Energy landscapes of the monomer and dimer of the Alzheimer’s peptide $\text{A}_\beta(1–28)$

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The cytotoxicity of Alzheimer’s disease has been linked to the self-assembly of the 40/42 amino acid of the amyloid-β ($\text{A}_\beta$) peptide into oligomers. To understand the assembly process, it is important to characterize the very first steps of aggregation at an atomic level of detail. Here, we focus on the N-terminal fragment 1–28, known to form fibrils in vitro. Circular dichroism and NMR experiments indicate that the monomer of $\text{A}_\beta(1–28)$ is $\alpha$-helical in a membranelike environment and random coil in aqueous solution. Using the activation-relaxation technique coupled with the OPEP coarse grained force field, we determine the structures of the monomer and of the dimer of $\text{A}_\beta(1–28)$. In agreement with experiments, we find that the monomer is predominantly random coil in character, but displays a non-negligible $\beta$-strand probability in the N-terminal region. Dimerization impacts the structure of each chain and leads to an ensemble of intertwined conformations with little $\beta$-strand content in the region Leu17-Ala21. All these structural characteristics are inconsistent with the amyloid fibril structure and indicate that the dimer has to undergo significant rearrangement en route to fibril formation. © 2008 American Institute of Physics. [DOI: 10.1063/1.2890033]

INTRODUCTION

One of the main signatures of Alzheimer’s disease is the extracellular deposition of amyloid plaques in brain tissue, composed of amyloid β-peptides ($\text{A}_\beta$). Although the exact relation between these plaques and the disease is still a matter of debate,1 it was shown recently that oligomeric assemblies of $\text{A}_\beta(1–40)$ or $\text{A}_\beta(1–42)$ are significantly more cytotoxic than the fibrils, raising the interest for the structures and dynamics of these early aggregates.2–4

It is difficult to gain insight into the details of the early steps of aggregation, either experimentally or theoretically, because of the transient nature of the oligomers. Since the first steps of $\text{A}_\beta$ aggregation in vitro, prior to the formation of a critical nucleus, can span several hours, experimentalists are faced with an ensemble of oligomers, ranging from monomers to dodecamers, in dynamical equilibrium, and trapping these intermediates for biophysical studies is a tour de force.5 Theorists, for their part, are often limited to studying only the very first moments of aggregation. All-atom molecular dynamics (MD), for example, can reach a time scale between 100 ns (Refs. 6 and 7) and 1 μs for a single full-length $\text{A}_\beta$ chain.8 To circumvent this limitation, it is essential to make approximation on the solvent or the protein representation and use accelerated sampling methods such as discontinuous molecular dynamics (DMD)9 or the activation-relaxation technique.10

To identify the necessary ingredients for full-length $\text{A}_\beta$ aggregation, many experimental and numerical works have been conducted on $\text{A}_\beta$ fragments that often form amyloid fibrils. Among the most studied fragments, we note $\text{A}_\beta(16–22)$,11–14 $\text{A}_\beta(21–30)$,15–17 $\text{A}_\beta(12–28)$,18,19 and $\text{A}_\beta(10–35)$.20–22 In this study, we focus on the human fragment 1–28 for four reasons. First, solid-state NMR spectroscopy of $\text{A}_\beta(1–28)$ shows a cross $\beta$-structure characterized by an in-register organization, parallel to the fibril axis23 as in full-length $\text{A}_\beta(1–40)$.24 Second, in a membranelike environment, the monomer forms a predominately $\alpha$-helical structure while the peptide is essentially random coil in solution at neutral pH or oscillates between a polyproline II helix (PPII) and disordered, random coil structure in solution at acidic $p$H.25–29 Third, inhibitors of $\text{A}_\beta(1–28)$ amyloidogenesis and cytotoxicity were successfully designed29,30 and antibodies raised against the region 1–28 can disaggregate $\text{A}_\beta(1–40)$ fibrils.31 Finally, there is experimental evidence that Zn2+ ions, which can be found in senile plaques, interact with the region 1–28 and notably through interactions with the side chains of His13 and His14.32

