Conformational Biases of $\alpha$-synuclein and Formation of Transient Knots

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Abstract

We study local conformational biases in the dynamics of $\alpha$-synuclein by using all-atom simulations with explicit and implicit solvents. The biases are related to the frequency of the specific contact formation. In both approaches, the protein is intrinsically disordered and its strongest bias is to make bend, and then turn, local structures. The explicit-solvent conformations can be substantially more extended which allows for formation of transient trefoil knots, both deep and shallow, that may last for up to 5 $\mu$s. The two-chain self-association events, both short- and long-lived, are dominated by formation of contacts in the central part of the sequence. This part tends to form helices when bound to a micelle.

Introduction

$\alpha$-Synuclein is a 140-residue protein that is found in the mammalian brain as both a soluble and membrane-associated molecule. It is highly expressed in the mitochondria and is active

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in the presynaptic region, where it is involved in the regulation of synaptic vesicle release, vesicle trafficking, and fatty-acid binding.\textsuperscript{2} It is also a chaperon protein that affects the degradation and assembly of the presynaptic SNARE complex.\textsuperscript{1} α-Synuclein has a propensity to form self-propagating fibrils that are present in the brain of most of the Parkinson-disease patients.\textsuperscript{3} It is unclear whether toxicity arises as a consequence of the fibers themselves or of the presence of oligomeric intermediates, which appear en route to fiber formation.\textsuperscript{2}

The structural properties of the monomeric α-synuclein are then of great interest and also a matter of uncertainty. When bound to a micelle, the protein is partially structured (the structure code PDB:1XQ8), as evidenced by NMR studies:\textsuperscript{4} the segments 2-37 (denoted here as $h_1$) and 45-92 ($h_2$) are α-helical. They form a hairpin linked by a loop between the helices. The N-terminal region is charged as it is lysine-rich. This feature enables interactions with a membrane\textsuperscript{4} and makes the region disordered. However, in the absence of binding to the micelle, both the tryptophan fluorescence\textsuperscript{5} and NMR studies\textsuperscript{6} indicate the existence of intrinsic disorder throughout the monomeric sequence.

α-Synuclein has also been studied by molecular dynamics simulations. Several of them,\textsuperscript{7–9} done within all-atom explicit solvent schemes, lead to the conclusion that α-synuclein is indeed disordered since the simulations reveal the existence of rapid temporal changes in the local conformational biases. The biases can be determined through the DSSP scheme.\textsuperscript{10} In particular, the occurrence of the $\beta$ structures in single chains is low\textsuperscript{9} which does not favor dimeric aggregation.

Here, we study the free protein by all-atom simulations of two kinds: with the explicit and implicit solvents. We determine the time-averaged probabilities for a residue $i$ to belong to a particular secondary structure and demonstrate that both approaches indicate the presence of the all-sequence disorder. These probabilities are found to be the largest for the
bend and turn local conformations whereas the helical ones rank the third.

The very sequential pattern of the probabilities is found to depend on the nature of the solvent. More importantly, we demonstrate that the interactions with the molecules of water generally introduce a qualitative difference to the physics of α-synuclein: the explicit solvent conformations tend to be substantially more extended. Such conformations allow for much more frequent global opening and closing events, resulting in higher probability to form knotted backbones. In the implicit-solvent case, we observe no knots. In contrast, in one trajectory calculated with the explicit solvent we observe a deeply knotted trefoil topology that lasts for about 5 μs. In another trajectory, we observe a series of shallow knots that emerge and disappear within a similar time span.

While the presence of knots in many structured proteins is well established by now, this is not so in the disordered case. One difficulty is related to the fact that the disordered conformations are not readily available and have to be generated through computer simulations. Another is that the knotted structures are only transient – in our simulations, they last for of order 5 μs. However, as we discuss at the end of the paper, even then they may impede proteasomal degradation and thus enhance accumulation of the proteins. We expect that other intrinsically disorder proteins may also tangle to the knotted forms, but the corresponding characteristic time scales for their stability should be protein dependent.

