Electronic Supplementary Information

of

Photo-switched self-assembly of Gemini α-helical peptide into supramolecular architectures

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Materials

2-Chlorotrityl chloride resin (100-200 mesh, loading: 1.18 mmol/g), *N*-Fluorenyl-9-methoxycarbonyl (FMOC) protected L-amino acids (FMOC-Ala-OH, FMOC-Glu(OtBu)-OH, FMOC-Arg(Pbf)-OH), *N*,*N'*-dissopropylcarbodiimide (DIC), 2-(1*H*-benzotriazol-1-yl)-1,1,1,1-tetramethyluroniumhexafluorophosphate (HBTU), benzotriazole-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), 1-hydroxybenzotriazole (HOBt) and triisopropylsilane (TIS) were purchased from GL Biochem. Ltd. (Shanghai, China) and used as received. Trifluoroacetic acid (TFA), piperidine, 4-dimethylamiopryidine (DMAP) and anhydrous ether were obtained from Shanghai Chemical Co. (China) and used directly. Diisopropylethylamine (DIEA) was acquired from GL Biochem. Ltd. (Shanghai, China) and used after distillation. *N*,*N*-Dimethylformamide (DMF), tetrahydrofuran (THF), methanol and dichloromethane (DCM) were provided by Shanghai Chemical Co. (China) and distilled prior to use. 4,4'-Azodialinine was obtained from ACROS (USA) and used as received. All other reagents and solvents were of analytical grade and used directly.

Synthesis of the Gemini α-helical peptide of AcARA$_4$EAA-Azo-AAEA$_4$RAAc

The Gemini peptide was synthesized *via* fragment condensation method between H-A-Azo-A-H and fully protected peptide Ac-AR(Pbf)A$_4$E(OtBu)A-OH. Fully protected peptide Ac-AR(Pbf)A$_4$E(OtBu)A-OH was synthesized manually in 3.0 mmol scale on the 2-chlorotrityl chloride resin (2.54 g) employing a standard FMOC chemistry SPPS. Cleavage of the expected peptide from the resin was performed using a cocktail of TFA, DI water, and DCM in the volume ratio of 0.2: 0.5: 99.3. After repeating the performance 10 times for each 20 min, the cleavage mixture and washing solution were collected and concentrated to precipitate the product in cold ether. Azobenzene with alanine modification (H-A-Azo-A-H) was synthesized via the condensation reaction between the amino of 4,4'-azodialinine and carboxyl group of FMOC-Ala-OH, using DIC and HOBt as activators.

Deprotection of FMOC group was performed by using 20% piperidine/DMF (V/V) solution for 30 min. Then the Gemini peptide of AcARA_4EAA-Azo-AAEA_4RAAc was synthesized *via* the condensation reaction between the amino of H-A-Azo-A-H and carboxyl group of Ac-AR(Pbf)A_4E(OtBu)A-OH, using PyBOP as a activator and DMAP as a base. Removing of side chain groups (Pbf and OtBu) was performed *via* using a cocktail of TFA, TIS and DI water with a volume ratio of 95 : 2.5: 2.5. After stirring 2 h, the cleavage mixture and subsequent TFA washing were collected and concentrated to precipitate the product in cold ether. The brown crude was dissolved in DMSO and further purified via dialysis against DMSO for 4 days and then DI water for 2 days using a dialysis membrane (MWCO = 1000 Da). The solvent (DMSO or DI water) was refreshed every 12 h to remove the impurities. After freeze-drying under vacuum for 3 days, the expected Gemini peptide was collected as brown solid. The detailed synthesis of the Gemini peptide is illustrated in Fig. S1-3.

