Supporting Information

Control of peptide assembly through directional interactions

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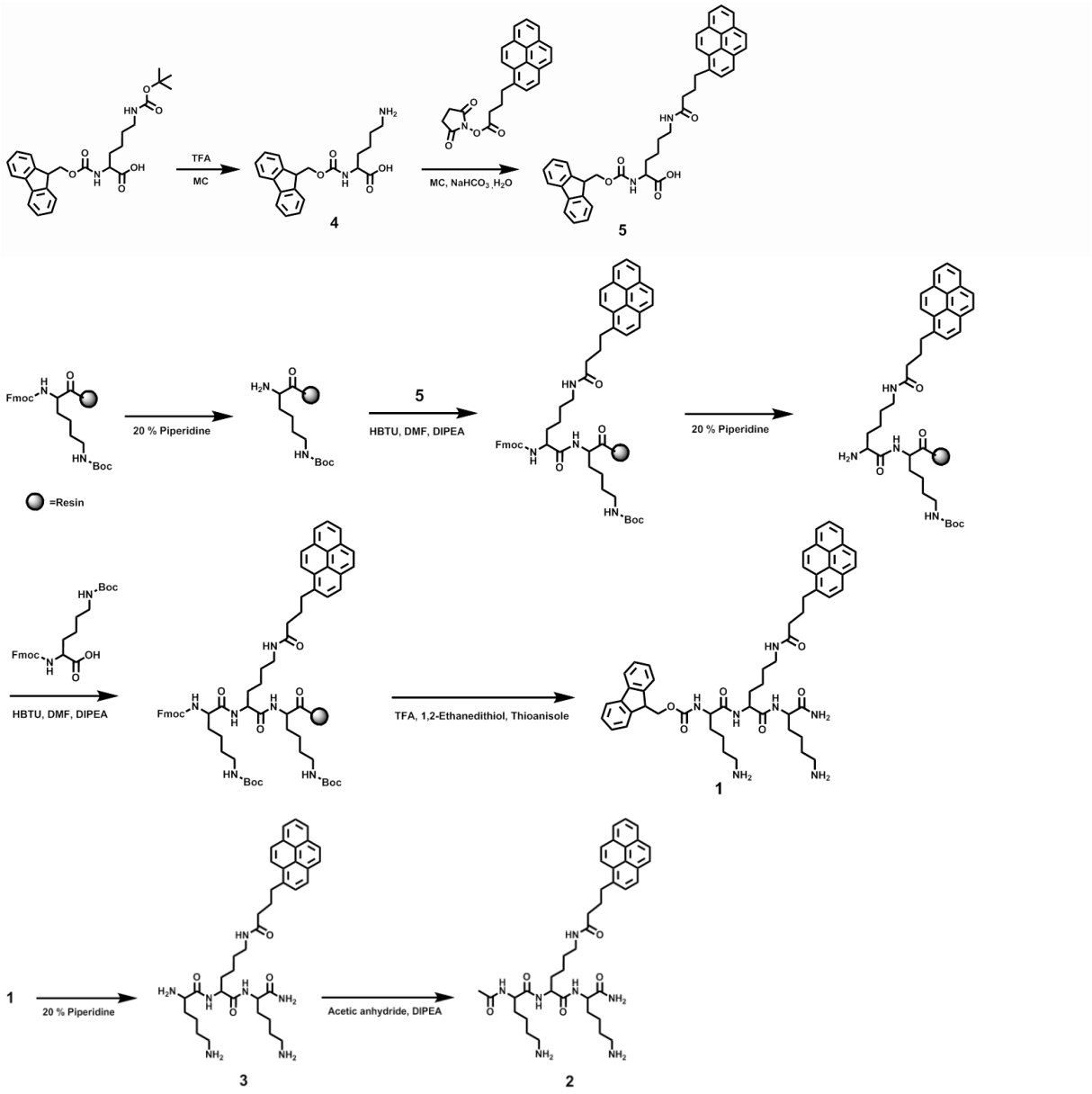
Materials and techniques

