Influence of temperature on formation of perfect tau fragment fibrils using PRIME20/DMD simulations

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Abstract: We investigate the fibrillization process for amyloid tau fragment peptides (VQIVYK) by applying the discontinuous molecular dynamics method to a system of 48 VQIVYK peptides modeled using a new protein model/force field, PRIME20. The aim of the article is to ascertain which factors are most important in determining whether or not a peptide system forms perfect coherent fibrillar structures. Two different directional criteria are used to determine when a hydrogen bond occurs: the original H-bond constraints and a parallel preference H-bond constraint that imparts a slight bias towards the formation of parallel versus antiparallel strands in a β -sheet. Under the original H-bond constraints, the resulting fibrillar structures contain a mixture of parallel and antiparallel pairs of strands within each β -sheet over the whole fibrillization temperature range. Under the parallel preference H-bond constraints, the β -sheets within the fibrillar structures are more likely to be parallel and indeed become perfectly parallel, consistent with X-ray crystallography, at a high temperature slightly below the fibrillization temperature. The high temperature environment encourages the formation of perfect fibril structures by providing enough time and space for peptides to rearrange during the aggregation process. There are two different kinetic mechanisms, template assembly with monomer addition at high temperature and merging/rearrangement without monomer addition at low temperature, which lead to significant differences in the final fibrillar structure. This suggests that the diverse fibril morphologies generally observed *in vitro* depend more on environmental conditions than has heretofore been appreciated.

Keywords: amyloid tau-fragment; protein aggregation; steric-zipper interface; coarse-grained model; discontinuous molecular dynamics

Introduction

The pathological hallmark of a number of severe neurological diseases, including Alzheimer's, Hun-

tington's, Parkinson's, and Creutzfeldt-Jakob diseases, is present in the brain of abnormal structures composed of ordered protein aggregates, called fibrils or amyloid.^{1–4} The specific reasons why amyloid forms in the brains of some people and not in others are unknown. Current thinking says that it is a consequence of protein misfolding^{5–7} which is induced by pathological trigger(s) whose identity is, unfortunately, still a mystery. Because misfolded or partially folded proteins have more exposed hydrophobic residues than usual,⁸ they tend to self assemble into small aggregates called oligomers, some with beta sheet characteristics, in a series of steps, eventually coming together to form insoluble aggregates—amyloid fibrils. Amyloid fibrils are ribbon-

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like layers of stacked β -sheets; this so-called cross- β structure^{9,10} is very stable due to backbone hydrogen-bonding which joins the strands into β -sheets, and hydrophobic interactions or shape-complementary van der Waals interactions between side chains which hold the sheets together.^{10,11}

Simulating the spontaneous formation of cross- β structures by partially folded or non-native peptides is one of the current challenges in computational biophysics. Atomistic simulations are generally not well suited for this purpose since the realistic detail that makes these simulations so accurate makes them too slow to access aggregation timescales. An alternative is coarse grained simulations in which the (hopefully) non-essential details of molecular geometry and energetics are removed, allowing the simulations to go faster and thereby access longer time scales. While there have been many significant works that shed light on the aggregation process, only a few have been able to get to the stage at which proteins spontaneously form ordered fibrillar structures. Monte Carlo simulations in conjunction with PROFASI, an all-atom protein representation with a simplified force-field developed by the Irbäck group, have been used to investigate the fibrillization of A β 16-22 peptides¹² and of amyloid tau fragment.¹³ Urbanc *et al.* used a coarse-grained model including hydrophobic and charge interactions as well as directionally dependent backbone hydrogen bonding to study A β oligomerization mechanisms.¹⁴ Auer *et al.* have employed a flexible tube model to examine the nucleation and structural changes associated with fibrillar assembly.^{15,16} Pellarin *et al.* designed a simple model peptide with a few backbone and side-chain spheres and studied the dependence of the fibril formation pathways on the energy difference between the two possible peptide conformers.¹⁷ The Shea group developed an intermediate-resolution peptide model with four types of single sphere side-chains (hydrophobic, polar, positive charge, negative charge) and looked at how side chain type influences the fibrillization pathway.^{18,19} Li *et al.* studied growth mechanisms in fibril formation based on lattice models.^{20,21} The Hall group examined fibril formation for polyalanine and polyglutamine peptide using the PRIME model.^{22,23}

While significant insight into the fundamentals of the fibrillization process have resulted from use of coarse grained protein models,¹⁹ most of the models which are capable of forming fibrils spontaneously in a simulation are too coarse grained to account for amino-acid specificity, that is, the unique features that distinguish each of the 20 amino acids. A notable exception to this is the work of Li *et al.* who used Monte Carlo simulations based on PROFASI¹³ to simulate the fibrillization of the tau fragment VQIVYK, the same as the fragment considered in this article. Starting from random peptide configurations,

they observed twisted fibrils or proto-filaments having cross- β structures in systems containing 12, 24, and 36 chains.

