Electronic Supplementary Information

of

Adjustable nanofibers self-assembled from an irregular conformational peptide amphiphile

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Experimental Section

1. Materials

N-Fluorenyl-9-methoxycarbonyl L-amino acids (Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Pro-OH), 2-chlorotrityl chloride resin (100-200 mesh, loading: 1.18 mmol/g), benzotriazole-N,N,N',N'-tetramethyluroniumhexa-fluorophosphate (HBTU), N-hydroxybenzotriazole (HOBt), and piperdine were purchased from GL Biochem (Shanghai) Ltd. (China) and used as received. Trifluorocaetic acid (TFA), N,N-Diisopropylethylamine (DIEA) and N,N-dimethylformamide (DMF) were obtained from Shanghai Reagent Chemical Co. and used after redistillation. Triisopropylsilane (TIS) was obtained from ACROS and used as received.Naproxen (Np) was purchased from Sigma-Aldrich. Matrix metalloproteinases-2 (MMP-2) was purchased from RD-SYSTEMS.

The other materials and chemicals were used as received without further purification.

2. Synthesis and characterization of the peptide amphiphile

Peptide Fmoc-GPLGLAGRGDFD was manually synthesized based on solid phase chemistry according to the literature procedure.[S1,2] After the completion of the peptide synthesis, Np was appended to the N-terminus of the peptide coupled by HBTU, HoBt and DIEA. The peptide amphiphile was cleaved from the resin with a cleavage cocktail comprised of 95% TFA, 2.5% TIS, and 2.5% H_2O (v/v) for 2 h at room temperature, and then collected after precipitated with cold ether. The molecular

weight of the peptide amphiphile was analyzed by electrospray ionization mass spectrometry (ESI-MS, LCQ Advantage, Finigan, USA) in a component solvent of H_2O and methanol. The theoretical molecular weight of the peptide was1385.7, which found in ESI-MS was 1386.7, corresponding to the pattern of $[M+H]^+$ (Fig. S1). The purity of peptide amphiphile was 91%, which was determined by high performance liquid chromatography (HPLC, Prominence LC-20A, Shimadzu, Japan) with a C18 column. The eluent consisted of a linear gradient from 5 to 95% of H_2O/acetonitrile containing 0.1% trifluoroacetic acid at 1 mL/min for 30 min (Fig. S2).

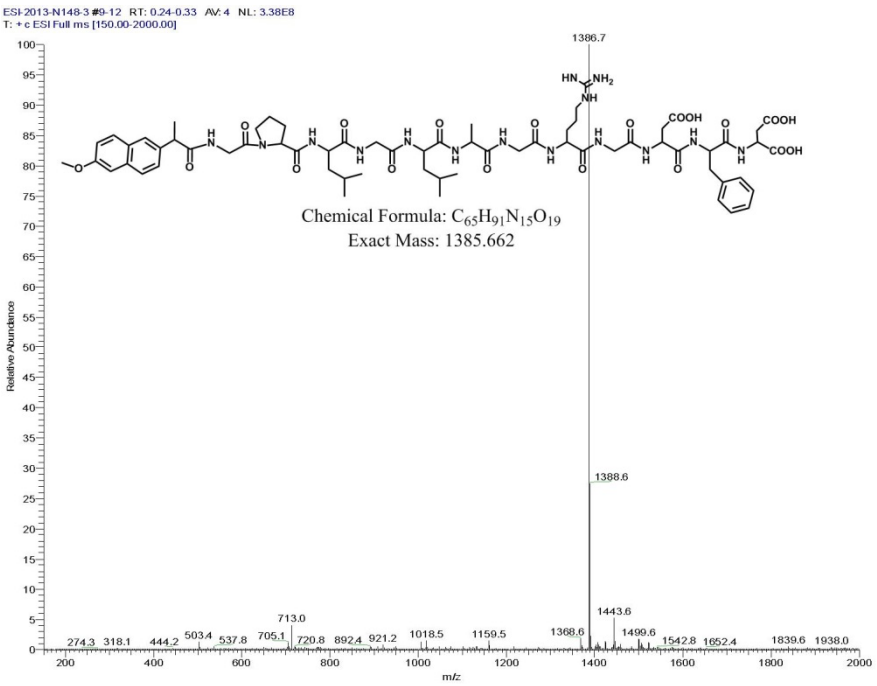

Fig. S1 ESI-MS of the peptide amphiphile. $[M+H]^+$at m/z 1386.7 was observed.

