

In Silico Studies to Predict the Role of Solvent in Guiding the Conformations of Intrinsically Disordered Peptides and Their Aggregated Protofilaments

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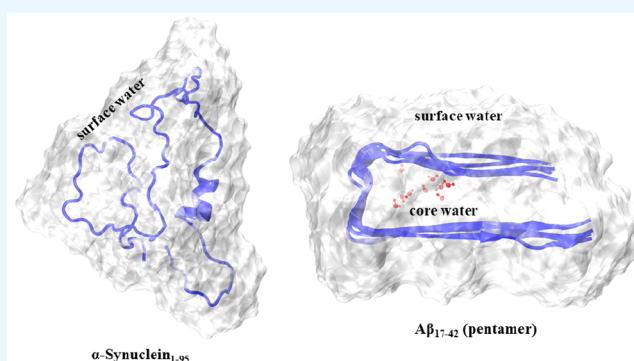
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ABSTRACT: The formation of amyloids due to the self-assembly of intrinsically disordered proteins or peptides is a hallmark for different neurodegenerative diseases. For example, amyloids formed by the amyloid beta ($A\beta$) peptides are responsible for the most devastating neuropathological disease, namely, Alzheimer's disease, while aggregation of α -synuclein peptides causes the etiology of another neuropathological disease, Parkinson's disease. Characterization of the intermediates and the final amyloid formed during the aggregation process is, therefore, crucial for microscopic understanding of the origin behind such diseases, as well as for the development of proper therapeutics to combat those. However, most of the research activities reported in this area have been directed toward examining the early stages of the aggregation process, including probing the conformational characteristics of the responsible protein/peptide in the monomeric state or in small oligomeric forms. This is because the small soluble oligomers have been found to be more deleterious than the final insoluble amyloids. This review discusses some of the recent findings obtained from our simulation studies on $A\beta$ and α -synuclein monomers and small preformed $A\beta$ aggregates. A molecular-level insight of the aggregation process with a special emphasis on the role of water in inducing the aggregation process has been provided.



1. INTRODUCTION

The dependence of the biological activity of a globular protein on its three-dimensional structured state has long been known. However, the discovery of intrinsically disordered proteins or peptides (IDPs) has unraveled that all proteins do not necessarily require unique three-dimensional structures to perform their functions, thereby breaking the traditional view of the protein structure–function paradigm.¹ While IDPs play crucial roles in several important biological processes, such as molecular recognition, signaling, etc.,¹ the induced conformational fluctuations of these IDPs due to their intrinsic disordered nature can often make them prone to misfolding, thereby causing various diseases. For instance, self-assembly of IDPs leading to the formation of amyloids is responsible for several neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), type II diabetes, Huntington's disease, etc.² Therefore, exploring the conformational behavior of the IDPs and the factors causing these diseases has become one of the most important current research areas for experimentalists as well as for theoretical and computer simulation researchers.

Two of the most prevalent neurodegenerative disorders are Alzheimer's disease and Parkinson's disease. AD is the most common form of dementia and is characterized pathologically

by the accumulation of amyloid deposits, senile plaques, and intracellular neurofibrillary tangles involving the $A\beta$ peptides. $A\beta$ peptides are 39–43 residues long and are produced by endoproteolytic cleavage of the amyloid β protein precursor (APP) by β and γ secretases.³ The variation in the residue lengths in $A\beta$ peptides is due to the ability of γ secretases to cleave the APP at multiple sites along the C-terminus. However, the chief component present in the senile plaques or the fibrillar deposits of the AD affected brain is the $A\beta$ protein containing 40 ($A\beta_{40}$) and 42 ($A\beta_{42}$) residues that trigger the nerve cell damage. $A\beta_{42}$ has even higher tendency than $A\beta_{40}$ to form amyloid fibrils.⁴ The prominent pathological hallmark of the other neurological disorder, PD, is the loss of dopaminergic neurons in the substantia nigra and the presence of intracellular inclusions called Lewy bodies and Lewy neuritis.⁵ These Lewy bodies are amyloid-like fibrils primarily comprised of the fibrillated form of an IDP, α -synuclein.⁶ α -

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Synuclein is a brain protein that consists of 140 residues. It is characterized by three distinct segments, namely, the two terminal segments (N-term (M61–K60) and C-term (K96–A140)) and the central hydrophobic segment (E61–V95) known as the “nonamyloid- β component” (NAC). It is reported that hydrophobic residues (G68–A78) present within the NAC region is crucial for α -synuclein aggregation.⁷

Although the symptoms and the responsible protein for the above-mentioned two neurodegenerative diseases are not the same, they share some commonalities. Moreover, it was recently found that patients affected with one particular neurodegenerative disease (say PD) are more prone to develop another disease, such as AD.⁸ This suggests that both diseases involve a common mechanism. The results based on various experimental and theoretical investigations indeed suggest that the formation of amyloid fibrils follows a nucleation-dependent polymerization mechanism.⁹ According to this mechanism, the key step is the formation of an aggregation-prone β -rich structure due to conformational fluctuation of the monomer. This initiates the aggregation process and leads to the formation of water-soluble oligomers that eventually self-aggregate to form insoluble fibrils at higher concentrations. While both soluble oligomers and mature insoluble fibrils are responsible for the etiology of the diseases, small oligomers and prefibrillar aggregates or protofilaments are reported to exhibit toxicity greater than that of the insoluble mature fibrils.¹⁰ The ideal strategy for therapeutic intervention is, therefore, to restrict the growth of the soluble oligomers at an early stage to combat the diseases. Thus, significant research efforts have been made to characterize the conformations of the monomeric state and identifying the intermediates along the fibrillation pathways.

Several factors can modulate the fibrillation pathways of IDPs. Here, we discuss the solvent environments that are reported to have a significant impact on the conformations of these IDPs and hence on their aggregation pathway. For instance, Thirumalai et al.¹¹ in an important work showed that the release of ordered water molecules from the interface of a protein into the bulk resulted in an increase in entropy change and hence is considered to be an important thermodynamic driving force for IDP aggregation. In a recent study, Camino et al.¹² demonstrated that the extent of solvation around α -synuclein plays a pivotal role not only in the nucleation process but also in governing the polymorphism and growth of the aggregates. In a series of studies, we used molecular dynamics simulations to examine various aspects of both $A\beta$ and α -synuclein peptides including the role of solvent for their aggregation. The present review summarizes the results obtained from these studies. This review discussion is organized mainly in two subsections: monomer (section 2.1) and protofilaments (section 2.2). In both of the subsections, the conformational aspects and the properties of interfacial water are described, with an emphasize on outlining the general mechanistic aspect on the role of water in the aggregation of the peptides.