The propensity to form transient local structures is related to the temporary establishment of inter-residue interactions that can be described, in the coarse-grained sense, as the establishment of inter-residue contacts. Our definition of a contact is based on the existence of overlaps between effective spheres associated with the heavy atoms belonging to separate residues (see the Methods section). We find that the most likely contacts are those that involve the N-terminus (e.g. by linking to the h2 region) and those that occur within the h1
region. The contacts in the latter region do not lead to any persistent helix.

We then analyze the self-association of two disordered α-synuclein chains and find that the most frequent contacts are those that connect $h_2$ in one chain with $h_2$ in another chain. The next in importance are those that connect $h_1$ with $h_2$. These findings are consistent with the expectation that the capacity for association is related to the central region 65-90. The absence of this region has been shown experimentally to impede oligomerization and fibrilogenesis. Interestingly, we also find that the dimeric inter-chain contacts are distinct from those that are likely to arise during the single-chain dynamics. The involvement of the $h_1$ and $h_2$ regions, that are helical when bound to the micelle, seems to echo the results of studies pertaining to the tetrameric aggregation which appear to lead to the emergence of a ring of helices (especially when the partially structured PDB:1XQR conformation is used to generate the starting states). Such ring states resist any further aggregation and may serve to store excessive α-synucleins in the cell.

**Methods**

Our implicit-solvent all-atom simulations were conducted using the NAMD code version CVS-2013-11-07 for Linux-x86_64-MPI with the CHARMM36n force field that is refined to be applicable to the intrinsically disordered proteins. The snapshots of the conformations were plotted by using the VMD package. The simulations were performed with the use of the Generalized Born Implicit Solvent method. In the single-chain simulations, we used free boundary conditions. The cut-off radius for non-bonded interactions was set to 1.4 nm. We have generated 5 trajectories, each lasting for 30 ns. The first part of the simulations involved energy minimization of the system for 5 000 conjugate gradient steps. In the second part, the system was heated up to $T$ of 298 K. The temperature was controlled by the
standard Langevin algorithm. The time step was 1 fs. The conformations of the protein were captured every 2 ps. The 5 starting conformations were obtained by random selection from 5 different points of the ANTON-derived trajectory.

When studying the association processes of multiple chains, we switch to periodic boundary conditions and use the smooth Particle Mesh Ewald procedure\textsuperscript{22} to treat the long range electrostatics. The procedure is a variant of the Particle Mesh Ewald method\textsuperscript{23} as implemented in NAMD. The grid spacing is 0.16 nm. In the simulation of two chains, we used the cubic simulation box with the edge length of 60.0 nm. We prepared 50 starting systems in the following way. We first chose all possible pairs from the set of the initial structures that were simulated as single chains. This yields 10 different sets of pairs. For each of them, we prepared 5 different conformations by changing the mutual orientations of the chains by using the PyMol software.\textsuperscript{24} Each starting system was simulated for 30 ns which yielded 15 000 frames. Altogether, we have considered 750 000 conformations.

Independent of the number of chains, the conformations can be characterized by their instantaneous contact maps. The contacts may link residues within chains and between chains. We determine their presence by checking if there are atomic overlaps between residues. Specifically, each non-hydrogen heavy atom is represented by a sphere of radius equal to the atom’s van der Waals radius enlarged by a factor of 1.24 to account for attractive interactions (the factor corresponds to the inflection point in the Lennard-Jones potential). The values of the van der Waals radii are taken from ref.\textsuperscript{25} A contact between residues $i$ and $j$ is said to exist if the two residues contain at least two spheres, one belonging to $i$ and the other to $j$, that overlap. In the terminology of ref. (Wolek et al.),\textsuperscript{26} this procedure is denoted as OV. We have successfully used such contact maps in the studies of protein stretching.\textsuperscript{27}
Results & Discussion