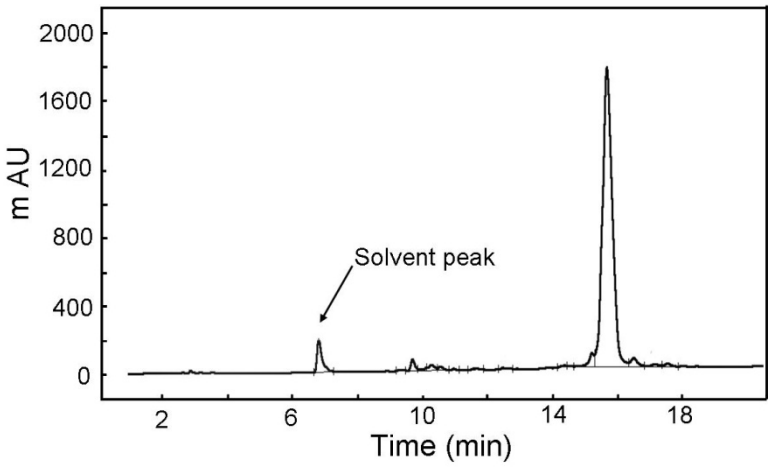

Fig.S2 The HPLC profile of the peptide amphiphile.

3. Acid-Base Titration

The peptide amphiphile Np-Gly-Pro-Leu-Gly-Leu-Ala-Gly-Arg-Gly-Asp-Phe-Asp was suspended in distilled water at a concentration of 4.0 mg/mL. With respect to the acid-base titration of the acidic peptide, 1M NaOH was added to adjust the pH around 11, and 0.01 M HCl aqueous solution was added in 5-10 μL equivalence. After each addition, the sample was constantly stirred for 3 min at room temperature, and the pH value of the solution was measured using a PB-10 Sartorius pH meter.

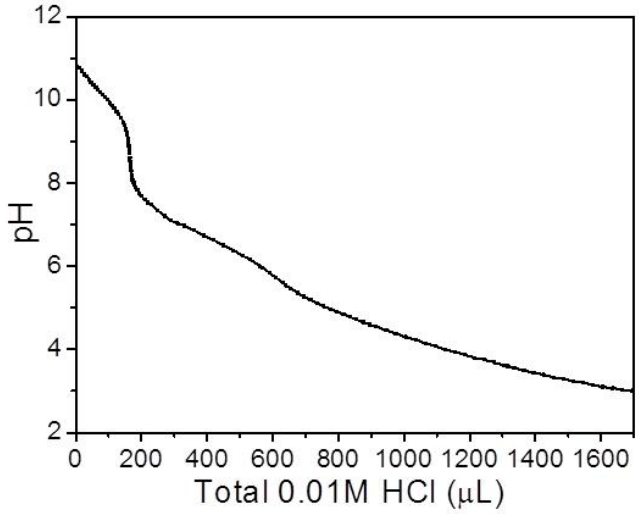

Fig. S3 Acid-base titration curve of the peptide amphiphile at a concentration of 4 mg/mL.

4. Formation of different self-assembled nanostructures

To obtain different self-assembled nanostructures, 1.0 mL of di-water was dropped into a 5 mL glass-vial which contained 4.0 mg of the lyophilized peptide powder. An aliquot of 1.0 M sodium hydroxide (NaOH) solution was added to the suspension to adjust the pH value to around 7.4. Ultrasonication was applied until the peptide was dissolved completely. Peptide self-assemblies formed under different pHs were prepared by gradiently adding 1.0 M hydrochloric acid (HCl). For preparing the peacock-feather-like nanofibers, saturated NaCl solution was added into the peptide solution containing dendritic nanofibers, resulting in 2.0 mM final NaCl concentration. Then the obtained solutions were stood for 12 h for the following experiments.

