Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Smart hydrogel from laterally-grafted peptide assembly

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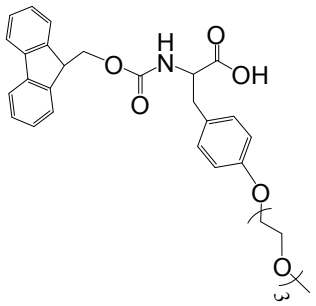
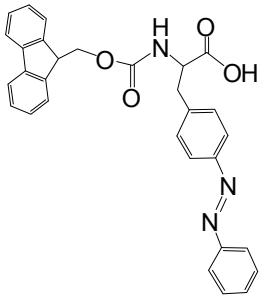
1. Synthesis of Fmoc-amino acids derivatives:



Fmoc-tyrosine derivative

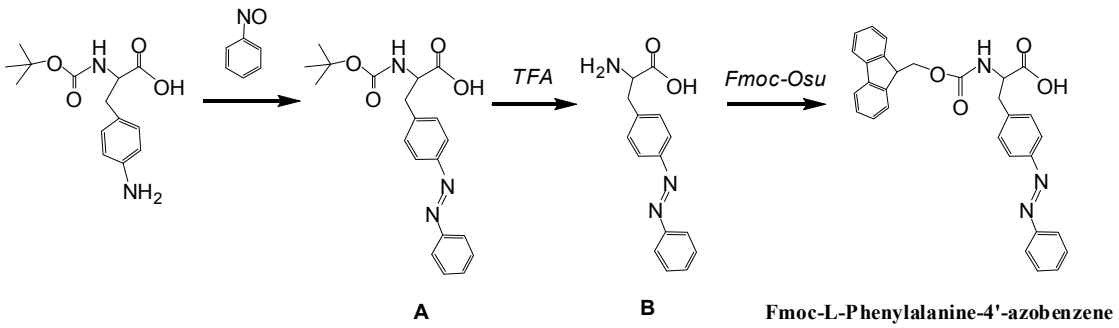


Fmoc-L-Phenylalanine-4'-azobenzene

Fig S1. Molecular structure of Fmoc-amino acids derivatives.

Fmoc-tyrosine derivative with tri(ethylene glycol) monomethyl ether was synthesized following the previous procedures.[1]

Fmoc-L-Phenylalanine-4'-azobenzene (Fmoc-Phe-Azo) was synthesized as the modified procedures.[2]



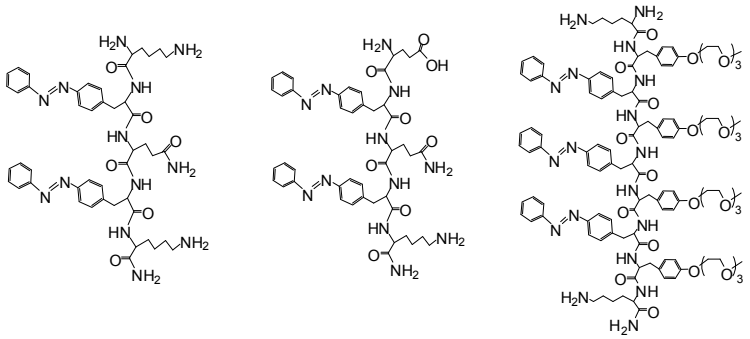
Scheme S1. The synthesis route of Fmoc-L-Phenylalanine-4'-azobenzene (Fmoc-Phe-Azo).

Detailed procedures:

Synthesis of A: L-N-tert-Butoxycarbonyl-p-aminophenylalanine (2 g, 7.2 mmol) was dissolved in glacial acetic acid (200 mL) at room temperature. Nitrosobenzene (1.156 g, 10.8 mmol) was added to this solution in one portion and allowed to stir for 8 h. The reaction mixture was then quenched with satd. $NaHCO_3$ solution (300 mL) and extracted with ethyl acetate for 3 times. The organic layers were then combined, dried over anhydrous $MgSO_4$ and concentrated on a rotary evaporator. The crude material was then purified by silica gel column chromatography (CH_2Cl_2: MeOH = 10:1, v/v), yielding 1.64 g (65 %) of **A** as an orange solid. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ = 1.42 (s, 9H, $(CH_3)_3C$), 2.95 (dd, 1H, $CH(CH_2)$), 3.2 (dd, 1H, $CH(CH_2)$), 4.65 (d, 1H, $CH(CH_2)$, 7.33 (d, 2Ar-H, J = 6 Hz)), 7.47 (m, 3Ar-H), 7.85 (m, 4 Ar-H).

