TWO-PEPTIDE AGGREGATION KINETICS AND β-SHEET FORMATION

Wonmuk Hwang (1,2), Shuguang Zhang (1), Roger D. Kamm (2,3)

(1) Center for Biomedical Engineering
Massachusetts Institute of Technology
Cambridge, MA 02139

(2) Biological Engineering Division
Massachusetts Institute of Technology
Cambridge, MA 02139

(3) Dept. of Mechanical Engineering
Massachusetts Institute of Technology
Cambridge, MA 02139

SUMMARY

Molecular dynamics was used to analyze the interaction of two short peptides, initially separated, and allowed to form a β-sheet. Backbone hydrogen bond patterns and their formation are monitored over time and analyzed. Unlike β-hairpin or β-sheet formations in a single polypeptide chain, the absence of constraint between the two peptides makes their mutual direction of approach random. However, the resulting alignment between the two after collision has strong directional preference. We attribute this to the inherent twist in the peptide backbone, which causes hydrogens (H) and oxygens (O) on one side to be more exposed than those on the opposite side. Our methods and results can be extended to subsequent nucleation and growth of peptide nanofibers.

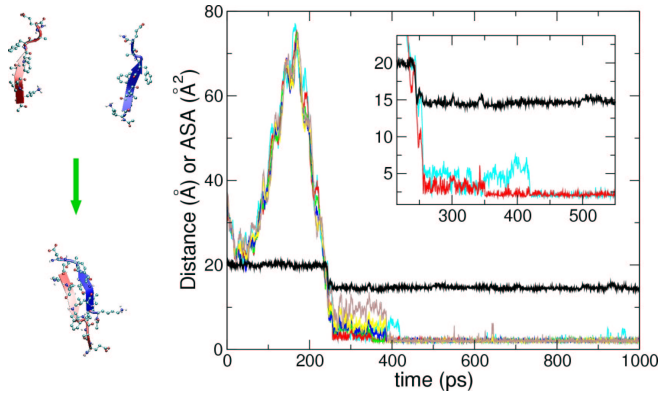
RESULTS

Two peptide sequences were tested: KLVFFAE (Aβ7 [1]), KLVFFAG (Aβ7g [2]). They are known to form amyloid fibrils. Initially, two peptides are placed 3nm apart with random orientations inside a reflecting sphere. Two hundred independent 1ns simulations were run for each sequence.

Upon initiating the simulation, the peptides move randomly, bouncing off the boundary, and finally coming together to form the backbone hydrogen bonds. Figure 1 is a sample run for the sequence Aβ7. From the change in H-O distance curves, one can see that the peptides rebound twice before coming together at 250ps. Between this time and the final stabilization time, 420ps, the backbone H and O 'search' for their partners to form hydrogen bonds. Also shown is the accessible surface area (ASA), illustrating that hydrophobic clustering is coupled with, and often precedes, hydrogen bond formation.

As peptides are asymmetric molecules, six different ways exist of forming β-sheets between them (Fig. 2). If a purely random approach is assumed, the configurations Aregl, Aregr, Pinvl, Pinvr should occur with equal probability, which in turn should be half the probability of forming either Ainv or Preg. However, left-faced configurations are more likely to occur than right-faced (Fig. 3). We attribute this to the inherent twist or bend of the peptide backbone (Fig. 4a). To measure the degree of asymmetry in the twist, we have conducted separate 1ns simulations for individual peptide monomers and measured average distance of the backbone H and O from the major axis through the peptide. Figure 4b shows that for each of the four sequences tested, backbone H and O on the left side are farther away from the major axis than those on the right side.

These findings provide new insight into peptide self-assembly and amyloid fibrillogenesis. Peptides in commonly observed anti-parallel β-sheet fibrils alternate with Aregl and Aregr configurations.



(a) **(b)**

Figure 1. Spontaneous formation of an anti-parallel β-sheet by Aβ7. (a) Configuration of the system before and after the simulation. (b) Analysis of the backbone hydrogen bonding events. Thin lines: distances between backbone H and O that eventually form hydrogen bonds. Thick black line: average ASA per atom. Inset: data near docking time. Only the earliest and the latest formed hydrogen bond distances are plotted for clarity.

