Molecular Dynamics Simulation of Amyloid β Dimer Formation

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ABSTRACT

Recent experiments with amyloid-β (Aβ) peptide suggest that formation of toxic oligomers may be an important contribution to the onset of Alzheimer’s disease. The toxicity of Aβ oligomers depends on their structure, which is governed by assembly dynamics. Due to limitations of current experimental techniques, a detailed knowledge of oligomer structure at the atomic level is missing. We introduce a molecular dynamics approach to study Aβ dimer formation: (1) we use discrete molecular dynamics simulations of a coarse–grained model to identify a variety of dimer conformations, and (2) we employ all-atom molecular mechanics simulations to estimate the thermodynamic stability of all dimer conformations. Our simulations of a coarse–grained Aβ peptide model predicts ten different planar β-strand dimer conformations. We then estimate the free energies of all dimer conformations in all-atom molecular mechanics simulations with explicit water. We compare the free energies of Aβ(1–42) and Aβ(1–40) dimers. We find that (a) all dimer conformations have higher free energies compared to their corresponding monomeric states, and (b) the free energy difference between the Aβ(1–42) and the analogous Aβ(1–40) dimer conformation is not significant. Our results suggest that Aβ oligomerization is not accompanied by the formation of stable planar β-strand Aβ dimers.

Keywords: Alzheimer’s disease, coarse–grained peptide model, monomer conformation, dimer conformation, free energy, stability

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I. INTRODUCTION

Alzheimer’s disease (AD) is neuropathologically characterized by progressive neuronal loss, extracellular amyloid plaques and intracellular neurofibrillary tangles (Yankner, 1996; Selkoe, 1997). Fibrillar amyloid plaques, a result of Aβ peptide aggregation, have been implicated in the pathogenesis of AD. Recent experimental studies on Aβ peptide (Lambert et al., 1998; El-Agnaf et al., 2000; El-Agnaf et al., 2001; Dahlgren et al., 2002) as well as various animal model studies (Hsia et al., 1999; Mucke et al., 2000; Dodart et al., 2002; Westerman et al., 2002; Walsh et al., 2002) suggest that soluble forms of Aβ assemblies cause substantial neuronal dysfunction even before the appearance of amyloid plaques. Hence, finding the conformation of these oligomeric forms of Aβ may be important for understanding of neurotoxicity in AD (Kirkitadze et al., 2002; Klein et al., 2001; Klein, 2002a; Klein, 2002b; Bucciantini et al., 2002; Kayed et al., 2003). At present, the precise nature, conformation, and time evolution from monomer Aβ peptides into intermediates is still unknown.

The fibrillar structure of Aβ peptide aggregates is relatively well established. Experiments have targeted the structure of Aβ fibrils using electron microscopy (Malinchik et al., 1998; Tjernberg et al., 1999; Tjernberg et al., 2002), X-ray diffraction (Malinchik et al., 1998; Serpell et al., 2000), electron paramagnetic resonance spectroscopy (Török et al., 2002) and solid state nuclear magnetic resonance spectroscopy (Balbach et al., 2002; Petkova et al., 2002; Antzutkin et al., 2002; Thompson, 2003; Antzutkin et al., 2003). The most common view is that Aβ(1-40) and Aβ(1-42) in fibrils form parallel β-sheets with a β-turn between residues Asp 23 and Lys 28. The most flexible regions of the peptide in a fibril are the first 10 amino acids of the N-terminus, last few amino acids of the C-terminus (residues 39-42), and the β-turn region between residues 23 and 28 (Petkova et al., 2002; Török et al., 2002).

The aggregation process from a monomer Aβ peptide via soluble oligomeric states to fibrils is a complex dynamic event which depends critically on the peptide concentration, pH, and solvent properties. Structural studies have shown that in vitro, Aβ fibril formation is preceded by formation of intermediates, spherical oligomeric states and protofibrils (Walsh et al., 1997; Hartley et al., 1999; Walsh et al., 1999; Kirkitadze et al., 2001; Yong et al., 2002). Structural studies on oligomeric states are in a less advanced stage compared to those in fibrils. The nature and structure of different oligomeric states may depend crucially on the specific amino acid sequence of the peptide (Nilsberth et al., 2001). The Aβ plaques
in AD brain are predominantly comprised of two Aβ alloforms, Aβ(1-40) and Aβ(1-42). Despite the relatively small structural difference between these two alloforms, they display distinct behavior, with Aβ(1–42) being a predominant component of parenchymal plaques (Suzuki et al., 1994; Iwatsubo et al., 1994; Gravina et al., 1995), associated with both early onset AD (Scheuner et al., 1996; Golde et al., 2000) and increased risk for AD (Weggen et al., 2001). The cause of the clinical differences between the two alloforms is still unknown. Recent experiments have shown that in vitro Aβ(1–40) and Aβ(1–42) oligomerize through distinct pathways with Aβ(1–42) forming spherical paranuclei which further assemble into higher order oligomers (Bitan et al., 2001; Bitan et al., 2003a; Bitan et al., 2003b).