We have obtained the implicit-solvent results through five 30 ns NAMD-based simulations, as described in the Methods section. The explicit-solvent trajectories have been derived by Robustelli et al. when developing a set of novel molecular force fields that would be adequate for both structured and disordered proteins. α-Synuclein was one of several proteins considered in ref. in the tests. These novel force fields were designed to be used on the special purpose supercomputer ANTON. For each of the considered force fields, α-synuclein comes out as a fully disordered protein. In particular, the local α-helical content, though non-zero in certain parts of the sequence (e.g. around residue 20), has been found never to exceed 45% for any of the force fields used. Here, we analyze two trajectories, one 30-µs and the other 20-µs long, that were obtained using the C22* force field and the TIP4P-D water molecules.

An earlier extensive explicit solvent study by Sethi et al. involved GROMACS, the OPLS-AA force field, and a division of the protein into weakly interacting modules. Our results on the propensities to form helices and β-bridges are consistent with this study (when the starting conformations are random). However, the novelty of our research in this context, also compared to that of ref., is showing that the dominant structural bias is for the T and S secondary structures and that the structural biases can be explained in terms of contact maps.

The transient secondary structures

Here, we consider the probabilities, $P_\lambda$, of forming secondary structures, $\lambda$, at the sequential residue $i$. In addition to the turn (T) and bend (S), the secondary structures detected are helix (H), β-bridge (B), and helix 3_{10} (G). We find no appreciable probabilities for strands (E) and helices-π (I). When there is no detectable structure at a residue, we refer to it as a
coil state (C).

The time-averaged data shown in the left panels of Figure 1 is based on the explicit-solvent 30 µs simulation performed on the special purpose ANTON supercomputer at D.E.Shaw Research in New York. The $P_H(i)$ function for the C22*/TIP4P-4D force field never exceeds 20%. Neither does $P_B(i)$. $P_G(i)$ does not exceed 10%. It is seen that the dominant tendency is to form the local S and T structures, sometimes in the same segment. The system is clearly disordered because the various structural propensities switch in time and compete at essentially all residue. At about 8% of the residues, $P_C(i)$ is under 50% (the level indicated by the dotted horizontal line) but at all remaining residues the lack of any detectable order is dominant behavior.

The right panels of Figure 1 show similar data for the all-atom $5 \times 30$ ns implicit-solvent simulation. They are qualitatively similar in that the strongest structural propensities are for the S and T local arrangements. However, the overall propensities for making secondary structures are stronger than those shown in Figure 1. In particular, at most residues, the propensity to show no structure (C) is smaller than 50%. In most cases, the starting conformations were chosen randomly from the ANTON-derived trajectory. In this way, the two kinds of simulations are expected to be in similar regions of the phase space. Thus we conclude that the larger structural propensities are due to the lack of the molecules of water. In order to quantify the differences between the explicit and implicit solvent results, we residue average $P_{\lambda}$ along the sequence. For the explicit solvent case, we obtain 0.026 (H), 0.096 (T), 0.179 (S), 0.028 (B), 0.022 (G), 0.000 (I), 0.033 (E), and 0.614 (C) where the letters in brackets indicate the type of the secondary structure. For the implicit solvent case, we obtain 0.062 (H), 0.161 (T), 0.243 (S), 0.025 (B), 0.029 (G), 0.000 (I), 0.025 (E), and 0.455 (C). The biggest shift is seen to be for the C-content. Independent of the model, the largest ordering tendency is for S.
The top-right panel of Figure 1 also shows results for $P_H(i)$ for 5 trajectories that start from the PDB:1XQ8 conformation. They are seen to boost $P_H(i)$ substantially, indicating a strong sensitivity to the initial conditions within the simualtional times considered.

Even though the implicit-solvent simulations yield results that are qualitatively similar to those of the explicit-solvent ones, other than the general shift toward more ordering, there is a sequential displacement in the patterns. When one plots the ratio of $P_{\lambda(i)}$ obtained with the implicit solvent to $P_{\lambda(i)}$ with the explicit solvent then, for any $\lambda$, there is a significant patterning as a function of $i$ (not shown).