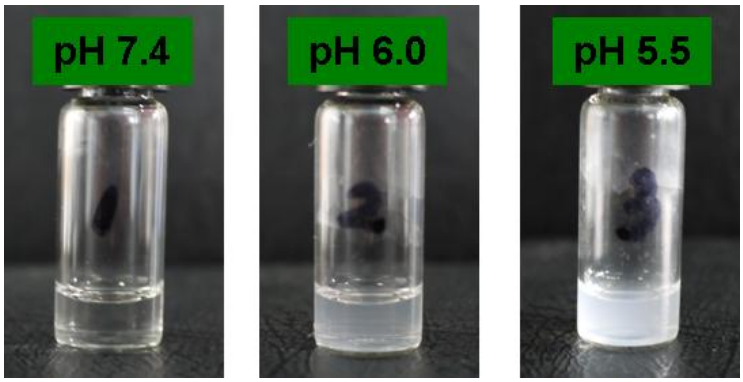

Fig. S4 Turbidity transformation of the peptide amphiphile solutions at different pHs.

5. ζ-Potential measurement

The ζ-potentials of different self-assemblies were measured with Nano-ZS ZEN3600 (Malvern Instruments) at room temperature with a 0.5 mm cell. 4.0 mg/mL peptide solutions containing different self-assemblies were used for the ζ-potential measurement.

6. TEM and EDX characterizations

The self-assembled peptide morphologies formed at different pHs were observed by transmission electron microscopy (TEM, JEM-2010, Japan) and the elemental composition of the knots within the peacock-feather-like nanofibers was investigated with an accelerating voltage of 100 kV. The samples were prepared by dipping a copper grid into the solutions containing self-assemblies (4.0 mg/mL). After the deposition, the samples were dried in air for the observation.

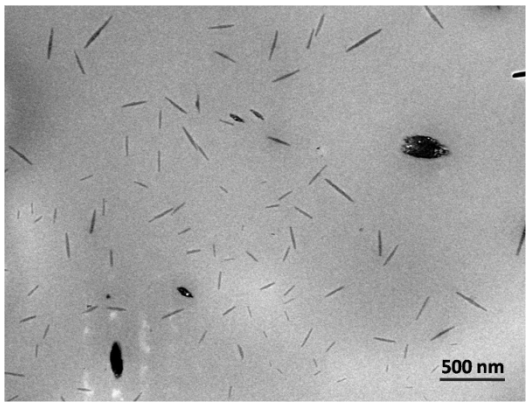

Fig. S5 TEM image of the short nanofibers self-assembled by the U-shaped peptide at pH 7.4.

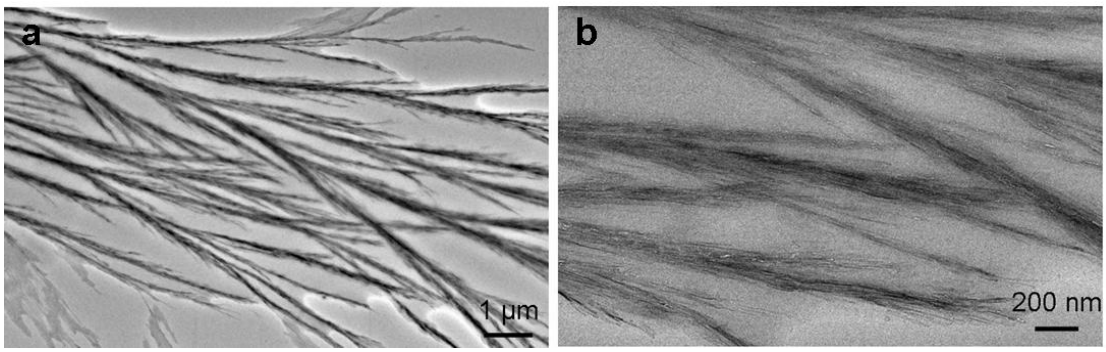

Fig. S6 TEM images of self-assembled dendritic nanofibers with different magnification times.