Synthesis of Fmoc-Phe-Azo: Freshly distilled methylene chloride (50 mL) and compound **A** (1 g, 2.71 mmol) were placed in a dry round bottomed flask under nitrogen atmosphere and cooled down to 0 ^{o}C in an ice bath. To this mixture, trifluoroacetic acid (40 mL) was added slowly and the reaction mixture was stirred for 5-6 h at room temperature. After the reaction, the mixture was evaporated to dryness, yielding compound **B**. Compound **B** was dissolved in 40 mL dioxane and cooled down to 0 ^{o}C in an ice bath. To this solution, 10% Na_2CO_3 solution (100 mL) was added and then the 9-fluorenylmethyl-N-hydroxysuccinimide (Fmoc-Osu, 0.76 g, 2.25 mmol) in dioxane (50 mL) was added at 0 ^{o}C. The resulting mixture was stirred at 0 ^{o}C for 1h and at room temperature for 20 h. After diluting the mixture with water (150 mL), the aqueous solution is extracted with ethyl acetate. The aqueous layer was then cooled down to 0 ^{o}C, acidified to pH 2 by the addition of 6 N HCl, and the resulted suspension was extracted by ethyl acetate for 3-4 times. The organic layers were combined, dried over Na_2SO_4 and concentrated by evaporation. The product was purified by column chromatography on silica gel using a mixture of ethyl acetate and methanol (20:1 v/v) as an eluent, yielding 0.67 g (50 %) of **Fmoc-Phe-Azo** as an orange solid. ^{1}H NMR (300 MHz, DMSO-d6, ppm) δ = 3.05 (d, 2H, $CH(CH_2)$), 4.15 (t, 1H, CH_2CH-Fmoc), 4.35 (d, 2H, CH_2CH-Fmoc, J = 4.5 Hz), 4.5 (m, 1H, $CHCH_2$), 7.29 (m, 3Ar-H), 7.39 (m, 3Ar-H), 7.59 (m, 5Ar-H), 7.76, (m, 2Ar-H), 7.87 (m, 4Ar-H). MS (MALDI) calc'd for $C_{30}H_{25}N_3O_4$ $(M+Na)^+$ 5514.53; found 514.86.

2. Synthesis of the peptides:



1 **2** **3**

All the peptides were synthesized with a microwave-equipped liberty peptide synthesizer (CEM). Rink amide resin was utilized and loaded using standard methods HBTU activation. Peptide synthesis was carried out using standard Fmoc methods. Briefly, the synthesis was initiated using 60 μmmol of Fmoc-Rink Amide MBHA resin (0.65 mmol/g, Advanced Chem

Tech), and the chain extension was accomplished using an insitu neutralization/HBTU activation procedure, where 3-fold excess of amino acid was pre-activated with 2.7 equiv of HBTU and 6 equiv of DIPEA in DMF (1 mL) for 5 min before it was added to the resin. Each coupling step took a minimum of 7 min. After completion of the assembly of the designed sequences, the peptide was cleaved from the resin manually by treatment with trifluoroacetic acid (TFA), 1,2-ethanedithiol, and thioanisole (95:2.5:2.5) for 3 h under constant agitation in a rotary shaker (37 ^{o}C, 200 rpm of constant agitation). The resultant peptide was precipitated with cold Hexane and tert-Butyl Methyl Ether (TBME), dissolved in acetonitrile: water (2:3), and filtered. The crude peptides were purified by reverse-phase HPLC on C18 column (Vydac, USA) using linear gradient of water to acetonitrile in the presence of 0.1% TFA, and the column eluents were monitored by UV absorbance at 230 and 320 nm. The molecular weight was confirmed by MALDI-TOF mass spectrometry.

Peptide **1:** (MALDI-TOF-MS) m/z 904.69 (905.07 calcd for [M+H$^+$]), 926.75 (927.06 calcd for [M+Na$^+$]), 912.91 (943.17 calcd for [M+K$^+$]).

