Globular state in the oligomers formed by A β peptides

Seongwon Kim, Takako Takeda, and Dmitri K. Klimov^{a)}

Department of Bioinformatics and Computational Biology, George Mason University, Manassas, Virginia 20110, USA

(Received 19 February 2010; accepted 14 May 2010; published online 9 June 2010)

Replica exchange molecular dynamics and implicit solvent model are used to study two oligomeric species of A β peptides, dimer and tetramer, which are typically observed in *in vitro* experiments. Based on the analysis of free energy landscapes, density distributions, and chain flexibility, we propose that the oligomer formation is a continuous transition occurring without metastable states. The density distribution computations suggest that A β oligomer consists of two volume regions—the core with fairly flat density profile and the surface layer with rapidly decreasing density. The core is mostly formed by the N-terminal residues, whereas the C-terminal tends to occur in the surface layer. Lowering the temperature results in the redistribution of peptide atoms from the surface layer into the core. Using these findings, we argue that A β oligomer resembles polymer globule in poor solvent. A β dimers and tetramers are found to be structurally similar suggesting that the conformations of A β peptides do not depend on the order of small oligomers. © 2010 American Institute of Physics. [doi:10.1063/1.3447894]

I. INTRODUCTION

Amyloid fibrils are ordered macromolecular assemblies resulting from the aggregation of polypeptide chains.¹ Because amyloidogenic polypeptides have low sequence homology, it appears that the propensity to form fibrils is a generic property of proteins. Amyloid assembly is a complex multistage conformational transition, which begins with the oligomerization of monomers and gradually leads to the appearance of protofibrils and mature fibrils as final products.^{1–5} The current interest in amyloidogenesis is two-fold. Self-assembled amyloid structures have potential technological applications, such as production of conductive nanowires or peptide-metal composites.^{6,7} On the other hand, amyloidogenesis is linked to more than 20 various medical disorders, including Alzheimer, Parkinson, and Creutzfeldt–Jakob diseases.⁸

There is a compelling experimental evidence suggesting that the onset of Alzheimer disease is related to the aggregation of A β peptides,⁹ which are produced in the course of proteolysis of the transmembrane amyloid precursor protein. Although these peptides are released in a variety of lengths, the 40-residue species, A β _{1–40}, are most abundant.¹ At micromolar concentration and normal physiological conditions (~300 K and normal pH) A β oligomers coexist with monomers¹⁰ and demonstrate a distinctive size distribution existing predominantly in the form of dimeric through tetrameric species.^{11,12} Higher order oligomers have significantly smaller frequency of occurrence. Recent biomedical studies have showed that A β oligomers are the primary cytotoxic species in Alzheimer disease.^{2,13–15} It has been reported that synaptic structure and function are impaired even by the smallest A β oligomers, dimers.¹⁶ There are also ex-

perimental indications of the dynamic equilibrium between soluble oligomeric species and amyloid fibrils.¹⁷ These observations implicate a crucial role of A β oligomers in amyloidogenesis.

A β monomers have been a focus of numerous experimental studies. In aqueous solution they are largely unstructured lacking stable secondary or tertiary structures.^{18,19} The end products of amyloid assembly, A β fibrils, are characterized by extensive and remarkably homogeneous β -sheet structure.^{20–22} In contrast, relatively little is known about the structures of A β oligomers. In part, experimental difficulties in obtaining structural information are due to rapid interconversion of oligomeric species and apparent lack of stable structure.¹² A useful alternative are computational methods, particularly, molecular dynamics (MD) simulations, which can probe the details of A β amyloid formation at all-atom resolution.^{23,24} Several studies have explored the conformational properties of A β _{1–40} monomers^{25,26} and its fragments^{27–29} as well as fibril growth and stability.^{30–32} The full-length A β _{1–40} was shown to have several structured regions, including short β -strands, helices, and β -turns, in generally random coil-like ensemble of conformations.^{25,26} MD investigations of A β oligomers, which are more computationally intensive, have been recently reported.^{33–37} In general, these studies have shown that aggregation leads to considerable conformational changes in A β peptides. For example, we have found that interpeptide interactions in A β dimers induce a profound shift in the secondary structure, resulting in the conversion of helical states into β -strand conformations.³⁵ Furthermore, the sequence region 10–23 in A β peptide was observed to form most of interpeptide interactions upon aggregation.^{35,36} Studies of coarse grained models of A β peptides showed that A β _{1–40} and A β _{1–42} species form oligomers via distinct pathways in agreement with experimental data.³⁷ However, many questions concerning oligomer structure and assembly remain unexplored. For ex-

^{a)}Author to whom correspondence should be addressed. Electronic mail: dklimov@gmu.edu.

ample, (1) what are the basic physicochemical characteristics of $A\beta$ oligomers? In particular, do oligomers resemble polymer globules? (2) What are the free energy landscapes of oligomer assembly and the nature of underlying structural transitions? (3) Are there differences between the conformational ensembles of oligomers of different orders? In other words, does the structure of small $A\beta$ oligomer undergo further changes upon the addition of new chains?