7. Investigation of MMP2 to cleave U-shaped linker

To investigate the influence of topology of U-shaped peptide on the self-assembly

behavior, a commercialized matrix metalloproteinases-2 (MMP2) was exploited to cleave the U-shaped linker. A droplet of concentrated MMP2 (20 μL, 2×10^{-2} mg/mL) solution was added into the self-assembled solution containing dendritic nanofibers, and then co-cultured for one day at 37°C to ensure the effective cleavage. The solution containing self-assembled dendritic nanofibers with the performance of adding same volume of buffer solution without MMP2 was located at 37°C for one day as control. TEM images in Fig. S7 indicated the different self-assembled morphologies co-incubated without and with MMP2, respectively.

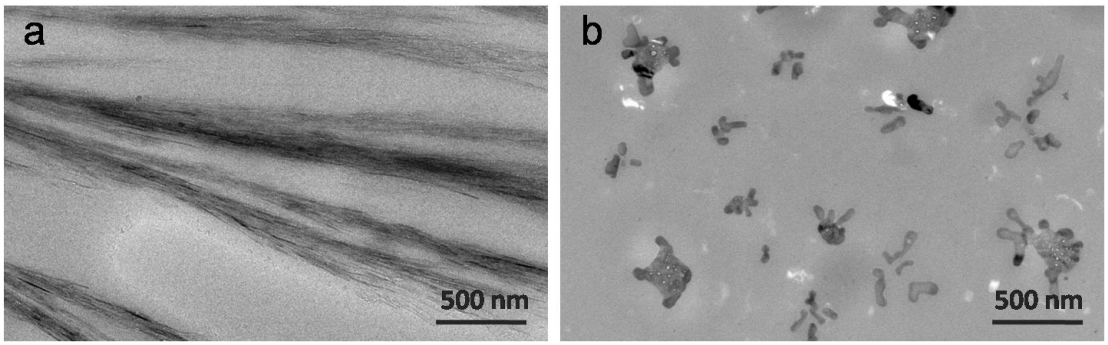

Fig. S7 TEM images of self-assembled dendritic nanofibers co-incubated without (a) and with MMP2 (b) for one day at 37°C, respectively.

8. SEM characterization

Scanning electron microscopy (SEM) was also carried out to synergistically investigate the self-assembled morphologies of the peptide. 4.0 mg/mL peptide solutions containing self-assemblies were dropped onto glass substrates, respectively. After dried in air, the specimens were coated with gold before examination. The examination was performed on a NovaNano SEM FEI (Holland) instrument with an accelerating voltage of 30 kV.

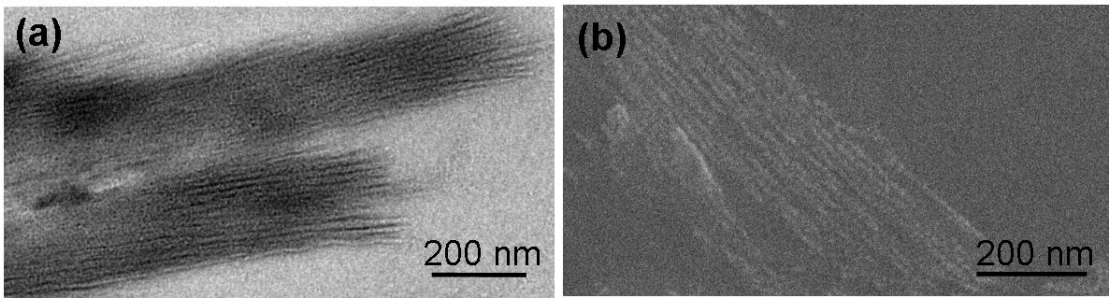

Fig. S8 TEM (a) and SEM images (b) of the self-assembled parallel nanofiber formed at pH 5.5 with a concentration of 4.0 mg/mL.

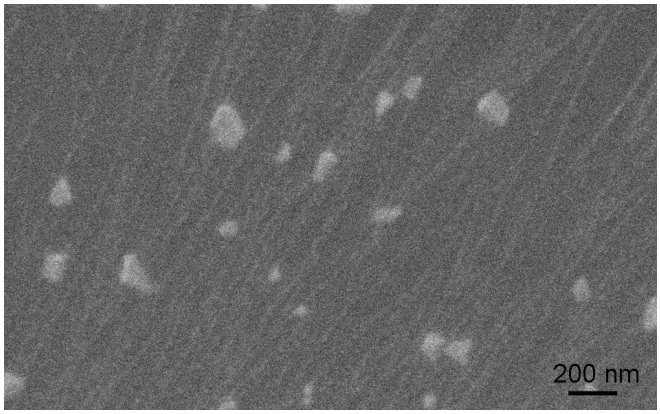

Fig. S9 SEM image of the self-assembled parallel nanofibers after the culture with 2.0 mM NaCl.

