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# Preformed template fluctuations promote fibril formation: Insights from lattice and all-atom models

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Fibril formation resulting from protein misfolding and aggregation is a hallmark of several neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Despite the fact that the fibril formation process is very slow and thus poses a significant challenge for theoretical and experimental studies, a number of alternative pictures of molecular mechanisms of amyloid fibril formation have been recently proposed. What seems to be common for the majority of the proposed models is that fibril elongation involves the formation of pre-nucleus seeds prior to the creation of a critical nucleus. Once the size of the pre-nucleus seed reaches the critical nucleus size, its thermal fluctuations are expected to be small and the resulting nucleus provides a template for sequential (one-by-one) accommodation of added monomers. The effect of template fluctuations on fibril formation rates has not been explored either experimentally or theoretically so far. In this paper, we make the first attempt at solving this problem by two sets of simulations. To mimic small template fluctuations, in one set, monomers of the preformed template are kept fixed, while in the other set they are allowed to fluctuate. The kinetics of addition of a new peptide onto the template is explored using all-atom simulations with explicit water and the GROMOS96 43a1 force field and simple lattice models. Our result demonstrates that preformed template fluctuations can modulate protein aggregation rates and pathways. The association of a nascent monomer with the template obeys the kinetics partitioning mechanism where the intermediate state occurs in a fraction of routes to the protofibril. It was shown that template immobility greatly increases the time of incorporating a new peptide into the preformed template compared to the fluctuating template case. This observation has also been confirmed by simulation using lattice models and may be invoked to understand the role of template fluctuations in slowing down fibril elongation *in vivo*. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4917073>]

## I. INTRODUCTION

Alzheimer's, Parkinson's, Huntington's diseases, type II diabetes, mad cow disease, and cystic fibrosis: these apparently unrelated diseases, the so-called protein structural diseases, are found to be a result of protein misfolding.<sup>1</sup> This has spurred many experimental<sup>1–11</sup> and theoretical studies<sup>12–27</sup> to understand factors and mechanisms that drive oligomer formation. Aggregation rates depend not only on protein sequence but also on the concentration of proteins and external conditions like temperature, pH, and presence of crowding agents. The observation that many proteins that are unrelated by sequence and structure can aggregate and form fibrils<sup>1</sup> with similar morphologies suggests certain generic aspects of oligomerization.

A number of mechanisms for fibril elongation such as the so called templated-assembly mechanism,<sup>28–31</sup> nucleation-

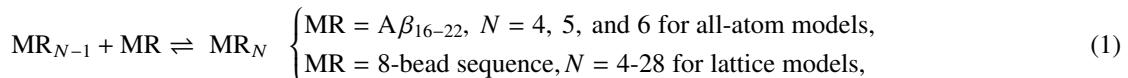
growth,<sup>32</sup> and nucleated conformational conversion<sup>2,33</sup> have been proposed. Experimental<sup>28,34</sup> and theoretical<sup>23,30,35</sup> studies suggest that in the templated-assembly scenario, the association of monomers to the preformed fibril follows the dock-lock mechanism, i.e., a nascent monomer can dock and then undergo the needed structural arrangement to lock onto the template. In the previous work,<sup>30</sup> it has been suggested that a template of a few peptides fluctuates a lot to accommodate a nascent monomer. However, the question as to what extent the fluctuation modulates the fibril formation rate remains open. In the present paper, we consider this problem assuming that the growth of fibrils occurs by addition of one unstructured monomer at a time<sup>34</sup> and that fluctuations of the preformed template are small provided its number of monomers exceeds the size of critical nucleus  $N_c$ .

Because in simulations we can only deal with a limited number of monomers to mimic weak fluctuations of the template, we kept  $C_\alpha$  positions of the template fixed during

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simulations. Such a template will be referred to as the fixed template (FT). To study the effect of fluctuations on fibril formation rates, we considered the non-fixed template (NFT) in which monomers of the preformed template are allowed to

move. Initial configurations of FT and NFT were chosen to be the same. The kinetics of association of an added monomer with the preformed FT and NFT is monitored by studying the following reaction (Eq. (1)):



where MR stands for the monomer. Because simulations of fibril formation by long peptides and proteins are very time consuming, for all-atom models we chose the Gromos96 43a1 force field<sup>36</sup> and short amyloid peptide  $\text{A}\beta_{16-22}$ .<sup>30</sup> In the lattice model, MR is an 8-bead monomer.<sup>37</sup> To assure the robustness of main results against template structures, we have also performed limited all-atom simulations for (8 + 1)-systems of fragment  $\text{A}\beta_{16-21}$  for which the double-layered protofibril structure was experimentally resolved.

Our study of reaction (1) with FT and NFT shows that the immobility of templates greatly slows down the fibril elongation process. This main result, based on both all-atom and coarse-grained lattice models, may be invoked to understand why fibril growth above the critical nucleus with small fluctuations of the preformed template is still very slow.

Overall, we present evidence that in the case of FT, the fibril state might be reached along alternative slow kinetics pathways. Since the fixation of backbone atoms makes the template more rigid, it mimics the decreased backbone entropy. An intriguing conclusion one might propose that fast kinetics in case of NFT is entropy-driven, e.g., an increase in backbone entropy facilitates the kinetics of fibril formation.

## II. METHODS/EXPERIMENTAL SECTION

### A. All-atom models

We used the GROMOS96 43a1 force field<sup>36</sup> to model  $\text{A}\beta_{16-22}$  peptides, and SPC water model<sup>38</sup> to describe the solvent. This model has been successfully used for studying protein folding,<sup>39</sup> unfolding,<sup>40</sup> and aggregation.<sup>41,42</sup> The simulations were performed for systems with FT, while the corresponding systems with NFT have been studied in our earlier work.<sup>30</sup> Gromacs version 4 was employed for the simulations.