1-Pyrenebutyric acid *N*-hydroxysuccinimide ester (95%) from Aldrich, Fmoc-Lys(Boc)-OH (98%) from Aldrich and Trifluoroacetic acid (99%) from Aldrich were used as received. Rink amide MBHA resin (100-200 mesh) from Beadtech, 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) from Beadtech, Diisopropylethylamine (DIPEA) (99%) from Aldrich, acetic anhydride (99 %) from Aldrich were used as received.
All atmosphere sensitive reactions were done under argon. Flash chromatography was carried out with Silica Gel 60(230-400 mesh) from EM science. Visualization was accompanied with UV light and iodine vapor. ^{1}H-NMR spectra were recorded from DMSO-d_6 on a Bruker AM 300 spectrometer. The purity of the products was checked by thin layer chromatography (TLC; Merck, silica gel 60). MALDI-TOF-MS was performed on a Perseptive Biosystems Voyager-DE STR using α-Cyano-4-hydroxycinnamic acid matrix. Preparative high performance liquid chromatography (HPLC) was performed at room temperature using a 20 mm × 600 mm poly styrene column on a Japan Analytical Industry Model LC-908 recycling preparative HPLC system, equipped with UV detector 310 and RI detector RI-5. Dynamic light scattering (DLS) were performed with an ALV/CGS-3 Compact Goniometer System. The fluorescence spectra were measured in a Hitachi F–4500 fluorescence spectrophotometer. The transmission electron microscopy (TEM) was performed at 120kV using JEM–2010. The atomic force microscopy (AFM) was performed with a Bruker Nanoscope V Multimode 8 instrument.



Scheme 1. Synthesis of compounds **1, 2** and **3**.

Experimental Section

Synthesis of compound 4

Fmoc-Lys(Boc)-OH (853.68 mg, 1.822 mmol) were dissolved in methylene chloride(50 mL). Trifluoroacetic acid (0.383 mL, 5 mmol) was added to the solution. The reaction mixture was stirred over 2h under argon. The purity of the products was checked by thin layer chromatography. It was used for next reaction without further purification.

Synthesis of compound 5

To a solution of compound **4** in methylene chloride (100 mL) was dropped in 1-Pyrenebutyric acid *N*-hydroxysuccinimide ester(668.3 mg, 1.734 mmol) dissolved in methylene chloride (50mL). Degassed 2 M aqueous $NaHCO_3$ (50mL) was added to the solution. The mixture was the aqueous layer was washed twice with ethyl acetate. The combined organic layer was dried over anhydrous magnesium sulfate and filtered. The solvent was removed in a rotary evaporator, and the crude product was purified by column chromatography (silica gel) using ethyl acetate/hexane as eluent to yield 730mg (88 %) of brown solid. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ = 11.0 (s, 1H; carboxylic acid-*H*), 8.12-7.71 (m, 9H; phenanthrene-*H*), 8.03 (s, 2H; sec. amide-*H*), 7.87-4.46 (m, 9H; fluoren-*H*),4.70-1.25 (m, 16H; methylene -*H*), 4.55 (m, 1H; methane-*H*)

Synthesis of compound 1, 2, and 3

Peptide was synthesized on Rink amide MBHA resin using standard Fmoc protocols on a microwave peptide synthesizer. Before addition to the resin, a mixture of HBTU (20.48 mg, 54 μmol), dimethylformamide (DMF) (1 mL), and 1% diisopropylethylamine(DIPEA) (21μL, 120 μmol) and amino acids was incubated for 10 min for carboxyl activation. The amino acid groups were employed, Fmoc-Lys(Boc)-OH and compound **5** (Fmoc-Lys (Pyrene)-OH. For deprotection of the Fmoc group from the resin, the resin was treated with 20% piperidine in DMF (3 mL) and microwave turned on for Fmoc deprotecting. Then the resin was washed successively with DMF and methylene chloride. Next, the mixture of Fmoc-Lys(Boc)-OH and DMF (3 mL) were added to the resin and microwave turned on for peptide coupling. Repeat this procedure of deprotecting, coupling and deprotecting, for three times, peptide synthesis ended. For synthesis of peptide **2**, acetic anhydride (3 mL) and DIPEA (21μL, 120 μmol) added to last product of deprotected peptide. For synthesis of peptide **1**, last process of Fmoc deprotecting was not needed. And the resin was precipitated by THF, and dried *in vacuo*. The dried resin was treated with cleavage cocktail (TFA: 1,2-ethanedithiol: thioanisole; 95 : 2.5 : 2.5) for 3 h, and was triturated with hexane and *tert*-butyl methyl ether (5 mL each). The peptides were purified by reverse-phase HPLC eluting with a linear gradient of acetonitrile/water (35/65 to 100/0 over 30 minutes, 0.1% TFA). The molecular weight was confirmed by MALDI-TOF mass spectrometry. The purity of the peptides was