This study focuses on the amyloid tau fragment (VQIVYK), residues 306 to 311 of the protein tau,^{24–28} which is found in the amyloid deposits that constitute the intracellular neurofibrillary tangles associated with Alzheimer's disease. The major components in the intracellular neurofibrillary tangle are paired helical filaments (PHF), which appear as twisted ribbons or pairs of proto-filaments. The hexapeptide VQIVYK has been found to be one of the core segments in PHF formation with twisted ribbon-like morphology.^{24–27} VQIVYK forms fibrils with parallel β -sheets and a steric zipper interface within the cross- β spine as has been shown by X-ray microcrystallography.²⁹ This six residue peptide is a nice model system for use in computational studies of spontaneous fibril formation. Fibrils form relatively easily for this peptide since the hydrophobic residues (V1, I3, Y5) and nonhydrophobic residues (Q2, K6) alternate in part. Recently D-amino-acid versions of VQIVYK have been designed and studied to see how well D-amino acid peptides inhibit fibril formation by their L-amino acid counterparts.²⁸

The goal of this article is to determine which factors are most important in determining whether or not a peptide system will form "perfect" fibrils, meaning highly ordered, coherent and (for the case of VQIVYK) containing perfectly parallel beta sheets. It is well known that the fibrillar structures formed experimentally are sensitive to the environment and are not necessarily perfect; they often have heterogeneous fibrillar morphologies. For these reasons, experimentalists intent on examining structure often use agitation, sonication, or consecutive seeding procedures to generate high grade fibrillar structures for X-ray, NMR and so forth, measurements.^{30–32}

In this article, we apply the newly developed PRIME20 model,^{33,34} an extension of the PRIME model^{22,35,36} that describes the pair interactions and model geometry for all twenty amino acids, to simulate spontaneous fibril formation by large systems of amyloid tau fragments (VQIVYK). In PRIME20, the protein is represented by three united atom spheres for the protein backbone (one each for the NH, CO, and C α H) and a single sphere for the side chain. Solvent molecules are modeled implicitly. The interactions between side chains are simple square-well potentials and the hydrogen bonding interactions between backbone NH and CO are directional square-well potentials (see Methods). This directionality is implemented by assigning four cutoff distances, henceforth referred to as the original H-bond distance constraints, between an NH on residue *i* and a CO on residue *j* (NH $_i$ -C $_{\alpha,j}$, NH $_i$ -NH $_{j+1}$, CO $_j$ -C $_{\alpha,i}$, CO $_j$ -CO $_{i-1}$). Hydrogen bonds form only when all four distances are larger than their respective cutoff

values. In this article we implement new H-bond distance constraints, the parallel preference H-bond constraints, to give a slight preference to the probability that the final fibrillar structures will have coherent cross- β spines, consistent with experiment. Our purpose in doing this is to make it a little easier to form these structures so that we can then examine if and how environmental conditions, notably the temperature, enhance or hinder the probability that a perfectly formed structure will result. These constraints are obtained by measuring the distributions for these four distances in 620 NMR PDBs and decomposing them into distributions for parallel and anti-parallel β -sheets. We perform discontinuous molecular dynamics (DMD) on systems containing forty eight tau fragment peptides at six reduced temperatures (using the original H-bond distance constraints) and at eight reduced temperatures (using the parallel preference H-bond distance constraints). Five independent runs at each temperature with up to 295 billion collisions are conducted at a molar concentration of 10mM. The simulations based on the new parallel preference H-bond cutoffs are fast and efficient, allowing us to quickly reach the expected structures by avoiding kinetic traps associated with mixed parallel and anti-parallel β -sheets.

Highlights of our results are as follows. As we have seen in simulations of other peptides,³⁴ there is a range of temperatures over which fibrils form for a given concentration; fibrils form most easily at a “marginal” temperature above which the system forms random coils, hereafter referred to as the “fibrillization temperature.” On the basis of the original H-bond distance constraints, we observe the formation of β -sheets containing both parallel and anti-parallel pairs of strands; these stack to form somewhat disordered or irregular proto-filaments with out-of-register pairs of β -strands. Using the parallel preference H-bond distance constraints at lower temperatures, $T^* = 0.17$ and 0.18 , we observe that both β -sheets and the cross- β spines are a mixture of parallel and antiparallel strands. This is likely due to kinetic trapping in a meta-stable state formed by rapidly associating small oligomers held together by strong hydrophobic pair-interactions which the thermal fluctuations cannot overcome. Using the parallel preference H-bond distance constraints at higher reduced temperatures, $T^* = 0.195$ – 0.198 , we observe perfectly-structured twisted structures with parallel β -sheets having coherent side-chain positions, with V1 I3 Y5 on one side of a β -sheet and Q2 V4 K6 on the other side. Moreover, the cross- β spines form coherent anti-parallel sheets containing the steric zipping residues V1 I3 Y3. We compare the positions of side-chains in our simulated structures to the steric-zipper spine seen in experiment. On the basis of our observations, we suggest that coherent fibrillar structures without mixed β -sheets and irregular

twist (the structures reported experimentally and theoretically) are mostly likely to occur at temperatures slightly below the fibrillization temperature. At these conditions fibrillization proceeds via a templated-assembly-by- monomer-addition mechanism.

The article is organized as follows. In the results section, we present the results from simulations with the original H-Bond distance constraints and with the parallel preference H-bond distance constraints. The discussion section is devoted to an analysis of the role played by the temperature in determining the fibrillization mechanisms and how this in turn influences the extent of order in the fibrillar structures that are formed. In the methods section, we describe the PRIME20 parameters and geometry for the tau fragment and present the parallel preference H-bond distance constraints.

Results

We begin by describing the results from simulations on 48-peptide systems with the original four distance cutoffs for the backbone hydrogen bonds. Table I shows the β -sheet content (fraction of chains that are in a beta sheet) and percent of neighboring pairs of strands in a β -sheet interacting by backbone hydrogen bonds that are parallel or anti-parallel for each simulated temperature at the end of independent runs. The values are averaged over the last 10% of the trajectories from 295 billion collisions. From the table it is apparent that the transition or fibrillization temperature (temperature above which fibrillization ceases) is near $T^* = 0.195$ since the β -sheet content is roughly 0.5. The β -sheets contain both parallel and antiparallel strands over all temperature regimes with no clear trend in the percentage of parallel or antiparallel content. Roughly speaking the fraction of parallel and antiparallel pair strands is 0.4 versus 0.6, although there are large fluctuations in these numbers at $T^* = 0.17$ and 0.18 . The fluctuations are due to the fact that at low temperature the relatively disordered β -sheets that are formed at initial stages in the simulation get trapped and undergo less of a rearrangement process than at high temperature. This ratio is similar to the observation of the medium size oligomers in Li *et al.*'s work.¹³