2. DISCUSSION

2.1. Peptide Monomers. An IDP does not possess a unique folded three-dimensional structure but rather adopts diverse conformations due to its inherent flexibility (Figure 1). IDPs are also highly prone to aggregation in aqueous solution, leading to various diseases. Extracting the precise details of the conformational features of such proteins or peptides in

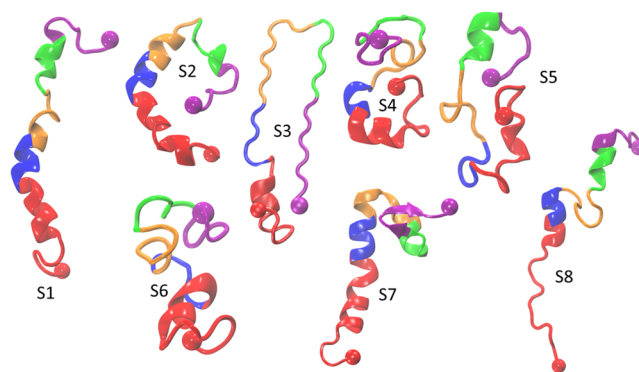


Figure 1. Representative configurations of an intrinsically disordered protein ($A\beta$ peptide). Distinct colors are used to display five segments of $A\beta$ peptide monomer: N-term (red), hp1 (blue), turn (orange), hp2 (green), and C-term (magenta). The peptide terminals are represented as spheres. Reprinted from ref 13. Copyright 2022 American Chemical Society.

aqueous solution is challenging in standard experimental techniques, such as X-ray crystallography, NMR, etc., although monomer conformations of the $A\beta$ peptide and α -synuclein were determined in the presence of cosolvents such as fluorinated solvents by NMR experiments.^{14,15} On the other hand, attempts have been made by researchers to probe the behavior of IDPs in the monomeric states in aqueous solutions using molecular simulations, and an exploration of complete conformational space within reasonable computer time remains a challenge in simulations, too. In our studies, we performed independent molecular dynamics (MD) simulations of $A\beta$ and α -synuclein monomer conformations with varying secondary structural contents using atomistic force fields. The primary objectives of these studies were to scrutinize the conformational aspects of important segments of these peptides and the hydration environment around those. In particular, we considered five segments for $A\beta$ peptides: two crucial hydrophobic patches, denoted as hp1 (Leu-17 to Ala-21) and hp2 (Ala-30 to Met-35), connected by a hydrophilic patch, denoted as turn (Glu-22 to Gly-29), a C-terminal segment, denoted as C-term (Val-36 to Ala-42), and a disordered N-terminal segment (N-term) containing 16 residues (Asp-1 to Lys-16). On the other hand, seven imperfect repeat unit segments with a consensus motif KTKEGV were considered for α -synuclein. These repeat units are denoted as R1 (Ser-9 to Ala-19), R2 (Glu-20 to Ala-30), R3 (Gly-31 to Gly-41), R4 (Ser-42 to Val-52), R5 (Glu-57 to Gly-67), R6 (Gly-68 to Ala-78), and R7 (Gln-79 to Ala-89). Among all of these repeat unit segments of α -synuclein, R6 is known to be the most hydrophobic in nature.

It may be noted that most of the atomistic force fields available in the literature are optimized for globular proteins. Thus, an appropriate refinement of the existing atomistic force fields is crucial to reproduce secondary structural characteristics of an IDP. In fact, it has been reported that the global and local properties of an IDP can change with a slight variation of a force field.¹⁶ The research groups of Mittal and co-workers^{16,17} and MacKerell and co-workers^{18,19} have made significant contributions in refining the existing force fields by introducing different backbone torsional terms to reproduce the properties of IDPs. As CHARMM force fields are known to better reproduce the radii of gyration of an IDP like the $A\beta$

peptide,²⁰ we have used CHARMM force fields in all of our simulation works.

2.1.1. Conformational Features of IDP. Differential hydrophobicity of different segments of an IDP can influence their flexibility and can have an impact on the overall conformational features. To probe such an effect, we computed the configurational entropy of different segments of the IDPs using a method developed by Schlitter.²¹ For clarity, Schlitter's method of calculating the configurational entropy (S) of a macromolecule is described in brief in the [Supporting Information](#). Here, we specifically discuss the ensemble-average configurational entropies of seven repeat units of the α -synuclein peptides calculated for the non-hydrogen atoms. Note that due to different numbers of non-hydrogen atoms in different repeat units, here we present the results as the normalized data obtained by dividing the total value with the corresponding number of atoms used in the calculation. [Figure 2a](#) shows the time evolution of per-atom configurational

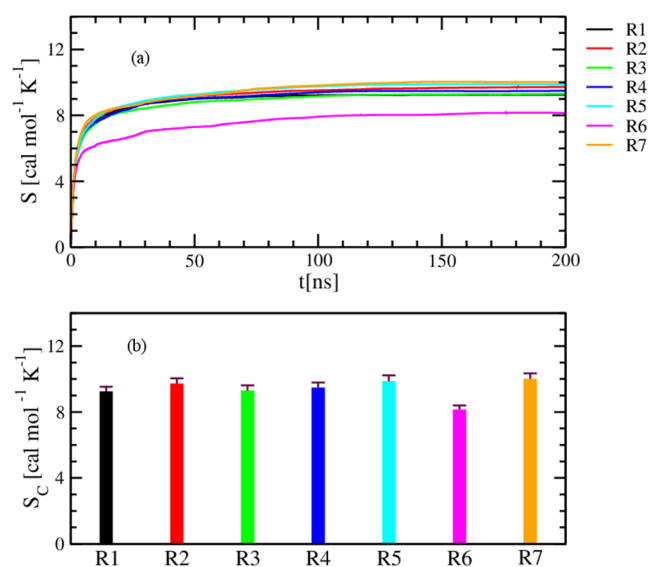


Figure 2. (a) Ensemble-average per-atom configurational entropies (S) of the α -synuclein peptide repeat units as a function of time, t . (b) Bar plot for the corresponding cumulative value (S_C). Reprinted from ref 22. Copyright 2021 American Chemical Society.