Despite extensive experimental studies, important questions remain regarding the conformational changes of $\text{A}_\beta(1–28)$ during oligomerization due, in part, to the lack of simulations on the dimer and higher order species. Only the stability of the $\alpha$-helical structure, found in membranelike environment, has been studied as a function of $p$H in short MD,33,34 and the equilibrium structures of the monomer have been studied by all-atom implicit solvent replica exchange molecular dynamics (REMD) simulations.35 In this study, we report both the monomeric and dimeric equilibrium structures of $\text{A}_\beta(1–28)$ in aqueous solution. To this end, we use...
the activation-relaxation technique (ART nouveau)\textsuperscript{10,36,37} coupled with the OPEP force field (version 3.2).\textsuperscript{38} Our simulations show that, although the monomer is mostly random coil, its structure is restricted by the N-terminal which has a non-negligible probability to form a $\beta$-sheet. Dimerization increases the $\beta$-sheet content and leads to two intertwined chains with overall parallel organization, but local antiparallel secondary structures, suggesting the need for the peptides to rearrange in the next steps of oligomerization.

**MATERIAL AND METHODS**

**ART-OPEP simulations in implicit solvent**

ART nouveau explores the space of conformations by moving the system from minimum to minimum going through a common first order transition point.\textsuperscript{10,37} One ART event consists of four steps. Starting from a local minimum, the atoms are moved in a randomly chosen direction until the lowest local curvature of the energy landscape, given by the Hessian matrix, becomes negative. At this point, the system is pushed against the force along the direction of negative curvature while the energy is minimized in the hyperplane perpendicular to this direction. This second step is iterated until the total force becomes smaller than a given threshold, indicating that the system has reached a transition point. The system is then displaced slightly over the saddle point and is relaxed into a new local minimum, using standard minimization techniques. Finally, the move is accepted using a Metropolis criterion: $P_{\text{accept}} = \min(1, e^{-\Delta E/k_B T})$, where $\Delta E$ is the difference between the new and previous energies. Because thermal vibration effects are not included in ART, the Metropolis temperature in the accept/reject criterion does not correspond to a real temperature and it is adjusted to ensure an efficient sampling of the conformational space. While ART does not sample according to a well-defined ensemble, the structures sampled sit at the bottom of the energy basin, often providing a clearer indication of the underlying important structures than standard techniques such as MD.

ART is coupled to the OPEP energy function (optimized potential for efficient peptide-structure prediction in solution) which uses a reduced off-lattice protein representation with implicit solvent.\textsuperscript{40,41} All amino acids are represented by their N, H, C$_{\alpha}$, C, and O atoms and a side chain modeled by one sphere with appropriate van der Waals radius and hydrophobic/hydrophilic character and a well-defined position with respect to the main chain.\textsuperscript{42} OPEP energy function is expressed by a linear combination of four interaction types: (1) harmonic potentials for maintaining the bond lengths and bond angles of all particles, the improper dihedral angles of the side chains and peptide bonds near equilibrium values, and $\phi$, $\psi$ potentials for generating Ramachandran plot of reduced protein structures in agreement with all-atom protein structures, (2) backbone two-body and four-body (cooperative) hydrogen bonding interactions, (3) side-chain–side-chain interactions represent by a 12-6 potential if the side chains are hydrophobic or oppositely charged and by a six potential, otherwise, and (4) excluded-volume potentials between all particles. OPEP has been shown to predict accurately the native structures of multiple polypeptides,\textsuperscript{42,43} and in this work, we use the OPEP version 3.2 parameter set refined on a total of 30 proteins,\textsuperscript{38} and providing a very good agreement with NMR for the A$\beta$(21–30) monomer.\textsuperscript{17}

**Details of the simulations and structural analysis**

The sequence of A$\beta$(1–28) is DAEFRHDSGYEVHHQKLVFFAEDVGSNK, where the cluster hydrophobic core (CHC) at position 17–21 is underlined. All simulations are carried out at neutral pH. Ten 15 000-event independent ART-OPEP simulations are performed on the monomer using various random seeds: Runs 1–5 are started from a fully extended state and runs 6–10 from a full $\alpha$-helix corresponding to the favored structure in a membranelike environment [PDB entry 1MAB (Ref. 25)]. The two initial points are shown in Figs. 1(a) and 1(b).