Several studies found stable soluble Aβ low molecular weight oligomers (Barrow and Zagorski, 1991; Barrow et al., 1992; Zagorski and Barrow, 1992; Soreghan et al., 1994; Shen and Murphy, 1995; Podlisny et al., 1995; Roher et al., 1996; Kuo et al., 1996; Garzon-Rodriguez et al., 1997; Xia et al., 1995; Enya et al., 1999; Funato et al., 1999; Huang et al., 2000). Low molecular weight oligomers were found in culture media of Chinese hamster ovary cells expressing endogenous or mutated genes (Podlisny et al., 1995; Xia et al., 1995). Aβ(1–40) and Aβ(1–42) oligomers, specifically dimers, were isolated from human control and AD brains (Kuo et al., 1996; Kuo et al., 1998; Enya et al., 1999; Funato et al., 1999). Dimers and trimers of Aβ were isolated from neuritic and vascular amyloid deposits and dimers were shown to be toxic to neurons in the presence of microglia (Roher et al., 1996). Experiments on synthetic Aβ peptides (Garzon-Rodriguez et al., 1997; Podlisny et al., 1998) showed that soluble Aβ(1–40) exists as a stable dimer at physiological concentrations which are well below the critical micelle concentration (Soreghan et al., 1994).

It has been shown that the β-sheet content of Aβ depends strongly upon the solvent in which the peptide is dissolved (Shen and Murphy, 1995). Various experimental studies (Barrow and Zagorski, 1991; Barrow et al., 1992; Zagorski and Barrow, 1992; Shen and Murphy, 1995) indicate that soluble Aβ has substantial β-sheet content. Huang et al. (Huang et al., 2000) reported on two types of soluble oligomers of Aβ(1-40) which were trapped and stabilized for an extended period of time: the first type was a mixture of dimers and tetramers with irregular secondary structure and the second type corresponded to larger spherical particles with β-strand structure. Despite some discrepancies in the experimental results, the studies mentioned above suggest that dimerization may be the initial event in amyloid aggregation and thus dimers may be fundamental building blocks for further fibril
assembly.

Experimental methods, such as circular dichroism, nuclear magnetic resonance and electron microscopy, provide only limited information on the structure of intermediate oligomeric states. Therefore, there is a motivation to develop new computational approaches to determine the exact conformation of oligomers at the atomic level and track the exact pathway from individual monomer peptides to oligomers and protofibrils in fast and efficient ways. With the dramatic increase of computer power in recent decades, it has become possible to study the behavior of large biological molecular systems by Monte Carlo and molecular dynamics (MD) simulations (Dinner et al., 2002; Fersht and Daggett, 2002; Karplus and McCammon, 2002; Thirumalai et al., 2002; Plotkin and Onuchic, 2002; Mendes et al., 2002; Mirny and Shakhnovich, 2001; Bonneau and Baker, 2001; Dill, 1999; Levitt et al., 1997; Wolynes et al., 1996; Snow et al., 2002; Vorobjev and Hermans, 2001; Zhou and Karplus, 1997). However, traditional all-atom MD with realistic force fields in a physiological solution currently remains computationally unfeasible. An aggregation process as allowed by all-atom MD can only be studied on time scales of up to $10^{-7}$ s using such advanced technologies as worldwide distributed computing (Snow et al., 2002; Zagrovic et al., 2002). However, in vivo and in vitro studies suggest that the initial stages of oligomerization occur on a time scale of one second (Bitan et al., 2003a), while further aggregation into protofibrillar and fibrillar aggregates may span hours (Kayed et al., 2003).

Here we conduct a two–step study of Aβ dimer conformations and their stability using a computationally efficient algorithm combined with a coarse–grained peptide model for Aβ. We apply a four–bead model for Aβ peptide to study monomer and dimer conformations of Aβ(1–42) peptide (Ding et al., 2003). We use fast and efficient discrete molecular dynamics (DMD) simulations (Dokholyan et al., 1998; Smith and Hall, 2001a). The DMD method allows us to find and study a large variety of dimer conformations starting from initially separated monomers without secondary structures. Our coarse–grained model combined with the DMD method predicts 10 different planar β-strand dimer conformations. In the second step, we estimate the free energy of Aβ(1–42) and Aβ(1–40) dimeric conformations in a stability study using all-atom MD simulations with explicit water and well-established force fields. This second step enables us to estimate the free energy of different dimeric conformations and to compare the free energies of Aβ(1–42) and Aβ(1–40) for each of the dimer peptides. Our results suggest that Aβ oligomerization is not accompanied by the
formation of stable planar $\beta$-strand $A\beta$ dimers, and that such dimers of both $A\beta(1-42)$ and $A\beta(1-40)$ are equally unlikely to represent stable oligomeric forms.