>95% as determined by analytical HPLC. Concentration was determined spectrophotometrically in water using a molar extinction coefficient of pyrene (54,000 $M^{-1}cm^{-1}$) at 335 nm.

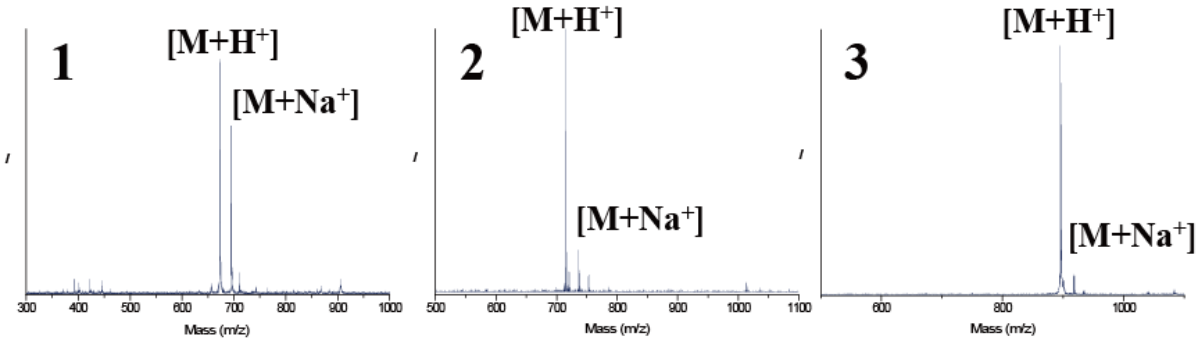


Figure S1. MALDI-TOF mass spectra of **1**, **2** and **3**. MALDI-TOF calculated mass for **1**($C_{38}H_{54}N_7O_4$–[M+H^+]) is 672.42, found 673.43. **2** ($C_{40}H_{56}N_7O_5$–[M+H^+]) is 713.43, found 713.93. **3** ($C_{53}H_{63}N_7O_6$–[M+H^+]) is 894.48, found 895.00.