entropies for these repeat units with the cumulative per-atom configurational entropy values included as the histogram in [Figure 2b](#) for easy reference. The entropy buildup for each of the peptide repeat units and its convergence to plateau at long times are evident from the figure. Importantly, we notice that all of the repeat units except R6 exhibit near-identical entropy gains. In particular, the distribution shown in [Figure 2b](#) reveals that the cumulative per-atom entropy of R6 is noticeably lower than that of the other repeat units. Such lower configurational entropies of the crucial hydrophobic segments (hp1 and hp2) of the $A\beta$ peptide compared to those of the other segments were also reported previously.²³ This thus suggests that the specific domains of an IDP having primary roles for driving the aggregation process are, in general, rigid compared to those of the other domains. The presence of such rigid domains is expected to initiate the self-assembly process at higher peptide concentrations by assisting the association of the nearby flexible unstructured regions of the peptide.

2.1.2. IDP–Water Interactions. Nonuniform flexibilities of various IDP segments as described in the previous section can influence the peptide–water interactions heterogeneously. We explored in detail such heterogeneous peptide–water interactions around the segments of both peptides by various calculations. Here, we present one such result for $A\beta$ peptides. [Figure 3](#) depicts the variation of ensemble-averaged tagged

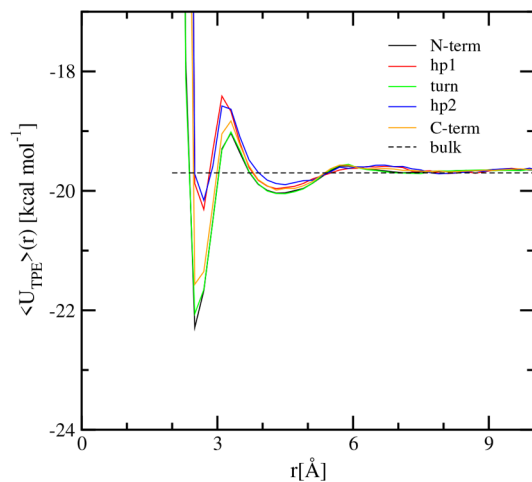


Figure 3. Ensemble-average tagged potential energy (TPE), $\langle U_{TPE} \rangle(r)$, of water molecules vs distance from five segments of the $A\beta$ peptide monomers. Bulk data are also shown for comparison. Adapted with permission from ref 23. Copyright 2016 Royal Society of Chemistry.

potential energy ($\langle U_{TPE} \rangle(r)$) as a function of distance from the five segments of the $A\beta$ monomer with the pure bulk data shown as a reference. The tagged potential energy (TPE) (U_{TPE}), which provides an estimate of the binding strength of water, is the interaction energy of a tagged water molecule with the rest of the system.^{22,23} The definition of U_{TPE} is provided in the [Supporting Information](#). The TPE plot is characterized by three distinct regions irrespective of the segments: (i) a minimum within 2–3 Å (bound water), (ii) a thin layer with a maximum within 3–4 Å (quasi-free water), and (iii) bulk-like water beyond 5 Å (free water). Here, we find a clear difference among the segments, especially in the first two regions. While TPE values for bound water around hp1 and hp2 are approximately -20 kcal mol⁻¹, the corresponding water around other segments are found to be relatively strongly bound (within -22 to -23 kcal mol⁻¹). Moreover, we see a difference in the maximum within 3–4 Å, suggesting a nonuniform barrier height for the water exchange rates between the “bound” and “free” water molecules around the $A\beta$ segments. Such a barrier height is found to be lower for the hydrophobic segments, hp1 and hp2. The calculation also revealed weakly bound water with a relatively lower barrier height for the R6 repeat unit of α -synuclein that is known to play an important role in α -synuclein aggregation.²² Given the fact that hp1, hp2, and R6 repeat units play unique roles in the aggregation, based on the above discussion, we conclude that weakly bound water molecules around these segments ease the water expulsion process required for the aggregation at a higher peptide concentration. This is a key finding that explains a plausible reason why these segments play special roles in peptide aggregation.

2.1.3. Dynamic Heterogeneity at the Interface of IDP. In this section, we discuss how nonuniform flexibility and hydrophobicity of peptide segments resulting in heterogeneous peptide–water interactions, as presented in the previous section, influence the dynamics of solvent (water) around these segments. The results presented here were obtained only for the water molecules that are present in close vicinity (within 5 Å) of the peptide segments, termed as “surface water”. Note that we present the results obtained for the α -synuclein peptide; however, we emphasize highlighting the commonality between $A\beta$ and α -synuclein peptides as deduced from our works. The dynamics of the surface water molecules were probed by measuring different time correlation functions (TCFs). For example, we measured the mean square displacement (MSD), $\langle \Delta r^2 \rangle$, and the dipole–dipole time correlation function (TCF), $C_\mu(t)$, of the surface water molecules. For clarity, the definitions of these functions are provided in the [Supporting Information](#). The data shown in [Figure 4a,b](#) suggest that nonuniform flexibilities of the peptide

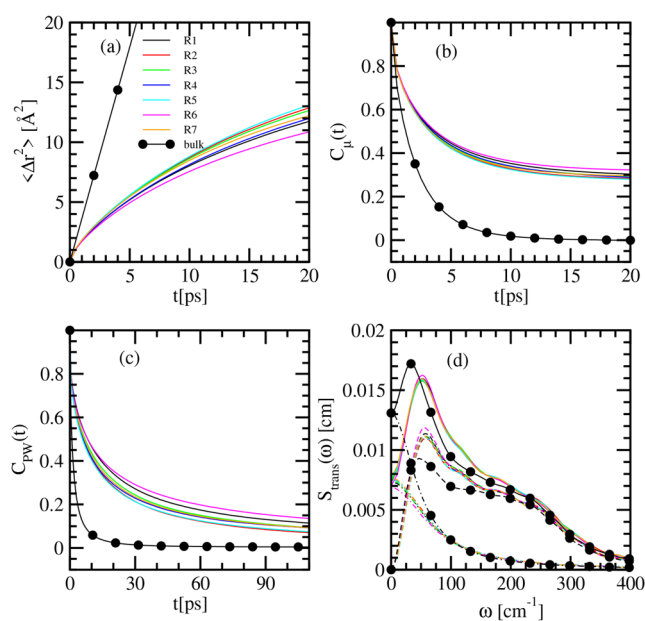


Figure 4. (a) Mean square displacement ($\langle \Delta r^2 \rangle$). (b) Reorientational time correlation function, $C_\mu(t)$, (c) Intermittent hydrogen bond time correlation functions, $C_{PW}(t)$, for the PW hydrogen bonds. (d) DOS corresponding to the translational ($S_{trans}(\omega)$) motions for water molecules present at the interface (within 5 Å) of different repeat units (R1–R7) of the α -synuclein peptide. The results are presented as an ensemble-average data. Reprinted from ref 24. Copyright 2022 American Chemical Society.