Panels A and B of Figure 2 show 20 of the most likely contacts that form during the single-chain evolution. The panels are for the explicit and implicit solvent cases respectively. There is a qualitative similarity in their looks but the differences in the details of the pattern are also evident. There are four contacts appear in both panels: 5-8, 19-22, 102-140, and 1-140. Furthermore, contact 121-139 in panel A is almost identical to contact 121-140 in panel B. However, 15 other contacts are distinct. Despite the differences, all of these top contacts are seen to be related to the attempts to construct the first helix and to connect the N-terminal part with the center region. The increased number of strong contacts in the N-terminal segment extending up to residue 36 explains the larger tendencies to form helices in the implicit-solvent case. The bottom-left panel of Figure 2 show the probability density corresponding to all contacts that have been detected in the single-chain simulations. The contact maps corresponding to the implicit- and explicit-solvent simulations are seen to be distinct.

The geometrical characteristics of conformations
A qualitative way to assess the free-energy landscape is by plotting the visited points on the $R_g-L$ plane, where $R_g$ denotes the radius of gyration and $L$ the end-to-end distance. The regions that are the most frequently visited are expected to correspond to the lowest free energy. The left panels of Figure 3 show such plots for the implicit- and explicit-solvent systems considered (top and bottom respectively). The landscapes are seen to have substantial regions that overlap but the highest frequency regions (marked in red) are shifted significantly between the two descriptions. This is seen especially in the values of $R_g$: the explicit-solvent conformations are much less globular. This feature is also reflected in the distributions of $R_g$ and $L$ shown in the right panels of Figure 3. The distribution of $R_g$ is double peaked for both systems but the maxima of the peaks are shifted upward in the explicit-solvent case. The distribution of $L$ is single-peaked but the peak is also upward-moved.

The top-right panel of Figure 3 allows for making comparisons with experiments. Recent SAXS study of α-synuclein obtained from human blood cells yields the average $R_g$ between 33.1 and 33.3 Å depending on the buffer used (pH=7.4). This is consistent with our explicit-solvent value of 36.2 Å compared to 25.8 Å indicating that the explicit-solvent approach is closer to the reality. It should be noted that the same SAXS study yields results that depend strongly on the buffer (between 27.2 and 42.7 Å) if one uses a recombinant α-synuclein. The authors attribute this fact to the ”harsh” treatment used in the preparatory work of such proteins.

**Dynamics of the knot formation**

We test the presence of knots by using the KMT algorithm. We have not spotted any knots in the implicit-solvent trajectories. However, the much more expansive (on the $R_g-L$ plane) explicit solvent trajectory is found to have a 3 µs long time segment in which a
A shallow trefoil knot keeps forming and disappearing very much like what happens with the shallowly knotted proteins at the air-water interface.\textsuperscript{35} This can be illustrated by showing the sequential locations of the knot ends \( n_1 \) and \( n_2 \). The knot ends are determined by truncating the sequence from both termini until coming to a stage at which the knot dissolves. Figure 4 shows that the locations of \( n_1 \) and \( n_2 \) fluctuate but their distances from the closest termini (N for \( n_1 \) and C for \( n_2 \)) never exceed the span of 10 residues and sometimes can be as short as 2 residues. It should be noted that for most of the discussed segment of time, \( L \) varies little and stays in the upper range of the values shown in the left panels of Figure 3.

Figure 5 shows an example of knot formation by direct threading (the panel corresponding to 3492) or slipknotting (5523-5526 ns) and unknotting by the slipknot mechanism. A slipknot conformation is one in which a bend segment of the backbone pierces through a backbone (knot) loop, It transforms into a knot on straightening the segment.\textsuperscript{14}

We have also considered a second available trajectory that is 20 \( \mu \)s long. Figure 6 shows that in nearly a quarter of the duration of this trajectory there is a stable deeply knotted topology. The knot ends of the corresponding conformations keep sliding to an extent, as shown and explained in Figure 7.