To answer these questions, we use exhaustive replica exchange MD (REMD) and implicit solvent model to study the structural properties of $A\beta$ dimers and tetramers. Because these two species represent the most abundant $A\beta$ oligomers, we can make some tentative conclusions about their generic properties. We demonstrate that the density distribution in $A\beta$ oligomer resembles the one in the globule formed by flexible polymer chains. Based on this analogy and the analysis of free energy landscapes, we propose that the formation of $A\beta$ oligomers is consistent with the continuous barrierless transition. We further show that the oligomer core is predominantly formed by the N-terminals of $A\beta$ peptides. By analyzing the radial density distributions, fluctuations, and solvent accessible surface areas along $A\beta$ sequence, we infer that the structural characteristics of dimers and tetramers are similar. We conclude the paper with the comparison of our *in silico* results with experimental data.

II. METHODS

A. Simulation model

To perform simulations of $A\beta$ peptides we used CHARMM MD program³⁸ and united atom force field CHARMM19 coupled with the SASA implicit solvent model.³⁹ Detailed description of the CHARMM19+SASA model and its extensive testing were reported in our previous studies.^{35,40–42} In particular, we have shown that the CHARMM19+SASA force field correctly reproduces the experimental distribution of chemical shifts for C_α and C_β atoms in $A\beta$ monomers.^{35,43} In the past, CHARMM19+SASA model has been used to fold α -helical and β -sheet polypeptides^{44,45} and to study aggregation of amyloidogenic peptides.^{46,47} Additional arguments supporting the use of CHARMM19+SASA implicit solvent model are presented in the supplementary material.⁴⁸

In this study we use $A\beta_{10-40}$ peptides, the N-terminal truncated fragments of the full-length $A\beta_{1-40}$ [Fig. 1(a)]. Solid-state NMR experiments have indicated that $A\beta_{1-40}$ and $A\beta_{10-40}$ form similar twofold symmetry fibril structures.^{21,49} According to the experiments¹¹ and simulations,⁴¹ $A\beta_{1-40}$ and $A\beta_{10-40}$ oligomers share significant similarities in terms of their size distributions and structure. It is also known that the first nine N-terminal residues in $A\beta_{1-40}$ fibril are disordered.²¹ Consequently, $A\beta_{10-40}$ is used as a model of the full-length $A\beta_{1-40}$ peptide.

Two $A\beta_{10-40}$ systems, tetramer and dimer, were considered [Figs. 1(b) and 1(c)]. The dimer system was introduced in our previous studies.^{35,41} The tetramer system, which is similar to the dimer, involves four identical unconstrained

FIG. 1. (a) The sequence of $A\beta_{10-40}$ peptide and the allocation of the N-terminal Nt and C-terminal Ct regions. [(b) and (c)] Typical structures of $A\beta$ oligomers, tetramer (b) and dimer (c), sampled at 360 K. The N- and C-terminals are colored in shades of red and yellow, respectively. The $A\beta$ Nt region tends to form the oligomer's core, whereas the Ct mostly makes up the surface layer. The structures are visualized using Chimera (Ref. 60).

$A\beta_{10-40}$ peptides. Tetramer and dimer were subject to spherical boundary condition with the radius $R_s=90$ Å and the force constant $k_s=10$ kcal/(mol Å²). The concentration of $A\beta$ peptides was therefore on the order of $\sim$ mM.

B. Replica exchange simulations

Conformational sampling was performed using REMD.⁵⁰ The REMD implementation is described in our previous studies.^{35,47} Briefly, 24 replicas were distributed linearly in the temperature range from 300 to 530 K with the increment of 10 K. This temperature range spans the spectrum of conformational states of $A\beta$ peptides from aggregated to completely dissociated. Small temperature increment provides significant overlap of energy distributions from neighboring replicas. The exchanges were attempted between all neighboring replicas with the average acceptance rate of 38%. Between replica exchanges, the system evolved using *NVT* underdamped Langevin dynamics with the damping coefficient $\gamma=0.15$ ps⁻¹ and the integration step of 2 fs. The simulation time between replica exchanges was set to 80 ps. Eight REMD trajectories were produced for the tetramer resulting in the cumulative simulation time of 154 μ s. Because the initial parts of REMD trajectories are not equilibrated and must be excluded from thermodynamic analysis, the cumulative equilibrium simulation time was reduced to $\tau_{\text{sim}} \approx 126$ μ s. The REMD trajectories were started with random distributions of peptides in the sphere. The REMD simulations for the dimer were described earlier.³⁵

C. Computation of structural probes

To probe the interactions between A β peptides, we computed the number of side chain contacts. A side chain contact is formed if the distance between the centers of mass of side chains is less than 6.5 Å. If both amino acids in contact are apolar, the contact is considered hydrophobic. Two peptides are considered aggregated if there is at least one hydrophobic side chain contact between them. The radial distribution function $g(r)$ reports the atom number density at the distance r from the center of mass of oligomer. To compute $g(r)$ we considered nonhydrogen atoms from all peptides. The histogram of atom distances r was constructed using the bin-size of 0.5 Å. The solvent accessible surface area of amino acid was computed using SASA module in CHARMM program.³⁹ To probe the extent of burial of amino acid X we considered the relative accessible surface area (rASA) defined as ASA/ASA_0 . Here ASA and ASA_0 are the accessible surface areas of amino acid X in a given conformation and in the reference extended trimer Gly-X-Gly.⁵¹

Throughout the paper, angular brackets $\langle \dots \rangle$ imply thermodynamic averages. Because tetramer (dimer) system includes several indistinguishable peptides, we report averages over four (two) peptides. The distributions of states produced by REMD were analyzed using multiple histogram method.⁵² The convergence of REMD simulations and error analysis are presented in the supplementary material.⁴⁸