Peptide **2:** (MALDI-TOF-MS) m/z 905.87 (906.01 calcd for [M+H$^+$]), 927.66 (928.0 calcd for [M+Na$^+$]), 945.06 (944.01 calcd for [M+K$^+$]).

Peptide **3:** (MALDI-TOF-MS) m/z 2266.11 (2265.65 calcd for [M+H$^+$]), 2287.98 (2287.64 calcd for [M+Na$^+$]), 2304.10 (2303.75 calcd for [M+K$^+$]).

3. Measurements:

Circular Dichroism Spectroscopy. Peptide aqueous solutions were analyzed by CD spectroscopy to characterize the secondary structures. Spectra were recorded on a JASCO model J-810 spectropolarimeter. CD spectra were obtained from 300-190 nm with a 1.0 nm step, 1.0 nm bandwidth, and a 3 s collection time per step at 25 ^{o}C in a 0.1 cm path length quartz cuvette (Hellma). The scans were repeated five times and averaged. The JASCO software was used for background subtraction, conversion to molar ellipticity per amino acid residue.

Fourier Transform Infrared Spectroscopy. FT-IR spectra were obtained using a Bruker Equinox 55 FT-IR spectrophotometer. The aqueous solution of the peptide samples was cast onto gold plate. Absorbance spectra were obtained from 1750 to 1550 cm^{-1} with a 4 cm^{-1} resolution. 502 scans were acquired and background spectra were also collected and subtracted.

Transmission Electron Microscopy. Samples (2 μL) were spotted directly onto a carbon-coated copper grid and dried completely. Then 2 % (w/v) uranyl acetate solution (2 μL) was added and allowed to stand for 1 min. Then, the excess amount of solution was carefully removed by capillary action (filter paper). Direct imaging was carried out with a 120 kV accelerating voltage, while acquiring the images with a SC 1000 CCD camera (Gatan, Inc., USA). The data were analyzed with Digital Micrograph software.

Scanning Electron Microscopy. SEM was performed by using a Hitachi S-4300 system with an accelerating voltage of 15 kV. Samples for SEM were prepared by casting the gels on silicon slices, and then freeze-dried under vacuum for 1 day. After that, all samples were coated with gold.