#### 1. Templates and an added peptide

The initial conformation for the nascent peptide  $\text{A}\beta_{16-22}$  used in the simulations was extracted from the structure of the  $\text{A}\beta_{10-35}$  peptide available in the Protein Data Bank (ID: 1hz3).<sup>43</sup> The terminal residues are oppositely charged (a positive charge on the lysine and a negative charge on the glutamic acid). For the templates, we used antiparallel configurations of  $\text{A}\beta_{16-22}$  peptides obtained by long molecular dynamics (MD) all-atom simulations in our previous work.<sup>30</sup>

The templates for the three systems studied in this paper are shown in Fig. 1. During all-atom FT simulations, we kept  $C_\alpha$  positions of the template frozen to prevent template disassembly. All other atoms of template were allowed to move without any restraints. The added monomer is randomly put next to the template, and the same starting conformations for both FT and NFT are used.

### 2. Details of MD runs

For each system we performed 8 MD runs (trajectories), the durations of which are given in Table I. The typical volumes of boxes used in the simulations are 62, 86, and 130 nm<sup>3</sup> for (3 + 1), (4 + 1), and (5 + 1) systems, respectively. This corresponds to peptide concentrations of 112, 99, and 80 mM which are about two orders of magnitude as high as those used in the experiments.<sup>1</sup>

### 3. Principal component analysis

We used dihedral principal component analysis (dPCA)<sup>44</sup> to represent the free energy landscape (FEL) of the 3*N*-dimen-

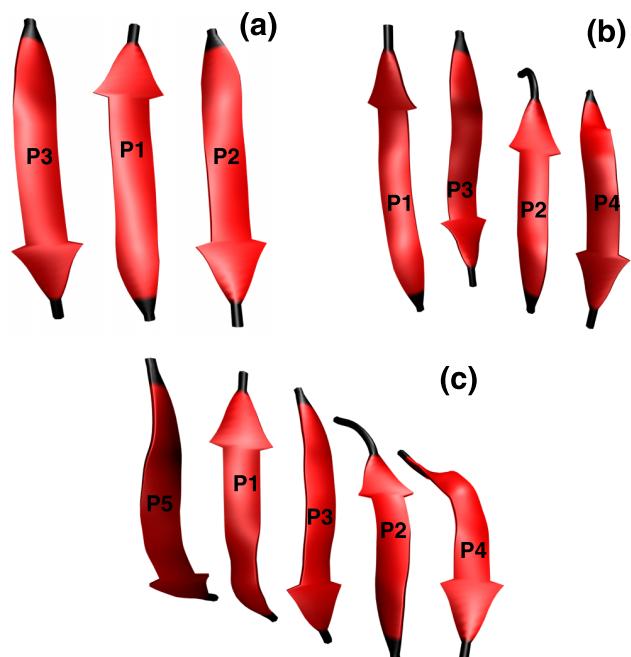


FIG. 1. The templates used in our simulations, where (a), (b), and (c) are for the (3 + 1)-, (4 + 1), and (5 + 1)-system, respectively. These configurations were obtained by long MD simulations with the Gromos 43a1 force field in our prior work.<sup>30</sup>  $P_i$  refers to peptide *i*.

TABLE I.  $\bar{\tau}_{\text{inc}}$  of individual trajectories of all-atom MD runs for three systems with FT. The whole simulation time is shown in parentheses.

System	Time (ns)		
	3+1	4+1	5+1
Tr1	19.4 (178)	>500 (500)	179.5 (500)
Tr2	383.5 (500)	138 (500)	321 (500)
Tr3	>500 (500)	247.5 (500)	>500 (500)
Tr4	>500 (500)	486.5 (500)	212 (500)
Tr5	411.7 (500)	>500 (500)	>500 (500)
Tr6	102.3 (200)	>500 (500)	>500 (500)
Tr7	43.7 (200)	31.5 (200)	>500 (500)
Tr8	13.5 (200)	426.5 (500)	>500 (500)
Tr9		96.4 (500)	
Tr10		>500 (500)	
Tr11		345.2 (500)	
Tr12		165.9 (500)	
Tr13		>500 (500)	
Tr14		498.5 (500)	
Tr15		>500 (500)	
Tr16		>500 (500)	

sional system. Free energy is calculated as a function of the first two eigenvectors  $V_1$  and  $V_2$  in dPCA. Note that FEL is not equilibrium FEL but rather to present probabilities of different structures to occur during MD simulations.

#### 4. Measures used in structure analysis

To characterize the fibril state of short peptides, we used not only nematic order parameter  $P_2$ <sup>30,45</sup> but also took into account the number of backbone hydrogen bonds (HBs) between nascent peptide and FT. This more strict condition prevents false signals of an ordered state including cases where nascent peptide has high  $P_2$  but is located either far from the template or above/below the template. If  $P_2$  is larger than 0.8 and the number of average backbone HBs is larger than 3, then the system is considered to be in an ordered state. A visual inspection was also performed to exclude the configurations with a parallel arrangement of  $\beta$ -strands. The time to incorporate a nascent peptide to the template,  $\tau_{\text{inc}}$ , is defined as the first passage time to reach an antiparallel ordered structure starting from a preformed template and a randomly added peptide. The median time serves as an estimate of the oligomerization time for each of studied systems. As we have 8 trajectories for each system, the median is defined as the mean of the 4th and 5th values.

#### B. Lattice model

To overcome the limits set by expensive all-atom modeling, coarse-grained lattice models might be successfully utilized for protein folding studies.<sup>46–48</sup> In this work, we use the toy lattice model which has been developed for studying oligomerization kinetics.<sup>37</sup> Typically, each chain consists of  $M$  connected beads confined to the vertices of a

cube. The simulations use  $N$  identical chains and  $M = 8$ . The sequence of a chain is +HHPPHH-, where + and - are the charged beads. H and P refer to hydrophobic and polar beads, respectively. Despite the simplicity of the lattice model, it has been proved to be useful in providing insights into fibril formation mechanisms.

The inter- and intra-chain potentials include excluded volume and nearest-neighbor contact interactions. Excluded volume is imposed by the condition that a lattice site can be occupied by only one bead. The energy of  $n$  chains is

$$E = \sum_{l=1}^N \sum_{i < j}^M e_{sl(i)sl(j)} \delta(r_{ij} - a) + \sum_{m < l}^N \sum_{i,j}^M e_{sl(i)sm(j)} \delta(r_{ij} - a), \quad (2)$$

where  $r_{ij}$  is the distance between residues  $i$  and  $j$ ,  $a$  is the lattice spacing,  $sm(i)$  indicates the type of residue  $i$  from the  $m$ th peptide, and  $\delta(0) = 1$  and zero, otherwise. The first and second terms in Eq. (2) represent intrapeptide and interpeptide interactions, respectively.