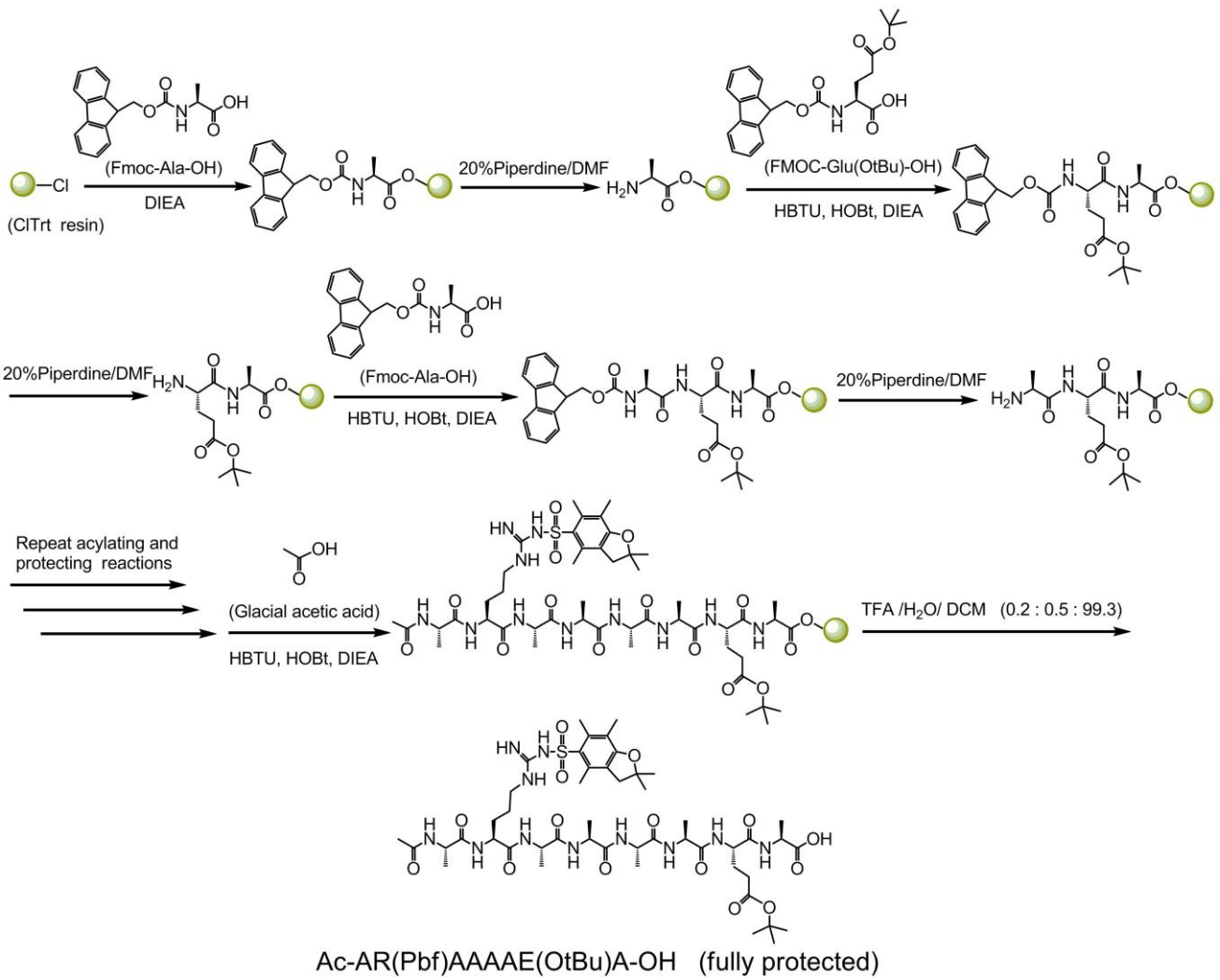

Fig. S1 Synthesis route of the fully protected peptide of AcAR(Pbf)A_4E(OtBu)A-OH.