Figure 1 shows typical structures at the end of simulations at $T^* = 0.15$, 0.17 , 0.18 , 0.185 , 0.19 , and 0.20 for the original H-bond distance constraints. Fibrillar structures containing two β -sheet layers with cross β -structures are observed at all temperatures below the transition temperature. The structures seen at lower temperatures are rather disordered. For example at $T^* = 0.15$ [Fig. 1(a)] the β -sheets are aggregated in an haphazard way and at $T^* = 0.17$ [Fig. 1(b)] the red β -sheet is not attached to the end of the orange β -sheet, interfering with the overall cross- β structure. At $T^* = 0.18$ [Fig. 1(c)] the

Table I. The β -Sheet Content and Fractions of Parallel and Antiparallel Pairs of Strands at Six Temperatures from Five Independent Runs

		1st run	2nd run	3rd run	4th run	5th run
$T^* = 0.17$	β -sheet content	0.982	0.954	0.972	0.951	0.988
	% of parallel	0.145	0.408	0.519	0.215	0.407
	% of antiparallel	0.847	0.583	0.475	0.781	0.588
$T^* = 0.18$	β -sheet content	0.965	0.963	0.955	0.895	0.963
	% of parallel	0.375	0.432	0.188	0.350	0.379
	% of antiparallel	0.623	0.565	0.801	0.634	0.617
$T^* = 0.185$	β -sheet content	0.868	0.863	0.861	0.899	0.875
	% of parallel	0.401	0.368	0.337	0.379	0.400
	% of antiparallel	0.594	0.622	0.656	0.616	0.597
$T^* = 0.19$	β -sheet content	0.736	0.811	0.760	0.776	0.863
	% of parallel	0.432	0.412	0.431	0.398	0.283
	% of antiparallel	0.562	0.584	0.565	0.596	0.714
$T^* = 0.195$	β -sheet content	0.560	0.323	0.434	0.451	0.476
	% of parallel	0.438	0.366	0.436	0.401	0.376
	% of antiparallel	0.551	0.589	0.546	0.578	0.607
$T^* = 0.20$	β -sheet content	0.018	0.028	0.031	0.046	0.025
	% of parallel					
	% of antiparallel					

Simulations are performed with the original H-bond distance constraints.

fibrillar structure is very curvy with an irregular twist between pair of strands. In contrast, simulations near the transition temperature at $T^* = 0.185$ and 0.19 show rather nice fibril structures without any disordered or bent sections as seen in Figure 1(d,e). Above the transition temperature at $T^* = 0.20$ [Fig. 1(f)], only random free monomers are observed. Interestingly the β -sheet contents at $T^* = 0.185$ and 0.19 shown in Table I are not close to 1.0 and there are free monomers around the oligomers [not shown in Fig. 1(d,e)]. The well-formed fibrillar structures seen in Figure 1(d,e) have β -sheets with mixed parallel and anti-parallel pairs of β -strands. Moreover not all pairs of β -strands are in-register. Instead β -sheets are composed of mixtures of in-register and out-of-register pairs of β -strands.

Examination of Table I gives us useful information on what factors can and cannot drive the system to form perfect coherent structures without mixed parallel and anti-parallel β -strands. With the original H-bond distance constraints (as in Table I), the fraction of parallel and anti-parallel pairs of β -strands in the final fibril structures are independent of temperature and perfect antiparallel protofilament structures are not found even at a high temperature near the transition temperature. There are two possible explanations for this. One is that our force field and the PRIME20 geometries for amino acids are not detailed enough to allow the system to relax to a global minimum so that the effect of temperature is not well captured. The other is that mixed parallel/antiparallel structures might actually be dominant in fibril formation experiments at high concentrations like 10 mM, especially in the absence of a growing process based on seeding. Improving PRIME20 geometry by incorporating more spheres into side-chains and designing a more detailed force-

field might help but it is beyond the scope of the present article. Instead we have introduced the parallel preference H-bond distance constraints—purposely making it a little easier for PRIME20 to form parallel β -sheets so that we can clarify the effect of temperature on structure formation.

Table II shows the β -sheet content and fractions of parallel and antiparallel pairs of strands at each simulation temperature from different independent runs based on the parallel preference H-bond distance constraints. The transition temperature is between $T^* = 0.20$ and 0.202 since the β -sheet content sharply decreases at $T^* = 0.202$. This transition temperature is a little higher than it was for the original H-bond distance constraints (see Table I), likely because a wider range of NHO and COH angles are allowed in parallel pairs. At the lower temperatures, $T^* = 0.17$ –0.19, the β -sheets contain a mixture of both parallel and antiparallel strands. The fraction of parallel strands increases as the temperature increases. Nearly-perfect parallel β -sheets are observed for a few independent runs such as the 2nd and 4th runs at $T^* = 0.195$ and the 4th run at $T^* = 0.20$.

Figures 2 and 3 show snapshots over the course of simulations with the parallel preference H-bond constraints at $T^* = 0.18$ and 0.195, respectively. Also shown are plots of the hydrogen-bonding energy, the pair-wise interaction energy between side-chains, and the total potential energy versus time. Figures 2(a) and 3(a) show the random starting configurations at the two temperatures. At the lower temperature, $T^* = 0.18$, four small oligomers are formed at $t^* = 1100$ as shown in Figure 2(b); these merge together to form the large, clearly fibrillar structure shown in Figure 2(c). The pair-wise interaction energy between side chains (red line) in

Figure 1. Final structures at the end of the simulations with the original H-bond distance constraints. Simulated temperatures are $T^* = 0.15, 0.17, 0.18, 0.185, 0.19$, and 0.20 . Different β -sheets are colored differently for ease of viewing.