Contact energy between H beads  $e_{HH}$  is  $-1$  (in hydrogen bond energy units  $\epsilon_H$ ). The propensity of polar (including charged) residues to be “solvated” is mimicked using  $e_{P\alpha} = -0.2$ , where  $\alpha = P$ , +, or -. “Salt-bridge” formation between oppositely charged beads is accounted for by a favorable contact energy  $e_{+-} = -1.4$ . All other contact interactions are repulsive. The generic value for repulsion  $e_{\alpha\beta} = 0.2$ . For a pair of like-charged beads, the repulsion is stronger, i.e.,  $e_{++} = e_{--} = 0.7$ . The chains are confined to the vertices of the three-dimensional hypercube. Monomer concentration is kept at  $\approx 6$  mM (the cubic size is roughly  $35a$  for  $N = 10$  monomers) for all systems. This is roughly one order of magnitude denser than that used in typical experiments.

Simulations were performed by enclosing  $N$  chains in a box with periodic boundary conditions. We used the Monte Carlo (MC) method to study the kinetics of fibril formation. MC moves include global and local ones. A local move<sup>49,50</sup> corresponds to tail rotation, corner flip, and crankshaft rotation. Global moves correspond to either translation of a peptide by  $a$  in a randomly chosen direction or rotation by  $90^\circ$  around one of the randomly chosen coordinate axes. Acceptance probabilities of global and local moves are 0.1 and 0.9, respectively (see Ref. 37 for more details). We measure time in units of Monte Carlo steps (MCS’s). The combination of local and global moves constitutes one MCS. Although the correspondence between real time and MC step is not clear, as shown previously, our MC method is still useful to compare kinetics of different systems.<sup>26,37,51,52</sup>

Initial conformations have a preformed template with  $N$  antiparallel chains and one chain which is randomly added next to the template (see below). As in the all-atom model case,  $\tau_{\text{inc}}$  is defined as the number of MCS’s needed to reach the mature  $N$ -chain fibril which has the lowest energy. The fibril state is characterized by the number of inter-chain contacts which are called fibril contacts.

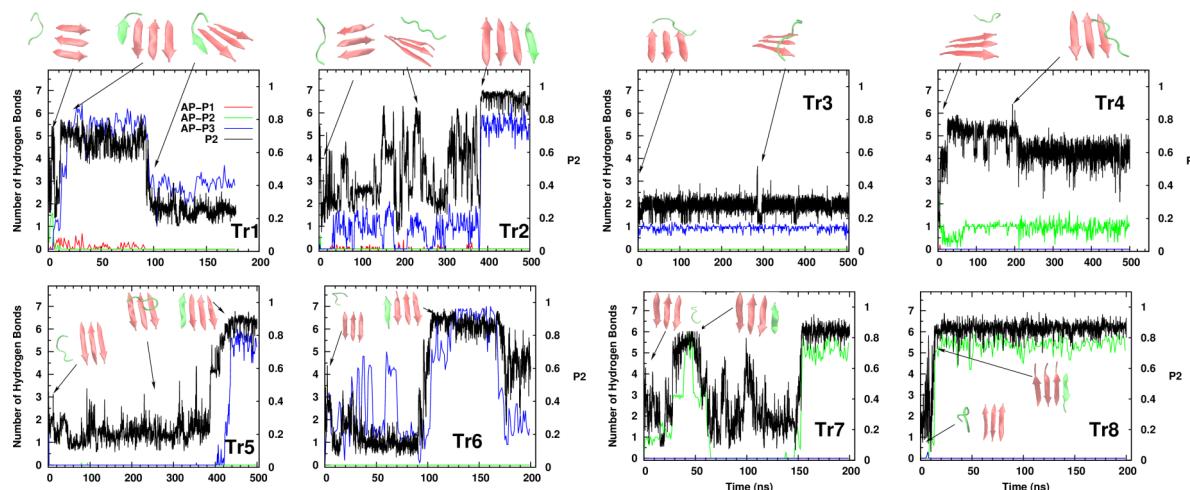


FIG. 2. The time dependence of order parameter  $P_2$  (right scale) and number of backbone HBs between nascent peptide and FT (left scale) for four trajectories of the (3 + 1)-system. Black curve corresponds to  $P_2$ , while the curves representing number of HBs between nascent peptide and one of peptides from FT are colored according to the legend on Fig. 2 for Tr1. Here, AP- $P_i$  refers to backbone HBs between added peptide and peptide  $i$  that belongs to the template. The nascent peptide  $\text{A}\beta_{16-22}$  shown on snapshots is colored in green. The fibril antiparallel arrangement occurs in all trajectories except the Traj3 and Traj4.

### III. RESULTS AND DISCUSSION

#### A. All-atom model

##### 1. Diversity of fibril formation kinetics: Peptide association may proceed via intermediates

As shown by experiments and simulations,<sup>30,31,34</sup> the addition of a new monomer onto a growing template obeys the two-stage dock-lock mechanism. It is worth noting that amyloid fibrils are characterized not only by ordered peptides perpendicular to the fibril axis but also by backbone HBs between them parallel to the fibril axis. For a mobile template, the kinetics is simple.<sup>30</sup> First, a nascent peptide rapidly docks to the edge of the preformed template, then it reaches the fibril state through a slower locking stage. The conformations with high beta content and  $P_2$  values are identified as fibril-like conformations where a nascent peptide is tied to the preformed template by backbone HBs. Although such correspondence

between high  $P_2$  values and a large number of HBs works for NFT, it becomes insufficient for FT. In this case, there exist a number of trajectories where the ordered peptide (with  $P_2$  values  $>0.8$ ) has no more than 1 backbone hydrogen bond with the template (Tr4 and Tr5 in Fig. 2; Tr4, Tr5, and Tr6 in Fig. 3; and Tr1, Tr3, Tr5, and Tr7 in Fig. 4). In such trajectories, a nascent peptide is directed into the position above the template in which it predominantly interacts with a template through side chain-side chain (SC-SC) interactions. In other words, although the nascent peptide is extended and oriented in the right direction, backbone HBs with the template are not formed and those conformations do not correspond to the fibril state. Thus, we use the number of backbone HBs between a nascent monomer and a template as an additional (unambiguous) indicator of the fibril state. The simple definition involving  $P_2 > 0.8$  and more than three backbone HBs between the monomer and the template defines the fibril state in a well-defined manner.