repeat units result in heterogeneous translational and rotational movement of the water molecules. The results further revealed that water molecules around the R6 unit exhibit relatively more sluggish dynamics compared to those of water around other repeat units. This correlates well with relative degree of rigidity of the repeat units. Note that we noticed similar correlation and relatively more retarded surface water around the hydrophobic hp1 and hp2 segments of the $A\beta$ peptide.¹³ This commonality of slower dynamics around the segments responsible for aggregation for the respective peptides is an important observation. This leads us to propose that distinctly slower dynamics of water around these crucial segments could

be a marker to track the early stages of the onset of the aggregation process of an IDP at higher peptide concentration.

Water molecules present at the interface of $A\beta$ or α -synuclein peptide can form hydrogen bonds, either with the peptide amino acid residues or with other water molecules. Such a network of peptide–water (PW) and water–water (WW) hydrogen bonds can be nonuniform around different peptide segments. Such nonuniformity is expected to depend on relative strengths of PW and WW hydrogen bonds and the heterogeneous dynamics at the peptide interface. The network of PW and WW hydrogen bonds is expected to be correlated with the dynamics of surface water. To probe such an influence of a hydrogen bonded network on surface water dynamics, we analyzed the hydrogen bond dynamics of PW hydrogen bonds by calculating the intermittent TCF, $C_{PW}(t)$. The definition of the function is provided in the [Supporting Information](#). Significantly longer relaxation time scales for the PW hydrogen bonds around the seven repeat units of α -synuclein compared to that for WW hydrogen bonds in pure bulk water as evident from [Figure 4c](#) are signatures of sluggish surface water dynamics, as discussed above. Besides, we find a direct one-to-one correlation between the overall relaxation of PW hydrogen bonds with the translational and rotational surface water motions, suggesting that the PW hydrogen bond strength directly controls the water dynamics. Here, we emphasize the results obtained for the R6 repeat unit that exhibits the maximum degree of slowness in the hydrogen bond dynamics. We also noticed a maximum degree of restricted PW hydrogen bond dynamics around hp1 and hp2 for $A\beta$ peptides.¹³ Note that sluggish water dynamics around these crucial segments (hp1 and hp2 for $A\beta$ peptide, and R6 repeat unit for α -synuclein peptide) considered in this study are anticipated to have a dominant role in the aggregation process at higher peptide concentration. The results for PW and WW hydrogen bonds thus illustrate that slower water dynamics around these segments originate from slower relaxation time scales of PW hydrogen bonds formed around those. Besides, rigid surface water layers around the peptide segments were found to originate from relatively more solid-like caging motions. This results in relatively lesser surface water entropy as reflected by noticeable blue shift in the O···O bending mode corresponding to the low-frequency vibrational spectra (see [Figure 4d](#)).

2.2. Aggregated Protofilaments. In the discussion so far, we have presented a general perspective about the behavior of surface water around some specific regions of two peptides, namely, $A\beta$ and α -synuclein peptides, that are known to play dominant role in their aggregation. In this section, we discuss the conformational features and the role of water in stabilizing the preformed aggregates and driving their further growth. For this purpose, here we emphasize highlighting the mechanism for the growth of such preformed aggregates in terms of conformational fluctuations and role of water as obtained from our size-dependent simulation studies of a series of $A\beta$ protofilaments of five different sizes, pentamer (O_5), octamer (O_8), decamer (O_{10}), dodecamer (O_{12}), and tetradecamer (O_{14}), that are formed by $A\beta_{17-42}$ monomers. These protofilament structures were designed based on the experimental pentameric model as reported in the protein data bank with identifier 2BEG.²⁵ The details are provided in our previously published work.²⁶

2.2.1. Amyloid Growth Mechanism. A careful analysis first suggested that the end-to-end distance of each of the monomers present in a protofilament and the degree of twisting measured at the C-terminal interface between the second and penultimate monomer can essentially capture the size-dependent conformational aspect of the $A\beta$ protofilaments. We noticed a one-to-one correlation between the free energy values for the selected configurations lying on the extremum of the free energy landscape based on the above-mentioned two properties and the degree of monomer binding estimated from binding free energy calculations. The data further revealed that a protofilament after attaining a particular critical size can transform from a relatively less stable configuration to a more stable one due to low barrier heights (Figure 5). The low-order protofilaments do not exhibit similar

protofilaments and the behavior of water molecules confined in and around those.

2.2.2. Role of Confined Water. A number of studies^{27,28} highlighted the presence of a set of water molecules confined within the amphiphilic cores of the $A\beta$ protofilaments, while none of the structures deposited in the protein data bank contains such water molecules.²⁵ Our simulations also indicated the presence of such types of water, which we termed “core” water (Figure 6a).

The presence of core water molecules was further qualitatively confirmed by the appearance of a depleted water layer beyond the first neighboring shell, i.e., around 4 Å from the peptide, as reflected in the oxygen–oxygen pairwise correlation function, $g(r)$ (Figure 6b). Such a depleted layer of water is prominent for the low-order aggregates O_5 and O_8 , further suggesting O_{10} to be the critical size. Structural characterization of these core water molecules, such as tetrahedral ordering, further added important insight about the size-dependent behavior of the protofilaments. The result illustrated simultaneous presence of a fraction of extremely ordered water molecules surrounded by two neighboring waters and randomly oriented three-coordinated disordered water molecules within the nanocores of the protofilaments (Figure 7). Further, we noticed a decrease in the fraction of ordered water with a consequent increase in the fraction of disordered water with the increase in the size of the protofilaments. This trend seemed to be true except for O_{10} that represented the critical size. We anticipate both types of water molecules confined within the protofilament core to play equally important roles in stabilizing the aggregate (by ordered water) and controlling its further growth (by disordered water) by assisting water expulsion in accommodating a new incoming peptide monomer.

