

Supporting Information for

Self-Coiling of Single-Stranded Protofibrils into Rings: A Pathway of Alzheimer's β -Peptide Amyloidosis on Lipid Membranes

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Experimental section

Materials. Amyloid- β peptide A β 40 was obtained from AnaSpec (USA) and its purity was confirmed by high performance liquid chromatography (HPLC). 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids (USA). Phosphate buffered saline (PBS) was freshly prepared from sodium and potassium salts: NaCl, KCl, Na₂HPO₄, and KH₂PO₄ to give a pH value of 7.4 at 25°C.

An ultrafiltration method was used to prepare A β 40 solutions without pre-aggregations and fibrils as described in the literature¹. Briefly, A β 40 was firstly dissolved in 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP) at a concentration of 1 mg/mL overnight. After removing HFIP by a gentle stream of argon gas followed by vacuum, the peptides were dissolved with PBS (50 mM, pH 7.4) under vortex. Then, the peptide solution was filtrated through a 20 nm nanopore filter (Whatman Anotop 10) under an elongation flow controlled by a GASTIGHT syringe and a pump. The non-uniform pore structure of the filter is the key to obtaining the real monomers. Finally, the peptide solution was centrifuged at 30000 g for 30 min

and the top two-thirds of the solution was used for fibril formation experiments. The concentration of final peptide solution was determined by UV absorption at 280 nm.

Fibril Formation. $A\beta$ 40 fibrillation experiments were carried out on DOPC supported lipid bilayer (SLB) platforms in a home-made vial. First, the DOPC SLB were generated on freshly cleaned mica via the vesicle fusion on the surface. Briefly, DOPC was dissolved in $CHCl_3$, dried, and re-suspended in PBS at a concentration of 1.0 mg/mL. Suspension (\sim 1 mL) of the lipids was forced through a polycarbonate filter with 100 nm pores 11 times to yield a transparent DOPC vesicle solution. Then, the mica was immersed under the DOPC vesicle solution (\sim 500 μ L) in the home-made vial for at least one hour at room temperature, allowing SLB formation on the mica. The excess vesicles in solution were removed by extraction with fresh PBS buffer at least 10 times. The final volume of the solution above the mica was kept as \sim 500 μ L. Finally, \sim 500 μ L of the freshly prepared $A\beta$ 40 PBS solution (2.0 μ M) was added into the vial. The final $A\beta$ 40 concentration in solution was kept as 1.0 μ M. The vial was then carefully sealed with a plastic cap to eliminate any air bubbles inside the vial. After incubation at 37 °C for different time windows, the mica substrate was carefully brought out, gently rinsed with Milli-Q water and allowed to dry in the air for further AFM characterization.

Atomic force microscope (AFM). The morphologies of amyloid fibrils were imaged on an atomic force microscopy (AFM, FM-Nanoview 1000) in a tapping mode. A silicon tip on nitride level (Budget Sensors Inc.) with 48 N/m spring constant and 190 kHz resonance frequency were used. The fibril morphologies were tracked through FiberApp² to extract the height and contour length information from AFM images with a step length \sim 1 pixel between two subsequent points along the fibril contour.