For the dimer, we launched a total of 20 runs of 30 000 events: Runs 1–10 from two fully extended chains in parallel register separated by 25 Å from each other, and runs 11–20 from a randomly chosen orientation of the lowest-energy structure generated by the ART-OPEP run 2 of the monomer (mR2). These initial points are shown in Figs. 1(a) and 1(b).

All simulations are carried out at a Metropolis temperature of 900 K. This temperature is selected to ensure a wide sampling of the conformational space and leads to an acceptance rate of 65% for the monomer and of 54% for the dimer. The generated structures are clustered according to the $Ca$ root-mean-square deviations (RMSDs) and normalized Holm–Sander scores (HSSs),\textsuperscript{44–46} given by
RESULTS

The ART-OPEP simulations sample extensively the energy landscape of the monomer and dimer of Aβ(1–28). For both systems, we identify the lowest-energy structures and discuss the marginally destabilized states because they may represent seeds for higher order species. We also determine the secondary structure probability along the sequence and the contact side-chain–side-chain map averaged over the lower-energy structures.

Aβ(1–28) monomer

The ten runs generated a total of 97 457 accepted conformations with an energy ranging from −4.3 to −50.8 kcal/mol. The lowest-energy conformation (mRX, m for monomer, RX for the Xth run) of each of the ten runs reaches values between −47.5 and −50.8 kcal/mol, without any correlation with the two starting states. As seen in Fig. 2, the overlap between the lowest energy states of each run is striking. The mR3, mR5, mR7, mR8, and mR10 states, generated from two distinct starting points, share a small three-stranded antiparallel β-sheet with the N-terminal placed in the middle. More precisely, mR7 and mR8 superimpose on each other within 0.04 Å RMSD, while mR7 and mR10 deviate from mR5 by 2.9 and 2.6 Å RMSD. The mR3 state, for its part, is at 7.7 Å RMSD from mR5 because the loop spanning Q16–A22 passes on the other side of the β-sheet. The lowest-energy structures are also characterized by β-hairpin-coil (mR1, mR2, and mR6), coil–β-hairpin (mR4), and fully disordered conformations (mR9).

Secondary structure composition of the mR1–mR10 states are given in Table I. We see that they are predominantly random coil in character, as computed with DSSP, with only 30%–40% of the amino acids in β-sheet. While the amino acids E22–S26 displays a τ-helix in mR2, none of the minima shows α-helix content.

Figure 3 reports the energy, $C_\text{g}$, RMSD, radius of gyration, end-to-end distance, HSS, and percentage of secondary structure as a function of the accepted events in run 5, locating the structure of lowest energy. The overall properties of this run are similar to those of the nine other runs and can therefore be considered as generic. In panel (a), we see that the energy goes down rapidly to about −35 kcal/mol and oscillates around this value for the first 5000 accepted events. At this point, the peptide reorganizes, as shown by the RMSD (panel b), and eventually visits its lowest-energy state at accepted even 8201. In this basin, the energy oscillates between −40 and −51 kcal/mol. Around event 8300, the peptide leaves this low-energy basin as is indicated by the jump in RMSD (panel b) and end-to-end distance (panel d). While the radius of gyration (panel c) fluctuates between 7 and 9 Å, the end-to-end distance and RMSD vary substantially, indicating the thorough sampling of conformations during the simulation. Note that HSS (panel e) mirrors closely the RMSD (panel b). We also show the evolution of secondary structures as computed using PROSS (panel f). The percentage of α-helix (in red, shifted by 70 for a better visualization) is very low, 0%–21%, with an average of only 0.15% at neutral pH. We observe a similar pattern in the runs 6–10 even though the starting conformation is a full helix. The percentage of β-sheet also fluctuates strongly, varying between 0% and 68%, with an averaged value of 16%. The β-sheet content is slightly higher in the lower-energy basin, reaching 32%. Finally, 0%–36% of the peptide adopts a PPII structure during the simulation with 9.4% on average. Not
surprisingly, the proportion of PPII is maximal when that of β-sheet is minimal. Overall, however, 69% of the residues are random coil in character during the simulation. Very similar β-sheet and PPII compositions are obtained with the other runs, and notably runs 6–10, excluding the first 1000 steps.