II. METHODS

A. Discrete molecular dynamics simulations

In a DMD simulation, pairs of particles interact by means of spherically-symmetric potentials that consist of one or more square wells. Within each well the potential is constant. Consequently, each pair of particles moves with constant velocity until they reach a distance at which the potential changes. At this moment a collision occurs and the two particles change their velocities instantaneously while conserving the total energy, momentum, and angular momentum. There are three main types of collisions. The simplest is when particles collide at their hard-core distance, the sum of the particle radii. In this case, the particles collide elastically, and their kinetic energy before and after the collision is conserved. In the second case, the particles enter a potential well of depth $\Delta U$. In this case, their total kinetic energy after the collision increases by $\Delta U$, their velocities increase, and there is a change in their trajectories. In the third case, particles exit a potential well of depth $\Delta U$. Here, total kinetic energy after the collision decreases by $\Delta U$. If the total kinetic energy of the particles is greater than $\Delta U$, they escape the well. If their total kinetic energy is smaller than $\Delta U$, the particles can not escape and simply recoil from the outer border of the well inwards. At low temperatures, which correspond to low average particle kinetic energies, particles whose potentials are attractive thus have a tendency to remain associated with each other.

DMD, unlike traditional continuous MD, is event-driven and as such it requires keeping track of particle positions and velocities only at collision times, which have to be sorted and updated. It can be shown that the speed of the most efficient DMD algorithm is proportional to $N \ln N$, where $N$ is the total number of atoms (Rapaport, 1997). In addition, the speed of the algorithm decreases linearly with the number of discontinuities in the potential and particle density. In our DMD simulations the solvent is not explicitly present, which reduces the number of particles in the system. Consequently, the DMD method is several orders of magnitude faster than the traditional continuous MD. The DMD simulation method has been so far successfully applied to simulate protein folding (Zhou and Karplus, 1997;
Dokholyan et al., 1998; Ding et al., 2002a; Borreguero et al., 2002) and aggregation (Smith and Hall, 2001a; Smith and Hall, 2001b; Ding et al., 2002b; Ding et al., 2003). In simulating protein folding and aggregation, coarse–grained models of proteins have been introduced. In a coarse–grain model the number of atoms per amino acid is reduced to one, two or four, which further speeds up the DMD simulation. While traditional continuous all-atom MD can simulate events on time scales of nanoseconds, the DMD method combined with a coarse–grained protein model can easily reach time scales of seconds or more, which is long enough to study oligomer formation of up to 100 Aβ peptides.

B. A coarse–grained model for Aβ peptide

The Aβ peptide is derived from its larger amyloid precursor protein by sequential proteolytic cleavages. In amyloid plaques, the two most common forms of the Aβ peptide are Aβ(1–40) and Aβ(1–42). The amino acid sequence of Aβ(1–42) is

\[DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA.\]

The amino acid sequence of Aβ(1–40) is the same as that of Aβ(1–42), but shorter by two amino acids at the C-terminus, Ile and Ala (IA in the above sequence).

In our DMD simulations we apply the simplest version of the four–bead model (Takada et al., 1999; Smith and Hall, 2001a; Smith and Hall, 2001b; Ding et al., 2003) for Aβ peptide. In this model, each amino acid in the peptide is replaced by at most four “beads”. These beads correspond to the atoms comprising the amide nitrogen N, the alpha carbon \(C_\alpha\) and prime-carbon \(C'\). The fourth bead, representing the amino acid side-chain atoms, is placed at the center of the nominal \(C_\beta\) atom. Due to their lack of side-chains, the six glycines in Aβ (positions 9, 25, 29, 33, 37 and 38) are represented by only three beads. Two beads that form a permanent bond can assume any distance between the minimum and maximum bond length. In addition to permanent bonds between the beads, the model introduces constraints between pairs of beads which do not form permanent bonds. These constraints are implemented in order to account for the correct peptide backbone geometry. The hard–core radii, minimum and maximum bond lengths, constraints’ lengths and their corresponding standard deviations are either calculated from distributions of experimental distances between pairs of these groups found in about 7700 folded proteins with known
crystal structures (Protein Data Bank), or chosen following the standard knowledge of the geometry of the peptide backbone (Creighton, 1993). The values of all these parameters have been reported previously (Ding et al., 2003).