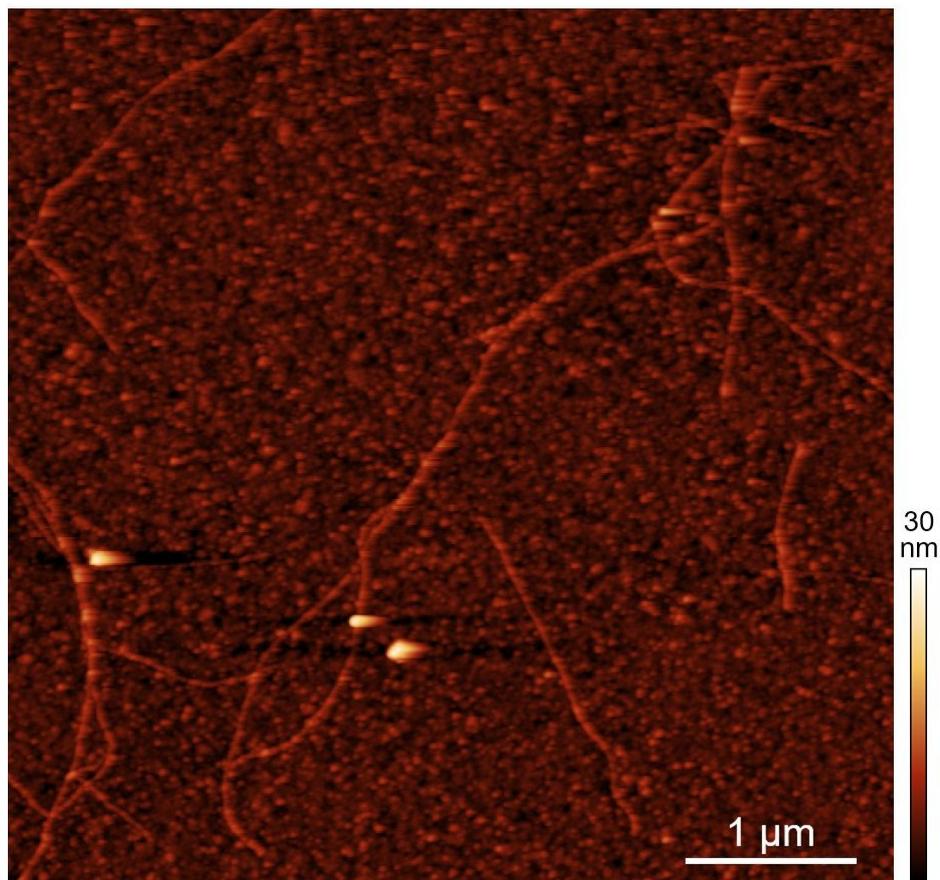


Figure S1. AFM height image of $A\beta_{40}$ amyloid fibrils formed on plasma treated polystyrene surface by incubation at 37 °C for 24 hours. The concentration of $A\beta_{40}$ in PBS solution is 1 μ M.

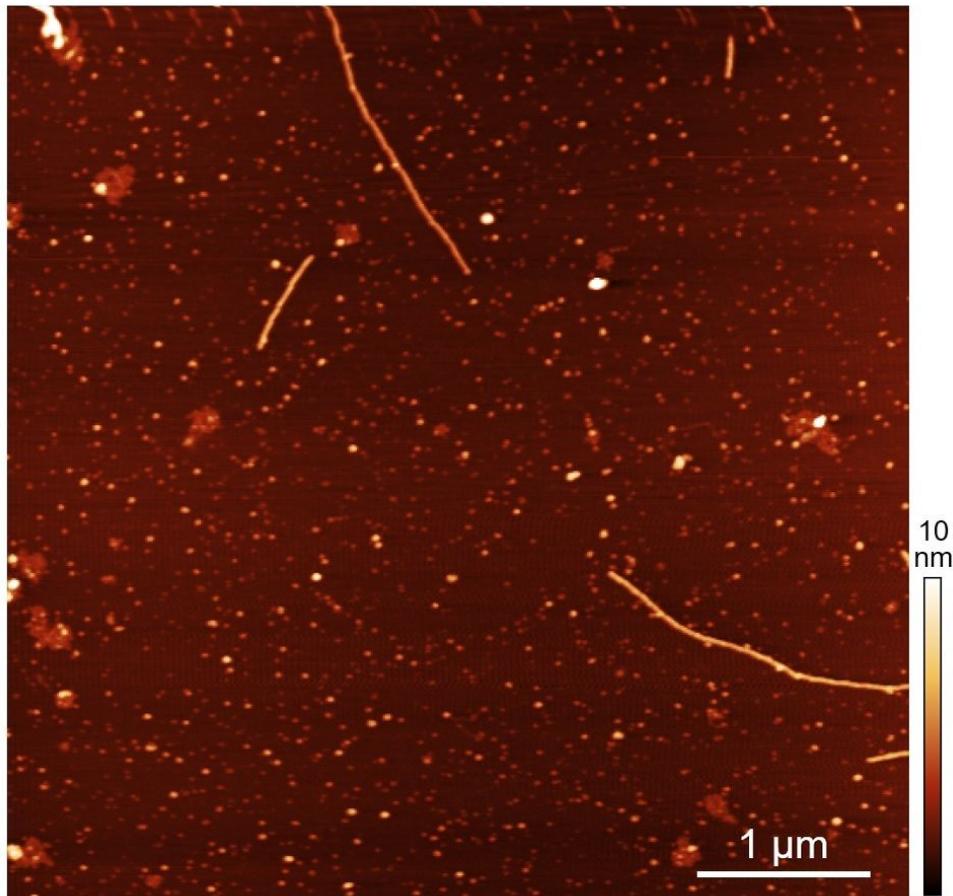


Figure S2. AFM height image of A β 40 amyloid fibrils formed on mica by incubation at 37 °C for 24 hours. The concentration of A β 40 in PBS solution is 1 μ M.

¹ Y. Wang, C. Wu, *Biochemistry* **2017**, *56*, 6575-6584.

² I. Usov, R. Mezzenga, *Macromolecules* **2015**, *48*, 1269-1280.