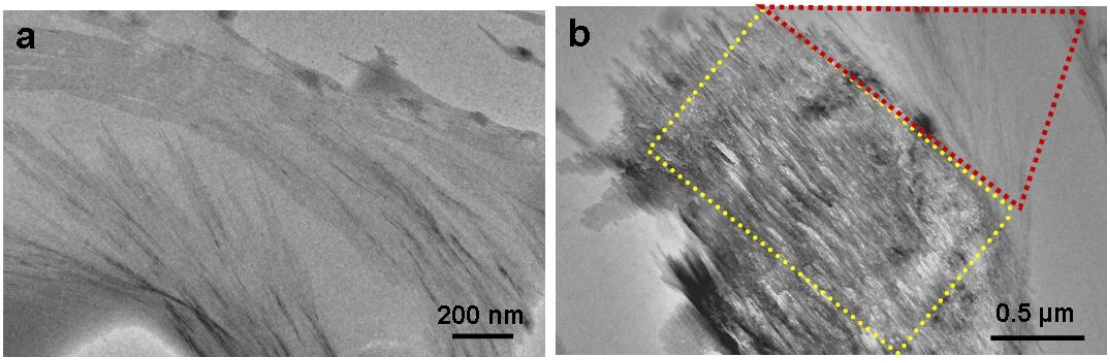

Fig. S10 TEM images of self-assembled transition state with the coexistence of dendritic and parallel nanofibers formed at pH 5.8.The dendritic nanofibers begun to rearrange when triggered by the addition of 1.0 M HCl (a); and the nanofibers marked

by yellow rectangle representing the parallel matrix and nanofibers marked by red triangle representing the dendritic matrix (b).

9. CD measurement

Circular dichroism (CD) was recorded on a J-810 spectropolarimeter (Jasco, Japan) for the purpose of investigating the secondary structure of peptide self-assemblies. 4.0 mg/mL peptide solution was fixed into a 0.5 mm quartz cell and analyzed with 4 s accumulations every 1 nm. The data range was collected from 180 nm to 320 nm.

10. FT-IR spectroscopy

Fourier transform-infrared (FT-IR) spectra of the lyophilized self-assembled nanofiber matrixes were performed on an AVATAR 360 spectrometer (Perkin-Elmer, USA). Prior to the measurement, the self-assembled peptide powder was pressed into potassium bromide (KBr) pellet with KBr powder. To investigate the conformation of the self-assembled peptide in aqueous environment, the aqueous FT-IR spectra of the peptide in different pHs (7.4, 6.0 and 5.5) were also obtained using D_2O as the solvent, and 1 M NaOH solution was used to adjust the pH value.

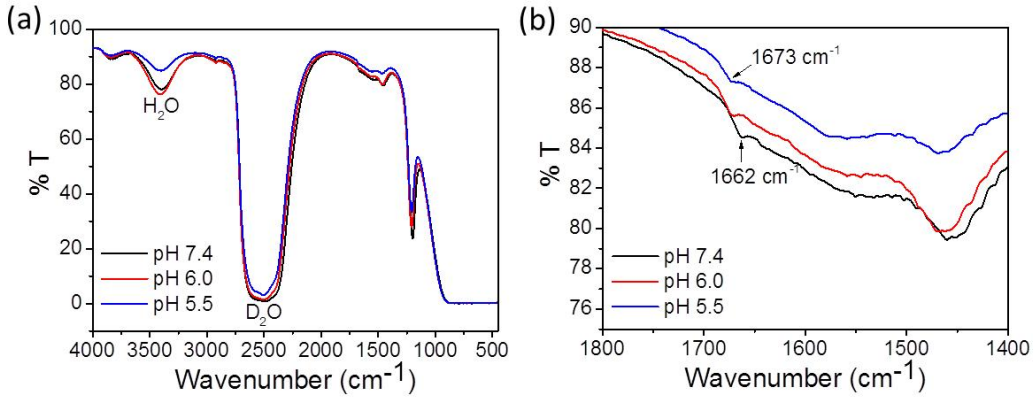

Fig. S11 FT-IR spectra of the peptide in different pH solutions. The peak at 1673 cm^{-1} was observed for the peptide solutions at pH 6.0 and 5.5.