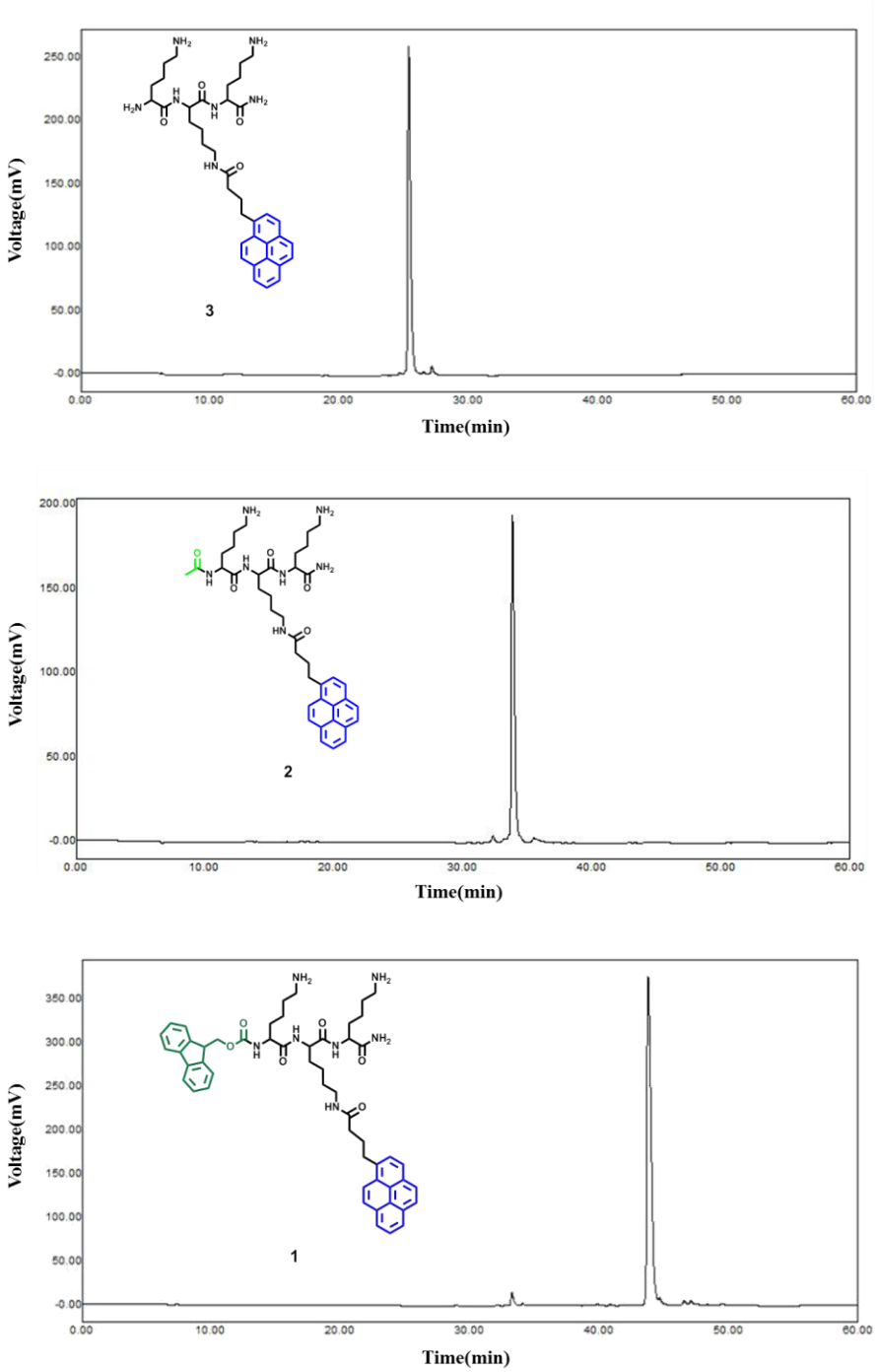


Figure S2. Analytical HPLC graph data of peptide **1**, **2** and **3**. Performed HPLC by VYDAC C18 column, eluent condition is water/acetonitrile (100: 0 to 30: 70 over 60 min, 0.1 % TFA) and loading 20 μL each. Increasing hydrophobicity at N-termini of the peptide from 3 to 1, the retention time also increased.

Circular dichroism

CD spectra were measured using a JASCO model J-810 spectropolarimeter equipped with temperature controller. Spectra were recorded from 500 nm to 190 nm using a 0.1 cm path-length cuvette. Scans were repeated five times and averaged. Molar ellipticity was calculated per amino acid residue. Peptide concentration was 200 μM. Sample solutions were incubated at least for 3 days before measurement, and essentially the same CD spectra were obtained after prolonged incubation, indicating thermodynamic equilibrium states.

Fourier Transform Infrared (FTIR) Spectroscopy Measurement

All FTIR spectra were collected on a Nicolet FTIR spectrometer at ambient temperature. The instrument was continuously purged with CO_2-free dry air. Interferograms were recorded between 1700 and 1600 cm^{-1} at a resolution of 4 cm^{-1}, and a total of 256 scanswere averaged. Samples for FTIR were dissolved in D_2O (about 10 mg/mL) and analyzed in a transmission cell having CaF_2 windows and a 0.025 μm path length.

Dynamic light scattering (DLS)

DLS experiment was performed at room temperature with LV/CGS-3 Compact Goniometer System equipped with He-Ne laser operating at 632.8 nm. The scattering angle was 90°. Before measurement, the sample was centrifuged at 10,000 × g for 20 min to sediment any dust particles. Sample concentrations were typically 200 μM in water. The size distribution was determined by using a constrained regularization method. The hydrodynamic radius (R_H) was determined from the DLS auto correlation functions by the cumulants and the CONTIN methods using the software provided by the manufacturer.