2.2.3. Role of Hydrogen Bonds. The “core” water molecules as mentioned above display extremely sluggish dynamics.³⁰ Such a slowness in water dynamics is a manifestation of confinement. The PW and WW hydrogen bonded network in such a confined region may not be similar to that one expects at the surface of a protein without confinement. We analyzed the PW and WW hydrogen bonds formed by the water molecules confined within the protofilament cores and compared the results with that formed by the external surface water molecules. The results provided interesting features about how PW and WW hydrogen bonded network by the core water gets modified with the confinement. This led us to propose a plausible role of water in $A\beta$ aggregation in terms of hydrogen bonds, as presented in Figure 8.

According to this, the PW and WW hydrogen bonded network within the amphiphilic core begins to be modified once a monomer approaches a preformed $A\beta$ aggregate. However, a fraction of PW hydrogen bonds remains unaffected, which we believe to be primarily responsible for stabilizing the $A\beta$ aggregate. It is the WW hydrogen bonds that disrupt and initiate the growth process by moving away from the core to bulk to accommodate the new incoming peptide monomer. After the new monomer docks to the existing prefibrillar aggregate, WW hydrogen bonds once again break, but this time, the free water molecules form PW hydrogen bonds with the new monomer instead of displacing themselves away from the core. Thus, the proposed mechanism highlights the equally important role of PW and WW core hydrogen bonds in stabilizing the aggregates and controlling their further growth.

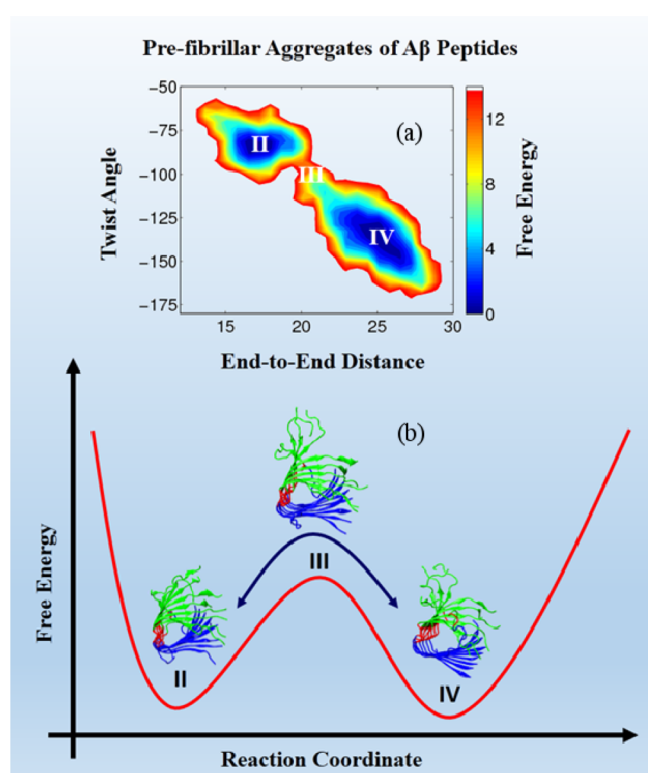


Figure 5. (a) Representative free energy landscape for $A\beta$ protofilaments with a size beyond a critical size based on end-to-end distance and twist angle. (b) Schematic minimum free energy pathway for these higher-order protofilaments with representative configurations corresponding to the extremum points. Adapted from ref 26. Copyright 2017 American Chemical Society.

conversion since they remain trapped within a minimum surrounded by high energy barriers. The idea is that such a critical size was also found to concur with another simulation study.²⁷ Based on our findings, we proposed a probable growth mechanism of the protofilaments. We proposed that the protofilament growth process passes through an intermediate with a relatively less stable twisted structure, facilitating the accommodation of a new incoming peptide monomer, and such a process occurs after a critical size (O_{10}) of the protofilament is attained (see Figure 5).²⁶ In the next section, we highlight the correlation of this critical protofilament size obtained based on the conformational features of the $A\beta$

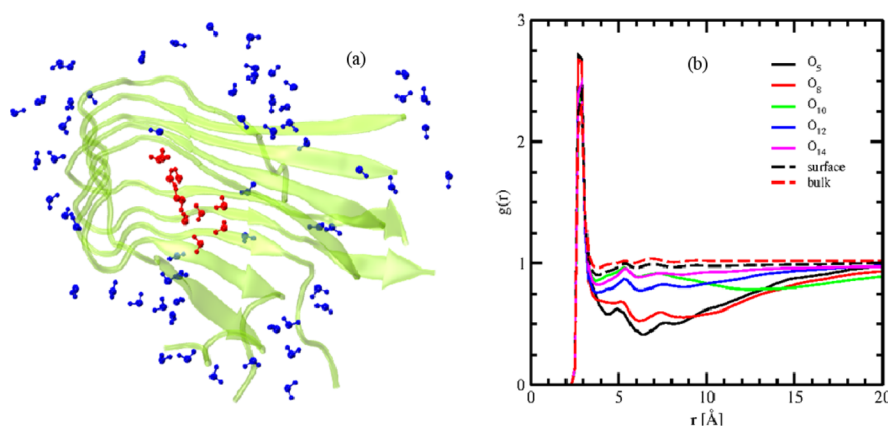


Figure 6. (a) “Core” (red color) and “surface” (blue color) water molecules in and around $A\beta$ protofilaments (green color). (b) Radial distribution functions, $g(r)$, for the “core” (solid lines) and “surface” (dashed lines) water in and around $A\beta$ pentamer (O_5), octamer (O_8), decamer (O_{10}), dodecamer (O_{12}), and tetradecamer (O_{14}). Reprinted with permission from ref 29. Copyright 2018 American Institute of Physics.