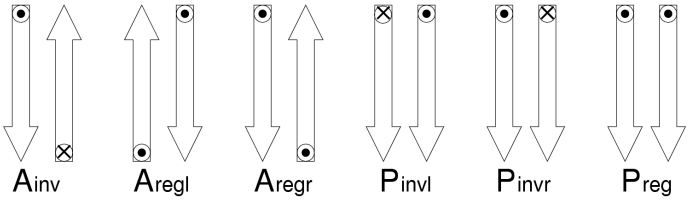


Figure 2. Six ways of aligning two peptides. Here, the sense of 'left' or 'right' is determined with respect to the direction of the peptide (N to C terminal) top to bottom and the first side chain placed out of the page (dotted circle). An inverted peptide has its first side chain facing into the page (crossed circle). For example, Aregl is an anti-parallel configuration with the left sides of the peptides facing each other.

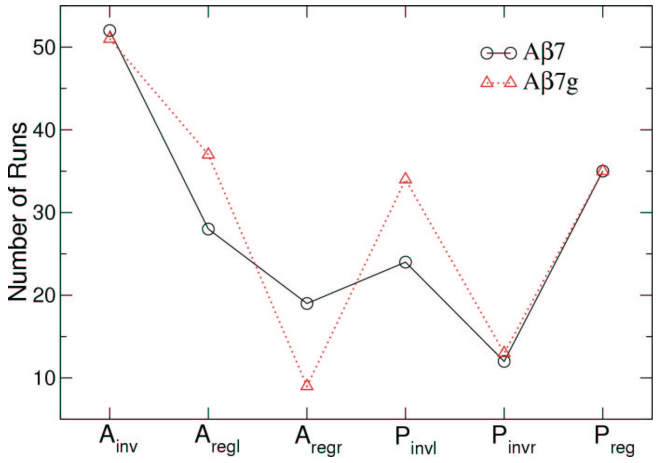


Figure 3. Number of runs yielding a given configuration. Overall the total number of anti-parallel configurations is up to 30% greater than parallel configurations.

As the Aregl configuration is more likely to form, dimer addition to the growing fibril may be an important step rather than simple monomer addition [3]. This is consistent with experiments showing that oligomers rather than monomers are observed during amyloid fibril growth [4]. We are currently investigating the interaction between an additional monomer with each of the dimers in Fig. 2, and dimer-dimer interactions. Our approach, based on statistical analysis of many independent runs, is suitable for studying nonequilibrium properties of early stage peptide self-assembly, where the ensemble average is more important than the time average.

ACKNOWLEDGEMENT

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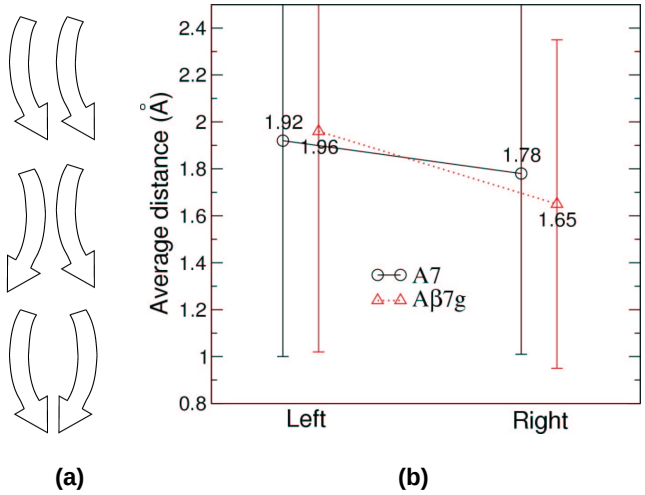

Figure 4. (a) Schematic showing the effect of backbone twist. Top: Ainv and Preg. Middle/bottom: left/right-faced configurations. (b) Average distances of H and O from the major axis on either side of the backbone.

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