It should be pointed out that the knot formation in both trajectories is kinetically driven and that the knots, though fairly long lasting, are transient.

In an effort to assess the consistency of the knotted conformations from the a99SB-disp and c22*/TIP4P-D explicit solvent trajectories with previous experiments,\textsuperscript{36} we back-calculated several NMR observables from the knotted conformational ensembles and compared them to a suite of previously reported NMR measurements in Table 1. These measurements include chemical shifts, residual dipolar couplings (RDCs) and backbone scalar...
coupling constants which report on local backbone conformations, as well as paramagnetic relaxation enhancements (PREs) which report on transient tertiary contacts. We found the knotted portions of the c22*/TIP4P-D and a99SB-disp trajectories show slightly worse agreement with experimental measurements compared to their parent trajectories, but are in substantially better agreement than simulations run with similar force fields and the TIP3P and TIP3P-CHARMM water models. This comparison suggests that a sub-population of knotted conformations similar to those observed in these simulations would not be grossly inconsistent with previous experimental measurements, though the populations may be smaller than those observed in these trajectories, particularly in the c22*/TIP4P-D trajectory.

Self-association in the implicit solvent approach

We have considered self-association of two α-synuclein chains under fairly dilute conditions as described in the Methods section. A dimer is considered to be formed if there is at least one contact that connects the chains. The C panel of Figure 2 shows that the residue-residue contacts that are the most engaged in coupling two chains together, as assessed throughout the evolution, are not those which drive the formation of transient secondary structures in the individual chains. However, they are consistent with the experimental findings discussed in the introduction. Figure 8 shows an example of association in which 7 of the 10 top most frequent contacts are present. They are indicated by the red lines and they link the center parts of the chains. The bottom-right panel of Figure 2 shows the full contact map – the over-all pattern is seen to be distinct from the one obtained for the single-chain implicit solvent calculation.

The top most frequent connecting contacts are shown in panel C of Figure 2. They are seen to be mostly within the $h_2$ regions of the two chains and then in the parts connecting $h_1$ with $h_2$. There are also important contacts between $h_2$ and residue 111.
Figure 9 shows the distribution of the duration times of the dimers. It is seen that most of the association events are short-lived: their life span does not exceed 0.2 ns. It seems that a power-law decay of the distribution describes the behavior better than an exponential low (see the caption of Figure 9). However, there are also events that last for tens of ns. These correspond to the data points shown in the inset of the figure. The corresponding most frequent contacts are different in each event indicating existence of many pathways to associate.

It is interesting to point out that one of the most frequent association contacts is 4-67. Thus, if one of the chains is in the knotted state then association involving residue 4 is expected to extend the duration of this state by making the knot deeper, similar to the effects of the procedures described in ref. 37.

Conclusions

When analyzing the structural propensities, we have found, not surprisingly, that the solvent increases the disorder substantially, which shows as an enhancement in the C-content. The types of the transient secondary structures that are detected are the same indicating that for most computational purposes the implicit-solvent approach is sufficient.

The nature of the solvent, however, may be important when assessing the topological features. This appears to be the case of α-synuclein. This protein supports formation of knots in the explicit-solvent case but not in the implicit-solvent one, or, at least, there is a reduction in the probability of making a knot. In any event, our results suggest that the intrinsically disordered proteins can generally support transient knots. It has been already demonstrated that sufficiently long poly-glutamine chains, 38 which are also disordered, can
support both deeply- and shallowly-knotted conformations (the implicit-solvent calculation; see also a coarse-grained study\textsuperscript{39}). The presence of the knots in these chains has been suggested\textsuperscript{40} to be responsible for the monomeric-level toxicity leading to the Huntington disease. The toxicity results from the fact that the knots may hinder or even jam the degradation by the proteasomes (see a related discussion in ref.\textsuperscript{37}) and thus enhance the concentration of the chains in the cytosol. In the case of $\alpha$-synuclein, the knots should enhance accumulation of the proteins which, in turn, leads to an enhancement of multimeric aggregation. It remains to be elucidated, however, whether the knotted states of $\alpha$-synuclein may lead to other physiologically relevant aspects.