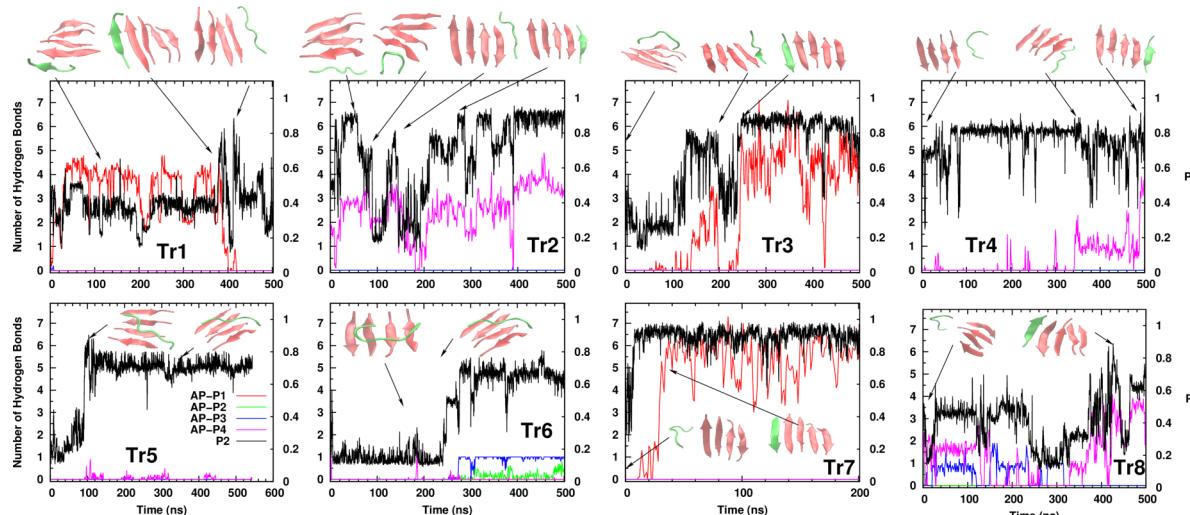


FIG. 3. The same as in Fig. 2 but for the (4 + 1)-system. Black curve corresponds to  $P_2$ , while the curves representing number of HBs between nascent peptide and one of peptides from FT are colored according to the legend on Fig. 3 for Tr5. The fibril antiparallel arrangement occurs in trajectories 2-4, 7, and 8 but not in the first, fifth, and sixth ones. Unexpected parallel ordering is observed in the first run, where the nascent peptide is docked by the edge of the template.

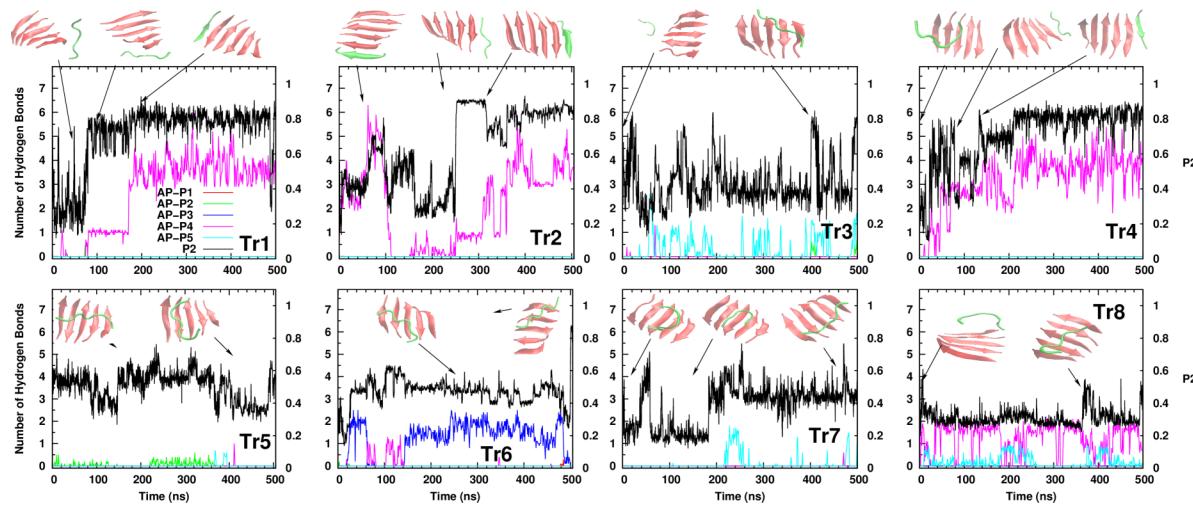


FIG. 4. The same as in Fig. 2 but for the (5 + 1)-system. Black curve corresponds to  $P_2$ , while the curves representing number of HBs between nascent peptide and one of peptides from FT are colored according to the legend on Fig. 4 for Tr1. The fibril arrangement occurs in the first and second trajectories but not in the third and fourth ones. In the second trajectory, the parallel orientation of the added peptide occurs (at  $t \approx 62$  ns) earlier than the antiparallel one (at  $t \approx 321$  ns).