Atomic Force Microscopy (AFM) Measurement

The AFM images were collected on a NanoScope V device at ambient temperature in tapping mode using silicon tips (NSC14/AIBS, MikroMasch). 10 μL of the sample solution(200 μM) was used and then was place on freshly cleaved silicon wafer. Adsorb sample for 1 day under dry conditions. The resultant substrates were rinsed with solvent (50 μl) twice to remove the loosely bound peptide and the samples were stored in a desiccator *in vacuo* for 1 h before imaging. The scanning speed was at a line frequency of 1.0 Hz, and the original images were

sampled at a resolution of 512 x 512 pixels.

TEM

Transmission electron microscopy observation was carried out with a JEOL JEM-2010 operated at 120 kV. For study of structure of laterally-grafted amphiphilic molecules in aqueous solution, a drop of aqueous solution (0.01 %) was placed on a carbon-coated copper grid and allowing the solution to evaporate under ambient conditions.

The cryogenic transmission electron microscopy experiments (cryo-TEM) were performed with a thin film of aqueous solution of molecule (5 μL) transferred to a lacey supported grid. The thin aqueous films were prepared under controlled temperature and humidity conditions (97-99 %) within a custom-built environmental chamber in order to prevent evaporation of water from sample solution. The excess liquid was blotted with filter paper for 2-3 seconds, and the thin aqueous films were rapidly vitrified by plunging them into liquid ethane (cooled by liquid nitrogen) at its freezing point. To investigate the effect of temperature, solution were sealed with Teflon tape and elevated the desired temperature in Daehan Scientific precision digital refrigerated circulator having an accuracy ± 0.1℃. The system was maintained for 1 hr. And then solution was placed on the lacey supported grid, and thin aqueous films were quickly quenched in liquid ethane. The grid was transferred, on a Gatan 626 cryo holder, using a cryo transfer device. After that they were transferred to a JEM-2010 TEM. Direct imaging was carried out at a temperature of approximately -175 ℃ and with a 120 kV accelerating voltage, using the images acquired with a Dual vision 300W and SC 1000 CCD camera (Gatan, Inc; Warrendale, PA)

XRD

Data was collected on an X-ray scattering 5D beamline in Pohang Accelerator Laboratory with a wavelength of 1.23984 Å. By varying the scattering angle the explored momentum transfer vector (q) was in the range of $5.3 < q\ (\mathrm{nm}^{-1}) < 18.3$, with $q = 4\pi \sin\theta/\lambda$, where θ is the scattering angle. The tripeptides at 5 mg/mL was spread on a silicon wafer slide as a film and allowed to air dry prior to data collection.