4. Characterizations:

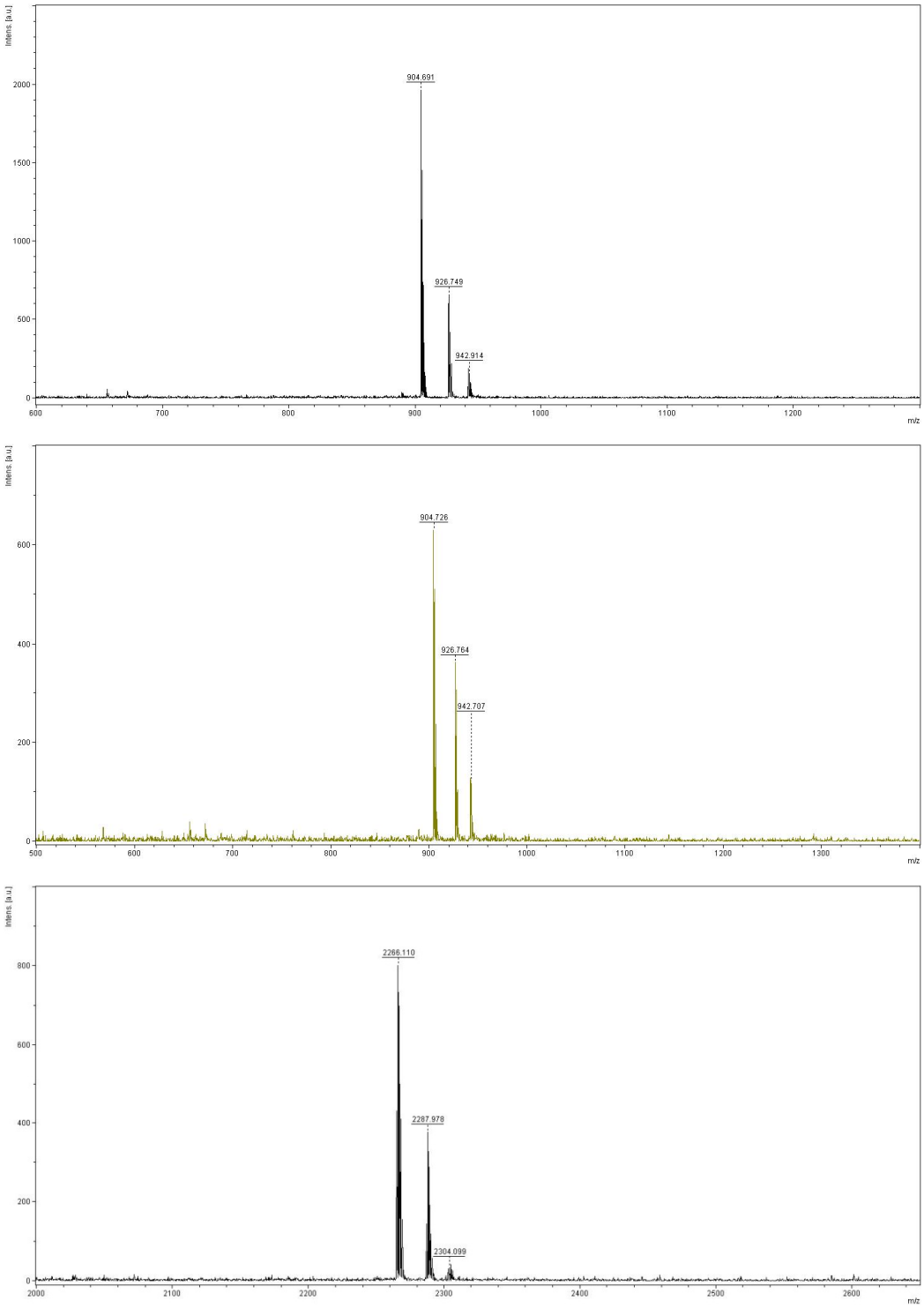


Fig S2. MALDI-TOF mass spectra of the synthesized peptides: **1** (top), **2** (middle), and **3** (bottom).

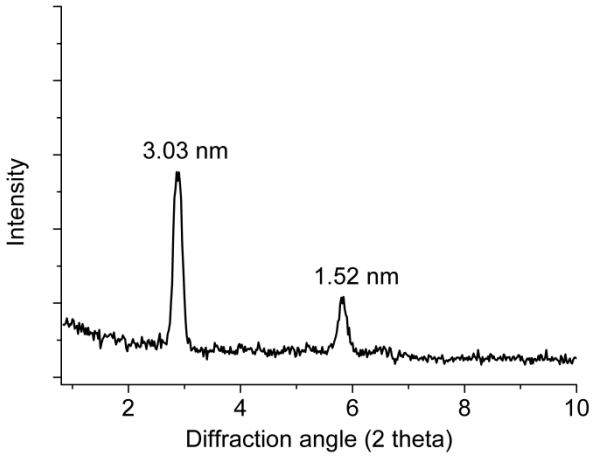


Fig. S3 X-ray diffraction of the peptide **1** xerogels.

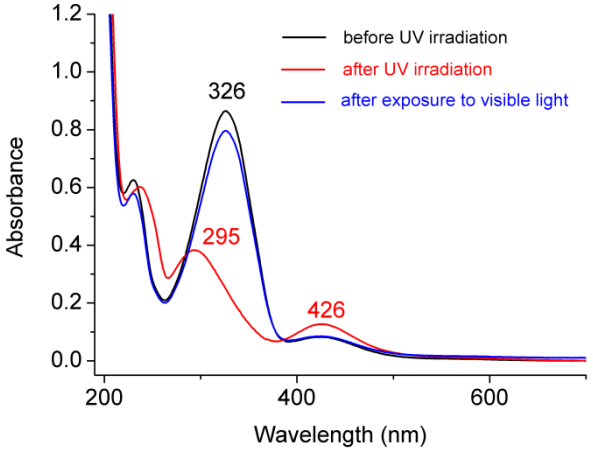
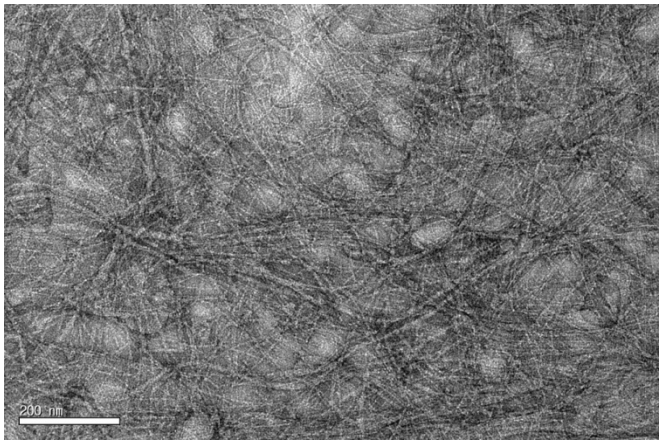