As evident from Figs. 2 to 4, there are fast and slow kinetics routes toward the fibril state. We interpret the slow kinetics pathways as a sign of the occurrence of the intermediate state which corresponds to a plateau on the curve of time dependence of the order parameter  $P_2$ . For (3 + 1) systems, intermediates occur in Tr3, Tr4, Tr5, and Tr6 (Fig. 2), while the fibril state was reached in other MD runs at relatively short time scales. In (4 + 1) systems, intermediate states were observed in Tr1, Tr5, and Tr6 where the ordered state did not appear during the whole simulation course (Fig. 3). Particularly, two intermediates with  $P_2 \approx 0.1$  and 0.65 occurred in Tr6. We also see short-lived intermediates in Tr2, Tr3, and Tr8. For (5 + 1) systems, there is clear evidence for the existence of intermediates in Tr3, Tr4, Tr5, Tr6, Tr7, and Tr8 where the fibril-like state did not appear during the whole MD run (Fig. 4). In Tr7, there are at least two intermediates. The anti-parallel configuration is reached relatively rapidly in the remaining trajectories.

We observe a parallel orientation of peptides (Fig. 3, Tr1 and Fig. 4, Tr2) which is one of the obstacles that complicates aggregation kinetics. In the case of 4 + 1 system (Fig. 3, Tr1), we do not observe transition from a parallel to antiparallel configuration for 500 ns, while for 5 + 1 system (Fig. 4, Tr2), it takes about 300 ns for the fibril state to occur. For the 4 + 1 system, the parallel orientation detected in our FT simulation is a sign of the intermediate state. However, because the average addition time for 5 + 1 system exceeds 220 ns, it is not clear whether such a conformation is on a pathway to intermediates but transitions from parallel to antiparallel configurations are apparently expected to slow aggregation.

## 2. Association of a new monomer with the fixed template depends on initial conditions

It is evident from Figs. 2 to 4 that  $\tau_{\text{inc}}$  greatly varies from trajectory to trajectory. One of the reasons for this is that we used different starting configurations for a nascent monomer for different runs keeping the same FT for all 8 trajectories. For trajectory 8 of the (3 + 1)-system, in which the fibril structure

is formed, the added monomer is initially located aside the template (Fig. 5, Tr8). This initial configuration is strikingly different from that of the slowest trajectory 3 (Fig. 5, Tr3). Here, the nascent monomer is located above (or below) the template and nearly perpendicular to the preformed chains. The difference in starting configurations leads to different FELs (Fig. 5). Typical free energy barriers separating main basins of the fast trajectory 8 are about 5 kJ/mol compared to  $\approx 14$  kJ/mol for the slow trajectory 3. In the former case, the high mobility of a nascent monomer caused by the flat FEL facilitates fibril formation. For trajectory 3, due to high free energy barriers, the system may get trapped in local minima that hinder the formation of ordered fibrils. The difference in free energy barriers,  $\Delta\Delta G \approx 14 - 5 = 9$  kJ/mol, leads to the difference in aggregation rates of about two orders of magnitude at room temperature.

The dependence of FEL on initial configurations is also illustrated in Fig. 5 for two trajectories 2 and 3 of the (5 + 1)-system. Similarly to the (3 + 1) case, if the added monomer is initially positioned aside the template, FEL is more flat (trajectory 2) than when the nascent peptide is positioned above/below the template (trajectory 3). In the latter case, FEL consists of isolated pieces leading to slow fibril elongation. As follows from Figs. 5(c) and 5(d), the difference in free energy barriers between main basins is also about 10 kcal/mol.

## 3. Immobility of the template slows down the fibril formation process

a. (3 + 1)-system. In contrast with NFT simulations,<sup>30</sup> our results indicate that kinetics is much more complex and diverse for FT. The fibril state occurs very fast with  $\tau_{\text{inc}} \approx 19.4$ , 43.5, and 13.5 ns for trajectories 1, 7, and 8, respectively. The fibril state is not stable for trajectories 1 and 7 because peak  $P_2$  drops at  $\approx 92$  and  $\approx 50$  ns, respectively, and fluctuates around a moderate value (Fig. 2, Tr1 and Tr7). Such instability is due to shallow free energy barriers (Fig. 5, Tr1) and a nascent peptide can easily jump from one basin to another. As a result, after