To account for the hydrogen bonding that normally occurs in proteins between the carbonyl oxygen of one amino acid and the amide hydrogen of another amino acid, the coarse-grained model implements a bond between the nitrogen of the $i-th$ amino acid, $N_i$, and the carbon of the $j-th$ amino acid, $C'_j$, as introduced previously (Ding et al., 2003). The planar geometry of the hydrogen bond is modeled by introducing auxiliary bonds between the left and the right neighboring beads of $N_i$ and $C'_j$. The hydrogen bond between $N_i$ and $C'_j$ will form only if all six beads are at energetically favorable distances. Once the hydrogen bond is formed, it can break due to thermal fluctuations, which can cause energetically unfavorable distances among the six beads involved in the hydrogen bond. When amino acids $i$ and $j$ belong to the same peptide, they can form a hydrogen bond only if at least three amino acids exist between them (to satisfy the 180° NH-CO bond angle). A more detailed description of the hydrogen bond implementation has been given elsewhere (Ding et al., 2003). Our current implementation differs slightly from before (Ding et al., 2003): one of the auxiliary bonds, namely the auxiliary bond between $N_i$ and the bead $N$ (the nearest neighbor of $C'_j$) has a shorter equilibrium distance: instead of $5.10 \pm 0.31\text{Å}$ used previously (Ding et al., 2003), it is $4.70 \pm 0.08\text{Å}$. This slight change in the auxiliary bond length stabilizes the \(\beta\)-hairpin monomer conformation in our model as described in the results section.

C. All-atom molecular dynamics in an explicit solvent

Next we detail how we use all-atom MD simulations in an explicit solvent to compute the conformational free energy of \(A\beta(1-40)\) and \(A\beta(1-42)\) monomer and dimer conformations.

1. Preparation of peptide conformations

For \(A\beta(1-40)\) monomer peptide structures we use ten NMR structures with coordinates (Coles et al., 1998) (ID code name 1BA4 of Protein Database Bank). For each of these ten \(A\beta(1-40)\) monomer structures, we construct a corresponding \(A\beta(1-42)\) monomer structure by adding two residues, Ile and Ala, to the C-terminus of the peptide using the SYBYL
All Aβ(1-42) dimer conformations in this study are generated by DMD simulations using the four–bead model as described above. Dimer conformations, initially in the four–bead representation, are converted into all–atom representation by using all–atom template amino acids. These templates are superposed onto the coarse–grained amino acids such that the four beads of the coarse–grained model coincide with the N, Cα, C' and Cβ groups of the all–atom template amino acids. The new template coordinates with increased number of degrees of freedom are optimized for preserving backbone distances as well as formation of peptide planes. This optimization is performed by rotating the template amino acid along two axes, the Cα–N and the C'–Cα axes using a Monte Carlo algorithm. After positioning the backbone atoms, the positions of side–chain atoms are determined by avoiding steric collisions with the backbone and other neighboring residues. Positioning of side–chain atoms also follows a Monte Carlo algorithm, during which side–chain atoms are rotated sequentially along the Cα–Cβ axis and the Cβ–Cγ axis in order to find the optimal combination of axes angles that prevent collisions. The backbone structure of the resulting peptide remains very close to the initial structure of the peptide: the lengths of bonds and constraints after the conversion are within the limits given by our coarse–grained model. The Aβ(1-40) dimer conformations corresponding to each Aβ(1-42) dimer are constructed by disposing of the last two amino acids of each Aβ(1-42) dimer conformation.

2. Calculation of the conformational free energy in water

We estimate conformational free energies of monomers and dimers in a water environment using all–atom MD simulations. All MD calculations are performed using the Sigma MD program (Hermans et al., 1994) with CEDAR force fields (Ferro et al., 1980; Hermans et al., 1984). We complete the all–atom reconstruction from above by adding hydrogen atoms and solvating the peptide(s) in a SPC water model bath (Berendsen et al., 1981). We use periodic boundary conditions on a cubic box whose sides extended 12Å beyond the leading edge of the peptide(s) on all sides. The MD method consists of two stages, equilibration and production (Vorobjev et al., 1998; Vorobjev and Hermans, 1999; Vorobjev and Hermans, 2001; Leach, 2001). Equilibration allows both the peptides and water to relax to a local energy minimum. The steps of equilibration, (1)-(7), and the production (8) are as follows:
(1) minimize the energy of the water—peptides are kept immobile;

(2) perform MD simulations on the water using the NVT ensemble at a temperature $T = 200K$ for 96ps (the time step is 1fs)—peptides are kept immobile;

(3) minimize the energy of the water a second time—peptides are kept immobile;

(4) minimize the energy of the peptides—water molecules are kept immobile;

(5) perform MD simulations of the peptide using the NVT ensemble at a temperature $(T = 100K)$—water molecules are kept immobile;

(6) minimize the energy of the peptides a second time—water molecules are kept immobile;

(7) minimize the energy of the peptides and water molecules simultaneously;

(8) perform the production run, i.e., unconstrained MD simulations on the peptides and water using the NPT ensemble at $T = 300K$ and $P = 1atm$ for 196ps.

At steps (1), (3), (4), (6) and (7) we use the steepest descent energy minimization method. During steps (2) and (5), which are parts of equilibration, peptide(s) and water coordinates have to reach a local energy minimum for the given force field and with respect to each other. The temperatures are kept low so that there are no conformational changes.

