, Y10 – I31 and D7 – K16. These interactions span major inter-zonal contacts between the core and the terminal residues. Hence, it is evident that the Zn\(^{2+}\) cation introduces more inter-zonal side chain contacts and induces more long-ranged interactions leading to a rather folded conformation. This is also consistent with the previous reported results (Shi et al., 2014). The contact maps corresponding to the other trajectories are given in the supplementary information (Figures S6 and S7).

### 3.4. DSSP analysis

The presence of the Zn\(^{2+}\) ion can alter the secondary structure content which has been traced by the direct secondary structure prediction algorithm. Table 3 shows the average secondary structure content of the two trajectories in two intervals, one at the beginning and another at the end of the simulation. The average over all the 1 \(\mu\)s simulations is also shown in the table.

The overall average \(\alpha\)-helix content is increased and the \(\beta\)-sheet, \(\beta\)-bridge content is decreased with the addition of the Zn\(^{2+}\). The increase in the overall \(\alpha\)-helix content is balanced by the turn content. During the first 100 ns, with the addition of Zn\(^{2+}\) ion, there is a decrease in the \(\beta\)-sheet and the \(\beta\)-bridge content with an increase in the coil structure. In the final 100 ns, the \(\alpha\)-helix content predominates over the other secondary structures with the inclusion of Zn\(^{2+}\). The \(\beta\)-sheet, \(\beta\)-bridge and 3–10 helix structures are less pronounced. The secondary structure propensity of residues for the two trajectories over the entire 1 \(\mu\)s is shown in Figure 4. The top plot shows the DSSP timeline for the trajectory without the Zn\(^{2+}\) cation. In case of the A\(\beta\) monomer, the beta sheet region is steadily observed at the C-terminal region. There is also an increase in the alpha helix content with time between the residues 10 – 20 in the N-terminal and the CHC region. In the trajectory of the A\(\beta\) monomer with Zn\(^{2+}\), there are some sporadic accounts of beta sheet at the C-terminal zones along with the overall predominance of the \(\alpha\)-helix. The \(\alpha\)-helix is found throughout the microsecond run in the presence of the Zn\(^{2+}\) cation. Previous studies on the metal amyloid interactions at a different ion concentration have found that the fraction of the \(\beta\)-sheet content decreases under the influence of the metal ions (Huy et al., 2016; Khatua et al., 2019). The inclusion of the Zn\(^{2+}\) ion leads to a more pronounced presence of \(\alpha\)-helix structure as found in the DSSP plots corresponding to the other independent trajectories given in the supplementary information (Figures S8 and S9). Along with \(\alpha\)-helix, there are few regions of 3–10 helix and \(\beta\)-sheet which are observed in the snapshots of the final structures from different independent simulations (Figure S2). The improved stability of the \(\alpha\)-helix in the presence of Zn\(^{2+}\) ion has been reported in case of an \(\alpha\)-helical conformation of Clavanin A peptide (Duay et al., 2019).

The representative structures generated from the simulations are shown in Figure 5. In case of the A\(\beta\) monomer + Zn\(^{2+}\), there is a clear predominance of \(\alpha\)-helix, 3–10 helix, \(\pi\)-helix and coil, but without the Zn\(^{2+}\) there is \(\beta\)-sheet, coil and \(\alpha\)-helix. The helix structure of the A\(\beta\) monomer + Zn\(^{2+}\) is maintained both at 500 ns and 1 \(\mu\)s. The final snapshots of the structures from the other independent simulations are given in the supplementary information (Figure S2). Presence of 3–10 helix and \(\beta\)-sheet in the monomer and Zn\(^{2+}\) systems is also observed, which is in accordance with their respective DSSP profiles (Figures S8 and S9).
3.5. Internal rotational dynamics

The influence of Zn$^{2+}$ cation on the structure and dynamics of the protein is significant as discussed earlier. Experimentally, it is found that the Zn$^{2+}$ binding sites are toward the N-terminal side. However, Zn$^{2+}$ ion exerts an appreciable influence over the entire stretch of the peptide.