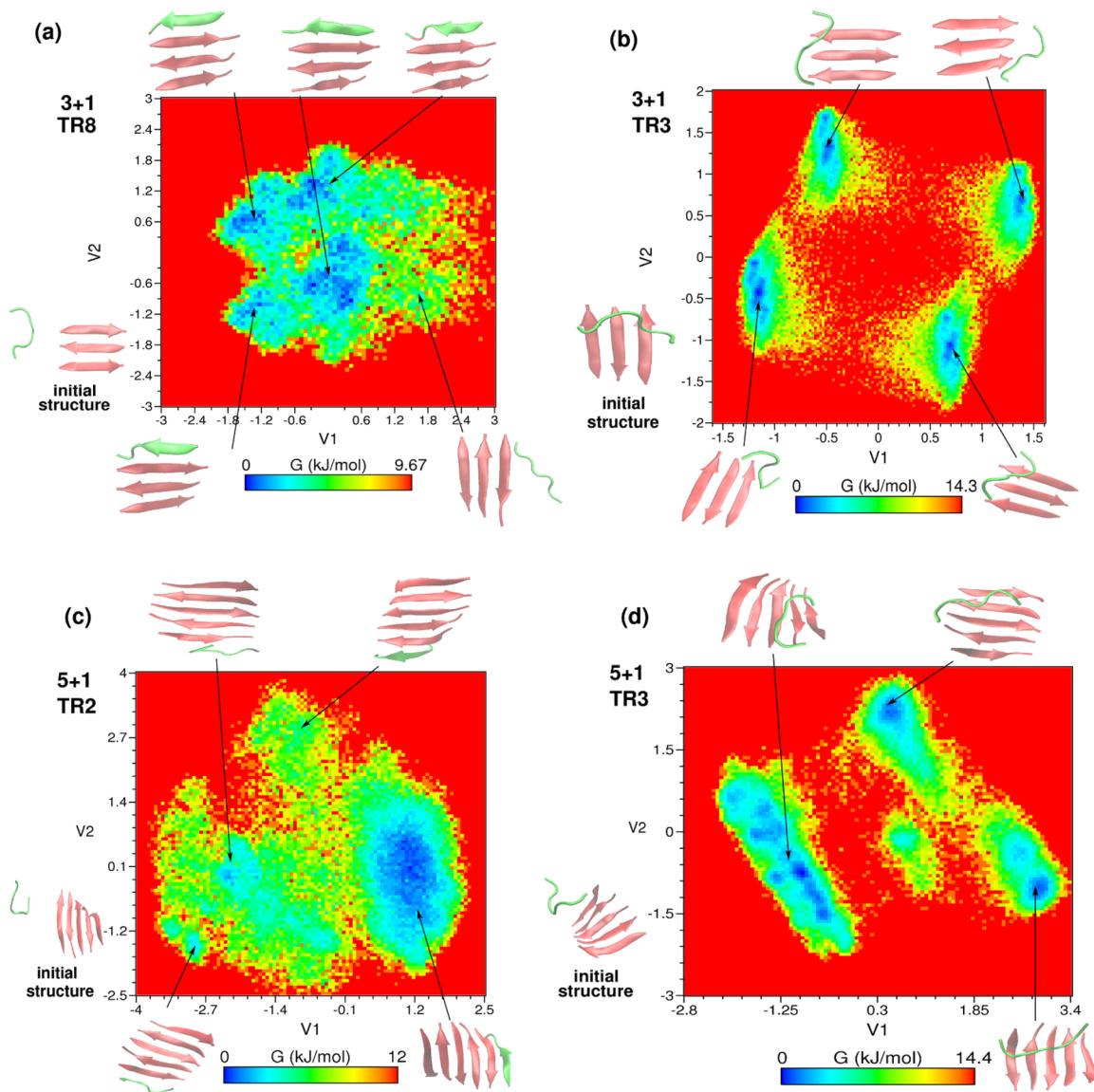


FIG. 5. Free energy surface of trajectory 8 (a) and trajectory 3 (b) for the (3 + 1) systems, and of trajectory 2 (c) and trajectory 3 (d) for the (5 + 1) systems. The first and second eigenvectors of the fluctuations covariance matrix used for construction of FEL's account for roughly 62% of the whole information about the systems studied. In trajectory 1 of (3 + 1) system and trajectory 2 of (5 + 1) system, the fast association of the nascent with the fixed template is observed. For trajectory 3 of both systems, the fibril state does not occur after 500 ns.

about 100 ns, the fibril state reoccurs in Tr7 but not in Tr1. The eighth trajectory represents an example of a fast aggregation pathway at which the fibril state remains stable. Remarkably, the fibril structure does not appear in MD runs 3 and 4. For the third run, the order parameter  $P_2$  and average number of HBs remain low for 500 ns. Much higher  $P_2$  values are observed for trajectory 4, but the low number of backbone HBs does not guarantee the occurrence of the fibril state (Fig. 2, Tr4). Typical snapshots shown in Fig. 2, Tr3 and Tr4, indicate that in cases where fibril structure is not formed or formed very slowly (Fig. 2, Tr2 and Tr5), the nascent peptide is directed into a state above the template in which it predominantly interacts with the template through SC-SC interactions. We have interpreted such slow kinetics pathways as a sign of an intermediate state, where the slow phase is associated with crossing over the high barrier from off-pathway intermediate states to the fibril-like ones. This can be also interpreted as

getting out of conformations above/below the template to the edge of the template before the docking phase begins.

Calculating the median time over eight trajectories, we obtain  $\bar{\tau}_{\text{inc}} \approx 242.9$  ns for the (3 + 1)-system. This value is much larger than  $\bar{\tau}_{\text{inc}} \approx 23$  ns obtained for the case where the template is not fixed<sup>30</sup> (Table I). Thus, template immobility considerably slows down the association of a nascent peptide with the preformed oligomer.

b. (4 + 1)-system. The nascent peptide and preformed template form a fibril in trajectories 2-4, 7, and 8, where  $\tau_{\text{inc}}$  varies from  $\approx 31.5$  to  $\approx 486$  ns (Fig. 3). For the first MD run, the fibril-like state is observed but with a parallel orientation. Thus,  $\tau_{\text{inc}}$  for the expected antiparallel ordering should be longer than the whole run of 500 ns. This is supported by the snapshot collected when  $P_2$  reaches one of the highest values.  $P_2$  becomes relatively high after 100 and 282 ns for trajectories 5 and 6. However, a fibril is not formed due to a

TABLE II.  $\bar{\tau}_{\text{inc}}$  obtained for FT and NFT cases. The results were averaged over 4 trajectories for NFT. The median time, i.e., the mean of the 4th and 5th values from 8 trajectories, is used for FT.

System	Not fixed template	Fixed template
3 + 1	23	242.9
4 + 1	114	456.5
5 + 1	>220	>500

very low value of backbone HBs between the added peptide and FT. Using the results shown in Table I, we obtain median time  $\bar{\tau}_{\text{inc}} \approx 456.5$  ns which is larger than  $\bar{\tau}_{\text{inc}} = 114$  ns for NFT<sup>30</sup> (Table II).

Interestingly, the off-pathway intermediate and parallel configurations typical for slow pathways were not observed in NFT simulations.<sup>30</sup> Thus, the reduced flexibility of a template with a lower level of complexity allows a nascent peptide to visit a larger number of conformation states compared to NFT. This includes both off-pathway intermediate and parallel ordered conformations, which requires an extra barrier to be overcome for fibrils to occur.

c. (5 + 1)-system. The slowing down of the peptide association process by template immobility is also seen in the (5 + 1)-case (Fig. 4). An antiparallel fibril occurs at  $\tau_{\text{inc}} \approx 179.5, \approx 321$ , and  $\approx 212$  ns for the first, second, and fourth trajectories, respectively. For trajectories 3 and 5–8, at high  $P_2$  values, the nascent peptide gets trapped in a conformation and it is typically located above (or below) the template without a significant number of backbone HBs formed with FT. The fibril state does not appear after 500 ns (Table I). The median time calculated from 8 trajectories exceeds the duration of MD runs,  $\bar{\tau}_{\text{inc}} > 500$  ns. Since for the NFT case the corresponding  $\bar{\tau}_{\text{inc}} > 220$  ns<sup>30</sup> (Table II), our result suggests that template fixation slows down the association of a peptide to the preformed template but this conclusion is not as transparent as in (3 + 1) and (4 + 1) systems. Therefore, to clarify this point, an additional simulation will be carried out using simple lattice models.