III. RESULTS

Using REMD we probe the conformational ensembles of A β_{10-40} dimers and tetramers (Fig. 1). Following experimental A β fibril structure,²¹ two sequence regions in A β_{10-40} are distinguished—the N-terminal (Nt, residues 10–23) corresponding to the first fibril β -strand and the C-terminal (Ct, residues 29–39) corresponding to the second fibril β -strand [Fig. 1(a)]. The thermodynamic quantities for A β oligomers are computed at the temperature 360 K, at which A β peptide locks into fibril-like state during fibril growth as reported earlier.^{40,47}

A. Thermodynamics of A β oligomer assembly

We start the investigation of A β_{10-40} oligomers by computing the probability P_4 of forming the largest oligomer, tetramer, in the system consisting of four peptides. Operationally, P_4 is defined as a probability of forming the four-peptide aggregate in which each chain makes at least one interpeptide hydrophobic side chain contact. At 360 K, $P_4 \approx 0.99$, suggesting that at the A β concentration of $\sim$ mM, the tetramer is a single aggregate present in the system. The probability P_2 of forming a dimer in the two-peptide system (defined similarly as P_4) is ≈ 0.98 at 360 K. Because the tetramer and dimer are stable at 360 K, we use these terms to refer to the systems consisting of four and two peptides, respectively.

To map the free energy landscape of A β aggregation we computed the free energy of a peptide as a function of the number of interpeptide hydrophobic contacts, $F(C_h)$ [Fig. 2(a)]. Because the oligomers are formed at 360 K ($P_4 \approx P_2 \approx 1.0$), $F(C_h)$ probes the free energy of incorporat-

FIG. 2. (a) Free energies of A β_{10-40} peptide $F(C_h)$ as a function of the number of interpeptide hydrophobic side chain contacts C_h at 360 K. The free energy of peptide incorporation into the oligomer is $\Delta F_{O-D} = F_O - F_D$, where F_O and $F_D = 0$ are the free energies of the associated (O) and dissociated (D, $C_h = 0$) states. F_O is obtained by integrating over the O states (shaded in gray) for which $F(C_h) \leq F_{\min} + 1.0RT$, where $F_{\min}$ is the minimum in $F(C_h)$. (b) Temperature dependence of the oligomer free energy $F(T)$. Quadratic fitting functions $F(T) = -\alpha(T - T_o)^2$ are shown by black continuous curves. The fitting parameters are $\alpha = -0.003$ kcal/(mol K²), $T_o = 371$ K (dimer) and $\alpha = -0.007$ kcal/(mol K²), $T_o = 382$ K (tetramer). Maximum values of $F(T)$ are set to zero. In panels (a) and (b) the data for dimer and tetramer are shown by open and filled circles, respectively. The figure suggests that the formation of oligomer is a continuous transition without intermediate states.

ing a peptide into oligomer. The free energy profiles for the tetramer and dimer in Fig. 2(a) are similar, although the dimer profile is shallower. According to Fig. 2(a), the free energy of incorporating a peptide into the tetramer ΔF_{O-D} is $\approx -6.4RT$ and is reduced to $-3.7RT$ for the dimer. Therefore, compared to the dimer the free energy of peptide associated state in the tetramer is lower by $\Delta\Delta F_{O-D} = \Delta F_{O-D}(\text{tetra}) - \Delta F_{O-D}(\text{dimer}) \approx -2.7RT$. Both free energy profiles $F(C_h)$ in Fig. 2(a) show no metastable states or barriers suggesting that oligomer formation is a continuous transition. It is also important that the plots $F(C_h)$ remain barrierless in the entire temperature range, in which the oligomer state is stable, i.e., P_4 or $P_2 > 0.5$ ($T \lesssim 490$ K).

The temperature dependence of the oligomer free energy $F(T)$ can be indicative of the nature of transition associated with the oligomer formation. For clarity, $F(T)$ includes the contributions from inter- and intrapeptide interactions and conformational entropy but omits the ideal gas term associated with translational entropy. If the structural transition is continuous, then $F(T)$ is expected to follow a quadratic dependence on temperature at $T < T_o$, i.e., $F(T) \sim -(T - T_o)^2$, where T_o is the temperature, at which oligomer formation is completed⁵³ (see Sec. IV). Figure 2(b) demonstrates that the tetramer $F(T)$ can be fit using the quadratic function with $T_o = 382$ K. The dimer $F(T)$ also follows the quadratic dependence, from which we obtained $T_o = 371$ K. Smaller value of T_o for the dimer is consistent with the smaller free energy gain associated with incorporating a peptide into the dimer compared to the tetramer.

The analysis of the density profile in the oligomer is presented in Fig. 3(a). This figure shows the normalized radial distribution function for the number density $g(r)$ in the tetramer and dimer. Two observations follow from this plot. First, the density profiles for both oligomers are qualitatively similar. As expected the dimer $g(r)$ decreases with r faster than for the tetramer indicating smaller spatial dimensions of the dimer. Second, the profiles of $g(r)$ suggest that there are two volume regions in the oligomer—the core with relatively constant density (especially in the tetramer) and the surface layer in which the density rapidly decays. To get better understanding of the density distribution, we plot in Fig. 3(b) the tetramer functions $g(r)$ computed in the temperature range of 330–490 K. It is seen that the inflection point at $r \approx 15$ Å does not depend on temperature and approximately corresponds to the midpoint of $g(r)$ profile. This suggests that with the increase in temperature, a redistribution of oligomer atoms between the core and the surface layer takes place. As a result, the surface layer (and the oligomer itself) expands with temperature.