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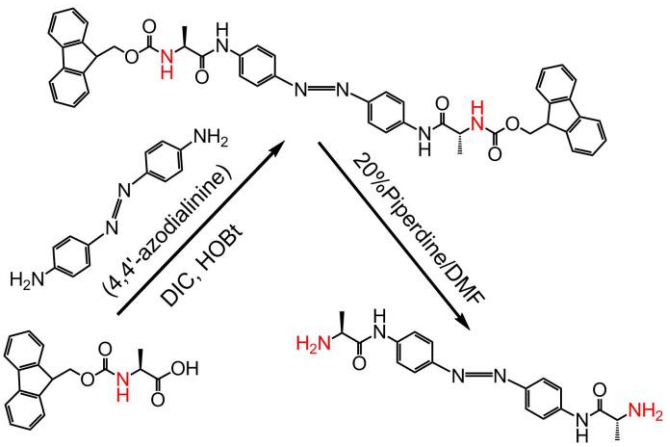

Fig. S2 Synthesis route of the alanine-modified azobenzene H-A-Azo-A-H.

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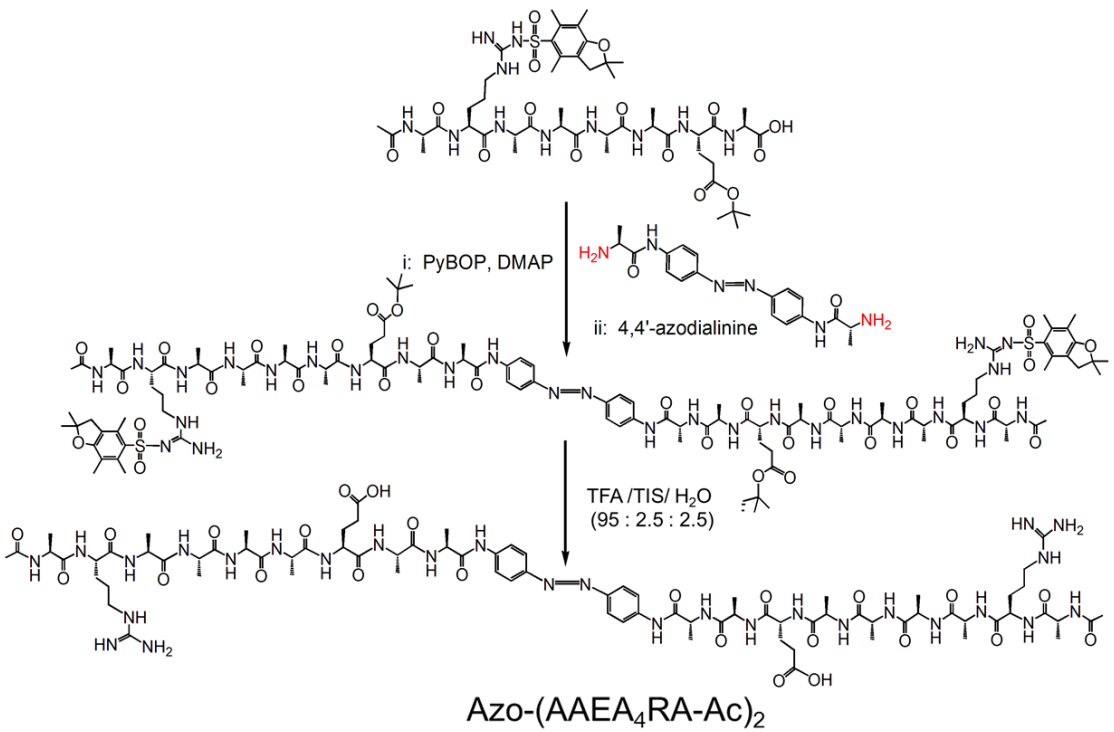

Fig. S3 Synthesis route of the Gemini α-helical peptide of $AcARA_4EAA$-Azo-$AAEA_4RAAc$.

Electrospray ionization-mass spectrometry (ESI-MS)

The molecular weights of the fully peptide of AcAR(Pbf)A_4E(OtBu)A-OH (Fig. S4), alanine modified azobenzene of Fmoc-A-Azo-A-Fmoc (Fig. S5) and Gemini α-helical peptide of AcARA_4EAA-Azo-AAEA_4RAAc (Fig. S6) were analyzed by ESI-MS (LCQ Advantage, Finigan, USA).