#### 4. Robustness of results against data sampling

So far we have performed 8 independent MD runs for each system. The important question emerges is if this sampling is sufficient enough to not bias our main conclusions on the impact of template mobility on the kinetics behavior of the system. Because the all-atom simulation in explicit water is very time consuming, we have carried 8 additional 500 ns runs for (4 + 1)-systems (Fig. S1 in the supplementary material<sup>58</sup> and Table I). Calculating the median time over 16 trajectories, we obtain  $\bar{\tau}_{\text{inc}} \approx 492.5$  ns. This value is comparable to the median time  $\bar{\tau}_{\text{inc}} \approx 456.5$  ns obtained for the first 8 trajectories of (4 + 1) system and which is larger than  $\bar{\tau}_{\text{inc}} = 114$  ns for NFT. Thus, the reduction of aggregation rates due to template immobility is robust against data sampling and this is expected to hold not only for the (4 + 1) system but also for other systems.

The diversity in kinetics routes to the fibril-like state is also observed in 8 additional trajectories (Fig. S1 in the supplementary material<sup>58</sup>). For Tr9, the antiparallel arrangement occurred without intermediates but it is not the case

for Tr11, Tr12, and Tr14 although their  $\bar{\tau}_{\text{inc}}$  is shorter than the whole simulation time. A long-lived intermediate was observed in Tr11 where the parallel configuration appears at about 90 ns. The ordered state did not appear during the 500 ns MD simulation in Tr10, Tr13, Tr15, and Tr16. Taken together, the overall picture about complex kinetics pathways remains the same as in the case of 8 trajectories suggesting that the reduced entropy plays a decisive role but not the number of sampling.

#### 5. Robustness of results against double-layered structure

Strictly speaking, the single layer structure of short peptides is neither amyloid fibril nor protofibril. To mimic protofibril in a more realistic way, we consider a double-layered structure as template. Because the double-layered structure of  $\text{A}\beta_{16-22}$  is not available, we used the atomistic model proposed by the Eisenberg group<sup>53</sup> for  $\text{A}\beta_{16-21}$  (KLVFFA). KLVFFA octamer (Fig. S2 in the supplementary material<sup>58</sup>), extracted from KLVFFA dodecamer structure (pdb code: 3OW9), was chosen as a template. As in the single-layered case, the added monomer was randomly put next to the template so that no intermolecular contacts presented and the same starting conformations for both FT and NFT cases were used. The combined (8 + 1)-system was placed in a dodecahedron box of such a size that the minimal distance from peptides and the box is 1.75 nm. This was followed by solvation with 7734–9565 water molecules and nine chloride ions were added to neutralize the system charge. To avoid improper structures, the whole system was minimized with the steepest-descent method, before being equilibrated at 300 K with two successive molecular dynamics runs of length 500 ps each; the first one at constant volume and the second at constant pressure (1 atm). The equilibrated conformations were used as the starting structures for 200 and 400 ns MD simulations for NFT and FT, respectively. The simulations were performed at  $T = 300$  K with the same force field and water model as in the single-layered structure case.

Out of 4 trajectories, the antiparallel conformation was observed only in Tr4 for the FT case (Fig. S3 and Table S1 in the supplementary material<sup>58</sup>). In contrast, for NFT, the protofibril occurred in all MD runs after relatively short times. We obtained  $\bar{\tau}_{\text{inc}} \approx 84.35$  and  $>500$  ns for NFT and FT double layer systems, respectively. Thus, regardless of sequence and protofibril structure, the template immobility reduces the aggregation rate and this effect is universal and holds for other systems.

#### B. Lattice model

In this section, we consider the kinetics of association of a new monomer to the preformed template using the lattice model.<sup>37</sup> The reason for doing this is that, as follows from Table II, it remains uncertain within the all-atom model whether template immobility slows down the oligomerization of the (5 + 1)-system and this is also unclear for larger systems. Therefore, our aim is to show that the irreversibility of aggregates affects the growth rate for large-size systems

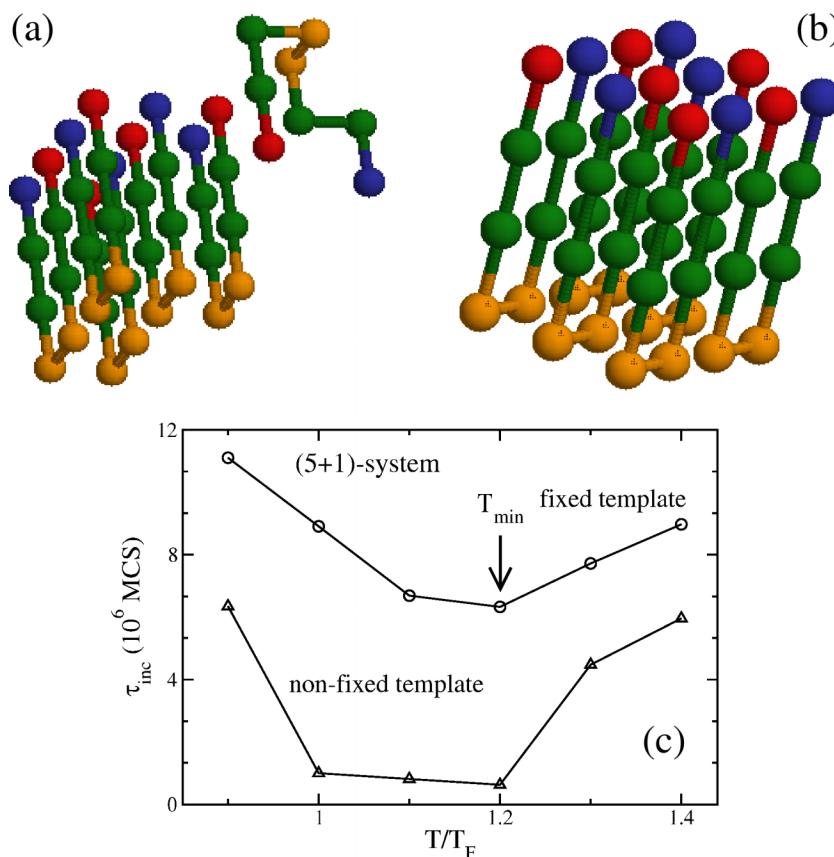


FIG. 6. (a) A typical initial conformation for the (5+1) system in the lattice model. Five template monomers are antiparallel, while the conformation of a nascent monomer is randomly generated. (b) The fibril conformation with the lowest energy  $E = -60$ . (c) The temperature dependence of  $\tau_{\text{inc}}$  for NFT and FT cases.  $T_F = 0.5\epsilon_H/k_B$  is the folding temperature for the monomer. The results are averaged over 50 MC trajectories.

using lattice model (2). The simplicity of this model allows us to study much larger systems compared to all-atom models.