To examine the dynamical fluctuations, we have analyzed the mean internal correlation time for all the backbone NH dipoles of the peptide residues. The internal motions of the protein backbone can be investigated by the NMR relaxation parameters. The spin relaxation rates have been used to validate the molecular dynamics (MD) simulations. The analysis is executed by separating the internal rotational fluctuations from the overall rotational fluctuations. It has been assumed that the motions related to the overall Brownian fluctuations are independent of the internal conformational fluctuations. The rotational correlation function is written as

$$ C(t) = \frac{C_i(t)}{C_o(t)} $$

where $C_i(t)$ is the internal rotational correlation function and $C_o(t)$ is the overall rotational correlation function. The internal rotation is removed from the overall rotation by using the gmx fit option. The internal rotational correlation functions are then expressed as the dipole correlation functions of the NH bond vectors across the trajectory.

$$ C_i(t) = \langle P_2(\hat{\mu}_i(t + \tau) \cdot \hat{\mu}_i(t)) \rangle $$

where $P_2(x) = \frac{1}{2}(3x^2 - 1)$ is the second order Legendre polynomial. $\tau$ is the time step for the computation of the correlation function and the angular brackets denote the average over the trajectory. The average lifetime is calculated by integrating over the entire time period using the formula,

$$ \langle \tau_e \rangle = \int_0^\infty C_i(t) \, dt $$

The evaluated $\langle \tau_e \rangle$ may be used to link the internal peptide backbone correlation time to the fluctuations in the peptide backbone.

The mean rotational correlation time for the residual backbone NH dipole moment are shown in Figure 6. From the figure, it can be noticed that the difference between the correlation time is more pronounced for the residues G-9 to F-19 and between I-31 and M-35 residues. This observation supports the earlier reports that conclude that the CHC (zone 2) and zone 3 are the aggregation prone segments in the full length Aβ peptide (Balbach et al., 2000). Aβ(29–42) and the region, K-16 to E-22 are the aggregation prone regions which are independently capable of aggregation (Barrow et al., 1992; Hilbich et al., 1991).

The mean lifetime (averaged over all the trajectories) with and without the Zn$^{2+}$ cation are found to be 0.52 ns and 0.47 ns respectively. The Zn$^{2+}$ cation not only imparts local changes in the residue binding site, but also alters the overall mean peptide internal correlation time. One can deduce

![Figure 3](image-url)  
Figure 3. The representative contact maps associated with the two trajectories. (a) Aβ monomer and (b) Aβ monomer + Zn$^{2+}$ ion.

![Table 3](image-url)  
Table 3. Average percentage of secondary structure content by averaging across all the trajectories.

|            | Aβ42          | Aβ42 + Zn$^{2+}$ | Aβ42 + Zn$^{2+}$ | Aβ42 + Zn$^{2+}$ |
|------------|---------------|------------------|------------------|------------------|
|            | Overall       | 0–100 ns         | 900–1000 ns      |                  |
| coil       | 34.78         | 34.12            | 26.25            | 32.63            | 43.78            | 35.84            |
| β-sheet    | 1.67          | 0.50             | 0.64             | 0.37             | 2.15             | 1.25             |
| β-bridge   | 1.80          | 1.15             | 2.42             | 0.18             | 3.24             | 3.04             |
| θ-helix    | 11.53         | 16.70            | 20.05            | 19.80            | 5.91             | 12.29            |
| 3-10-helix | 0.57          | 0.90             | 1.44             | 0.22             | 0.21             | 1.88             |

The evaluated $\langle \tau_e \rangle$ may be used to link the internal peptide backbone correlation time to the fluctuations in the peptide backbone.
that the changes in the H-bond network are responsible for the considerable changes observed in the backbone rotational correlation lifetimes.

![Figure 4](image1.png)

**Figure 4.** The representative timeline of DSSP plots for (a) Aβ monomer and (b) Aβ monomer + Zn^{2+}.

![Figure 5](image2.png)

**Figure 5.** The structures (a) and (b) are the peptide configurations obtained at the 500 ns and structures (c) and (d) represent the configurations at 1 μs.