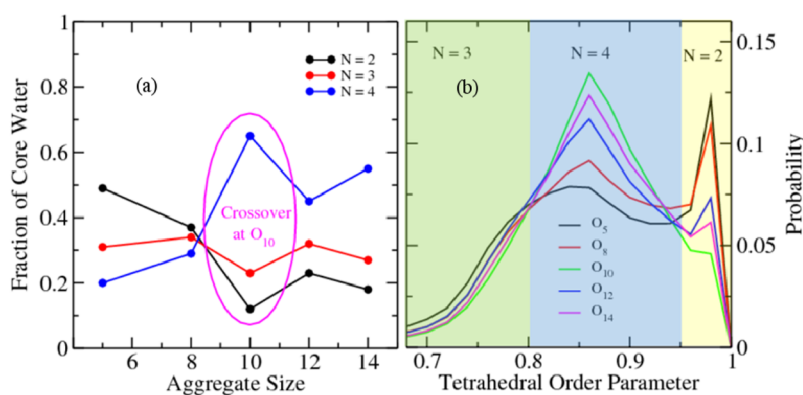


Figure 7. (a) Fraction of core water as a function of the size of the protofilaments. O_{10} shows a crossover that is marked in a circle. (b) Distribution of generalized tetrahedral order parameter for the “core” water present in the $A\beta$ protofilaments with different sizes. Number of neighboring water within the hydration shell (within 3.5 Å) or the coordination number (N) of these core water can vary from 2 to 4. Shaded regions represent the “core” water with a predominant coordination number. Adapted with permission from ref 29. Copyright 2018 American Institute of Physics.

3. CONCLUSIONS

Intrinsically disordered proteins and/or peptides have received significant attention over the years due to their connection with the pathogenesis of various diseases. For example, formation of amyloids due to self-assembly of IDPs is linked with various neurodegenerative diseases. In this review, we presented and discussed some of our recent findings on two IDPs, namely, $A\beta$ and α -synuclein peptides, which are responsible for Alzheimer’s and Parkinson’s diseases, respectively. Here, we primarily focused on highlighting the conformational aspects and the role of water crucial for aggregation of such peptides based on the data obtained from simulation studies on $A\beta$ and α -synuclein peptides in their monomeric states, as well as that on aggregated protofilaments of $A\beta$ peptides.

It has been observed that the peptide segments vital for aggregation process (hp1 and hp2 for $A\beta$ peptide and R6 repeat unit for α -synuclein peptide) exhibit relatively rigid conformations compared to that of the other regions or segments of the peptides. We conjecture that such rigid conformations of these specific regions play pivotal roles in the aggregation at higher peptide concentrations, since these segments are expected to initiate the self-assembly process by entropically favoring the association of adjacent unstructured/disordered regions. The analysis further revealed that water

molecules in the vicinity of these crucial segments compared to those around the other segments of both $A\beta$ and α -synuclein peptides are relatively weakly bound with relatively low energy barriers along the exchange pathway between the bound and the bulk-like free water molecules. This was an important finding, which convinced us to propose that relative ease of water expulsion around these segments is the driving force for the aggregation of both the peptides at higher peptide concentration.

The review also highlighted the growth mechanism of $A\beta$ protofilaments based on size-dependent conformational characteristics of $A\beta$ preformed protofilaments and the role of water. We showed that end-to-end separation of $A\beta$ monomers present in the protofilament and the degree of twisting essentially describe the size-dependent conformational aspect of the protofilaments. Based on the binding free energy calculation for the protofilament conformations we were further able to predict the growth mechanism for the $A\beta$ protofilaments. We also identified a set of water molecules confined inside the amphiphilic core region of the protofilaments, termed as “core” water, which display extremely sluggish dynamics. The core water molecules were found to be either extremely ordered or disordered, depending on their coordination number. Based on the correlation of variation of fraction of these two types of core water molecules with

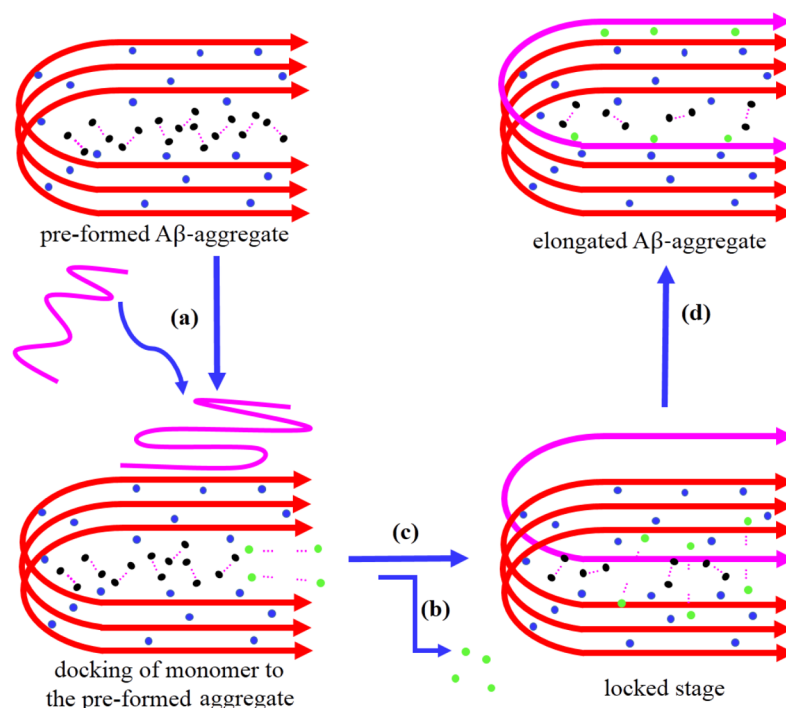


Figure 8. Proposed role for water in $A\beta$ aggregation, shown in a schematic diagram. Blue and black spherical beads represent protein–water (PW) hydrogen bonded and water–water (WW) hydrogen bonded “core water”, respectively, while those shown in green are the ones with no hydrogen bonds. The proposed mechanism has four steps: (a) approach of $A\beta$ monomer (shown in magenta) to a preformed $A\beta$ aggregate (shown in red) and breaking of WW hydrogen bonds (shown in green), (b) diffusion of free water from the core to bulk due to the breaking of WW hydrogen bonds in the preceding step, (c) docking of the approached monomer, and (d) breaking of few more WW hydrogen bonds (shown in green) and formation of PW hydrogen bonds between the free water with the newly added monomer. Reprinted with permission from ref 30. Copyright 2018 Royal Society of Chemistry.

protofilament size, we predicted that both types of water molecules play an equally important role for stabilizing the protofilaments and driving their further growth. We demonstrated that protein–water and water–water hydrogen bonded network within the core gets modified in a way that can be correlated with the degree of confinement of the protofilaments. This led us to predict that such protein–water hydrogen bonds are solely responsible for the stability of the protofilaments, while the breaking of water–water hydrogen bonds and simultaneous formation of protein–water hydrogen bonds by the free water controls the growth process.