To probe the changes in tetramer structure with temperature, we assume that the tetramer core is confined to the sphere with the radius R_c defined from the condition $g(R_c) = 0.7g(0)$. The surface layer is restricted to the concentric volume with the radii (R_c, R_s) , where R_s is obtained from $g(R_s) = 0.3g(0)$. The inset to Fig. 3(b) demonstrates that the core radius R_c remains almost constant at $330 \text{ K} < T \leq 490 \text{ K}$ by changing merely 14%. At 360 K the core size is $R_c \approx 14.0$ Å. At high temperatures ($T \geq 490 \text{ K}$), the core dramatically expands due to the dissociation of the tetramer. In contrast, the surface thickness $\Delta R = R_s - R_c$ monotonically increases threefold in the temperature range of 330–490 K. At $T \geq 490 \text{ K}$, ΔR approaches R_c . The temperature dependencies of R_c and ΔR for the dimer are very similar to those of the tetramer. At 360 K the size of the dimer core R_c is 11.0 Å, which is 3.0 Å smaller than of the tetramer. This difference in R_c is consistent with the difference in the number of atoms in the dimer and tetramer.

Using the radial distribution functions $g(r)$ and the values of core radii R_c , it is straightforward to compute the average number density in the oligomer core n_c . The temperature dependence $n_c(T)$ for the tetramer and dimer shown in Fig. 4 demonstrates that the oligomer core becomes more

FIG. 3. (a) Normalized radial distribution function for the number density $g(r)$ in the A β tetramer (thick line) and dimer (thin line) at 360 K. The distance r is measured with respect to the center of mass of the oligomer. The profiles of $g(r)$ suggest that the oligomer volume can be divided into two regions—the core ($r < R_c$) and the surface layer ($R_c < r < R_s$). (b) Normalized radial distributions $g(r)$ for the tetramer computed in the temperature range from 330 to 490 K with 5 K increment. The plots implicate the expansion of the oligomer with the increase in temperature. Inset: Temperature dependencies of the core radius R_c (in black) and the thickness of surface layer ΔR (in gray): tetramer (filled circles) and dimer (open circles). Monotonic increase in ΔR with temperature coupled with relatively constant R_c suggests that the oligomer expansion is due to the swelling of the surface layer. In (a) and (b) dotted lines mark the minimum density levels in the core ($0.7g(0)$) and in the surface layer ($0.3g(0)$).

dense with the decreases in T . Because the core dimensions remain almost constant with temperature [Fig. 3(b)], this implies that the peptide atoms are “pumped” (redistributed) into the core from the surface layer. This figure also reveals that the tetramer and dimer densities at $T \leq 400 \text{ K}$ are almost the same. As the core absorbs more peptide atoms, it is of interest to consider the temperature dependence of the fraction of atoms in the core, $\Phi_c(T)$. The inset of Fig. 4 reveals that more than half of tetramer and dimer atoms make up the core at the temperatures $T \leq T_c \approx 370 \text{ K}$. One can interpret T_c as the temperature at which the formation of oligomer is completed. We note that T_c are in agreement with the temperatures of oligomer formation T_o estimated from the temperature dependence of the free energy [Fig. 2(b)].

FIG. 4. The temperature dependence of the core number density $n_c(T)$ in $A\beta$ oligomer. The plot shows that the atom packing in the core increases at low temperatures. Inset: the fraction of oligomer atoms in the core, $\Phi_c(T)$, as a function of temperature. The temperature, at which half of $A\beta$ atoms are confined to the core, approximately coincides with the temperature of oligomer formation T_o [Fig. 2(b)]. Dotted line marks the level $\Phi_c=0.5$. Data for the dimer and tetramer are shown by open and filled circles, respectively.

B. Structure of $A\beta$ oligomers

The analysis of the density distribution presented above suggests that $A\beta$ oligomer is a two-tier system in which the core region of nearly constant density is surrounded by the surface layer of rapidly decreasing density. Because $A\beta$ peptide is a heteropolymer, it is conceivable that the sequence regions Nt and Ct have different propensities to form the core and surface layer. To explore this possibility, we computed the distribution of relative solvent accessible surface areas ($\langle rASA(i) \rangle$) of $A\beta$ amino acids i in the tetramer and dimer [Fig. 5(a)]. There are considerable variations in $\langle rASA(i) \rangle$ along the sequence, but two conclusions can be drawn. First, although there is a small systematic decrease in $rASA$ in the tetramer compared to the dimer, their profiles $\langle rASA(i) \rangle$ remain qualitatively similar. More importantly, the distribution of $rASA$ values indicates that the C-terminal is exposed to solvent to a larger extent than the N-terminal. Indeed, for the dimer the ratio $\langle rASA(Ct) \rangle / \langle rASA(Nt) \rangle \approx 1.2$, whereas for the tetramer it increases to 1.3. (Here $\langle rASA(Nt) \rangle$ and $\langle rASA(Ct) \rangle$ are the average $rASA$ values for the Nt and Ct sequence regions.)