**3.6. Steered molecular dynamics and end-to-end pull**

The potential of mean force (PMF) is the free energy computed across the specified degree of freedom with the other degrees of freedom being Boltzmann-averaged and is exclusive of that specific reaction coordinate. Thus PMF succinctly reveals the free energy preferences in many biologically relevant processes such as stretching the proteins, RNA, DNA (Cuendet & Michielin, 2008; Marin-Gonzalez et al., 2017; Ozer et al., 2012; Wang et al., 2009; Zacharias, 2006), pulling small molecules or ions through ion channels (Chen & Kuyucak, 2011; Zhang et al., 2013) and crown ethers (Benay & Wipff, 2016). Jarzynski has put forward an equality which connects
the non-equilibrium steered MD with the equilibrium property, PMF for a particular system (Jarzynski, 1997a, 1997b). It connects the equilibrium free energy with the work done through the non-equilibrium (or pseudo-equilibrium) processes.

\[
\langle \exp^{-\beta W} \rangle = \exp^{-\beta \Delta F}
\]

where \(\beta = 1/k_B T\), \(\Delta F\) is the change in free energy, \(W\) is the work done and \(\langle \cdot \cdot \cdot \rangle\) denotes the average over the conformations. The other method is the weighted histogram analysis method (WHAM) (Kumar et al., 1992) to extract the equilibrium data from the non-equilibrium steered MD trajectory. The Jarznyski’s method involve the generation of the PMF profiles using multiple steered MD simulations whereas the WHAM method involves the generation of the PMF using a single steered MD simulation. We have used the latter method here. Two snapshots of the pulling is shown in the Figure 7.

![Figure 7](image)

**Figure 7.** An \(\alpha\)-helix A\(\beta_{42}\)-monomer during the pulling simulation across the terminal residues. (a) Is the initial starting configuration and (b) is the structure during the course of the pulling simulation.

3.6.1. Pull DSSP profile

The terminal C-\(\alpha\) carbon atoms were pulled away from each other to obtain an entirely unfolded peptide structure having a coil conformation. We have taken the A\(\beta\) monomer pdb file and pulled across the end-to-end degree of freedom. Then, by storing the subsequent conformational states across this chosen degree of freedom we have extracted the PMF results.

The pull DSSP profiles in Figures 8 and 9 show a few notable observations. The DSSP profile without the Zn\(^{2+}\) ion is shown in Figure 8. We find that all the alpha helical zones are transformed to the turn conformation and subsequently to the coil conformation with some trace of 3–10 helix and bend coming up sporadically. Secondly, the C-terminal region (zone 4 according to our convention) is less rigid with respect to other parts of the peptide. The total uncoiling of the C-terminal \(\alpha\)-helix happens within the first 20 ns of pull simulation with a few traces of the turn, bend and 3–10 helix conformation. For the other region, the uncoiling took longer time, with the maximum time taken for the uncoiling near the zone 1 (N-terminal) and the zone 2 (CHC) being nearly 38 ns. Thus, the C-terminal undergoes unwinding readily compared to the other regions.

In the A\(\beta\) monomer the unwinding pattern appears to proceed along two regions simultaneously, characterized by the uncoiling of the \(\alpha\) helices across the two different regions (one from D-7 to S-26 and the other from I-31 to V-40) that are separated by a turn portion. For the unwinding of the A\(\beta\) monomer + Zn\(^{2+}\), the DSSP profile in Figure 9 shows some significant changes. With the Zn\(^{2+}\) ion, the unwinding is continuous, characterized by the smooth transformation of \(\alpha\)-helix to the coil structure. There is no distinction of the pull profile of the \(\alpha\)-helix at the two terminal regions. Thus, end-to-end pulling reveals the distinction in the nature of the \(\alpha\)-helices being found in the A\(\beta\) monomer structure, both inclusive and exclusive of the Zn\(^{2+}\) cation.

The PMF is then extracted by taking the conformations across the stretching co-ordinate. We have taken two sets of WHAM data, one by simulating 5 ns per window and another
with 10 ns per window. The PMF for the Aβ monomer with and without Zn$^{2+}$ is shown in Figure 10.

The evolution of the secondary structure across the α-helix pull has been quantified using the DSSP profile shown in Figures 8 and 9. The free energy profiles are shown in the Figure 10. The corresponding free energy is calculated to be 46.64 kcal/mol in case of the α-helix Aβ monomer without Zn$^{2+}$ cation and 37.09 kcal/mol in case of the Aβ monomer + Zn$^{2+}$. Literature studies have shown that the PMF calculations for the helix-coil transition of the alanine deca-peptide gives a value of nearly 22 kcal/mol in vacuum and nearly 7 kcal/mol in explicit solvent (Ozer et al., 2012; Park & Schulten, 2004). In another study using the adaptive steered molecular dynamics (ASMD) the deca-alanine PMF for the helix to coil stretching has been calculated (Bureau et al., 2015). The pull distance in those cases are also defined with respect to the C-α atoms of the terminal atoms. The computational complexity in our case stems from the fact that the end-to-end distance of the Aβ peptide is larger and hence the corresponding box dimensions.