Based on the discussion in this review, it is apparent that despite the importance of the problem, only a handful of studies are reported on exploring the role of solvent in forming aggregated protofilaments by IDPs and their stabilities. It would be worthwhile to design suitable experimental studies to verify the mechanism of solvent-mediated aggregation characteristics of IDPs as presented in this review. We envisage that further systematic studies on the molecular level understanding of the problem will open up new horizons in combating human neurodegenerative disorders caused by aggregation of different IDPs.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.2c06235>.

Definition of configurational entropy, tagged potential energy, mean square displacement, dipole–dipole time

correlation function, and intermittent time correlation function (PDF)

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Author Contributions

All the authors have made contributions in preparing the manuscript.

Notes

The authors declare no competing financial interest.

Biographies

Prabir Khatua received his Ph.D. from Indian Institute of Technology (IIT) Kharagpur in 2018 under the supervision of Prof. Sanjoy Bandyopadhyay. His Ph.D. thesis work was on Alzheimer's disease with a primary focus on exploring the role of water in the aggregation of amyloid β peptide responsible for this disease and also predicting the aggregation mechanism by exploring the conformational landscapes of the peptide monomers and aggregates using molecular simulation techniques. He was a postdoctoral fellow with Prof. Ulrich H. E. Hansmann at the University of Oklahoma (2018–2020), where he worked on developing improved techniques for biomolecular simulations and applying those to study folding and aggregation of proteins. He next moved to the University of Utah and worked with Prof. Valeria Molinero for about a year, where he worked on developing coarse-grained force fields for small polymers with antifreeze and ice recrystallization inhibition activity. During this period, he also worked on anion-exchange-membrane fuel cell (AEMFC) with an aim to investigate the effect of degradation on diffusivity and conductivity of the AEMFCs. He next moved to the College of Staten Island, The City University of New York, and worked with Prof. Sharon M. Loverde until September 2022, where his research work was on examining the effect of sequence on structure and dynamics of nucleosome, the basic building block of chromatin. He has recently joined Department of Chemistry, GITAM School of Science, GITAM (Deemed to be University), Bengaluru Campus, where he is working as an Assistant Professor.

Souvik Mondal obtained his M.Sc. degree in Chemistry from IIT Guwahati in 2015. Currently, he is pursuing his Ph.D. at IIT Kharagpur under the supervision of Prof. Sanjoy Bandyopadhyay. His area of research involves microscopic investigations of intrinsically disordered peptides in their monomeric and aggregated states using state-of-the-art MD simulation tools.

Madhulika Gupta received her Ph.D. in computational studies of free energy landscapes of peptides, hydration behavior, and metal surface deactivation in 2018 from IIT Delhi, India. She worked jointly under the supervisions of (Late) Prof. Charusita Chakravarty (IIT Delhi), Prof. Sanjoy Bandyopadhyay (IIT Kharagpur) and Prof. M. Ali Haider (IIT Delhi). She worked with Prof. Jeremy C. Smith, Governor's chair professor at Oak Ridge National Lab (ORNL), USA until 2020 as a postdoctoral fellow where her research was focused on understanding biomolecular recognition, designing antagonists to overcome selective protein-receptor interactions, and understanding interactions in plant cell walls. In 2021, she was appointed Assistant Professor in the Department of Chemistry and Chemical Biology at IIT Dhanbad, Jharkhand, India. Her current research efforts are focused on understanding interactions in plant cell walls, harvesting biomass for bioenergy, understanding protein-receptor interactions in diseases, and designing small ligands to inhibit these interactions.

Sanjoy Bandyopadhyay received his Ph.D. from Indian Institute of Science, Bangalore in 1997 under the supervision of Prof. Subramanian Yashonath. During his graduate studies, he worked on the diffusion properties of small molecules within the confined regions of microporous solids using Monte Carlo (MC) and MD simulations. After postdoctoral studies at the University of Pennsylvania (USA) under the supervision of Prof. Michael L. Klein, he joined the Department of Chemistry, IIT Kharagpur to begin his independent academic career. At present, he is a Full Professor at IIT Kharagpur. His current research interests focus on exploring the microscopic structure and dynamics of biomolecules and the interfacial solvation properties using state-of-the-art computational methods.