Fig. S4 UV-vis absorption spectra for the trans/cis photoisomerization of peptide **1** in aqueous solution.



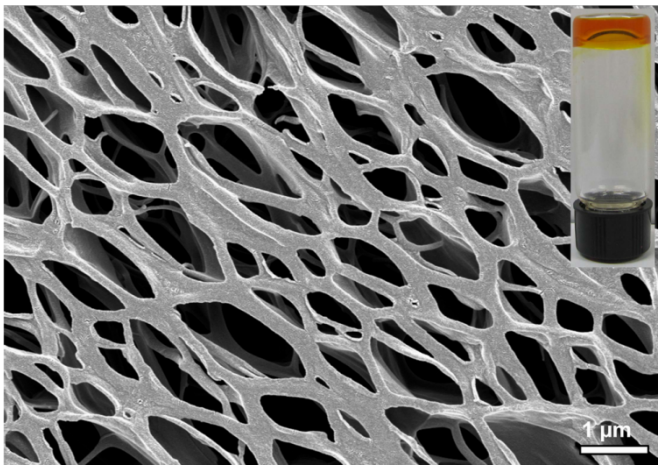


Fig. S5 TEM (top) and SEM (bottom) images of the recovered peptide **1** after aging the cis-conformation samples under sunlight for 2 hours.

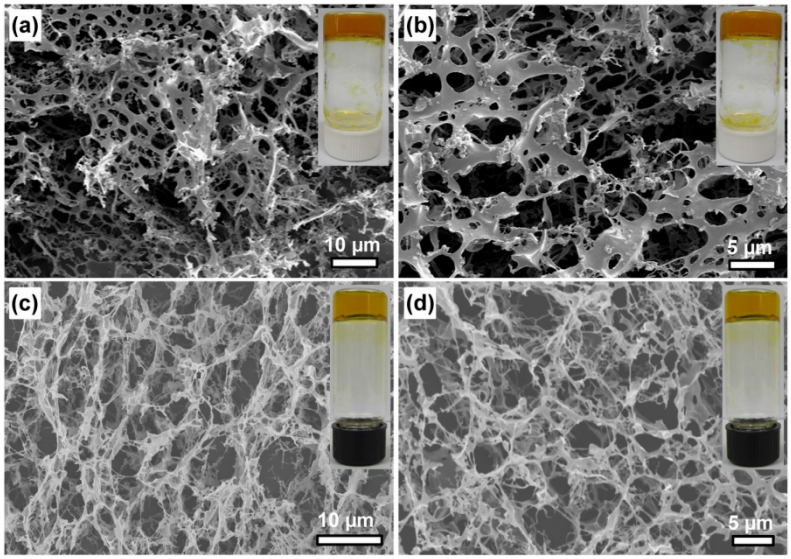


Fig. S6 SEM (bottom) images of peptide **1** gels: (a) gel formed in basic condition (pH~10.5), (b) basic gel sample after UV irradiation for 30 min, (c) gel formed in salt condition (10 mM NaCl), (d) salt gel sample after UV irradiation for 30 min. (Inset: optical images of the gels)