Figure 2(d) decreases very rapidly and does not change noticeably over long times while the hydrogen-bonding energy (blue line) and total potential energy (green line) decrease very slowly in time. Thus at low temperature the fibril formation mechanism is merging and rearrangement without monomer addition. At higher temperature $T^* = 0.195$, the free monomers in Figure 3(a) evolve into ordered oligomers plus free monomers as shown in Figure 3(b). Eventually a clearly fibrillar structure is formed as shown in Figure 3(c). The three types of energies at the higher temperature [Fig. 3(d)] decrease more slowly with time than they do at the lower temperature [Fig. 2(d)] indicating that at high temperature the fibril grows by a templated assembly mechanism plus monomer addition. In this mechanism, the monomer attaches to the ordered fibril template and then has sufficient time and

space to rearrange and become part of the ordered structure. This slow fibril growth process is conducive to the formation of perfect structures with parallel β -sheets.

The numbers of parallel and anti-parallel pairs of β -strands with the parallel preference H-bond constraints evolve with time. Figure 4 shows the time evolution of the probabilities that pairs of β -strands will be parallel and anti-parallel and the time evolution of the number of parallel and antiparallel pairs connected by four and five hydrogen bonds at two temperatures. At $T^* = 0.18$, the probability of being in a parallel pair [blue line in Fig. 4(a)] is almost 0.7. It shows no variation with time which implies that the parallel and antiparallel pairs formed at an early stage in the simulations might remain thus because they are trapped in metastable configurations or alignments. The in-register parallel pairs

Table II. The β -Sheet Content and Fractions of Parallel and Antiparallel Pairs of Strands at Eight Temperatures from Five Independent Runs

		1st run	2nd run	3rd run	4th run	5th run
$T^* = 0.17$	β -sheet content	0.985	0.976	0.996	0.913	0.939
	% of parallel	0.612	0.712	0.736	0.654	0.680
	% of antiparallel	0.385	0.284	0.264	0.328	0.316
$T^* = 0.18$	β -sheet content	0.968	0.974	0.941	0.975	0.983
	% of parallel	0.723	0.630	0.700	0.657	0.705
	% of antiparallel	0.275	0.368	0.295	0.341	0.293
$T^* = 0.185$	β -sheet content	0.944	0.956	0.958	0.962	0.951
	% of parallel	0.882	0.937	0.819	0.707	0.776
	% of antiparallel	0.115	0.061	0.179	0.291	0.222
$T^* = 0.19$	β -sheet content	0.912	0.955	0.952	0.920	0.912
	% of parallel	0.799	0.900	0.787	0.771	0.750
	% of antiparallel	0.199	0.099	0.211	0.227	0.249
$T^* = 0.195$	β -sheet content	0.917	0.897	0.947	0.862	0.942
	% of parallel	0.948	0.978	0.904	0.989	0.858
	% of antiparallel	0.050	0.020	0.095	0.008	0.141
$T^* = 0.198$	β -sheet content	0.873	0.791	0.771	0.904	0.753
	% of parallel	0.968	0.962	0.901	0.943	0.924
	% of antiparallel	0.029	0.035	0.096	0.054	0.073
$T^* = 0.20$	β -sheet content	0.680	0.671	0.736	0.765	0.714
	% of parallel	0.881	0.902	0.981	0.983	0.971
	% of antiparallel	0.114	0.093	0.016	0.014	0.025
$T^* = 0.202$	β -sheet content	0.059	0.015	0.021	0.034	0.015
	% of parallel					
	% of antiparallel					

Simulations are performed with the parallel preference H-bond distance constraints.

Figure 2. Snapshots over a simulation at $T^* = 0.18$ with the parallel preference H-bond distance constraints and the time evolution of energies. Snapshots are selected at the reduced times (a) $t^* = 0$ (initial random configuration) (b) $t^* = 1100$, (c) $t^* = 15,000$. (d) Time evolution of the backbone hydrogen-bonding energy (blue line), the pair interaction between side-chain centroids (red color), and total potential energy (green line), all averaged over five independent runs.

Figure 3. Snapshots over a simulation at $T^* = 0.195$ with the parallel preference H-bond distance constraints and the time evolution of energies. Snapshots are selected at the reduced times (a) $t^* = 0$ (initial random configuration) (b) $t^* = 2300$, (c) $t^* = 15,000$. (d) Time evolution of the backbone hydrogen-bonding energy (blue line), the pair interaction between side-chain centroids (red color), and total potential energy (green line), all averaged over five independent runs.

[pairs sharing five hydrogen bonds—blue line in Fig. 4(b)] increase with time, implying a slow relaxation process over time, while the out-of register parallel pairs [pairs sharing four hydrogen bonds—green line in Fig. 4(b)] show no variations. The in-register antiparallel pairs (red line) decrease slowly and the out-of-register antiparallel pairs (violet line) increase, which implies that the in-register antiparallel pairs are slowly destabilized with the parallel preference H-bond distance constraints. The small oligomers formed at an early stage [e.g., Fig. 2b] merge and become a large oligomer and finally a fibril structure. This merging and structural reorganization process does not, however, include changing antiparallel to parallel pairs of β -strands but instead is based solely on relaxation to in-register parallel β -sheets. At $T^* = 0.195$, the probability of being in parallel pairs [blue line in Fig. 4(c)] increases with time and the probability of being in antiparallel pairs [red line in Fig. 4(c)] decreases. The in-register parallel pairs [blue line in Fig. 4(d)] and the out-of register parallel pairs (green line) increase slowly with time, while the antiparallel pairs (red line for in-register and violet line for out-of-register) decrease with time. This is consistent with fibril growth via templated assembly; attaching

by pair interactions or detaching free monomers by thermal fluctuations respectively are slow processes because the system is trying to find the path to the perfect parallel β -sheet structures observed experimentally. Clearly thermal fluctuations make it possible for attached monomers to rearrange on an ordered template to form perfectly in-register parallel β -sheets.