To directly check the residue distribution in the oligomer volume, we computed the probability of occurrence of the residue i in the core, $P_c(i)$. In these computations the residue position is given by the location of its side chain center of mass. The plot of $P_c(i)$ shown in Fig. 5(b) reveals that the probability of finding the N-terminal Nt in the tetramer core is significantly higher than of the C-terminal Ct. Specifically, the average probabilities of occurrence in the core of the Nt and Ct residues are $P_c(Nt) \approx 0.59$ and $P_c(Ct) \approx 0.33$, respectively, i.e., these probabilities differ nearly twofold. Similar results were obtained for $A\beta$ dimer ($P_c(Nt) \approx 0.57$, $P_c(Ct) \approx 0.40$). Therefore, the oligomer core is predominantly composed of the amino acids from the $A\beta$ N-terminal.

Further support for the burial of the N-terminal in the tetramer core comes from the distribution of fluctuations in

FIG. 5. (a) The distribution of relative solvent accessible surface areas of amino acids i , $\langle rASA(i) \rangle$, in $A\beta$ peptide. (b) Probabilities of occurrence of the residues i in the oligomer core, $P_c(i)$. Data for the dimer and tetramer are shown by open and filled circles, respectively. Inset: standard deviations in the backbone dihedral angles $\phi(i)$, $\delta\phi(i)$ (black bars), and $\psi(i)$, $\delta\psi(i)$ (gray bars) in the tetramer as a function of the amino acid index i . The figure shows that the N-terminal Nt is mostly buried in the oligomer core, whereas the C-terminal Ct forms the surface layer. The Nt and Ct amino acids in $A\beta$ peptide are boxed. The plots are computed at 360 K.

the backbone dihedral angles $\phi(i)$ and $\psi(i)$ along $A\beta$ sequence (i is a residue index). It follows from the inset of Fig. 5(b) that the standard deviation in the ψ dihedral angle, $\delta\psi(i)$, has little variation with i . For example, the average values of $\delta\psi(i)$ for the Nt and Ct sequence regions are 82° and 88° . However, there is more than twofold difference in the standard deviations in the ϕ dihedral angle, $\delta\phi(i)$, between the Nt and Ct (30° versus 64° , respectively). Large values of $\delta\phi(i)$ in the Ct region are attributed to Gly residues. Therefore, the C-terminals in $A\beta$ tetramer experience larger structural fluctuations than the N-terminals. Similar results have been obtained for the dimer (data not shown).

IV. DISCUSSION

A. $A\beta$ oligomer resembles polymer globule

Our results suggest that $A\beta$ oligomer formation is described by a continuous structural transition. This conclusion is based on two lines of evidence. First, we showed that the

free energy landscape associated with oligomer formation appears to be barrierless. Indeed, the peptide free energy $F(C_h)$ computed as a function of the number of interpeptide hydrophobic contacts C_h reveals no metastable states [Fig. 2(a)]. This observation holds in the entire temperature range in which oligomer (dimer or tetramer) is stable. Second, the oligomer free energy $F(T)$ follows a quadratic temperature dependence near the temperature of oligomer formation T_o [Fig. 2(b)]. In statistical mechanics a quadratic dependence of the free energy of the low symmetry phase on the external parameter (in our case, temperature $T < T_o$) is associated with continuous phase transition.⁵³

The continuous nature of oligomer formation is also consistent with the density distribution in the oligomer. Figure 3 shows that the oligomer volume can be separated into the core with relative flat density profile and the surface layer in which density rapidly decreases. Importantly, the core dimensions remain fairly constant with temperature, whereas the surface layer expands with temperature increase. According to Fig. 4, as temperature decreases the peptide atoms are pumped from the surface layer into the core, causing a steady increase in the core density. This density distribution in the oligomer strongly resembles the one observed in the polymer globule near the coil-globule transition.⁵⁴ According to the mean field theory of coil-globule transition polymer globule expands with the increase in temperature due to swelling of the surface layer. Through the volume of the core the density remains constant, but its value decreases as the system approaches the coil transition.

The globule formation is a continuous (barrierless) transition as long as a polymer remains flexible,⁵⁴ i.e., its persistence length l is comparable with the chain thickness d . We check if this requirement is satisfied for $A\beta$ tetramer. The thickness d can be estimated from the packing of $A\beta$ peptides in the experimentally resolved fibril structure.^{21,47} In this structure $A\beta$ peptides are arranged in parallel in-register β -sheets. The average interpeptide distance between the $C\alpha$ atoms from matching residues is $d \approx 4.8$ Å. To compute the persistence length l we define the backbone vectors $\vec{r}(i) = \vec{R}_{C\alpha}(i+1) - \vec{R}_{C\alpha}(i)$, where $\vec{R}_{C\alpha}(i)$ is the radius vector of the $C\alpha$ atom in the residue i . The autocorrelation function $s(k) = \overline{\vec{r}(i) \cdot \vec{r}(i+k)}$, where the bar indicates an average over i , peptide, and all equilibrated tetramer structures, probes the correlations in the chain orientation as a function of the residue distance k . Consistent with the polymer theory,⁵⁴ $s(k)$ can be fit with an exponential function $s(k) \sim \exp(-k/k_0)$, where k_0 is a characteristic distance of correlation decay. The persistence length l can be computed using $l = k_0 \langle r \rangle$, where $\langle r \rangle$ is the average distance between successive $C\alpha$ atoms. At $T = 490$ K, which approximately corresponds to the tetramer dissociation, $l \approx 4.8$ Å. The persistence length is reduced to 3.8 Å at 360 K due to entanglement of $A\beta$ chains in dense oligomer core. It is important that at both temperatures $l \lesssim d$, indicating that the backbone of $A\beta$ peptides is flexible. Therefore, exploiting the analogy with the polymer globule, we propose that the oligomer formation is consistent with continuous transition. The continuous transition is likely the result of low structural ordering in small $A\beta$ oligomers.