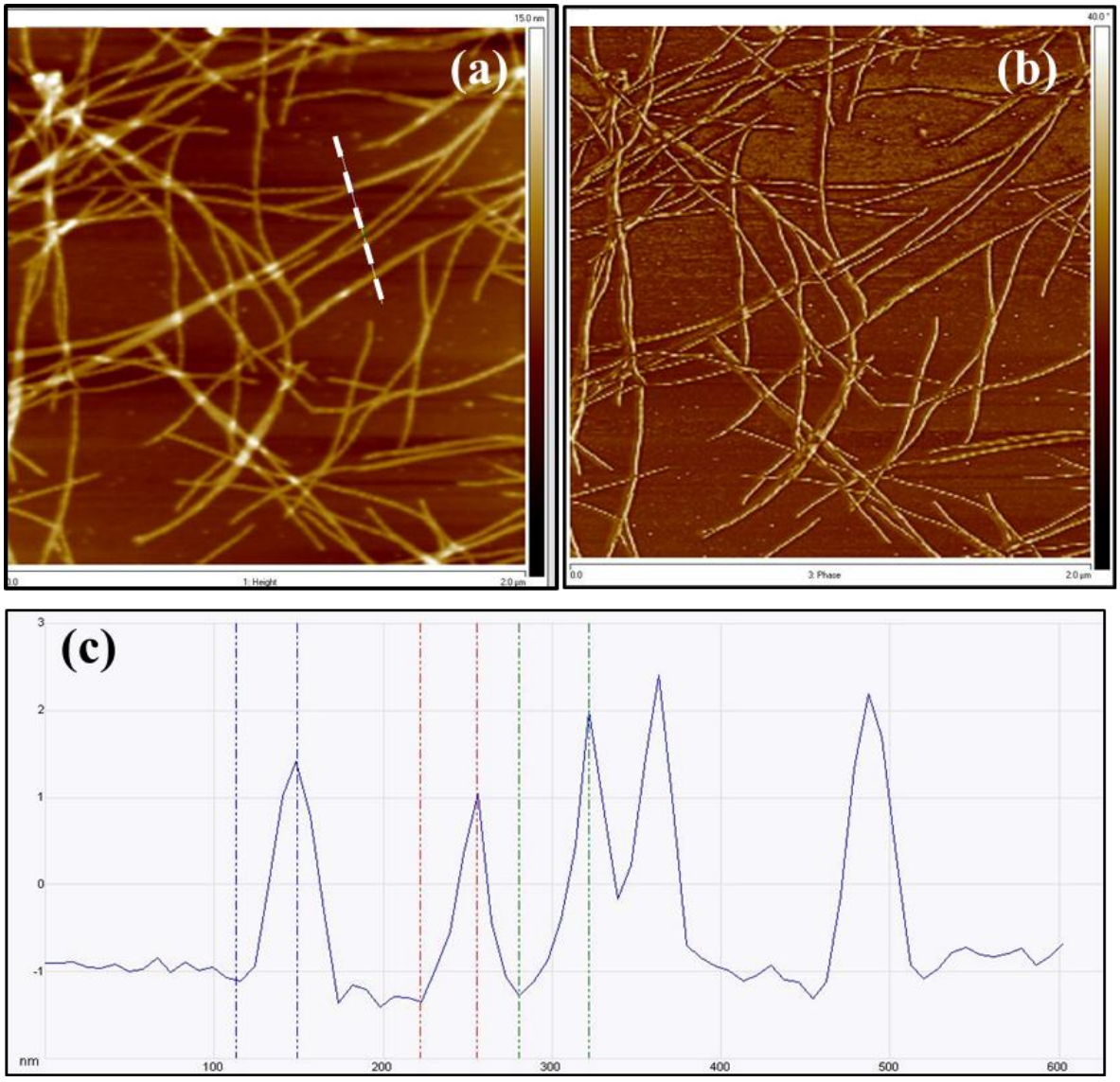


Figure S3. AFM image of tripeptide **1** with (a) height and (b) phase image at 200 μM in aqueous solution. (c) Section analysis along the line showed regular height of 3.2 nm.

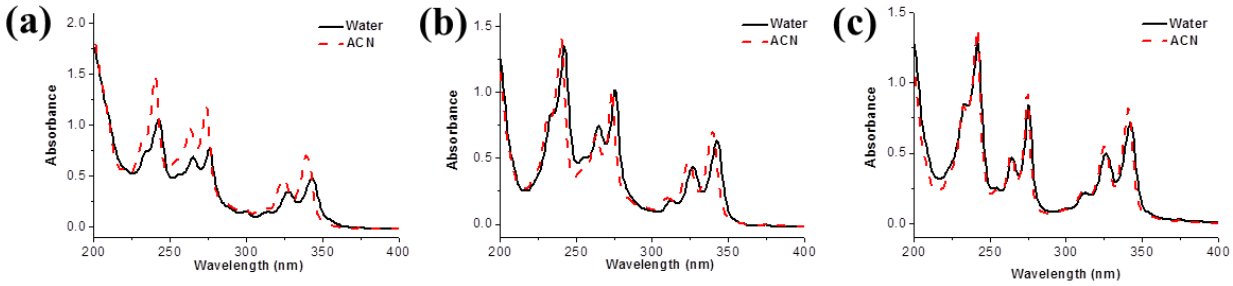


Figure S4. UV spectra of tripeptides **1**(a), **2**(b) and **3**(c). UV absorption maxima of tripeptides in aqueous solution were red-shifted with respect to those recorded in acetonitrile which tripeptides did not assemble. It indicates *J*-type aggregation of the pyrene segments within the ribbon structure. All solutions were at 200 μM.