The final fibril structures at $T^* = 0.18$ and $T^* = 0.195$ are shown in Figures 5(a,b). While mixed parallel and anti-parallel pairs are seen in Figure 5(a) for $T^* = 0.18$, the β -sheets shown in Figure 5(b) are almost perfectly parallel and mostly in-register. A view down the fibril axis shown in Figure 5(c) clearly shows the very regular twist of the cross- β spine. The positions of side-chain spheres in four selected in-registered peptides have a strikingly coherent pattern as shown in Figure 5(d).

To facilitate detailed comparison with the steric-zipper spine found in experiment, we present in Figure 6(a) snapshot of an eight-strand portion of the bilayered sheet in Figure 5(b) viewed down the fibril axis. The two β -sheets (purple and green) in Figure 6(a) are anti-parallel to each other, as can be seen by locating the C-terminal residue K (pink) spheres in the upper left on the purple β -sheet and in the

Figure 4. For parallel preference H-bond constraints, the time evolution of (a) the probability of having parallel (blue line) pairs and anti-parallel (red line) pairs in β -sheets at $T^* = 0.18$ and (b) the number of parallel strand pairs having five hydrogen-bonds (blue line) and four hydrogen-bonds (green line) and the number of antiparallel strand pairs having five hydrogen-bonds (red line) and four hydrogen-bonds (purple line). (c,d) The same as in (a) and (b) but at $T^* = 0.195$. All values are averaged over five independent runs at each temperature.

lower right on the green sheet. The pair interactions within the inter-sheet steric-zipper spine are illustrated in Figure 6(b) indicating side-chain-side-chain interactions among V1, I3, and Y5 (blue on right (green) sheet, red on left (purple) sheet). Those results are consistent with the computational work of Li *et al.*¹³ and the experiment by Sawaya *et al.*²⁹ However the dominant pair interactions in our steric-zipper spine are between I3 (small red sphere on left sheet) and Y5 (large blue sphere on right sheet), and between Y5 (large red sphere on left sheet) and I3 (small blue sphere on right sheet) which is different from the result of Sawaya's structure where the dominant pair interactions were between V1 (on one sheet) and I3 (on the other sheet), and between I3 (on one sheet) and V1 (on the other sheet). This may be an inevitable result of using PRIME20 which models each side-chain as a sphere. We cannot capture atomic scale shape-complementarity in side chains, a significant consequence of the van der Waals interaction between atoms. However we can produce very detailed structures for cross- β spines within a coarse-grained model. Another interesting feature of our results is that the two β -sheets shown in Figure 6(c) are tilted

Figure 5. Final fibrillar structures at (a) $T^* = 0.18$ and (b) $T^* = 0.195$. (c) The view down the fibril axis at $T^* = 0.195$. (d) Four in-register peptides selected from (b) (red) are shown with backbone NH, CO and $C_\alpha H$ spheres (purple) reduced in size for ease of viewing. The side-chain spheres, whose sizes are scaled based on $R_i - C_\alpha$ distances, are colored turquoise (V), red (Q), green (I), blue (Y), and pink (K).

Figure 6. Fibril-axis view of twisted β -sheets showing only four strands per sheet taken from middle of fibrillar sheets in Figure 5(b). (a) Fibril structure showing locations of individual side chains, V(turquoise), Q(red), I(green), Y(blue) and K(pink). (b) The same structure with different coloring scheme. Side-chains for Q2,V4,K6 are turquoise spheres which are located outside of bi-layered sheets. Side-chains for V1, I3, Y5 are colored: blue for right (green) sheet, red for left (purple) sheet, which are located inside forming steric-zipper interactions. (c) Side view with inside side-chain spheres only.

with respect to each other. This tilt is not seen in the crystal structure but is consistent with other observations based on computational works for A β 16-22 peptides.³⁷

The temperature dependence of the steric zipper spine can be further understood by analyzing Figure 7 which shows the time evolution of five observables that measure structure at two temperatures $T^* = 0.18$

Figure 7. Time evolution of five observables at two temperatures $T^* = 0.18$ and 0.195 . (a–b) Black lines are the number of peptides in a monomer state and red lines are the number of peptides in fibril structure. (c–d) Blue lines are the number of peptide pairs forming cross- β spines having V1 I3 Y5 residues inside arranged as both parallel and antiparallel intersheets. Purple lines represent other cross- β spines with Q2 V4 K6 residues on the inside of at least one of the two β -strands. Green lines are the number of peptide pairs forming cross- β spines having V1 I3 Y5 on the inside arranged as anti-parallel intersheets only. (e) Magnified plot for the number of peptide in fibrils of Figure 7(b) at early time.