It is also useful to mention that continuous structural transition is predicted to occur during denaturation of double-stranded heterogeneous DNA.⁵⁵ Furthermore, according to spectrometry experiments DNA oligomers formed by short strands (from 15 to 60 bases) demonstrate although sharp but still continuous denaturation transition at the concentration of 100 mM.⁵⁶ These experimental and theoretical results lend support to our findings that the oligomer formation is continuous at least in the range of millimolar polymer concentrations. Additional studies will be needed to establish if continuous nature of oligomer assembly remains valid at lower peptide concentrations.

B. Structural properties of $A\beta$ dimers and tetramers are similar

Analysis of $A\beta$ dimer and tetramer suggests that their properties are similar. Above we have shown that the tetramer and dimer assembly resembles the formation of polymer globule. The temperatures T_o , at which the assembly of these oligomers is completed, differ only by about 10 K. Now we further compare $A\beta$ dimers and tetramers by considering specific structural quantities. First, it is noteworthy that according to Fig. 4, the tetramer and dimer densities at $T \lesssim 400$ K are similar. In addition, the thicknesses of surface layers ΔR in $A\beta$ dimer and tetramer are almost identical [Fig. 3(b)]. Second, computations of accessible surface areas of $A\beta$ residues suggest very similar trends in the exposure of individual amino acids in both oligomers [Fig. 5(a)]. In the dimer and tetramer, the C-terminal is mostly found in the surface layer and is exposed to solvent, whereas the N-terminal tends to be buried in the oligomer core [Fig. 5(b)]. $A\beta$ peptides in both oligomers have uneven distribution of structural fluctuations along the sequence, with the C-terminal being more flexible than the N-terminal. Third, the analysis of the energetics of $A\beta$ peptides in the dimers and tetramers also points out to the similarities in their conformational ensembles. For example, at 360 K the average energy of intrapeptide nonbonded interactions in the dimer is -229 kcal/mol, which is very close to the tetramer value (-216 kcal/mol).

In our previous studies we have reported that the aggregation interface in $A\beta$ dimers predominantly involves the N-terminal.^{35,41} Specifically, the numbers of interpeptide side chain contacts formed by the Nt and Ct regions, $\langle C(Nt) \rangle$ and $\langle C(Ct) \rangle$, differ twofold at 360 K. For the tetramer, the values of $\langle C(Nt) \rangle$ and $\langle C(Ct) \rangle$ are 30.2 and 14.9, respectively, which imply almost identical ratios of these quantities for the tetramer and dimer. Therefore, the conformational ensembles of $A\beta$ peptides sampled in the dimer and tetramer appear to be similar.

Our conclusion about the importance of the Nt region for aggregation is consistent with some of the recent implicit solvent MD studies.^{36,57,58} In particular, Urbanc *et al.* showed that the central hydrophobic cluster (residues 17–21) plays a dominant role in $A\beta_{1-40}$ aggregation.⁵⁸ However, given a complexity of $A\beta$ oligomer assembly, studies with explicit solvent models will be needed to ascertain the role of the Nt region in aggregation.

C. Comparison of experiments and simulations

It is important to compare our structural analysis of $A\beta$ oligomers with experimental data. Although we are not aware of the experiments probing the thermodynamics of $A\beta$ oligomer formation, few recent studies have explored the distribution and sizes of these mobile aggregates. Gel electrophoresis experiments have shown that $A\beta$ peptides exist as monomers or oligomers of the sizes up to tetramer at micromolar concentration.^{12,59} It follows from the electron microscopy studies that the dimers and tetramers have overall spherical shape with the diameter D increasing from 18 Å (dimer) to 220 Å (tetramer).¹² The experimental size of the dimer is in a good agreement with our estimate of the size of the dimer core ($D=2R_c=22$ Å). Further experimental and simulation studies will clarify the discrepancy in the tetramer sizes.

Our conclusion that the structural properties of the dimers and tetramers are similar is in accord with the experimental analysis of the secondary structure of $A\beta$ oligomers.¹² The fractions of the residues in β -sheet conformation in the dimer and tetramer are 0.39 and 0.45, whereas the α -helix fractions are 0.11 and 0.13, respectively. These experimental data do not reveal considerable variation in the structures of $A\beta$ oligomers. It is important to emphasize that the conformational ensemble of $A\beta$ oligomers is distinct from those associated with $A\beta$ monomers or fibrils as shown in recent simulations of O'Brien *et al.*³¹

Finally, we discuss an apparent discrepancy in the oligomer size distribution between experiments and simulations. In our two- and four-peptide systems, the largest possible oligomers (dimer or tetramer) are formed with an overwhelming probability (~ 1.0). At the same time, experiments detect a continuous distribution of $A\beta$ species from monomers to tetramers.^{12,59} The likely source of this discrepancy is an elevated $A\beta$ concentration used in our study. The experimental measurements are performed at micromolar concentrations, whereas the $A\beta$ concentration in the simulations is in the millimolar range (that constitutes a 10^3 difference in concentrations). *In silico* elevated $A\beta$ concentration is used for computational efficiency to enhance sampling of aggregated states. It is therefore reasonable to suggest that with the increase in $A\beta$ concentration, the thermodynamic equilibrium is shifted to higher order oligomers disfavoring monomeric states or small oligomers.