During the production run (8) we maintain constant temperature and pressure by Berendsen coupling (Berendsen et al., 1984) and calculate electrostatic forces using the particle-mesh Ewald procedure (Darden et al., 1993). We record a snapshot of the configuration every picosecond. We calculate the free energy for each conformation by averaging the instantaneous free energy for each of the 196 snapshots. Each of these snapshots represent a microconfiguration. We calculate the free energy for each configuration by the ES/IS method (Vorobjev and Hermans, 1999), which uses an explicit solvent simulation with an implicit solvent continuum model:

$$ G_A = \langle U_m(x) \rangle_A + \langle W(x) \rangle_A - TS_{conf,A}, \quad (1) $$

where $\langle \ldots \rangle_A$ denotes an average over all recorded microconfigurations of the conformation A, $U_m$ is the intra–protein conformational energy, and $S_{conf,A}$ is the entropy of conformation
A. The intra–protein conformational energy, $U_m$, is a sum of two terms: one is the short–range energy of packing, $U_{m,pack}$, and the other is the electrostatic energy due to coulombic interactions, $U_{m,coul}$. The solvation free energy, $W(x)$, is the sum of three terms: the first one, $G_{cav}$, is the energy required to form a cavity in the solvent; the second one, $G_{s,vdw}$, is a contribution of the van der Waals interactions between solvent and protein; and the third one, $G_{pol}$, is a contribution of the electrostatic polarization of the solvent and polar components of the solute. Thus the above equation becomes

$$G_A = \langle U_{m,pack} \rangle_A + \langle U_{m,coul} \rangle_A + \langle G_{cav} \rangle_A + \langle G_{s,vdw} \rangle_A + \langle G_{pol} \rangle_A - TS_{conf,A}. \quad (2)$$

We determine $U_m$ and $G_{s,vdw}$ from the MD trajectory, calculate $G_{cav}$ as proportional to the accessible surface area for a given micro-configuration, and evaluate $G_{pol}$ using an implicit model for the solvent (in our case water) as described elsewhere (Vorobjev and Hermans, 1999).

III. RESULTS

A. Characterization of monomer conformations

The secondary structure of both Aβ(1–40) and Aβ(1–42) monomer peptides, as determined by NMR conformational studies in an apolar environment that mimics the lipid phase of membranes, is predominantly α-helical. Two α-helical regions exist at residues 8-25 and 28-38, and these regions are separated by a flexible hinge. The rest of the peptide adopts random coil-like conformation (Coles et al., 1998; Crescenzi et al., 2002).

In order to characterize the monomer conformations in our coarse–grained model, we calculate an average potential energy in dependence on the temperature. The energy unit corresponds to the potential energy of one hydrogen bond in our model, so that the absolute average of the potential energy is equal to the average number of hydrogen bonds in the monomer conformation, and the temperature unit is equal to the energy unit. At each temperature $T$, $0.080 < T < 0.155$, we perform $35 \times 10^6$ time steps long simulation runs. We start each run with an initial conformation equal to the observed NMR conformation with predominantly α-helical secondary structure (Crescenzi et al., 2002). The first $15 \times 10^6$ steps we allow for equilibration, whereas we calculate the time average of the potential energy $\langle E \rangle$ over the last $20 \times 10^6$ time steps.
Our monomer peptide experiences a structural transition from a predominantly α-helix conformation into a β-strand conformation at $T_{α,β} = 0.107 ± 0.002$, in agreement with previous work (Ding et al., 2003). At a higher temperature, $T_{β,RC} = 0.128 ± 0.002$, the monomer undergoes a transition from a β-strand into a random coil conformation with no particular secondary structure. Between $T_{α,β}$ and $T_{β,RC}$ our simulations show various types of β-strand rich conformations.

At temperatures $T, T < 0.107$, we observe an α-helix conformation which is consistent with the observed solution monomer conformation in an apolar microenvironment (Crescenzi et al., 2002). This conformation (Fig. 1a) has a random coil-like tail about 10 amino acids long at the N-terminus and another random coil-like tail about 2-4 amino acids long at the C-terminus. At residues 11-40 there are two α helices, separated by a hinge at residues 25-28. The average potential energy of this conformation is $⟨E⟩ = −28 ± 2$. At temperatures $0.107 < T < 0.117$, we observe various β-strand conformations, mostly with two or three β-turns, corresponding to 3 or 4 β-strands (Figs. 1b-c). The average potential energy of these conformations is $⟨E⟩ = −17±1$. The β-hairpin conformation, i.e. a 2-β-strand conformation with one β-turn, shown in Fig. 1d, is found as a predominant conformation at temperatures $T, 0.117 < T < 0.126$. This conformation is characterized by a random coil tail at residues 1-9 and by a well defined and localized β-turn which is positioned at residues 23-28. The average potential energy of this conformation is $⟨E⟩ = −13 ± 1$.