and 0.195. The black lines in Figure 7(a) for $T^* = 0.18$ and (b) for $T^* = 0.195$ are the number of peptides existing in a monomer state and the red lines are the number of peptides existing in a fibril state. The latter is defined to be a state in which each peptide forms at least three hydrogen bonds with the nearest β -strand on the same sheet and also forms at least three side-chain pair interactions with a β -strand on the opposite β -sheet. It can be seen that the number of monomers diminishes rather quickly at low temperature $T^* = 0.18$ [Fig. 7(a)] and very slowly at high temperature $T^* = 0.195$ [Fig. 7(b)]. The slow decay at high temperature supports the monomer addition mechanism. The blue lines in Figure 7(c,d) are the number of peptide pairs which form at least three intermolecular pair interactions among V1 I3 Y5 residues and no hydrogen bonds between them. These include both parallel inter-sheets (where VIY on one strand stacks next to VIY on the other strand) and antiparallel sheets (where VIY on one strand stacks next to YIV on the other strand). This curve confirms the existence of the V1 I3 Y5 cross- β spine in the fibril structure at later times. The peaks in the value of the blue curve (V1 I3 Y5) at early times and low temperature are associated with the presence of somewhat disordered oligomers which precede the formation of fibril structures. The purple lines represent another possible cross- β spine in which the Q2 V4 K6 residues are on the inside of at least one of the two β -strands. The green lines (VIY-AP) are the number of peptide pairs in which V1 I3 Y5 residues are on the inside but form anti-parallel inter-sheets (VIY stacks next to YIV); this corresponds to the steric zipper spine in experiment and is associated with the perfect fibril structure shown in Figure 6(b). At low temperature $T^* = 0.18$ [Fig. 6(c)], the green curve (VIY-AP) is significantly smaller than the blue curve (VIY) so that mixed cross- β interfaces have both parallel inter-sheets and anti-parallel inter-sheets. At high temperature $T^* = 0.195$ [Fig. 7(d)], the blue curve (VIY) is similar to the green curve (VIY-AP) at later times, which means that most peptide pairs in our simulation form the perfect cross- β spines found experimentally. The inset Figure 7(e) is a magnified version of the red curve in Figure 7(b) at early times which provides clear evidence for a lag-phase and nucleation event occurring about $t^* = 200$; it tells us that it takes time to form a nucleated template. Hence we can observe very clear correlation between the templated assembly mechanism by monomer addition and the perfect fibril structure.

Discussion

Our success in simulating the formation of fibrillar structures by 48 short amyloid tau fragments starting from a random configuration demonstrates how a coarse-grained model can contribute to our understanding

of protein aggregation. It illustrates the strengths of the recently developed PRIME20 protein model and suggests that it could be useful in examining other aggregation problems and protein systems. It also documents its weaknesses. Reproducing perfect β -sheets and steric-zipper interfaces structures consistent with experiments is not easy. We can (and will) work to improve our force fields and geometric representation but we expect that we would still see mixtures of parallel and anti-parallel strands. The very nice structures with parallel β -sheets and regular twist that we observe in our tau fragment simulations are due in large part to our use of the parallel preference H-bond distance constraints for the backbone hydrogen-bonds. These allow wider distributions for NHO and COH angles, promoting the formation of in-register parallel pairs of strands. This is a kind of bias, slight (2–5% difference in the cutoff distances) but nevertheless real, similar to enhancing native contacts in the protein folding problem. It effectively reduces the ruggedness of the energy landscape. In contrast mixed parallel and anti-parallel pairs of β -strands are observed when using the original H-bond distance constraints. Those angle constraints are actually very close to the hydrogen-bonds in α -helix structure and qualitatively similar to the values over all hydrogen-bonds in native PDBs.

Analysis of the results presented here provides insight into the effect of temperature on fibrillization. By simulating at the high end of the temperature range considered, we essentially speed up the code making it possible to access the long-time-scale phenomenon of fibrillization. This is, in a sense, equivalent to lowering the concentration, giving the peptides enough space and time for structural reorganization of small oligomers since nucleation and oligomerization are retarded at low concentration. Experimentalists also adjust the conditions that they look at to access fibrillization time scales. For example, the protein concentrations examined *in vitro* are in the micro- to millimolar range but the physiological protein concentration is in the nanomolar range. We find that the best fibrils, by which we mean fibrils that are in-register, parallel and have a regular twist, are formed at or near the fibrillization temperature, the temperature above which fibrils cease to form. This temperature range allows for nucleation and templated assembly through monomer attaching and detaching, providing enough kinetic energy and time for strands to rearrange to form the correct alignments. We expect that mixtures of parallel and anti-parallel β -sheets may also occur in experiments at certain conditions such as high concentration and rather low temperatures. Protein aggregation is apparently very sensitive to the environment (temperature, concentration, pH, sequence etc) and exhibits diverse fibrillization

Figure 8. (a) Geometry of a backbone hydrogen bond. Gray lines show four distances. Four distance distributions for (b) all, (c) α -helix, (d) parallel β -sheet, and (e) anti-parallel β -sheet structures. Distributions are evaluated over 620 NMR PDBs having 12,251 NMR models for hydrogen bonds with 4.5-Å cutoff distance between NH and CO united spheres.

pathways with long-lived meta-stable structures and polymorphism regarding final structures. The slight differences in the free energies for parallel and anti-parallel pairs of strands lend credence to the idea of having mixed β -sheets at high concentration and low temperature, where there is not enough time and space for rearrangement of peptides to form the expected structures.

Li *et al.* suggested that if they grow the size of β -sheets, more parallel β -sheets for the tau fragment would be obtained, especially if the simulations were run longer. However based on the simulation results in our article for 48 peptides at low temperatures (where we get a similar ratio of parallel and anti-parallel pairs in β -sheets to theirs) we think that having larger systems is not the “answer.” Instead the “answer” seems to be to have large thermal fluctuations because these facilitate the search for the minimum free energy. In a high temperature environment, the fibril grows by monomer attachment to the template and subsequent rearrangement to fit in to the perfect fibril structure. For optimum structure development, monomer rearrangement should be completed before the next monomer attaches to the template. This means that the attaching monomer needs enough time and space to do this; if new monomers attach before the old ones have finished rearranging, they wind up as strands in a mixed beta-sheet, making it hard to reorganize into perfect structures. The thermal fluctuations associated with high temperature favor this one-by-one attachment, because they make it easier to resist hydrophobic

collapse and they allow monomers to attach and detach constantly to the template.