### 1. Template fixation increases $\tau_{\text{inc}}$ by one order of magnitude

The temperature dependence of  $\tau_{\text{inc}}$  for the (5+1)-system with FT and NFT is shown in Fig. 6. As in the protein folding problem,<sup>54,55</sup> the U-shape comes from the interplay between

energy and entropy factors. At low  $T$  (energy driven regime), as  $T$  lowers, the probability of escaping local minima decreases due to reduced thermal energy, resulting in higher  $\tau_{\text{inc}}$ . At high  $T$ , where entropy dominates over energy, the thermal fluctuations are so high that the motion of chains becomes chaotic and the probability of acquisition of the lowest energy state becomes low resulting in increase of  $\tau_{\text{inc}}$  with  $T$ . The optimal aggregation rate is reached at  $T_{\min}$  (Fig. 6), where the entropy and energy factors reach a compromise.

The effect of template fixation is clearly seen in Fig. 6(c) for the (5+1)-system, where incorporation time onto FT,  $\tau_{\text{inc}}^{\text{FT}}$ , is nearly one order of magnitude as high as the incorporation time onto NFT,  $\tau_{\text{inc}}^{\text{NFT}}$ . The reason for the difference in incorporation times is the same as in the case of all-atom models, i.e., thermal fluctuations of NFT accommodate the added monomer.

The effect of template immobility for larger systems is shown in Fig. 7, where results were obtained at  $T_{\min}$ . The influence on a template of three chains is minor, but for  $N \geq 6$ , fibril elongation on the fixed template slows down by one order of magnitude. Thus, within lattice models, template fluctuations also speed up fibril growth. However, this result is more convincing than that based on all-atom models as it has been obtained for much larger system sizes.

If the number of chains in the template exceeds 11, both  $\tau_{\text{inc}}^{\text{FT}}$  and  $\tau_{\text{inc}}^{\text{NFT}}$  become scale invariant. Therefore, we can consider  $N_c = 11$  as the size of a critical nucleus at which the turn-over in system free energy occurs.<sup>51</sup> The same result has been obtained for other temperatures and fluctuating templates.<sup>51</sup> Thus,  $N_c$  seems to weakly depend on  $T$ , and template immobility does not have any effect on it.

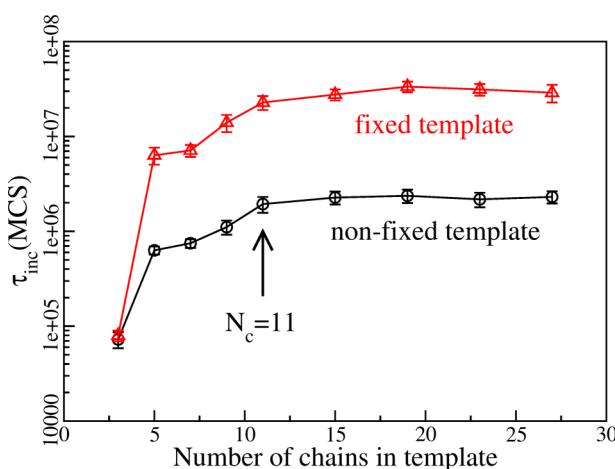


FIG. 7. Dependence of  $\tau_{\text{inc}}$  on the number of chains of FT and NFT in the lattice model. The values of  $\tau_{\text{inc}}$  are collected at  $T = T_{\min}$  (see Fig. 6). The arrow refers to the size of critical nucleus  $N_c = 11$  where  $\tau_{\text{inc}}$  starts to saturate. For each value of  $N$ , the results are averaged over 50 MC runs.

#### IV. CONCLUSIONS

Using all-atom and lattice models, we have compared the kinetics of association of a nascent monomer with FT and NFT. It is shown that the immobility of the preformed template greatly hinders oligomer growth. Since fluctuations of the preformed template are expected to be small beyond the critical nucleus size, one can partially understand why fibril formation is a very slow process. Thus, together with other intrinsic and environmental properties, template flexibility is one of the important factors governing oligomerization rates.

Due to the existence of intermediates on some pathways toward the fibril state, kinetics can be described by the kinetic partitioning mechanism,<sup>56</sup> where the fraction of trajectories without intermediates ( $\Phi$ ) reaches the ordered state rapidly, while the remaining fraction ( $1 - \Phi$ ) gets kinetically trapped following different slow pathways. Consequently, the free energy landscape includes additional valleys representing intermediate states.

We speculate that our study demonstrates that a backbone entropy loss introduced through the fixation of  $C_\alpha$  atoms opens up new kinetics routes with high energy barriers between intermediate and fibril states. Template rigidity deforms the FEL in such a way that a nascent peptide can explore newly available regions of energy landscape. Interestingly, instability of a mobile template has been pointed out as one of the factors governing oligomer growth.<sup>30</sup> However, template rigidity does not eliminate the possibility of aggregation, but reduces the kinetics rate. This result proves that the flexibility of the preformed template has a significant impact on aggregation kinetics and is one of the general determinants of aggregation rates. Since template flexibility is very important, it would be interesting to check how a change of peptide flexibility caused by mutations might affect oligomerization kinetics. For example, a Phe-Leu(Ile) mutation will enhance(decrease) peptide flexibility<sup>57</sup> while preserving hydrophobicity comparable to Phe. We are testing this idea of using the effects of amino acids substitution in the  $A\beta_{16-22}$  sequence to fine-tune oligomerization rates in ongoing simulations.

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