The observed β-turn between residues Asp 23 and Lys 28 is in agreement with recent NMR studies of Aβ fibrillar structure (Petkova et al., 2002). In the following, we provide an empirical explanation for the occurrence of this well-defined β-turn in our model. We hypothesize that within our model the occurrence of a β-turn at residues 23-28 is induced by the particular location of the six glycines in the Aβ(1-42) peptide. In order to test this hypothesis, we replace all glycines within the Aβ(1-42) peptide with alanines and perform simulation runs as described above. Our results (Fig. 2) show the probability for the amino acid at a certain position to be part of a β-turn both for the original Aβ peptide model (with glycines) and the one without glycines (42 amino acids long polyalanine chain). The results show that (i) the presence of glycines on average shifts the center of the β-turn from residue 20-22 for the chain with no glycines to 25-27 for the chain with six glycines, and (ii) the probability distribution in the presence of glycines is strongly peaked at residues 25-27, which makes these three residues part of the β-turn with more than 95% probability and
thus β-turn is well-defined.

The residues 25-27 of the Aβ peptide correspond to glycine, serine and asparagine, the residues which have according to the classical phenomenological approach of Chou and Fasman (Chou and Fasman, 1974) the highest probability to be within a β-turn. In our coarse-grained model the occurrence of the β-turn at 23-28 can be understood as a consequence of two tendencies: (1) a tendency to maximize the number of hydrogen bonds, which prefers a β-turn at the middle of the peptide chain, centered at residues 20-22; and (2) a tendency of 6 glycines to be associated with more flexibility, thus a β-turn. Consequently, the center of the β-turn is shifted from residues 20-22 to residues 25-27, and is well-defined.

B. Planar β-strand dimer conformations of Aβ(1–42)

We investigate next dimer formation of Aβ(1–42) peptides. The initial monomer conformations are taken from the Protein Database Bank and correspond to the observed NMR structures of Aβ(1-42) monomers in an apolar environment (Crescenzi et al., 2002). In order to obtain different starting random coil conformations, we place two monomers with mostly α-helical secondary structure in a cubic box with a side length of 100 Å. The centers of masses of the two monomers are initially about 50 Å apart and their orientations parallel. Next, we heat the system up to a temperature $T = 0.50$, which is far above the observed $T_{\beta,RC}$ temperature. The α-helical secondary structure of individual monomer peptides is dissolved in about 200 simulations steps, producing two peptides with different random coil conformations. We use many similarly generated pairs of peptides with random coil conformations as initial configurations in our study of dimer formation. Dimer formation runs are done at a constant temperature and volume. We perform 20 runs at a fixed temperature. Each run is $20 \times 10^6$ time steps long. In this way we explore temperatures $T = 0.120, 0.125$ and 0.130.

From the above simulations we find six possible dimer conformations with the following characteristics: (i) each peptide in a dimer is in a β-hairpin conformation with two β-strands and (ii) all four β-strands (two per peptide) are planar. We name those dimers according to the inner two strands of the dimer (each strand is either closer to the N-terminus or the C-terminus and the two inner strands are either parallel or antiparallel): NN-parallel, NC-parallel, CC-parallel, NN-antiparallel, NC-antiparallel, CC-antiparallel. These conforma-
tions are schematically presented in Fig. 3a-f. We find four additional dimer conformations with the characteristic (ii) described above. Only the inner peptide has also the characteristic (i), while the outer peptide is bent around the inner one, forming a “nest”. We term them nested parallel, nested antiparallel, anti-nested parallel and anti-nested antiparallel (in anti-nested conformations the termini of the two peptides are in the opposite directions). They are shown in Fig. 3g-j.

At $T = 0.12$, we find NC-parallel and NC-antiparallel conformations each in 3 out of 20 runs. The conformations NN-parallel, CC-parallel, CC-antiparallel, nested antiparallel, anti-nested parallel and anti-nested antiparallel, each occur in 2 out of 20 runs. The conformations NN-antiparallel and nested parallel each are found in 1 out of 20 runs. At $T = 0.13$, the most common dimer peptide conformation is NC-parallel (occurring in 8 out of 20 runs) and the next most common conformation is NN-parallel (occurring in 5 out of 20 runs). We find the NC-antiparallel conformation in 3 out of 20 runs. There are four more conformations found, each in 1 out of 20 runs: NN-antiparallel, CC-parallel, CC-antiparallel and a nested-antiparallel conformation.

Our dimer simulation runs at temperatures $T \geq 0.14$ show no dimerization within the first $20 \times 10^6$ simulation steps, even though typically one of the two peptides adopts one of the $\beta$-strand conformations. We thus conclude that at temperatures $T > 0.14$ there is no dimerization. At temperature $T = 0.11$, we observe a large number of different planar and non-planar $\beta$-strand dimer conformations, which are a mixture of 2-, 3-, 4-$\beta$-strand conformations. At temperatures $0.08 < T < 0.11$, the dimer conformations are an amorphous mixture of $\beta$-strand and $\alpha$-helical secondary structure. All these are omitted from the present all-atom free energy calculation study.