We have already mentioned that the SAXS experiments\textsuperscript{32} at the physiological pH yield the average $R_g$ of the human blood derived $\alpha$-synuclein to be close to the value obtained by our explicit-solvent calculations. While about 20\% of the durations of the explicit-solvent trajectories indicate the presence of the knots, the close agreement is not yet a proof of their existence. It would be interesting to perform single-molecule stretching experiments, similar to those done for structured proteins,\textsuperscript{42} to determine the fully stretched lengths. We expect the distributions of such data to be two-peaked, with the shorter $L$ peak corresponding to the knotted conformations.

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Table 1: Comparison of calculated RMSD from experimental measurements for simulations of α-synuclein. We compare the agreement of 30 µs explicit solvent trajectories of α-synuclein from ref.28 run with c22*/TIP4P-D, c36M/TIP3P-CHARMM, a99SB-disp, and a99SB*-ILDN/TIP3P and the knotted portions of the c22*/TIP4P-D and a99SB-disp trajectories with previously reported experimental solution NMR measurements. Rg penalties were computed using an experimental value of 31.0 ± 5.0 from ref.41. All classes (Cα, Hα, HN, C', Cβ) of chemical shifts (CS) are reported in ppm; residual dipolar couplings (RDCs) and indirect dipole–dipole couplings (J-couplings) are in Hz; Rg is in Å; paramagnetic relaxation enhancements (PREs) and the scores are unitless. NMR observables and force field (FF) scores were calculated as previously reported,28 using only the subset of trajectories considered here. The Combined FF Score is defined as (CS\text{Score} + NMR\text{Score}) / 2 + Rg\text{Penalty}, Rg\text{Penalty} = (|Rg_{\text{Exp}} - Rg_{\text{Sim}}| - Rg_{\text{Exp error}}) / Rg_{\text{Exp}}, where the CS\text{Score} is determined by normalizing the RMSD for each class of chemical shift by the smallest RMSD observed for the seven force fields and taking an average of the normalized RMSDs over all sets of experimental chemical shifts. The NMR\text{Score} is computed analogously for all additional classes of NMR measurements and Rg\text{Exp error} is an experimentally estimated error of Rg. We note that the knotted portions of the c22*/TIP4P-D and a99SB-disp trajectories show marginally worse agreement with experimental measurements compared to their parent trajectories, but still show large improvements relative to simulations run with similar force fields and the TIP3P and TIP3P-CHARMM water models.