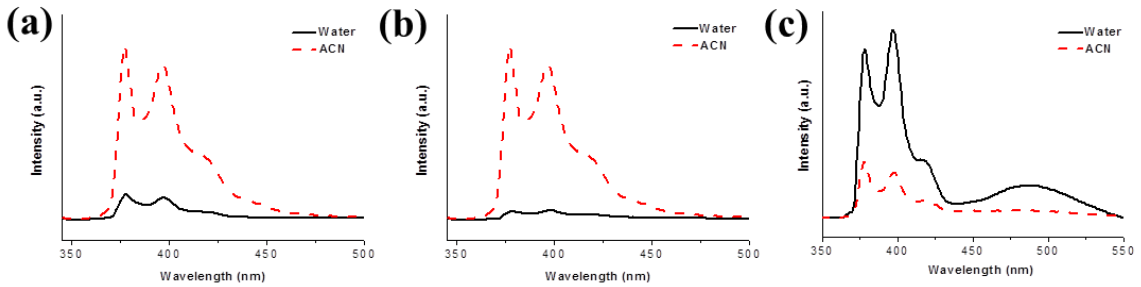


Figure S5. Changes in the emission (λ_{ex} = 340 nm) spectra of peptides, (a) **1** in aqueous solution (1 mM, solid) and **1** in acetonitrile (1 mM, dash). (b) **2** in aqueous solution (1 mM, solid) and **2** in acetonitrile (1 mM dash). The fluorescence spectra were quenched with respect to those recorded in acetonitrile, which supported UV data. But **3** had opposite tendency in emission spectrum, indicating that peptide **3** might have aggregates in organic solvent.

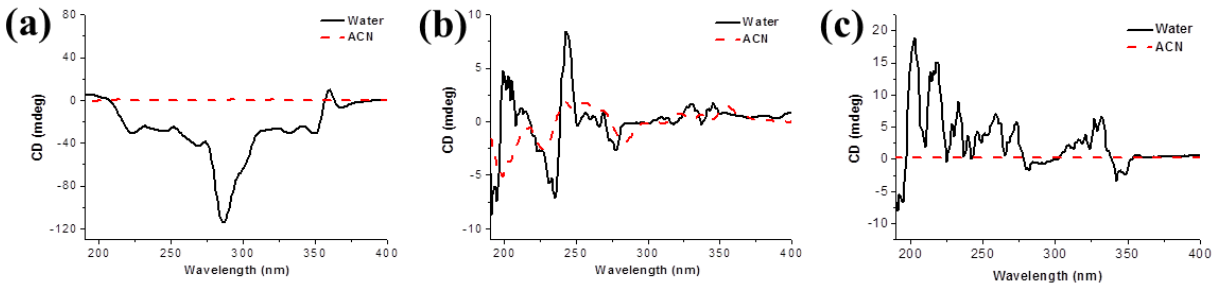


Figure S6. CD analysis of peptide secondary structures in 1 mM aqueous solution (solid) and in 1 mM acetonitrile (dash). (a) Trieptide **1**, (b) **2** and (c) **3**. Compared to other homologue **2** and **3**, which are random structures, CD spectrum of tripeptide **1** is shown to consist predominantly of β-sheet (Figure S6a). But **1** displayed flat signals in acetonitrile, it means that secondary structures of **1** were disassembled.

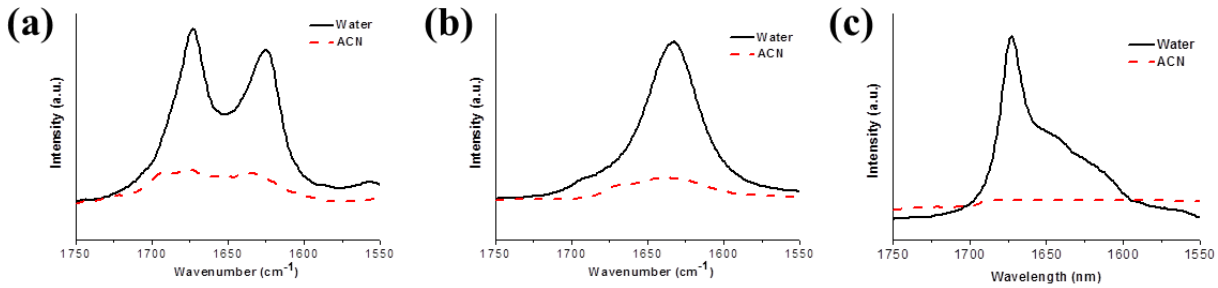


Figure S7. FTIR spectra of tripeptides in D_2O (1 mM, solid) and in acetonitrile (1 mM, dash). (a) **1**, (b) **2** and (c) **3**. Similar with the CD spectra, FTIR spectra of tripeptides showed flat signals in acetonitrile rather than in aqueous solutions.