C. Free energy calculations: $A_\beta(1-40)$ versus $A_\beta(1-42)$ monomer conformations.

In order to validate the free energy calculation method, we first analyze monomer peptides of $A_\beta(1-40)$ and $A_\beta(1-42)$. We choose 10 different NMR $A_\beta(1-40)$ monomer structures (Coles et al., 1998). The secondary monomer structure is mostly $\alpha$-helical, similar to Fig. 1a. To each of these structures we add 2 amino acids, Ile and Ala, to find the corresponding $A_\beta(1-42)$ monomer conformation. The estimate free energies are presented in Table I and show that all the monomer conformations, of $A_\beta(1-40)$ and $A_\beta(1-42)$, have on average the
same conformational free energy, $-1034.68 \pm 17.75 \text{kcal/mol}$ and $-1029.47 \pm 10.80 \text{kcal/mol}$, respectively. These results show that addition of two amino acids to the C-terminus does not alter the conformational free energy in a water environment at physiological conditions.

### D. Stability analysis of Aβ(1-40) and (1-42) dimer conformations.

The planar β-strand dimer conformations predicted by our coarse–grained model (Fig. 3) are tested for stability in our all–atom MD simulations in an explicit water environment at atmospheric pressure and room temperature. From ten different Aβ(1-42) dimer conformations, we create the corresponding Aβ(1-40) dimers by deleting the last two amino acids at the C-terminus. For each stable dimer configuration we then calculate the free energy as described in the Methods section. The free energies of all the stable dimers are presented in Table II. One dimer conformation, e.g. nested antiparallel of Aβ(1-40), is determined to be only marginally stable and does not allow for the free energy calculation. Columns 3 and 6 of Table II represent the free energy differences, $\Delta G_{A\beta-40}$ and $\Delta G_{A\beta-42}$, between a dimer conformation and two monomer conformations. For all stable dimer conformations $\Delta G$ is positive, indicating that in a water environment, planar β-strand dimer conformations are energetically unfavorable compared to α-helical monomer peptide conformations. The average conformational free energies of Aβ(1-40) and Aβ(1-42) dimers are $-2000.81 \pm 46.94 \text{kcal/mol}$ and $-1967.63 \pm 52.85 \text{kcal/mol}$, respectively. Although Aβ(1-40) dimers have on average lower conformational free energies than Aβ(1-42) dimers, the difference is not statistically significant.

### IV. DISCUSSION AND CONCLUSIONS

We introduce a coarse–grained peptide model for the Aβ peptide in order to study Aβ dimer formation. Our model predicts a thermally–induced conformational change between a predominantly α-helix to a predominantly β-strand monomer peptide. The prediction of our model is indirectly supported by recent experiments (Gursky and Aleshkov, 2000) on temperature–dependence of the Aβ conformation in aqueous solutions, which show that thermally induced coil to β-strand transition is not coupled to aggregation and can occur at the level of monomers or dimers. In a temperature range below the structural transition
into a random coil our model Aβ monomer peptide adopts a β-hairpin conformation with a β-turn between Asp 23 and Lys 28. The presence of this β-turn is consistent with a structural model for Aβ fibrils based on solid state NMR experimental constraints (Petkova et al., 2002).

Within our coarse–grained model we study Aβ dimer formation, which may be a pathway to higher oligomer and protofibril formation. In our model Aβ dimers are formed as a consequence of hydrogen-bond interactions between residues. Our model predicts that dimer conformations are β-sheet-like planar structures. We show by using all-atom simulations that these planar, β-sheet-like dimer conformations are energetically unfavorable compared to the α-helical monomer conformations in water environment. Moreover, the free energy comparison of Aβ(1-40) and Aβ(1-42) dimer conformations shows that there is no significant free energy difference between these two alloforms, thus suggesting that Aβ oligomerization is not accompanied by the formation of stable planar β-strand Aβ dimers.

Planar β-strand Aβ dimers as predicted by our coarse–grained model cannot account for experimentally observed differences in Aβ oligomer formation between Aβ(1-40) and Aβ(1-42) alloforms (Bitan et al., 2003a). It is not understood yet at which stage of oligomer formation those differences occur and what is the exact mechanism which drives the two alloforms along different pathways. In order to account for oligomer formation differences between the two Aβ alloforms, our coarse–grained model may need to include other interactions between the residues, in particular the ones that originate in polar versus apolar character of the side–chains as suggested by recent experiments (Bitan et al., 2003c; Bitan et al., 2003b).