### 3.6.2. Hydrogen bonding profiles

The hydrogen bonding profiles for the helix to coil transition gives interesting information about the unfolding of the amyloid monomer (Bureau et al., 2015). The H-bonding profiles along with the PMF profiles help in detailed understanding of the H-bond rupture taking place during the course of the pull simulations. The i – i + 4 (α-helix) contacts (where i refers to the residue index) are thoroughly disrupted in the course of the pulling simulation. The i – i + 4 contacts are ruptured along with the i – i + 3 (3–10 helix), i – i + 5 (α-helix) contacts, with the tearing of the residue contacts being different in the presence and absence of Zn$^{2+}$. The intra-helical contact distances (i – i + j distance where j = 3 to 5) are traced to account for the H-bond breaking. The monomer + Zn$^{2+}$ system shows a more slow or delayed
breaking of the H-bonds which is evident from both the intra-helical distance and the H-bond count profile shown in Figure 11. The $\alpha$-monomer + $\text{Zn}^{2+}$ system takes longer time to reach the coil conformation due to the increased network of H-bonding.

The $i - i + 5$ contacts ($\alpha$-helix) are slightly more prominent inclusive of the $\text{Zn}^{2+}$ unlike for the $\alpha$-monomer. For the higher contacts $i - j$ ($j > i + 6$) the hydrogen bonding is more prominent in case of the $\alpha$-monomer + $\text{Zn}^{2+}$ but the overall H-bond count for the $i - i + j$ (for $j = 3, 4, 5, 6$) is less with the inclusion of $\text{Zn}^{2+}$. From the Figure 11, it is clear that the change in the H-bond count for $i - i + 3$ and $i - i + 5$ contacts is more in presence of the $\text{Zn}^{2+}$ ion which further proves the presence of more intra-peptide contacts. Hence, along with the DSSP profile, the H-bond profiles also show the differential rupturing preference of the amyloid peptide. Thus, it is evident from the helix to coil transition that the $\text{Zn}^{2+}$ cation influences the dynamics and structural properties.

The profiles of the intra-helical distances also show a clear difference upon the addition of the $\alpha$-monomer + $\text{Zn}^{2+}$ (Figure 11). The $i$ to $i + j$ ($j = 3, 4, 5$) distance which is defined as the intra-helix distance increases along the course of the pulling simulation as seen in the Figure 11. Increase in the intra-helical distance is observed to be faster in case of $\alpha$-monomer compared to the $\alpha$-monomer + $\text{Zn}^{2+}$ ion. The breaking of the H-bonds (intra-peptide) for the $\alpha$-monomer + $\text{Zn}^{2+}$ occurs at around 40 ns during the course of pulling. However, in case of $\alpha$-monomer, the intra-peptide H-bonds are broken earlier which is seen as a steady increase of the intra-helical distance in the Figure 11. Hence, the influence of $\text{Zn}^{2+}$ ion on the intra-peptide H-bonding is evident. The rupture of the intra-peptide H-bonds disrupts the secondary structure, and these H-bonds are replaced by new peptide-water H-bonds formed in the solution. The rupture of the favorable intra-peptide H-bonds leads to the formation of a totally coiled state in going from $\alpha$-helix to coil.