V. CONCLUSIONS

We have investigated two oligomeric species of $A\beta$ peptides, dimer and tetramer, which are typically observed in *in vitro* experiments. Based on the analysis of free energy landscapes, density distributions, and chain flexibility, we proposed that the oligomer assembly is a continuous transition occurring without metastable states. The density distribution computations revealed that $A\beta$ oligomer consists of the core with fairly flat density profile and the surface layer with rapidly decreasing density. The core is mostly formed by the N-terminal residues, whereas the C-terminal tends to occur in the surface layer. Lowering the temperature results in the redistribution of peptide atoms from the surface layer

into the core. Taken together these findings led us to argue that $A\beta$ oligomers resemble polymer globules in poor solvent. $A\beta$ dimers and tetramers are found to be structurally similar suggesting that the conformations of $A\beta$ peptides do not depend on the order of small oligomers. The available experimental data appear to support the conclusions of our study.

ACKNOWLEDGMENTS

This work was supported by the National Institute on Aging (NIH) under Grant No. R01 AG028191. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Aging or NIH.

- ¹C. M. Dobson, *Nature (London)* **426**, 884 (2003).
- ²M. D. Kirkitadze, G. Bitan, and D. B. Teplow, *J. Neurosci. Res.* **69**, 567 (2002).
- ³R. M. Murphy, *Annu. Rev. Biomed. Eng.* **4**, 155 (2002).
- ⁴R. Nelson, M. R. Sawaya, M. Balbirnie, A. O. Madsen, C. Riek, R. Grothe, and D. Eisenberg, *Nature (London)* **435**, 773 (2005).
- ⁵O. S. Makin, E. Atkins, P. Sikorski, J. Johansson, and L. C. Serpell, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 315 (2005).
- ⁶M. Reches and E. Gazit, *Science* **300**, 625 (2003).
- ⁷Y. Song, S. R. Challa, C. J. Medforth, Y. Qiu, R. K. Watt, D. Pea, J. E. Miller, F. van Swolab, and J. A. Shelnutt, *Chem. Commun. (Cambridge)* **2004**, 1044.
- ⁸D. J. Selkoe, *Nature (London)* **426**, 900 (2003).
- ⁹J. Hardy and D. J. Selkoe, *Science* **297**, 353 (2002).
- ¹⁰S. Narayanan and B. Reif, *Biochemistry* **44**, 1444 (2005).
- ¹¹G. Bitan, S. S. Vollers, and D. B. Teplow, *J. Biol. Chem.* **278**, 34882 (2003).
- ¹²K. Ono, M. M. Condron, and D. B. Teplow, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 14745 (2009).
- ¹³R. Kaye, E. Head, J. L. Thompson, T. M. McIntire, S. C. Milton, C. W. Cotman, and C. G. Glabe, *Science* **300**, 486 (2003).
- ¹⁴C. Haass and D. J. Selkoe, *Nat. Rev. Mol. Cell Biol.* **8**, 101 (2007).
- ¹⁵M. T. Pastor, N. Kmmere, V. Schubert, A. Esteras-Chopo, C. G. Dotti, M. L. de la Paz, and L. Serrano, *J. Mol. Biol.* **375**, 695 (2008).
- ¹⁶G. M. Shankar, S. Li, T. H. Mehta, A. Garcia-Munoz, N. E. Shepardson, I. Smith, F. M. Brett, M. A. Farrell, M. J. Rowan, C. A. Lemere, C. M. Regan, D. M. Walsh, B. L. Sabatini, and D. J. Selkoe, *Nat. Med.* **14**, 837 (2008).
- ¹⁷N. Carulla, G. L. Caddy, D. R. Hall, J. Zurdo, M. Gair, M. Feliz, E. Giral, C. V. Robinson, and C. M. Dobson, *Nature (London)* **436**, 554 (2005).
- ¹⁸W. P. Esler, E. R. Stimson, M. J. Lachenmann, J. R. Ghilardi, Y. Lu, H. V. Vinters, P. W. Mantyh, J. P. Lee, and J. E. Maggio, *J. Struct. Biol.* **130**, 174 (2000).
- ¹⁹R. Riek, P. Guntert, H. Dobeli, B. Wipf, and K. Wuthrich, *Eur. J. Biochem.* **268**, 5930 (2001).
- ²⁰T. S. Burkoth, T. Benzinger, V. Urban, D. M. Morgan, D. M. Gregory, P. Thiagarajan, R. E. Botto, S. C. Meredith, and D. G. Lynn, *J. Am. Chem. Soc.* **122**, 7883 (2000).
- ²¹A. T. Petkova, W.-M. Yau, and R. Tycko, *Biochemistry* **45**, 498 (2006).
- ²²T. Luhrs, C. Ritter, M. Adrian, D. R. Lohr, B. Bohrmann, H. Dobeli, D. Schubert, and R. Riek, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 17342 (2005).
- ²³B. Ma and R. Nussinov, *Curr. Opin. Struct. Biol.* **10**, 445 (2006).
- ²⁴D. B. Teplow, N. D. Lazo, G. Bitan, S. Bernstein, T. Wytenbach, M. T. Bowers, A. Baumketner, J.-E. Shea, B. Urbanc, L. Cruz, J. Borreguero, and H. E. Stanley, *Acc. Chem. Res.* **39**, 635 (2006).
- ²⁵N. G. Sgourakis, Y. Yan, S. A. McCallum, C. Wang, and A. E. Garcia, *J. Mol. Biol.* **368**, 1448 (2007).
- ²⁶M. Yang and D. B. Teplow, *J. Mol. Biol.* **384**, 450 (2008).
- ²⁷M. Cecchini, R. Curcio, M. Pappalardo, R. Melki, and A. Caflisch, *J. Mol. Biol.* **357**, 1306 (2006).
- ²⁸A. Baumketner and J.-E. Shea, *J. Mol. Biol.* **362**, 567 (2006).
- ²⁹B. Tarus, J. E. Straub, and D. Thirumalai, *J. Am. Chem. Soc.* **128**, 16159 (2006).
- ³⁰N.-V. Buchete and G. Hummer, *Biophys. J.* **92**, 3032 (2007).