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TABLE I: Free energies of monomer conformations. Comparison of calculated free energies, $G_{A\beta-40}$ and $G_{A\beta-42}$, and the corresponding standard deviations, $\sigma_G$, of A$\beta$ monomer conformations as determined by the NMR experiment (Coles et al., 1998). The names of different monomer structures follow the ID code name 1BA4 of Brookhaven Protein Database Bank (http://www.rcsb.org/pdb/). The free energy unit is kcal/mol.

| Monomer | $G_{A\beta-40}$ | $\sigma_G$ | $G_{A\beta-42}$ | $\sigma_G$ |
|---------|----------------|------------|----------------|------------|
| 1BA4-01 | -1036.58       | 74.97      | -1026.23       | 73.48      |
| 1BA4-02 | -1050.25       | 77.16      | -1034.13       | 75.12      |
| 1BA4-03 | -1045.88       | 78.46      | -1028.07       | 73.56      |
| 1BA4-04 | -1045.93       | 75.66      | -1032.92       | 78.13      |
| 1BA4-05 | -1030.62       | 75.01      | -1008.66       | 73.68      |
| 1BA4-06 | -997.14        | 72.41      | -1017.85       | 74.81      |
| 1BA4-07 | -1043.71       | 75.81      | -1039.30       | 75.11      |
| 1BA4-08 | -1016.94       | 73.36      | -1027.37       | 75.40      |
| 1BA4-09 | -1038.70       | 75.97      | -1032.68       | 75.13      |
| 1BA4-10 | -1052.29       | 78.13      | -1044.28       | 74.37      |
TABLE II: Free energies of dimer conformations. Comparison of calculated free energies $G_{A\beta-40}$ and $G_{A\beta-42}$, the corresponding standard deviations $\sigma_G$, and the free energy differences $\Delta G_{A\beta-40}$ and $\Delta G_{A\beta-42}$ of $A\beta$(1-42) and $A\beta$(1-40) dimer conformations. The free energy unit is kcal/mol.

| Dimers     | $G_{A\beta-40}$ | $\sigma_G$ | $\Delta G_{A\beta-40}$ | $G_{A\beta-42}$ | $\sigma_G$ | $\Delta G_{A\beta-42}$ |
|------------|-----------------|------------|-------------------------|-----------------|------------|-------------------------|
| NN-para    | -1983.51        | 159.91     | 88.10                   | -1994.72        | 147.09     | 63.58                   |
| NN-anti    | -2061.09        | 147.16     | 10.52                   | -2019.14        | 145.00     | 39.15                   |
| NC-para    | -1935.59        | 138.00     | 136.02                  | -1937.60        | 137.21     | 120.70                  |
| NC-anti    | -1999.38        | 149.00     | 72.23                   | -1982.94        | 142.48     | 75.36                   |
| CC-para    | -2000.17        | 143.05     | 71.44                   | -1871.82        | 131.86     | 186.48                  |
| CC-anti    | -2043.70        | 147.02     | 27.91                   | -2022.44        | 144.31     | 35.86                   |
| nest-para  | -1964.90        | 139.27     | 106.71                  | -1989.43        | 143.00     | 68.87                   |
| nest-anti  | unstable        | N/A        | N/A                     | -1950.07        | 228.23     | 108.23                  |
| anti-nest-para | -2028.34 | 204.34     | 43.27                   | -2022.27        | 207.26     | 36.03                   |
| anti-nest-anti | -1988.27 | 205.89     | 83.34                   | -1972.38        | 205.40     | 85.92                   |
Fig. 1: Conformations of an Aβ(1–42) monomer peptide model as a function of temperature, (a) mostly α-helix conformation at $T = 0.100$ with two α helices at residues 12-23 and 29-38, and a hinge at residues 23-28; (b) 3-β-strand conformation at $T = 0.108$, (c) 4-β-strand conformation at $T = 0.115$, and (d) β-hairpin conformation at $T = 0.120$ characterized by a β-turn at residues 23-28.

Fig. 2: Two distributions that give the probability for an amino acid at a residue number (position in the chain) to be within a β-turn. These simulations are done at temperature $T = 0.125$, where our model for Aβ(1-42) yields a stable β-hairpin conformation. The curve with open circles corresponds to the altered chain (no glycines) and the curve with triangles corresponds to the original Aβ(1-42) model with six glycines. The distributions are calculated on the basis of 28 (the model with no glycines) and 38 (the Aβ(1-42) model) different β-hairpin configurations. For each β-hairpin conformation we use VMD (Humphrey et al., 1996) visualization package to determine and count all the residues with a β-turn. The probability to be in the β-turn is determined as a ratio between the number of conformations, in which the amino acid is part of a β-turn, and the total number of conformations.

Fig. 3: Schematic conformations of an Aβ(1–42) dimer peptide model. All the conformations are based on a β-hairpin conformation with a β-turn at residues 23-28. In our model the energies of all these conformations are approximately the same, however, the probability of the occurrence varies.
Fig. 1

(a) $T = 0.100$
(b) $T = 0.108$
(c) $T = 0.115$
(d) $T = 0.120$
Fig. 2

![Graph showing the probability for a residue to be in a β-turn.]

- With 6 Glys: \(<R> = 26.5 \pm 2.5\)
- No Gly: \(<R> = 22.9 \pm 4.6\)
Fig. 3

(a) NN parallel  (b) NN antiparallel  (c) NC parallel

(d) NC antiparallel  (e) CC parallel  (f) CC antiparallel

(g) nested parallel  (h) nested antiparallel  (i) anti-nested parallel  (j) anti-nested antiparallel