Clustering analysis provides a similar picture. For clarity, we include only the 78 728 structures with an energy at most one standard deviation above the average energy, i.e., with an energy of −33.1 kcal/mol and below, putting aside the other very energetic states [see the energy distribution in Fig. 4(a)]. With this set, we find five dominant structural families which are associated, in decrease order of frequency, with mR7, mR4, mR9, mR5, and mR2.

Because thermal fluctuations are not taken into account in ART nouveau, it is important to examine the fluctuations around the minima. As a first step, we focus on the conformations with energy less $\bar{E} - \sigma$, i.e., 6570 conformations with $E$ between −44.7 and −50.7 kcal/mol. Note that the exact position of the cutoff does not impact the qualitative results. Figures 5 and 6(a) show the contact map and the secondary structure probability averaged over the 6570 low-energy structures. The $\text{Aβ}(1–28)$ monomer is clearly stabilized by the strong tendency of A2–F4 to form an antiparallel β-sheet with Y10–V12 and, to a much lesser degree, with D23–S26. However, the second half of the peptide amino acids 13–28 is essentially random coil, although it can visit PPII helix with a probability slightly above 10% at the positions H13–L17 and D23–N27. To our surprise, the CHC region residues 17–21 has a very small signal for β-strand even though it has a strong tendency to form hydrophobic contacts with the amino acids A2, F4, V12, and V24. Clearly, interactions

FIG. 3. (Color online) Evolution of the $\text{Aβ}(1–28)$ monomer during run 5 as a function of accepted events. (a) Energy; (b) RMSD calculated from the lowest-energy conformation, mR5; (c) radius of gyration; (d) Cα end-to-end distance; (e) HSS calculated from the structure of lowest energy; (f) secondary structure given in percentage: Blue, polyproline II; green, β-sheet; red, α-helix (the zero of this curve is shifted to 0.70 for clarity).
between the CHC and the rest of the chain are essential to populate, among other states, a small $\beta$-sheet as seen in Fig. 6a.

These results can be compared with the secondary structure probability obtained from a single 100 ns MD-OPEP simulation\textsuperscript{49} at 300 K, rejecting the first 10 ns and starting from mR7. Identical properties are obtained starting from mR2, fully indicating the transient character of the minima. We note two points in Fig. 6b. First, the probability of forming PPII is almost identical between the ART and MD simulations, oscillating around 10%. Second, the probability of forming $\beta$-structure is almost systematically reduced by 0.30 over all residues, bringing the per-residue $\beta$-strand probability from 30% to 20%. Thermal vibrations are therefore responsible for destabilizing the C-terminal $\beta$-strand and bringing down the probability of $\beta$-strand within residues A2–R5 and E11–H13 to about 50%.

**$\alpha\beta(1–28)$ dimer**

The lowest-energy conformations found in runs 1–10 starting from two fully extended chains vary from $-128.6$ to $-144.9$ kcal/mol, while runs 11–20, started from two low-energy monomeric states, locate low-energy states between $-126.5$ and $-140.7$ kcal/mol. Details of the lowest-energy structure attained by each run can be found in Supplemental material (Supplementary Table 1).\textsuperscript{50} The five lowest-energy structures are shown in Fig. 7.