The coiled conformation has no intra-peptide H-bonding to have the stability and it forms more peptide-water H-bonds for its stability in the solution. We define the change in the number of H-bonds as $\Delta N_{\text{peptide-water}}$ which denotes the difference between the final and the initial number of H-bonds (peptide-water). For the helix-coil transition for $\alpha$-monomer the $\Delta N_{\text{peptide-water}}$ is 88 and for the $\alpha$-monomer + $\text{Zn}^{2+}$ it is 74. The $\Delta N_{\text{peptide-water}}$ being positive for both the cases show that the number of peptide-water H-bonds are increasing during the course of the helix-coil transition. The $\alpha$-monomer + $\text{Zn}^{2+}$ requires less number of peptide-water H-bonds in the solution for the stability. This is due to the fact that the $\text{Zn}^{2+}$ cation increases the number of intra-peptide contacts with the presence of more number of $i - i + j$ contacts as observed in Figure 11. The contact map in the case of the unbiased simulations also proves the case. The net lowering of the PMF values with the $\text{Zn}^{2+}$ ion can be attributed to the increased co-operativity of the H-bond breaking during the alpha helix unfolding. The earlier reports show that the alpha helices break the hydrogen bonds in a cooperative manner (Li et al., 2016; Morozov et al., 2006; Wieczorek & Dannenberg, 2003). We assume that with the inclusion of the $\text{Zn}^{2+}$ ion, the co-operativity of the hydrogen bond breaking increases and hence the work done for the unfolding decreases. From the fact that there are more number of intra-peptide hydrogen bonds and more number of intra-peptide contacts in the contact map, in the presence of $\text{Zn}^{2+}$ ion, we can conclude that the $\text{Zn}^{2+}$ ion leads to the formation of more intra-peptide networks in the solvent. This increase in intra-peptide networks leads to the increased possibility of co-operativity in the hydrogen bond breaking, resulting in the decrease in net work done for unfolding in the presence of $\text{Zn}^{2+}$.

If we consider the overall difference of the number of intra-peptide H-bonds, $\Delta N_{\text{intra-peptide}}$ (defined as number of intra-peptide H-bonds in final coil configuration minus the initial $\alpha$ helix), it is $-33$ in case of $\alpha$-monomer and $-27$ for the $\alpha$-monomer + $\text{Zn}^{2+}$. The negative $\Delta N_{\text{intra}}$ shows that the number of the H-bonds are decreasing during the course of the transition. The number of intra-peptide H-bonds is higher in case of the $\alpha$-monomer compared to the $\alpha$-monomer + $\text{Zn}^{2+}$. The difference in the number of peptide-water H-bonds is higher in case of the $\alpha$-monomer (88) than that in the $\alpha$-monomer + $\text{Zn}^{2+}$ (74). Thus there exists an interplay of the intra-peptide and peptide-water H-bonding network in the solution for the stability during the course of the helix-coil transition. This observation has also been found consistent with the rat amylin peptide and also for the deca-alanine pull that is reported elsewhere (Ozer et al., 2012; Reddy, Wang, Lin, et al., 2010; Reddy, Wang, Singh, et al., 2010).

The well depth for the binding of the $\text{Zn}^{2+}$ is calculated to be $-9.11 \pm 0.10 \text{kcal/mol}$ given in Figure 12. The PMF proves the significant binding of the $\text{Zn}^{2+}$ cation in the peptide.

4. Conclusion
All-atom molecular dynamics simulations of $\alpha$-monomer with and without the $\text{Zn}^{2+}$ ion were carried out to study the effect of $\text{Zn}^{2+}$ binding on an intrinsically disordered protein.
Unbiased simulations reported here shows the differential hydrophobicity/hydrophilicity with the presence of the Zn$^{2+}$ cation. The interplay of the discriminating affinity of water from zone 1 to zone 4 are evident from the corresponding RDFs. The structural differences can also be attributed to the Zn$^{2+}$ binding to the Aβ monomer, where an increasing number of the long ranged contacts are established upon binding. The NH backbone dipoles show a longer mean lifetime in the presence of Zn$^{2+}$ and more variation at the N-terminal region. For the steered molecular dynamics simulations, the unfolding of the complex Aβ monomer to the coil state gives us an idea of the relative flexibility and intra-peptide H-bonding in the presence of metal ion. The N-terminus of the Aβ monomer is more rigid compared to the C-terminus. The difference in the mobility among the terminal residues is reduced with the inclusion of the Zn$^{2+}$ cation. The competitive intra-peptide and peptide-water H-bonding stabilizes the peptide in the protic solvent medium. Further studies using varying pull rates and pull force are underway in our group.

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Supporting information
The supporting information consists of the initial snapshots (S1), final snapshots of the protein structures (S2), hydrogen bonding profiles (S3–S5), contact maps (S6–S7) and the DSSP timelines for the multiple simulations (S8–S9), the mdp file of the pull simulations along with the histograms of the PMF calculations (S10–S12).

Disclosure statement
No potential conflict of interest was reported by the authors.

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