The parallel versus antiparallel issue is a challenge for coarse-grained modelers and others since even all-atom simulations at present do not give clear preferences in forming correct β -sheets. In our simulations of A β (16-22) peptide systems³⁴ using the original H-bond distance constraints we observed perfect antiparallel β -sheets in simulations at a temperature that was slightly below the transition temperature and this agreed with experimental observations. This suggested to us that the backbone geometry in PRIME20 with the original H-bond constraint could have a slight preference for anti-parallel versus parallel pair of strands in registered β -sheets. This may be realistic since there are more anti-parallel populations than parallel populations in the PDB. Our rough estimation of over 620 NMR PDBs shows that antiparallel pairs of strands are three times more likely than parallel pairs of strands. This can be seen in Figure 8 (which is introduced in the methods section). When we started our VQIVYK simulations we had hoped that the accuracy and specificity of the PRIME20 force field with the original H-bond constraints would be sufficient to yield perfectly parallel β -sheets, overcoming the slight PRIME20 preference for antiparallel sheets, but this did not happen. Our observation of mixed parallel and anti parallel β -sheet structure for VQIVYK peptides with the original H-bond distance constraints at all temperature ranges suggests that we might need more detailed side-chain geometry. This

Table III. Pair Interaction Energy Between Side-Chain Centroids on Amino Acids in VQIVYK Peptides in Units of $|\epsilon_{HB}|$

	V	Q	I	Y	K
V	−0.200	0.015	−0.200	−0.203	0.015
Q	0.015	−0.080	0.015	−0.086	−0.086
I	−0.200	0.015	−0.200	−0.203	0.015
Y	−0.203	−0.086	−0.203	−0.201	−0.086
K	0.015	−0.086	0.015	−0.086	0.073

may be the case but there is also a strong possibility that having more spheres in side-chains may not be sufficient to yield perfect fibril structures in our kinetic approach. For example, all-atom simulations^{38,39} of GNNQQNY peptide systems, which form parallel β -sheets experimentally, show very small free energy differences between parallel and anti-parallel pairs of strands. This small free energy difference is one reason why it is hard to find unique parallel β -sheet structure in kinetic simulations starting from random configurations. Nevertheless the atomic scale details (N, C, O) of the interactions which contribute to shape complementarity could make a difference by encouraging the alignment of β -strands as well as enhancing the thermodynamic stability of the steric-zipper interface in cross- β spine. This is beyond our one-sphere per side chain model; we are considering incorporating more spheres in some of the side-chains and detailed force-fields among these spheres.

In summary, we simulate the spontaneous fibrillization of 48 amyloid tau-fragments (VQIVYK) at temperatures below and near the fibrillization temperatures. Mixed parallel and anti-parallel pairs of β -sheets are observed based on the original H-bond distance constraints. The parallel preference H-bond distance constraints are suggested for enhancing the formation of parallel pairs of β -sheets. At temperatures just below the transition temperature, the final fibrillar structures show nearly-perfect parallel β -sheets and cross- β spines with antiparallel inter-sheets, consistent with the experiment. We can observe the coherent locations of side-chain spheres in registered parallel β -sheets which is qualitatively consistent with the steric-zipper spine. The high temperature environment near the fibrillization temperature encourages the formation of perfect fibril structures.

Methods

We apply our new intermediate-resolution force field, PRIME20^{33,34} in discontinuous molecular dynamics⁴⁰ simulations of the aggregation of the amyloid tau fragment, VQIVYK. PRIME20 is an extension of PRIME (Protein Intermediate-Resolution Model)^{22,35,36} which we have previously used to simulate the aggregation of large systems of polyaniline peptides, (KA14K), and polyglutamine peptides (Q16

to Q48).²³ PRIME20 was designed to be applicable to all twenty amino acid residues. It has recently been tested in simulations of systems containing A β (16-22) a key fragment of the Alzheimer's peptide,³⁴ 6-10 amino-acid-fragments of the prion proteins,⁴¹ and the designed sequences of Lopez de la Paz *et al.*^{42,43}

In PRIME20, peptides are modeled using a 4-sphere-per-residue representation (backbone united atoms NH, C α H, and CO, and a single sphere side chain). All forces between the united atom spheres are modeled with discontinuous potentials, for example, hard-sphere and square-well interactions. We assigned mass to each united atom as C α H(0.866), NH(0.999), CO(1.863), side-chain spheres V(2.866), Q(4.795), I(3.799), Y(7.126), K(4.865) in units of alanine side-chain mass CH₃(15amu = 1.0). We used the 19 energy parameter set from Table II of Ref. 30, in these simulations. All pair interactions were represented in terms of reduced energies, $\epsilon^*(ij) = \epsilon(ij)/\epsilon_{HB}$, where ϵ_{HB} is the hydrogen-bonding energy. The pair-wise interaction energy parameters between the side-chain centroids of VQIVYK peptides are given in Table III. The interaction ranges (inner and outer diameter of the square well) between two side-chain centroids are given in Table IV. These are evaluated based on the distribution between side-chain pair distances described in Ref. 30. The C α to side-chain distances in the simulations reported here are 2.002Å (V), 3.300Å (Q), 2.400Å (I), 3.843Å (Y), and 3.550Å (K).