We first follow the trajectory R6 locating the lowest-energy structure. This trajectory does not present any specific feature and is representative of the 19 others. It is analyzed in Fig. 8 by following the evolution of energy, $\text{Ca}$ RMSD and HSS with respect to the lowest-energy structure, radius of gyration, end-to-end distance of both chains, and secondary structure composition as a function of accepted events. After a rapid drop, the energy hovers around $-110$ kcal/mol before

![FIG. 5. (Color online) Contact map averaged over the lower-energy structures (minimum to $E-\sigma$). The scale indicates the normalized probability of finding a given contact.](image)

![FIG. 6. (Color online) (a) Probabilities of the residues to participate in $\beta$ strand (red) and PPII helix (blue) within the lower energy conformations (minimum to $E-\sigma$). (b) Same, but for the MD-OPEP simulation. Here results are averaged over the last 90 ns of the simulation.](image)
falling rapidly to its low-energy basin, at around even 10 000. As with the monomer, however, the dimer is not locked into this basin and manages to escape within the next 5000 steps, as seen in the fluctuations of the RMSD (panel b) and of the radius of gyration (panel c). The percentage of secondary structure fluctuates significantly during the simulation but shows, on average, a higher order than for the monomer: The β-strand content slightly exceeds 50% in the lowest-energy basins, while the PPII signal oscillates around 10%. As with the monomer we observe almost no α-helix during the simulation.

The 20 runs generate 327 266 accepted events, with an energy ranging from −144.9 to −7.9 kcal/mol. The energy distribution for the sampled conformations follows a Gaussian [Fig. 4(b)] and we keep the 300 030 structures with an energy of at most one standard deviation above the average energy (from −99.93 to −144.93 kcal/mol) for clustering. Using the thresholds described in the method section, we identify 8692 clusters of which 566 contain 100 or more structures. These clusters can be further regrouped into superclusters with similar structural geometry. Table II lists the 16 largest superclusters (dSa to dSp) along with their lowest energy, RMSD and HSS with respect to the center of the largest supercluster. Even though the number of clusters is large, the structures of lowest energy, below −140 kcal/mol, are concentrated into two superclusters clusters, dSa and dSp, showing that the low-energy structures are rather very well defined. The energy of the clusters increases rapidly and only five other superclusters (dSbc, dSe, dSn, and dSo), among the 16 most populated ones, have a lowest-energy structure above −135 kcal/mol. Figure 9 shows the lowest-energy structure for the 16 superclusters.

All superclusters, except dScg with two side-by-side monomers, display intertwined chains. The interchain interactions explain the very small structural overlap with the lowest-energy monomeric states as the energy gain upon dimerization is significant (about 45 kcal/mol). After the first 1000 steps, only 145 dimers in runs R15, R17, and R20 show at least one chain deviating within 2.25 Å RMSD from one of the lowest-energy monomeric structure, with the smallest overlap at 1.9 Å RMSD from mR2, used as the initial states in runs R11–R20. Overall, all the dimers, with one chain similar to the minimum energy monomeric structure, are very destabilized (energies between −124.6 and −104.7 kcal/mol), and display a second chain deviating by 4.0–9.1 Å RMSD from the mRs states, suggesting that the monomeric structure is not favored in the dimer.

Following the monomer analysis, we focus on the structures with energies below $\bar{E} - \sigma$, i.e., about 17 kcal/mol above the energy minimum. If we look at the second structure probability along the sequence (Fig. 10), we note a number of similarities with the monomer. In particular, the amino acids A2-E3-F4-R5 and V24-G25-S26 have a high β-strand signal (−0.7) while the CHC region is predominantly random coil (β-strand signal <0.2). Since the two termini of both chains are locked in a β-sheet, only the central parts, mostly residues 16–21, can form PPII helix with a probability above 10%. If we include the thermal vibration effects, using a 0.30 correction similar to what is observed between ART and MD for the monomer, only two regions form a β-strand with probability near 50%: A2-R5 and V24-S26.

While the structured regions in the dimer are similar to those found in the monomer, the two chains are intertwined, in a very symmetric fashion, creating a significant number of interchain contacts. Interestingly, the chains are mostly parallel in their overall orientation, but with a local antiparallel organization. This is clearly seen in the contact map (Fig. 11) obtained by averaging over all lower-energy structures (−127 kcal/mol and less). We observe, for example, that the region E22–K28 of chain 1 is in contact with the residues K28–E22 of chain 2 (56–50 in Fig. 11), while at the same...
time residues L17 and F19 of chain 1 form contact with residues F19 and L17, respectively, of chain 2 (47 and 45 in Fig. 11). The two chains are not perfectly imbricated, however, and a number of intrachain contacts are also important. For example, in both chains residues 2–6 often bind to residues 17–13, while residues 26–28 bind to residues 24–22. Residues 4–5 also bind with residues 12–10. The hydrophobic region 17–21, while mostly unstructured, also assembles into an antiparallel organization between the two chains with contacts 17–19 or 18–20 being formed more than 80% of the time.