|     | Cα CS | Hα CS | HN CS | C' CS | Cβ CS | RDC (Q) | Rg   | PRE | Backbone 3J_{HNH} | Backbone 3J_{CC} | CS\text{Score} | NMR\text{Score} | Rg\text{Penalty} | Combined FF Score |
|-----|-------|-------|-------|-------|-------|---------|------|-----|-------------------|------------------|---------------|-----------------|----------------|------------------|
|     | 0.43  | 0.15  | 0.90  | 0.43  | 1.06  | 0.47    | 23.34| 0.18 | 0.66              | 0.15             | 1.10          | 1.06            | 0.09            | 1.16             |
|     | 0.51  | 0.18  | 1.09  | 0.44  | 1.04  | 0.59    | 21.69| 0.22 | 0.69              | 0.17             | 1.22          | 1.23            | 0.14            | 1.37             |
|     | 0.61  | 0.20  | 1.63  | 0.57  | 1.27  | 0.64    | 18.39| 0.32 | 1.13              | 0.30             | 1.54          | 1.81            | 0.25            | 1.92             |
|     | 0.51  | 0.14  | 1.46  | 0.31  | 1.04  | 0.41    | 36.76| 0.17 | 1.11              | 0.18             | 1.16          | 1.23            | 0.02            | 1.22             |
|     | 0.59  | 0.20  | 1.89  | 0.44  | 1.22  | 0.52    | 24.76| 0.23 | 1.31              | 0.24             | 1.50          | 1.23            | 0.04            | 1.57             |
|     | 0.88  | 0.31  | 3.73  | 0.69  | 1.60  | 0.93    | 15.53| 0.39 | 1.06              | 0.46             | 2.43          | 2.33            | 0.34            | 2.72             |
Figure 1: Probabilities $P_\lambda$ for the monomer of $\alpha$-synuclein to adopt the local secondary structures $\lambda$ at residue $i$. The structures shown are T, S (the upper panels, green and blue respectively) and H, B, G, and C (the lower panels, black, purple, magenta, and red respectively). The dotted lines (not shown for G and B for clarity of the presentation) indicate the size of the error bars. They were obtained by splitting the whole trajectory into two halves. The data points in the left panels have been obtained by using the C22*/TIP4P-4D force field with the explicit solvent. The panels on the right correspond to the NAMD-derived implicit solvent simulations. The black broken line shows the helical content if the starting conformations is the PDB:1XQ8 structure.
Figure 2: The top three panels: The 20 most likely contacts that participate in the α-synuclein dynamics. Panels A, B and C correspond to: the single-chain dynamics with the explicit solvent, single-chain dynamics with the implicit solvent and the two-chain dynamics with the implicit solvent respectively. The horizontal lines indicate locations along the sequence. Contacts link locations in the upper line with the locations in the lower line: contacts are either within the same chain (the top part) or with another chain (the bottom part). The higher the ranking, the thicker the line. The occupations are counted in the cumulative fashion throughout the simulation. We show contacts corresponding to locations $i$ and $j$ where $i < j$. For simplicity, we assume the symmetry between $i$ and $j$. In panel A, the most highly occupied contacts is 95-112. The other contacts shown are 102-140 (rank 2), 39-42 (3), 81-140 (4), 12-15 (5), 80-140 (6), 34-37 (7), 45-140 (8), 121-139 (9), 19-22 (10), 94-112 (11), 108-140 (12), 5-8 (13), 128-140 (14), 95-113 (15), 20-23 (16), 80-83 (17), 102-105 (18), 101-136 (19), and 103-106 (20). The total number of contacts identified was 8606. In panel B, the most highly occupied contact is 4-7. The other contacts shown are: 27-30 (rank 2), 102-140 (3), 19-22 (4), 22-25 (5), 76-140 (6), 118-140 (7), 5-8 (8), 128-140 (9), 18-21 (10), 11-14 (11), 96-140 (12), 33-36 (13), 93-140 (14), 53-140 (15), 8-11 (16), 4-8 (17), 69-140 (18), 96-99 (19), and 121-140 (20). The total number of contacts identified was 9454. In panel C, the most highly occupied interchain contact is 72-73. The other contacts shown are: 65-89 (rank 2), 4-67 (3), 51-111 (4), 49-111 (5), 48-111 (6), 64-86 (7), 71-73 (8), 73-73 (9), 66-87 (10), 8-68 (11), 49-110 (12), 78-87 (13), 4-66 (14), 50-111 (15), 65-87 (16), 71-75 (17), 72-74 (18), 71-74 (19), and 66-81 (20). The total number of contacts identified was 8596. The bottom panel: The time averaged contact map corresponding to the situations A, B, and C. The color code, with the scale on the right, indicates the probability of the occurrence of a contact. The white spots corresponds to regions in which no contacts were found. The contact maps are symmetric with respect to the diagonal so only a half of any map is shown. The digits indicate the rankings of the top three contacts.