We increased the value of the square well outer-diameter for the hydrogen-bonding interaction from 4.2Å in the PRIME polyaniline model to 4.5Å in the PRIME20 model. This enabled us to more accurately describe hydrogen-bonds in simulations on proteins containing larger side-chains like V, Q, I, Y, K as well as in 711 native PDB structures. The PRIME20 values for the four-distance cutoffs (N_i-C α _j (5.00Å), N_i-N_{j+1} (4.74Å), C_j-C α _i (4.86Å), C_j-C_{i-1} (4.83Å)) are the same as in PRIME. However by changing the value of the outer diameter for the hydrogen bond square well interaction, the NHO and COH angles

Table IV. Hard Sphere (Upper Value) and Well (Lower Value) Diameters in Å Units Used to Describe the Square Well Potentials Between Amino Acid Side-Chain Centroids in VQIVYK Peptides

	V	Q	I	Y	K
V	3.300 6.306	3.300 6.467	3.300 6.384	3.000 6.521	3.100 6.594
Q	3.300 6.467	3.600 6.597	3.100 6.577	3.400 6.736	3.400 6.666
I	3.300 6.384	3.100 6.577	3.300 6.647	3.000 6.775	2.900 6.679
Y	3.000 6.521	3.400 6.736	3.000 6.775	3.000 6.953	3.500 6.677
K	3.100 6.594	3.400 6.666	2.900 6.679	3.500 6.677	3.500 6.887

Table V. The Original H-Bond Distance Constraints, Average Values of the Four Distance Distributions Shown in Figure 7(b–e), and the Parallel Preference H-Bond Distance Constraints

	$N_i-C\alpha_j$ (Å)	N_i-N_{j+1} (Å)	$C_j-C\alpha_i$ (Å)	C_j-C_{i-1} (Å)
Original H-bond distance constraints	5.00	4.74	4.86	4.83
Average for all structures	4.94	4.80	4.86	4.78
Average for α -helix	5.07	4.76	4.91	4.80
Average for parallel β -sheets	4.95	4.71	4.86	4.73
Average for antiparallel β -sheets	4.73	4.87	4.78	4.75
Parallel preference distance constraints	5.10	4.54	4.96	4.58

in PRIME20 wind up having a wider distribution than in PRIME. Figure 7(a) shows how the allowed values for the four distances translate to allowed ranges for the NHO and COH angles associated with backbone hydrogen-bonds.³⁶ We call the aforementioned PRIME20 values for the four cutoff distances the original H-bond distance constraints.

Figure 8(b–e) show the distributions of the populations for the four backbone hydrogen bond distances associated with: (b) all, (c) α -helix, (d) parallel β -sheet, and (e) antiparallel β -sheet structures collected from over 620 NMR PDBs having 12,251 NMR models. These were selected from among the 711 single-domain globular proteins without ligands or non-standard amino acids listed in Table I of Ref. 30; they were taken from a 3693 PDB database having at most 25% sequence homology. This is the set of proteins that we used previously to estimate pair interaction parameters in developing PRIME20.³³ Table V shows the original and the parallel-preference H-bond distance constraints for the four hydrogen bond distances along with the average values from the distributions of these distances in all, α -helical, parallel β -sheet, and anti-parallel β -sheet structures. The original cutoff values are closest to the values for the α -helical structures in terms of their general trends. We observe a clear difference between parallel and antiparallel β -sheets. The first ($N_i-C\alpha_j$) and the third ($C_j-C\alpha_i$) average distances in parallel β -sheets are larger than the respective values in anti-parallel β -sheets. The second (N_i-N_{j+1}) and the fourth (C_j-C_{i-1}) average distances in parallel β -sheets are smaller than the respective values in anti-parallel β -sheets.

The parallel preference set of distance cutoffs introduced in this article was designed to mimic and enhance the hydrogen bond directionality that is represented in Table V by the parallel β -sheets. By increasing the $N_i-C\alpha_j$ and $C_j-C\alpha_i$ distances by 0.1Å, decreasing the N_i-N_{j+1} distance by 0.2Å, and

decreasing the C_j-C_{i-1} by 0.25Å compared to the original H-bond distance cutoff values, we define a new set of four distances. These are the parallel preference H-bond distance constraints: $N_i-C\alpha_j$ (5.10Å), N_i-N_{j+1} (4.54Å), $C_j-C\alpha_i$ (4.96Å), C_j-C_{i-1} (4.58Å). Although the changes in the cutoff distances are quite small, only 2–5%, they make it easier for parallel β -sheets to form in our simulations. This introduces a very slight bias in our simulations towards the formation of parallel, as opposed to anti-parallel, pairs of β -strands.

The simulations proceeded in the following way. Forty eight peptides were placed at random locations in the simulation box with random coil conformations; this was accomplished by performing DMD simulations at high reduced temperature ($k_B T/\epsilon_{HB}$) = T^* = 0.50. The temperature was then lowered to the desired simulated temperature at which point the constant temperature simulation began. The simulated reduced temperatures were selected to be below and near the transition temperatures for fibrillization, $T^* = 0.17, 0.18, 0.185, 0.19, 0.195, 0.20$ when using the original four-distance cutoffs for the hydrogen-bond and $T^* = 0.17, 0.18, 0.185, 0.19, 0.195, 0.198, 0.20, 0.202$ when using the parallel preference four-distance cutoffs. Extensive runs of up to 295 billion collisions (reduced time $t^* = t/\sigma(k_B T/m)^{1/2} \approx 15,000$) were conducted with periodic boundary conditions at a molar concentration of 10mM with box size (200Å).³ Five independent runs at each temperature were performed.

In this study, we analyze and measure the β -sheet content, and the probability of having parallel and anti-parallel neighboring β -strands within a β -sheet. A β -sheet is defined to exist when two peptides are interacting by at least three hydrogen bonds and the dihedral angles (ϕ, ψ) for their residues belong to the β -sheet region ($-180 < \phi < -30$ and $0 < \psi < 180$) in a Ramachandran plot. The β -sheet content is the fraction of peptides belonging to β -sheets. To determine if neighboring peptides in a β -sheet are parallel or antiparallel we calculate the angle θ between two interacting peptide vectors extending from the C_α position on V1 to the C_α position on K6. We identify ($\theta < 60^\circ$) with parallel and ($\theta > 120^\circ$) with antiparallel.

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