**DISCUSSION AND CONCLUSIONS**

The peptide Aβ(1–28) forms fibrils in vitro with the chains in parallel register as in full-length Aβ. Aβ(1–28) contains the region 1–10 which is unstructured in Aβ40 fibrils, and the central hydrophobic core (residues 17–21: LVFFA) which is believed to play an important role in full-length Aβ fibril formation. Circular dichroism (CD) and NMR studies indicate that the human Aβ(1–28) monomer is α-helical in a membranelike environment and mostly disordered in water. Similarly, the rat Aβ(1–28) monomeric variant with the R5G, Y10F, and H13R substitutions has been characterized as random coil by NMR in fully deuterated dimethyl sulfoxide solution. We find that the lower-energy monomeric structures display about 75% of residues in random coil, 20% in α-sheet, and 5% with PPII character. This high percentage of random coil is analogous to what is observed for the monomer under neutral and acidic pH conditions. Recent circular dichroism and 1H-NMR experiments by
Graslund et al. suggest, however, a higher proportion of PPII helix on the order of 40% at 300 K and pH 7.4, contrary to what is predicted here. The difference in the β-sheet and PPII populations derived from simulations and experiments is well known and is associated with a very small free energy barrier separating these two states for many empirical potentials. DFT calculations on trialanine show, however, that the total percentage of extended (β+PPII) states obtained by most empirical potentials is very close to that derived from quantum mechanical calculations. We can there-

### Table II

The largest 16 superclusters of dimer Aβ1–28. 300,030 conformations are included in the clustering and their energies vary from −144.9 to 99.9 kcal/mol. The RMSD and HSS are measured with respect to the lowest-energy structure of supercluster dSa and the energy of the lowest-energy structure is indicated. The last column indicates the energy of the lowest-energy structure of each cluster.

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<th>id</th>
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<th>RMSD (Å)</th>
<th>HSS</th>
<th>$U_{\text{min}}$ (kcal/mol)</th>
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FIG. 9. (Color) Lowest-energy structure for the 16 largest superclusters of the dimer of Aβ1–28. Chain 1 is colored green and chain 2 yellow. Also indicated are residues 2–5 (blue) and 16–22 (red).

FIG. 10. (Color online) Aβ1–28 dimer: Secondary structures of the lower energy conformations (minimum to $\bar{E}−\sigma$) using the program PROSS. Probabilities of residues to have β-strand (a) and polyproline II (b) character. (Note the difference in scale.)

FIG. 11. (Color online) Contact map averaged over the lower-energy structures (minimum to $\bar{E}−\sigma$). Residues 1–28 belong to the first chain and residues 29–56 to the second chain. The scale indicates the normalized probability of finding a given contact.
fore conclude that our 25% of extended secondary structure is consistent with the recent experimental data reported by Graslund et al.\textsuperscript{52}

The high percentage of residues with random coil character does not mean that the monomer is fully disordered. Rather we find that the peptide is in equilibrium between transient states displaying a coil-\(\beta\)-hairpin or a \(\beta\)-hairpin coil, a small three-stranded \(\beta\)-sheet and a fully disordered state. This ensemble of states is reminiscent of the free energy landscape of a 20 amino acid peptide which displays similar topologies.\textsuperscript{55} This conformational ensemble is also consistent with recent experimental evidence that the full-length \(\alpha\beta\) monomer adopts multiple conformations with very high or very low \(\beta\)-sheet contents.\textsuperscript{58}