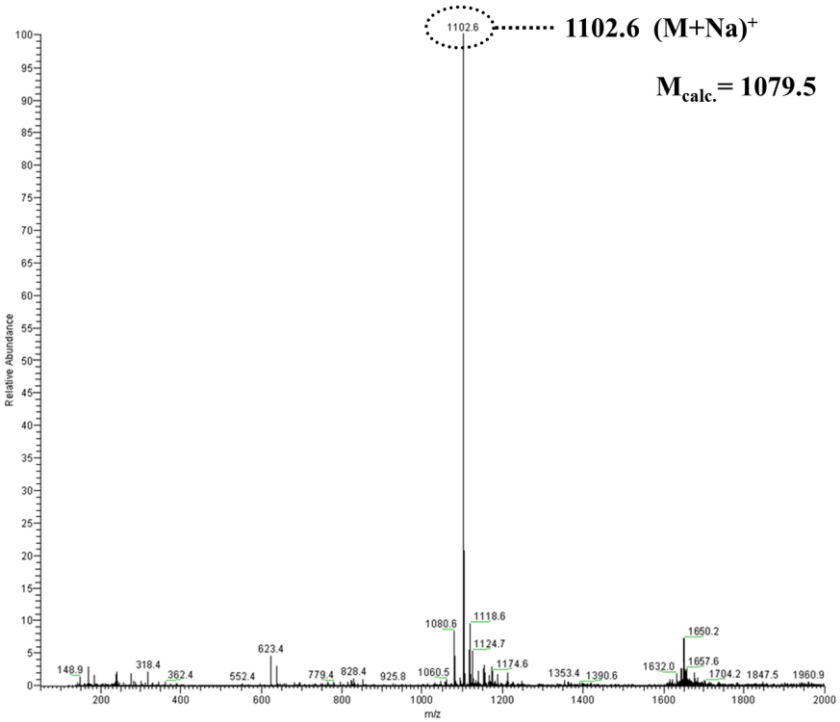

Fig. S4 ESI-MS profile of the fully protected peptide of AcAR(Pbf)A_4E(OtBu)A-OH.

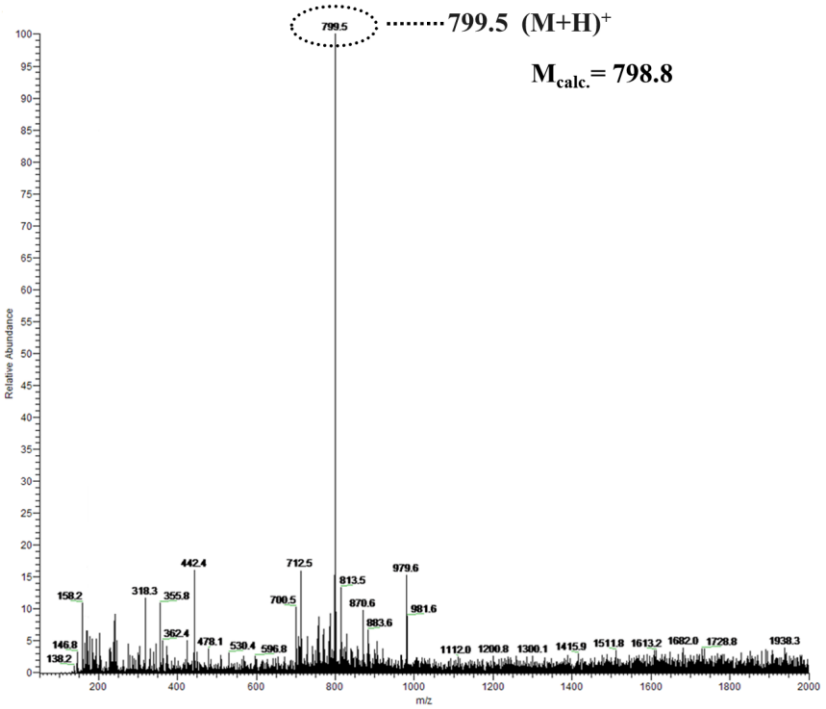

Fig. S5 ESI-MS profile of Fmoc-A-Azo-A-Fmoc.