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REFERENCES

- (1) Uversky, V. N. Intrinsically Disordered Proteins and Their "Mysterious" (Meta)Physics. *Front. Phys.* **2019**, DOI: 10.3389/fphy.2019.00010.
- (2) Uversky, V. N. Intrinsically Disordered Proteins and Their (Disordered) Proteomes in Neurodegenerative Disorders. *Front. Aging Neurosci.* **2015**, *7*, 18.
- (3) Haass, C.; Selkoe, D. J. Soluble Protein Oligomers in Neurodegeneration: Lessons from the Alzheimer's Amyloid β -Peptide. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 101–112.
- (4) Selkoe, D. J. Translating Cell Biology into Therapeutic Advances in Alzheimer's Disease. *Nature* **1999**, *399*, A23–A31.
- (5) Spillantini, M. G.; Crowther, R. A.; Jakes, R.; Hasegawa, M.; Goedert, M. α -Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 6469–6473.
- (6) Bartels, T.; Ahlstrom, L. S.; Leftin, A.; Kamp, F.; Haass, C.; Brown, M. F.; Beyer, K. The N-terminus of the Intrinsically Disordered Protein α -synuclein Triggers Membrane Binding and Helix Folding. *Biophys. J.* **2010**, *99*, 2116–2124.
- (7) Bodles, A. M.; El-Agnaf, O. M.; Greer, B.; Guthrie, D. J.; Irvine, G. B. Inhibition of Fibril Formation and Toxicity of a Fragment of α -synuclein by an N-methylated Peptide Analogue. *Neurosci. Lett.* **2004**, *359*, 89–93.
- (8) Mandal, P. K.; Pettegrew, J. W.; Masliah, E.; Hamilton, R. L.; Mandal, R. Interaction between $A\beta$ Peptide and α Synuclein: Molecular Mechanisms in Overlapping Pathology of Alzheimer's and Parkinson's in Dementia with Lewy body Disease. *Neurochem. Res.* **2006**, *31*, 1153–1162.
- (9) Straub, J. E.; Thirumalai, D. Toward a Molecular Theory of Early and Late Events in Monomer to Amyloid Fibril Formation. *Annu. Rev. Phys. Chem.* **2011**, *62*, 437–463.
- (10) Bucciantini, M.; Giannoni, E.; Chiti, F.; Baroni, F.; Formigli, L.; Zurdo, J.; Taddei, N.; Ramponi, G.; Dobson, C. M.; Stefani, M. Inherent Toxicity of Aggregates Implies a Common Mechanism for Protein Misfolding Diseases. *Nature* **2002**, *416*, 507–511.
- (11) Thirumalai, D.; Reddy, G.; Straub, J. E. Role of Water in Protein Aggregation and Amyloid Polymorphism. *Acc. Chem. Res.* **2012**, *45*, 83–92.
- (12) Camino, J. D.; Gracia, P.; Chen, S. W.; Sot, J.; de la Arada, I.; Sebastián, V.; Arrondo, J. L.; Goñi, F. M.; Dobson, C. M.; Cremades, N. The Extent of Protein Hydration Dictates the Preference for Heterogeneous or Homogeneous Nucleation Generating either Parallel or Antiparallel β -sheet α -synuclein Aggregates. *Chem. sci.* **2020**, *11*, 11902–11914.
- (13) Khatua, P.; Gupta, M.; Bandyopadhyay, S. Exploring Heterogeneous Dynamical Environment around an Ensemble of $A\beta_{42}$ Peptide Monomer Conformations. *J. Chem. Inf. Model* **2022**, *62*, 3453–3462.
- (14) Eliezer, D.; Kutluay, E.; Bussell, R.; Browne, G. Conformational Properties of α -Synuclein in its Free and Lipid-associated States. *J. Mol. Biol.* **2001**, *307*, 1061–1073.
- (15) Tomaselli, S.; Esposito, V.; Vangone, P.; van Nuland, N. A.; Bonvin, A. M.; Guerrini, R.; Tancredi, T.; Temussi, P. A.; Picone, D. The α -to- β Conformational Transition of Alzheimer's $A\beta(1-42)$ Peptide in Aqueous Media is Reversible: A Step by Step Conformational Analysis Suggests the Location of β Conformation Seeding. *ChemBioChem.* **2006**, *7*, 257–267.

- (16) Zerze, G. H.; Zheng, W.; Best, R. B.; Mittal, J. Evolution of All-Atom Protein Force Fields to Improve Local and Global Properties. *J. Phys. Chem. Lett.* **2019**, *10*, 2227–2234.
- (17) Tang, W. S.; Fawzi, N. L.; Mittal, J. Refining All-Atom Protein Force Fields for Polar-Rich, Prion-Like, Low-Complexity Intrinsically Disordered Proteins. *J. Phys. Chem. B* **2020**, *124*, 9505–9512.
- (18) Best, R. B.; Zhu, X.; Shim, J.; Lopes, P. E.; Mittal, J.; Feig, M.; MacKerell, A. D., Jr Optimization of the Additive CHARMM All-Atom Protein Force Field Targeting Improved Sampling of the Backbone ϕ , ψ and Side-Chain χ_1 and χ_2 Dihedral Angles. *J. Chem. Theory Comput.* **2012**, *8*, 3257–3273.
- (19) Best, R. B.; Mittal, J.; Feig, M.; MacKerell, A. D., Jr Inclusion of Many-Body Effects in the Additive CHARMM Protein CMAP Potential Results in Enhanced Cooperativity of α -helix and β -Hairpin Formation. *Biophys. J.* **2012**, *103*, 1045–1051.
- (20) Rosenman, D. J.; Wang, C.; García, A. E. Characterization of A β Monomers through the Convergence of Ensemble Properties among Simulations with Multiple Force Fields. *J. Phys. Chem. B* **2016**, *120*, 259–277.
- (21) Schlitter, J. Estimation of Absolute and Relative Entropies of Macromolecules Using the Covariance Matrix. *Chem. Phys. Lett.* **1993**, *215*, 617–621.
- (22) Mondal, S.; Mondal, S.; Bandyopadhyay, S. Importance of Solvent in Guiding the Conformational Properties of an Intrinsically Disordered Peptide. *Langmuir* **2021**, *37*, 14429–14442.
- (23) Khatua, P.; Jose, J. C.; Sengupta, N.; Bandyopadhyay, S. Conformational Features of the A β_{42} Peptide Monomer and Its Interaction with the Surrounding Solvent. *Phys. Chem. Chem. Phys.* **2016**, *18*, 30144–30159.
- (24) Mondal, S.; Ghanta, K. P.; Bandyopadhyay, S. Dynamic Heterogeneity at the Interface of an Intrinsically Disordered Peptide. *J. Chem. Inf. Model.* **2022**, *62*, 1942–1955.
- (25) Lührs, T.; Ritter, C.; Adrian, M.; Riek-Loher, D.; Bohrmann, B.; Döbeli, H.; Schubert, D.; Riek, R. 3D Structure of Alzheimer's Amyloid- β (1–42) Fibrils. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 17342–17347.
- (26) Khatua, P.; Sinha, S. K. J.; Bandyopadhyay, S. Conformational Features of the A β_{42} Peptide Monomer and Its Interaction with the Surrounding Solvent. *J. Chem. Inf. Model.* **2017**, *57*, 2378–2392.
- (27) Kahler, A.; Sticht, H.; Horn, A. H. C. Conformational Stability of Fibrillar Amyloid-Beta Oligomers via Protofilament Pair Formation—A Systematic Computational Study. *PLoS One* **2013**, *8*, No. e70521.
- (28) GhattyVenkataKrishna, P. K.; Mostofian, B. Dynamics of water in the amphiphilic pore of amyloid β fibrils. *Chem. Phys. Lett.* **2013**, *582*, 1–5.
- (29) Khatua, P.; Bandyopadhyay, S. Understanding the Microscopic Origin behind Heterogeneous Properties of Water Confined in and around A β_{17-42} Protofilaments. *J. Chem. Phys.* **2018**, *149*, 065101.
- (30) Khatua, P.; Bandyopadhyay, S. Dynamical crossover of water confined within the amphiphilic nanocores of aggregated amyloid β peptides. *Phys. Chem. Chem. Phys.* **2018**, *20*, 14835–14845.