11. Fluorescent spectroscopy

Fluorescent spectra of the self-assemblies at different pHs were recorded on a LS55 luminescence spectrometry (Perkin-Elmer), with excitation wavelength at 265 nm. Emission data range from 280 nm to 650 nm was collected to detect the π-π stacking interaction of Np groups.

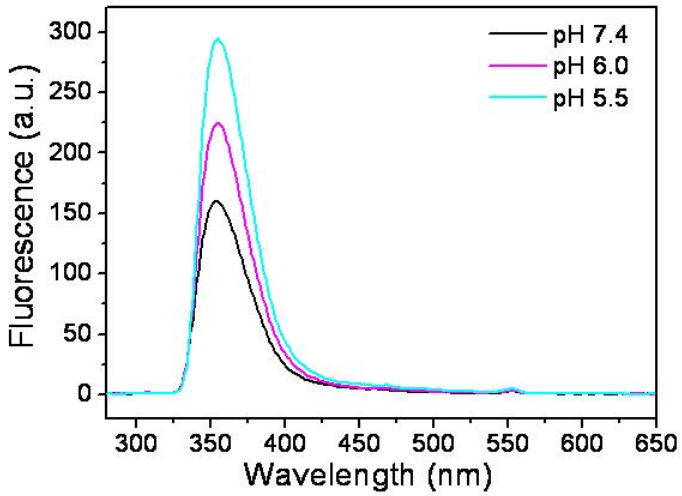

Fig. S12 Fluorescence emission (λex=265nm) spectra of the peptide solutions at different pHs.

12. UV-Vis spectroscopy

Ultraviolet-visible (UV-Vis) absorption spectra of the self-assemblies at different pHs were recorded with a spectrophotometer (Perkin-Elmer Lambda Bio 40 UV-Vis spectrometer, USA) from 200 nm to 400 nm.

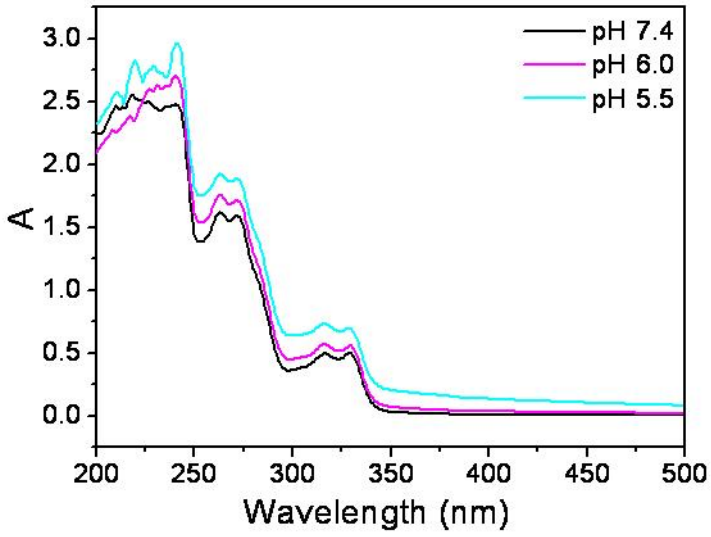

Fig. S13 Ultraviolet-visible absorption spectra of the peptide solutions at different pHs.

References

S1 S. Y. Qin, Y. F. Chu, L. Tao, S. S. Xu, Z. Y. Li, R. X. Zhuo and X. Z. Zhang, *Soft Matter*, 2011, **7**, 8635.

S2 S. Y. Qin, S. S. Xu, R. X. Zhuo and X. Z. Zhang, *Langmuir*, 2012, **28**, 2083.