Supplemental Information

Enzymatically Triggered Self-assembly of Poly(ethylene glycol)-attached Oligopeptide into Well-organized Nanofibers

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Experimental section

Materials.

The poly(ethylene glycol)-attached β-sheet peptide having thrombin-cleavage site, $L_4K_8L_4$-VPRGS-*PEG* (**1**), and its control peptide, $L_4K_8L_4$-SGRPVL-*PEG* (**2**), were prepared from solid phase peptide synthesis using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry. These peptides were synthesized on a TentaGel PAP (Papp Polymere), by using Fmoc-L-amino acid derivatives (3 equiv), 1-hydroxy-7-azabenzotriazole (3 equiv) and 1,3-diisopropylcarbodiimide (3 equiv) in *N,N*-dimethylformamide (DMF) for coupling, and piperidine (25%)/DMF for Fmoc removal. To cleave the peptide from the resin and remove the side chain protecting groups, the peptide-resin was treated with trifluoroacetic acid (TFA)/CH_2Cl_2 (9/1 v/v). The peptides were identified by matrix-assisted laser desorption ionization-time of flight MS (MALDI-TOFMS) (Shimazu KOMPACT MALDI) and ^{1}H-NMR spectroscopy (400 MHz, JEOL FX-400) (Fig. S2). The peptides **1** and **2** were dissolved in 2,2,2-trifluoroethanol (TFE) as a stock solution before the aggregation assay. The aggregation solutions of the peptides (final peptide concentration: 40 μM, TFE content: 5 %) were prepared by diluting the stock solution with the 5 mM Tris/HCl buffer. All the incubations for the aggregation of the peptides were performed at room temperature.

The reference peptide, $L_4K_8L_4$-VPR, was also synthesized by solid phase peptide synthesis on a CREAR (cross-linked ethoxylate acrylate resin, PEPTIDE INSTITUTE, INC.) in a manner similar to that described above, and purified by reversed-phase HPLC (Bio-Rad, DuoFlow) on a YMC-Pack Pro C18 (20 x 150 mm) by using a linear gradient of water-acetonitrile (containing 0.1% TFA). MALDI-TOFMS: found $[M+H]^+$ (calcd. $[M+H]^+$); $L_4K_8L_4$-VPR: *m/z* 2303 (2302.1). The thrombin (from bovin plasma) was purchased from Nacalai Tesque, Inc. and used without further purification.

Measurements.

CD spectra were recorded on a J-720 spectropolarimeter (JASCO Ltd.) under a nitrogen atmosphere. Experiments were performed in a quartz cell with a 5 mm path length over the range of 190-250 nm at room temperature. The AFM images were collected at ambient temperature on a Nanoscope IIIa (Digital Instrument, Inc.) operated by tapping using a silicon tip (tip radius 5-10 nm). A 10 μm x 10 μm scanner was used for imaging. The scanning speed was at a line frequency of 1 Hz, and the original images were sampled at a resolution of 512 x 512 points.

Thrombin-cleavage Study.

The poly(ethylene glycol)-attached peptide containing an enzyme-cleavage site was digested by thrombin under the following conditions. The peptide **1** (40 μM) was first dissolved in 1.5 mL of Tris/HCl buffer (5 mM, pH 9.0, 5% TFE), and then 15 μL of thrombin aqueous solution (0.8 NIH unit) was injected into the **1**-solution. Reaction mixture was incubated at room temperature for 30 min and the resulting solution was analyzed by MALDI-TOF MS spectroscopy (Fig. 1). Matrix: 2, 5-Dihydroxy benzoic acid.

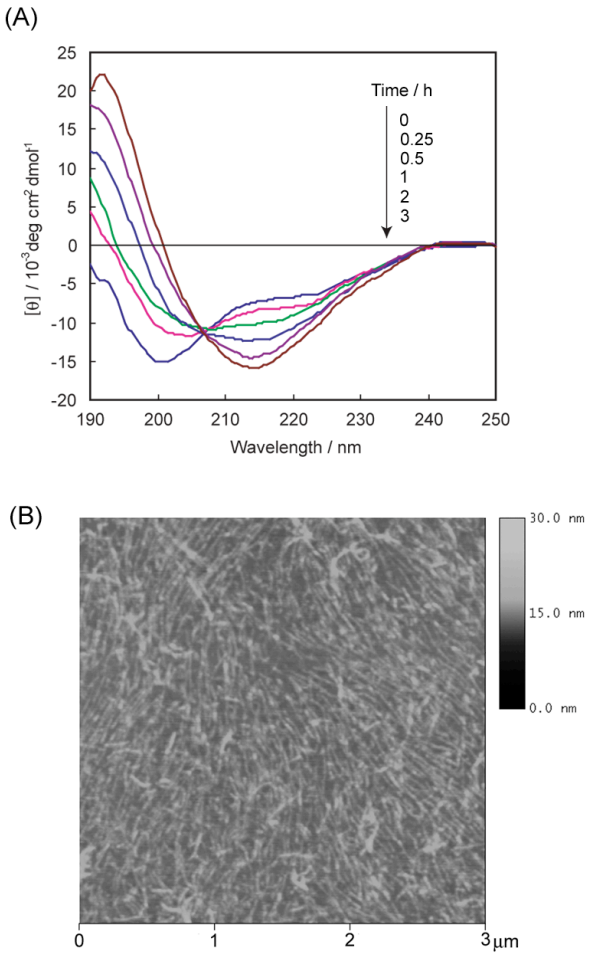

Fig. S1. (A) Time dependence of CD spectra for the independently and chemically synthesized **$L_4K_8L_4$-VPR** in Tris buffer at pH 9.0 (containing 5% TFE). The peptide was incubated at room temperature for the time indicated (0-3 h). [**$L_4K_8L_4$-VPR**] = 40 μM. (B) Tapping-mode AFM image (3 μm x 3 μm) of **$L_4K_8L_4$-VPR** at 24 h, pH 9.0. z-scale: 30 nm.

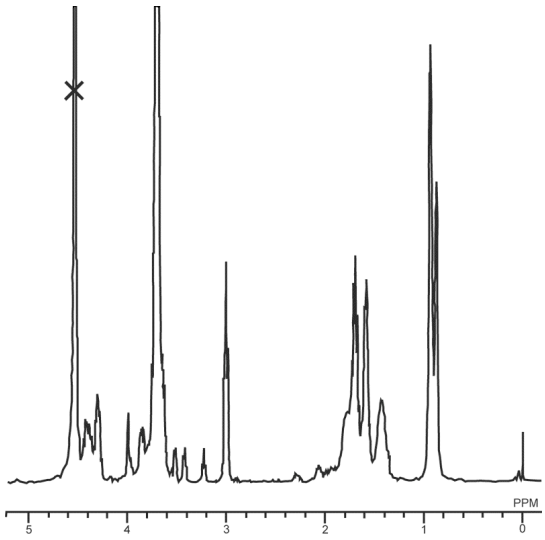

Fig. S2. ^{1}H-NMR spectrum of **1** in D_2O.