AGGREGATION PATHWAYS OF THE AMYLOID β(1–42) PEPTIDE DEPEND ON ITS COLLOIDAL STABILITY AND ORDERED β-SHEET STACKING

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Thioflavin-T (ThT) assay

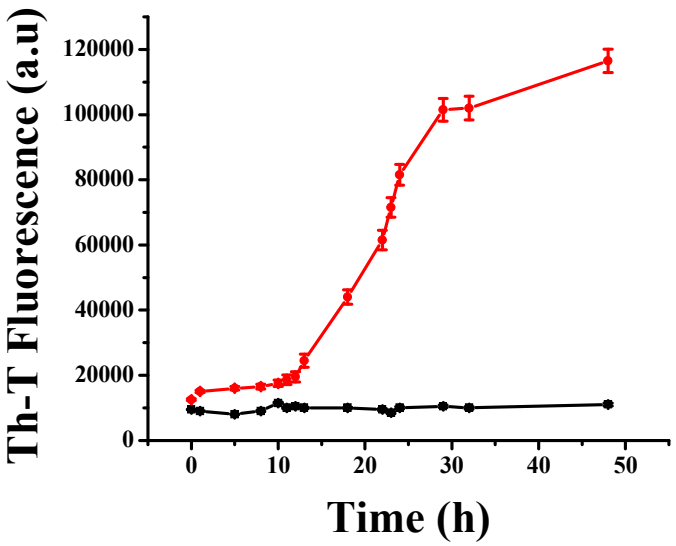

Figure S1. ThT fluorescence enhanced by 25 μM Aβ(1–42) (red curve) in a solution that had been incubated at pH 7.4 and 25 ^{o}C for different times. The ThT fluorescence signals in a mixture of Aβ(1–42) and Cu^{2+} (25 μM each) are shown in the black curve. Detailed experimental parameters are provided in the main text.

In addition to AFM and CD spectroscopy, the aggregation processes of Aβ(1–42) in the absence and presence of Cu^{2+} were also studied by thioflavin-T (ThT) assay following published procedures.[1] ThT fluorescence was not significantly enhanced in the Cu^{2+}-containing Aβ(1–42) solution throughout the entire incubation (black curve). By correlating with the morphological study by AFM, we conclude that the ThT assay is not indicative of the formation of amorphous aggregates of Aβ(1–42). In contrast, enhancement of the ThT fluorescence in the Cu^{2+}-free solution is significant after a 10-h incubation, in line with results from other studies and our CD data (cf. Figure 5A in the text). Although both types of aggregates are composed of the β-sheet structure, stacking of the β-sheets in the Aβ(1–42) fibrils is substantially more ordered than that in the amorphous aggregates. Consequently, the former significantly enhances ThT

signals and the enhancement of the ThT signals is semi-quantitatively related to the quantity of fibril aggregates in the solution.

References

1. LeVine, H., Tioflavine T interaction with synthetic Alzheimer's disease β-amyloid peptides: Detection of amyloid aggregation in solution. *Protein Sci.* **1993**, *2*, 76–83.