Figure 3: The left panels: The $R_g$-$L$ cross plot for $\alpha$-synuclein. The data points have been obtained by summing the values belonging to 1 Å × 1 Å squares and showing only the squares with the probability of occupation that exceeds a threshold. For the red data points, the threshold is 0.01. For the black data points it is 0.001, 0.0001, and 0.00001, in the diminishing order of the size of the symbols. The right panels: The probability distributions of $R_g$ (the top panel) and $L$ (the bottom panel). The histogram in black is for the implicit-solvent data. The histogram in blue, also denoted by A, is for the explicit-solvent case. The bin size is 1 Å. The arrows indicate the corresponding mean values.
Figure 4: A 6-µs fragment of the explicit solvent trajectory. The upper panel shows the end-to-end distance. The lower panel shows the locations of the knot ends.
Figure 5: Examples of conformations in the fragment of the trajectory shown in Figure 4. The conformation at 2704 ns is the state just before the knotting process begins. The panels for 3492 and 5526 ns correspond to situations in which the knot exists. In the remaining panels, there is no knot. In the panel corresponding to 5526 ns, the knotted segment is in green and the purple lines indicate the segments between the knot ends and the nearest termini. In this example, the purple segments are the longest that were found. In the panel corresponding to 3492 ns the sequence within the knot is longer: it combines the segments in green and blue. The segments in purple are outside of the knots and are shorter than the maximal ones (for 5526 ns). In the remaining (unknotted panels) the segments in blue are equal in length to the maximal segments in purple shown for 5526 ns. They merely indicate the regions to look at when a knot is about to form. The conformation at 3492 ns is obtained by performing moves indicated by the red arrows. The last stage here corresponds to the direct threading. In the panel for 5523 ns, a slipknot is formed and it further indicated motion leads to knotting (5526 ns). Further unknotting takes place by driving the slipknot out of the knot loop (5532 ns). The conformation at 5540 ns represents the completely unknotted state. The knot was no longer observed during this trajectory after this stage.
Figure 6: A 6-µs fragment of the explicit solvent trajectory. The upper panel shows the end-to-end distance. The lower panel shows the locations of the knot ends.
Figure 7: A snapshot of a knotted trefoil structure in which the sequential distance between the knot ends is 73 – the shortest observed in the second trajectory. The knotted segment is in green and blue and the knot ends are at the boundary of blue and purple. These are residues 52 and 126. In other conformations, the knot ends may move into the core of the knot along the segments shown in blue but the separation between them will become larger than the minimal value. The motion of the ends along the purple segments eventually leads to the dissolution of the knot.
Figure 8: An example of a two-chain associated state. One chain is shown in the shades of blue and another in the shades of green. The interfacial residues are shown as spherical beads, in blue and green correspondingly. Other residues are not shown. There are 40 inter-chain contacts. Seven of these contacts are marked as red lines. They belong to the top-ten most probable contacts. These are: 65-89 (rank 2), 4-67 (3), 51-111 (4), 49-111 (5), 48-111 (6), 64-86 (7), and 66-87 (10) (see also panel C in Figure 2). The ranking is based on all association events. The event shown is a part of the longest lasting dimer (see Figure 9). In this particular dimer, the most frequent contact is 4-67 (marked in red) and then 51-112. In the snapshot shown, 51-112 does form a contact but, unlike 51-111, it is not marked because it does not belong to the list of top 20 contacts derived from all events, independent of the duration of the corresponding dimer. The figure in the center shows full two chains. The panel on the right shows an enlargement of the interfacial region.
Figure 9: The distribution of the duration times, $t_2$, of dimers (the solid black line). The thinner blue line corresponds to the power law fit $(t_2/t_{2p})^{-3/2}$ with $t_{2p}=0.002$ ns. It provides a better fit than exponential (not shown) with the characteristic time scale of 0.005 ns. The inset shows the same distribution in a different scale that is focused on the long-lasting and separate association events. Some of such events still persisted at the end of the simulations. The numbers show the two longest lasting contacts corresponding to the particular long-lived events.