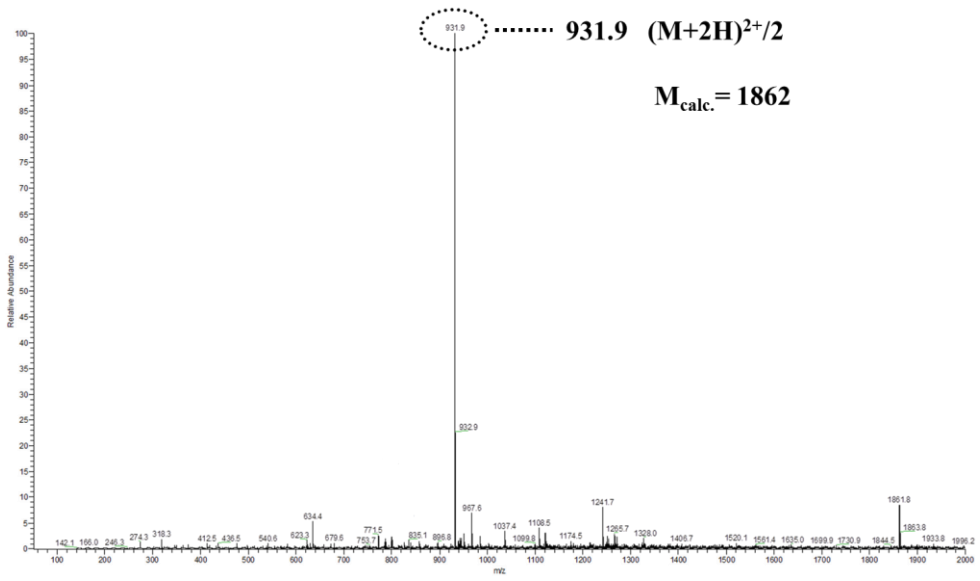

Fig. S6 ESI-MS profile of the Gemini α-helical peptide of AcARA4EAA-Azo-AAEA4RAAc.

^{1}H Nuclear magnetic resonance (^{1}H NMR)

The ^{1}H NMR spectra of FMOC-A-Azo-A-FMOC and H-A-Azo-A-H were recorded on a Mercury VX-300 spectrometer at 300 MHz (Varian, USA) using DMSO as a solvent and TMS as an internal standard. As shown in Fig. S7, three major groups of peaks emerged in the chemical shift. The chemical shift around δ 7-8.5 ppm is attributed to the benzene protons. The chemical shift around δ 4.3 ppm is assigned to the protons of the methane and methylene. And the chemical shift around δ 1.3 ppm is attributed to the protons of the methyl. The ratio of the protons in benzene group and that in methyl group (benzene: -CH_3) is 4:1, demonstrating the successful modification of Fmoc-Ala-OH. It was further confirmed from the ratio of the protons in benzene group and that in methane and methylene group (benzene: -CH and –CH_2, which is 3:1). The disappearance of chemical shift around 7.4 (blue line) demonstrated the cleavage of Fmoc group. And the presence of chemical shift around δ 2.0 ppm can be attributed to the protons of amide group of H-A-Azo-A-H.