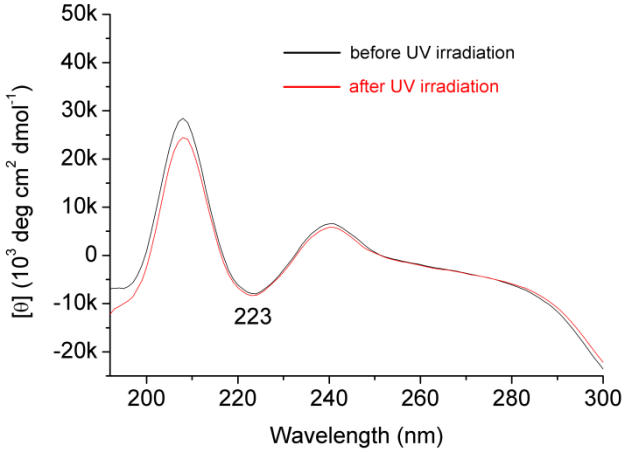


Fig. S7 Circular dichroism (CD) spectra of peptide **2** aqueous solution before (black line) and after (red line) UV irradiation.

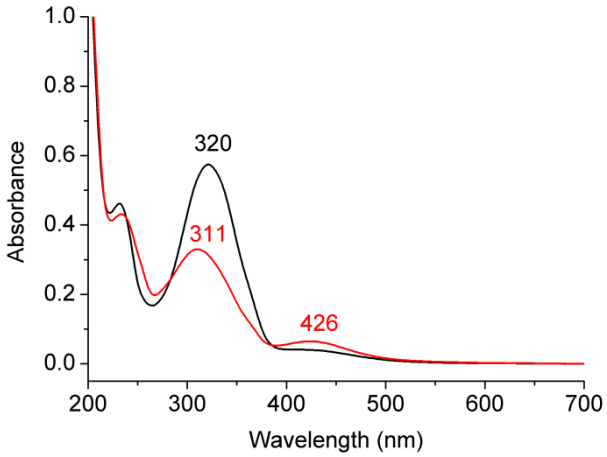


Fig. S8 UV-vis absorption spectra for the trans/cis photoisomerization of peptide **2** in aqueous solution.

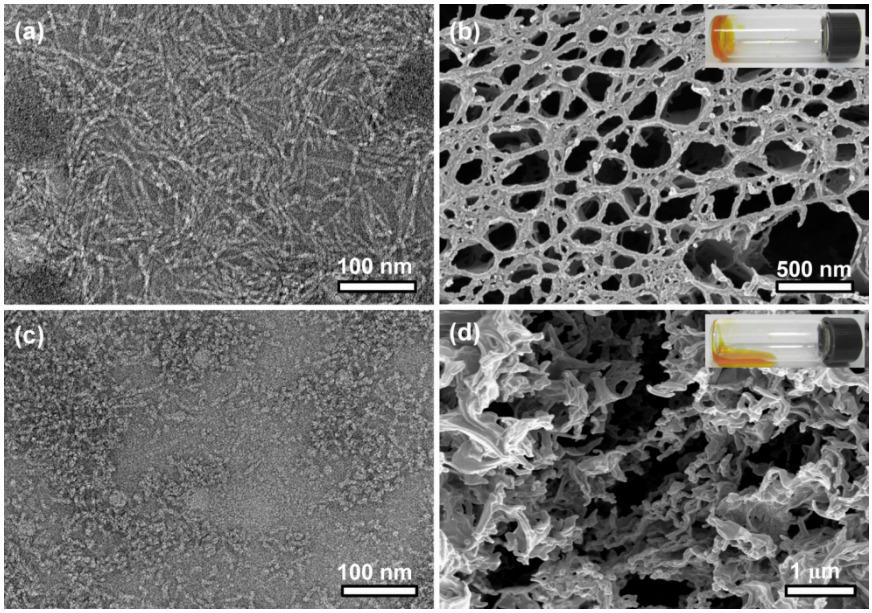


Fig. S9 Photo-responsive self-assembly structures of peptide 3: (a) TEM image of 3 in 40 μM aqueous solution; (b) SEM image of 3 gel (inset, optical image of 3 gel); (c) TEM image of 3 solution and (d) SEM image of 3 gel sample (inset, optical image of 3 sol) after exposing to 360-nm UV light for 20 min.

References:

[1] I-S. Park, Y-R. Yoon, M. Jung, K. Kim, S. B. Park, S. Shin, Y-B. Lim, and M. S. Lee, *Chem. Asian J.*, 2011, **6**, 452–458.

[2] M. Bose, D. Groff, J. Xie, E. Brustad, and P. G. Schultz, *J. Am. Chem. Soc*. 2006, **128**, 388–389.