- ³¹E. P. O'Brien, Y. Okamoto, J. E. Straub, B. R. Brooks, and D. Thirumalai, *J. Phys. Chem. B* **113**, 14421 (2009).
- ³²G. Reddy, J. E. Straub, and D. Thirumalai, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 11948 (2009).
- ³³Y. Lu, P. Derreumaux, Z. Guo, N. Mousseau, and G. Wei, *Proteins* **75**, 954 (2009).
- ³⁴G. Bellesia and J.-E. Shea, *Biophys. J.* **96**, 875 (2009).
- ³⁵T. Takeda and D. K. Klimov, *Proteins: Struct., Funct., Bioinf.* **77**, 1 (2009).
- ³⁶P. Anand, F. S. Nandel, and U. H. E. Hansmann, *J. Chem. Phys.* **129**, 195102 (2008).
- ³⁷B. Urbanc, L. Cruz, S. Yun, S. V. Buldyrev, G. Bitan, D. B. Teplow, and H. E. Stanley, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 17345 (2004).
- ³⁸B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, and M. Karplus, *J. Comput. Chem.* **4**, 187 (1983).
- ³⁹P. Ferrara, J. Apostolakis, and A. Caflisch, *Proteins* **46**, 24 (2002).
- ⁴⁰T. Takeda and D. K. Klimov, *Biophys. J.* **96**, 4428 (2009).
- ⁴¹T. Takeda and D. K. Klimov, *J. Phys. Chem. B* **113**, 6692 (2009).
- ⁴²T. Takeda and D. K. Klimov, *J. Phys. Chem. B* **113**, 11848 (2009).
- ⁴³L. Hou, H. Shao, Y. Zhang, H. Li, N. K. Menon, E. B. Neuhaus, J. M. Brewer, I.-J. L. Byeon, D. G. Ray, M. P. Vitek, T. Iwashita, R. A. Makula, A. B. Przybyla, and M. G. Zagorski, *J. Am. Chem. Soc.* **126**, 1992 (2004).
- ⁴⁴P. Ferrara and A. Caflisch, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 10780 (2000).
- ⁴⁵A. Hiltbold, P. Ferrara, J. Gsponer, and A. Caflisch, *J. Phys. Chem. B* **104**, 10080 (2000).
- ⁴⁶M. Cecchini, F. Rao, M. Seeber, and A. Caflisch, *J. Chem. Phys.* **121**, 10748 (2004).
- ⁴⁷T. Takeda and D. K. Klimov, *Biophys. J.* **96**, 442 (2009).
- ⁴⁸See supplementary material at <http://dx.doi.org/10.1063/1.3447894> for the applicability of implicit solvent model and REMD convergence.
- ⁴⁹A. K. Paravastu, A. T. Petkova, and R. Tycko, *Biophys. J.* **90**, 4618 (2006).
- ⁵⁰Y. Sugita and Y. Okamoto, *Chem. Phys. Lett.* **314**, 141 (1999).
- ⁵¹G. D. Rose, A. R. Geselowitz, G. J. Lesser, R. H. Lee, and M. H. Zehfus, *Science* **229**, 834 (1985).
- ⁵²A. M. Ferrenberg and R. H. Swendsen, *Phys. Rev. Lett.* **63**, 1195 (1989).
- ⁵³L. D. Landau and E. M. Lifshitz, "Course of Theoretical Physics," *Statistical Physics* (Butterworth-Heinemann, Oxford, 1984), Vol. 5.
- ⁵⁴A. Y. Grosberg and A. R. Khokhlov, *Statistical Physics of Macromolecules* (AIP, Woodbury, 1994).
- ⁵⁵D. Cule and T. Hwa, *Phys. Rev. Lett.* **79**, 2375 (1997).
- ⁵⁶G. Zocchi, A. Omerzu, T. Kuriabova, J. Rudnick, and G. Gruner, e-print arXiv:cond-mat/0304567v1 (2003).
- ⁵⁷A. Melquiond, X. Dong, N. Mousseau, and P. Derreumaux, *Curr. Alzh. Res.* **5**, 244 (2008).
- ⁵⁸B. Urbanc, M. Betnel, L. Cruz, G. Bitan, and D. B. Teplow, *J. Am. Chem. Soc.* **132**, 4266 (2010).
- ⁵⁹S. K. Maji, R. R. Ogorzalek, M. Inayathullah, S. M. Spring, S. S. Vollers, M. M. Condron, G. Bitan, J. A. Loo, and D. B. Teplow, *J. Biol. Chem.* **284**, 23580 (2009).
- ⁶⁰E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, and T. E. Ferrin, *J. Comput. Chem.* **25**, 1605 (2004).