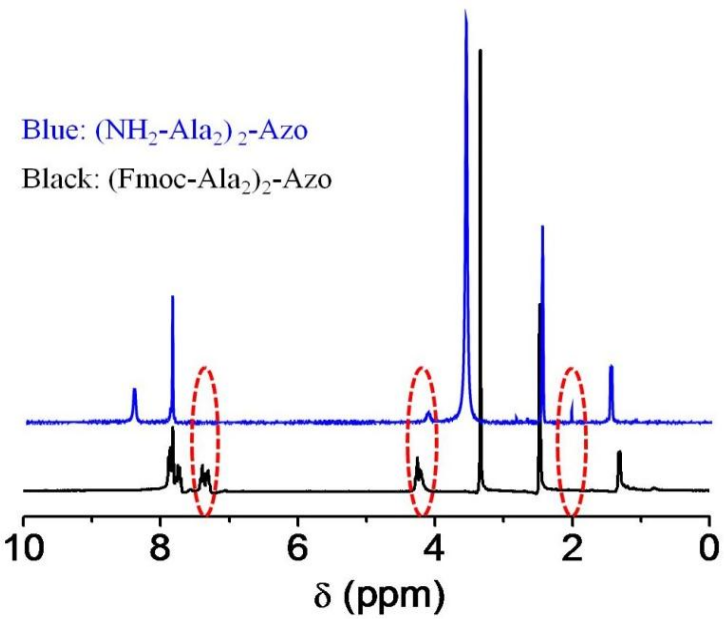

Fig. S7 ^{1}H NMR spectra of Fmoc-A-Azo-A-Fmoc and H-A-Azo-A-H.

UV-visible absorption

UV-visible absorption of the light-switched Gemini α-helical peptide was performed on a Lambda Bio 40 UV-vis spectrometer (Perkin-Elmer, USA) ranging from 550 to 220 cm^{-1}.

Light-switched self-assembly of the Gemini α-helical peptide

The light-switched self-assembly behavior of the Gemini α-helical peptide was respectively investigated under acidic (pH 3.0) and basic (pH 8.0) mediums. In brief, the Gemini peptide was directly dissolved in DI water to form a homogenous solution with a concentration of 0.5 $mg \cdot mL^{-1}$ (0.25 mM) and a solution pH of 5.5. And then, the solution pH was adjusted to a value of 3.0 or 8.0 and allowed to stand for 12 h for self-assembly. Subsequently, the solution was exposed to UV irradiation (λ=365 nm, 120 W) for another 12 h to realize the UV light-switched self-assembly. Thereafter, this solution was further exposed to visible light for 12 h to conduct the visible light-recoverable self-assembly. All these solutions were stored and used for the next characterizations. Noted that, when dissolving the Gemini α-helical peptide in DI water, it can self-assemble into nanofibers (Fig. S8a), in which α-helix and β-sheet conformations co-exist (Fig. S9). After 12 h UV light irradiation, these nanofibers can change into nanospheres (Fig. S8b and d) with dominate α-helix conformation (Fig. S9). If placing under visible light for 12 h, the self-assembled Gemini peptide can recover from nanospheres to nanofibers (Fig. S8c).