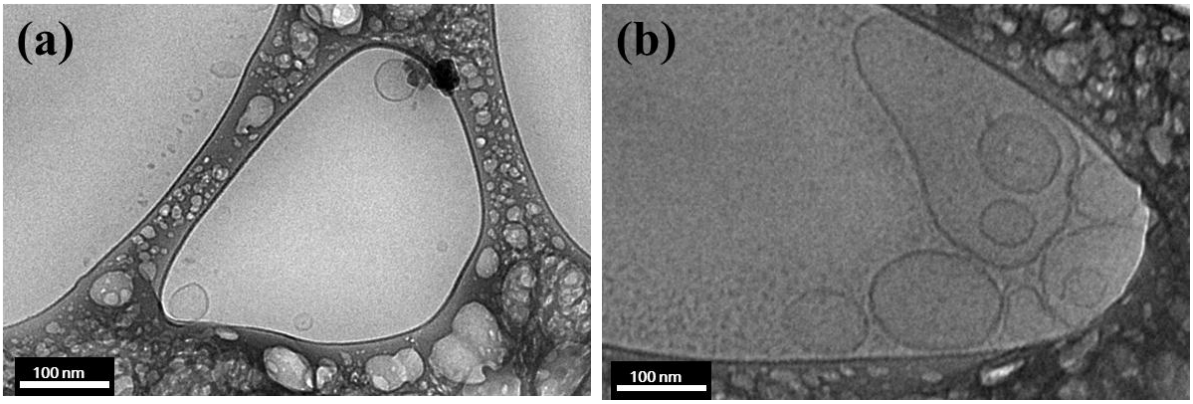


Figure S8. Cryo-TEM images of peptide **3**. The radius of the vesicles is about 20 nm to 100 nm.

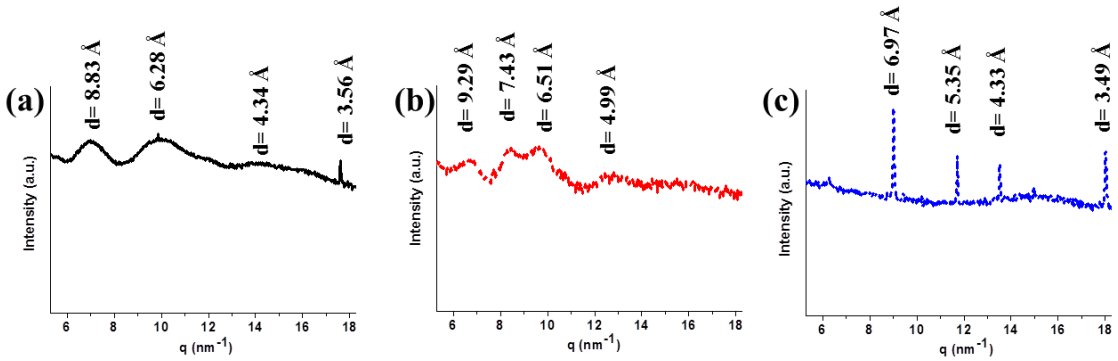


Figure S9. Wide Angle X-ray Scattering data of peptides (a) **1**, (b) **2** and (c) **3** spread as a film on a silicon wafer slide. The XRD peaks at 3.56 and 4.34 Å corresponding to the spacing between pairs of pyrene groups and the spacing of peptide within a β-sheet structure, respectively.[3] Peptide **1** and **2** show broad pattern indicating irregular packing of the aromatic segments. In contrast, **3** shows sharp XRD pattern, indicating that aromatic segments aligned with ordered structure through strong π-π interactions. However, the driving forces that lead to the well-defined nanostructures are not clear at this point.

Reference

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