We also find that the amino acids E3-F4-R5 have a non-negligible probability to form a \(\beta\)-sheet with amino acids E11-V12-H13, as can be seen in many clusters in Fig. 9. This N-terminal propensity for \(\beta\)-strands has been suggested by Danielsson et al. using NMR measurements on \(\alpha\beta(1-9)\), \(\alpha\beta(12-28)\), and \(\alpha\beta(1-28)\) monomers.\textsuperscript{52} Our prediction disagrees, however, with all-atom implicit solvent REMD simulations, where the \(\alpha\beta(1-28)\) monomer is found mainly unfolded with three nascent helices at positions 2–5, 10–13, and 18–22, whose populations decrease from \(pH\) 2 to \(pH\) 7.\textsuperscript{35} This REMD-based prediction in implicit solvent is rather surprising, since \(\alpha\)-helical content was not observed by all-atom REMD simulations on the monomer of \(\alpha\beta(10-35)\), \(\alpha\beta(1-40)\), and \(\alpha\beta(1-42)\) in explicit solvent.\textsuperscript{59}

The CHC core is essentially random coil and its amino acids V18, F19, and F20 can adopt multiple conformations, but they do not display any significant propensity for \(\beta\)-strand, and 18–22, whose populations decrease from \(pH\) 2 to \(pH\) 7.\textsuperscript{35} This REMD-based prediction in implicit solvent is rather surprising, since \(\alpha\)-helical content was not observed by all-atom REMD simulations on the monomer of \(\alpha\beta(10-35)\), \(\alpha\beta(1-40)\), and \(\alpha\beta(1-42)\) in explicit solvent.\textsuperscript{59}

There is no experimental information available on the structures of \(\alpha\beta(1-28)\) dimer. Our simulation results provide a first atomic picture of dimerization. The percentage of amino acids with \(\beta\)-sheet character increases from the monomer to the dimer, from about 20% to 35%. Most of the structured regions of the monomer form \(\beta\)-sheets in the dimer and the amino acids E3-F4-R5 and V24-G25-S26 have a \textasciitilde 50% probability of forming a \(\beta\)-strand in the dimer. Such a prominent \(\beta\)-strand structure within the N-terminal region was detected in DMD simulations of \(\alpha\beta(1-40)\) oligomers.\textsuperscript{60} In addition, in line with the monomer results, the hydrophobic CHC regions 17–21 do not participate into \(\beta\)-sheets and are not particularly shielded from the solvent (Fig. 7).

Interestingly, the dimer displays an overall parallel orientation, but with antiparallel interchain \(\beta\)-sheets. The dimer is also characterized by intertwined chains, rather than side-by-side chains. It is clear therefore that the dimer must undergo significant reorganization \textit{en route} to amyloid fibril structure. This propensity for intertwined chains is not totally surprising. It has been discussed for the \(\alpha\beta(1-42)\) dimer using coarse grained protein simulations\textsuperscript{61} and observed for the dimer of the \(\beta_2\)-microglobulin(83–89) peptide using all-atom MD in explicit solvent.\textsuperscript{62}

Finally, in both the monomer and the dimer, we find that the amino acids E3-F4-R5 have a high propensity for \(\beta\)-strand, while the CHC region has not. This picture runs in contrast to that provided by the full-length \(\alpha\beta\) fibril models\textsuperscript{24,63} where the N-terminal tails are disordered and the CHC regions form a mutimeric \(\beta\)-sheet. The predicted influence of the N-terminus on the monomer and dimer structures of \(\alpha\beta(1-28)\) is supported by the finding of the two novel D7N and H6R mutations which accelerate \(\alpha\beta(1-40)\) fibril formation by promoting the elongation phase.\textsuperscript{64} The effect of these mutations on our \(\alpha\beta(1-28)\) structures remains to be explored. We stress that our simulations do not mean the amino acids 17–21 do not play a significant role. Rather, they indicate that the dimer is not sufficient to stabilize amyloid-competent CHC interactions and, in line with a previous MD study,\textsuperscript{19} the solvent accessibility of the CHC core makes it an ideal spot for further oligomerization.

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48See EPAPS Document No. E-JCPSA6-007812 for supplementary Table I: Secondary structure composition of the lowest-energy structure of the 10 dimer runs. For more information on EPAPS, see (http://www.aip.org/pubservs/epaps.html).