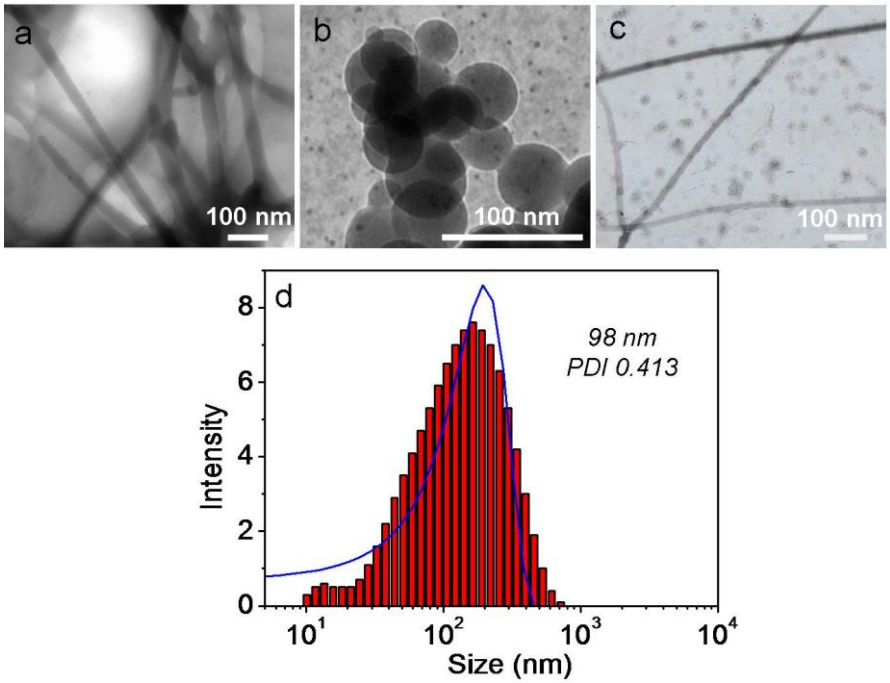

Fig. S8 (a-c) TEM images of the self-assembled Gemini α-helical peptide at pH 5.5; (a): before UV light irradiation; (b): after 12 h UV light irradiation; (c): after 12 h visible light irradiation; (d) Size distribution of the self-assembled Gemini α-helical peptide at pH5.5 after 12 h UV light irradiation.

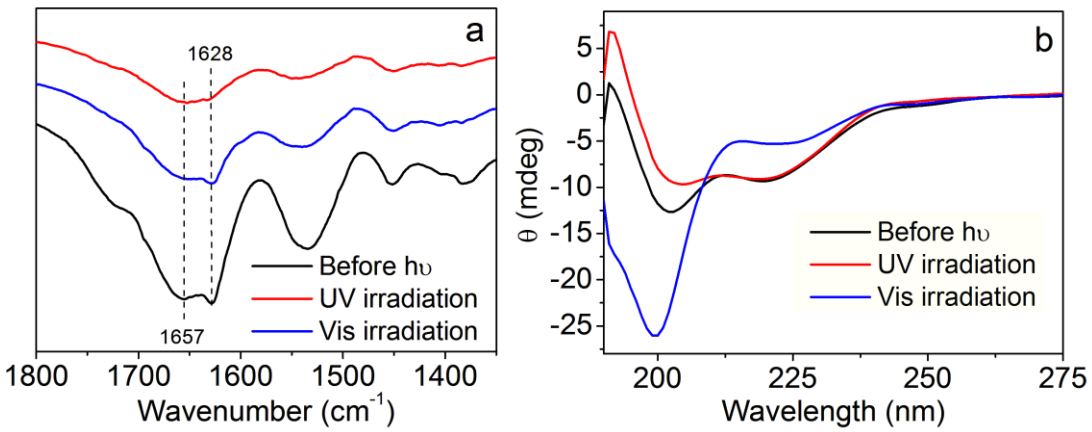

Fig. S9 FT-IR (a) and CD (b) spectra of the self-assembled Gemini α-helical peptide at pH 5.5.

Transmission electron microscope (TEM)

The morphology of the self-assembled Gemini α-helical peptide was observed on a JEM-2010 TEM (JEOL Ltd. Japan). Before the observation, the aqueous solution of the self-assembled Gemini

α-helical peptide was applied to a copper grid with Formvar film and then air dried.

Dynamic light scattering (DLS)

The average size of the self-assembled Gemini α-helical peptide and the corresponding size distribution in aqueous solution were determined using dynamic light scattering (DLS) techniques with a Nano-ZS ZEN3600 instrument (MALVERN Instruments).

Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectrum of the self-assembled Gemini α-helical peptide was collected on a Perkin-Elmer spectrophotometer. Before the measurements, the aqueous solution of the self-assembled Gemini α-helical peptide was freeze-dried and then pressed into KBr pellets.

Circular dichroism (CD)

The solution of the self-assembled Gemini α-helical peptide was fixed in a 0.5 mm quartz cell and analyzed on a Jasco J-810 spectropolarimeter with 4 s accumulations every 1 nm and averaged over three acquisitions.