Supporting Information

Light-powered and transient peptide 2D assembly driven by trans-to-cis isomerization of azobenzene side chains

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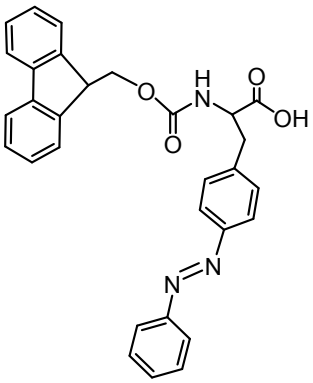
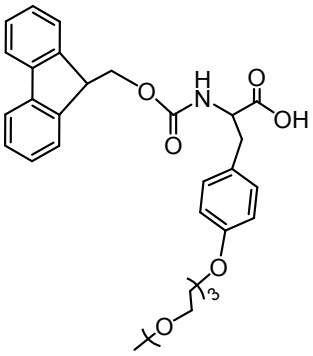
1. Materials



Fmoc-L-Phenylalanine-4'-azobenzene



Fmoc-tyrosine modified with tri(ethyleneglycol) monomethyl ether

Fmoc-L-phenylalanine-4′-azobenzene and Fmoc-tyrosine modified with tri(ethylene glycol) monomethyl ether were synthesized following our previous procedures.[1] Fmoc-L-lysine, Fmoc-Rink Amide resin (MBHA, 0.64mmol/g), 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-

tetramethyluronium hexafluorophosphate (HATU, 99%), and N-Methylmorpholine (NMM, 98%) were purchased from GL Biochem (Shanghai) Ltd. Trifluoroacetic acid (TFA, 98%), anisole (98%) and triisopropylsilane (TIPS, 98%) were purchased from Tokyo Chemical Industry (TCI). Piperidine (99.9%) was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai). Dimethyl formamide (DMF, 99.8%) was purchased from J&K Chemical Technology Company (Beijing), and acetonitrile (99.9%) was purchased from Spectrum Chemical Manufacturing Corp-China. Thioflavin T (ThT) was purchased from the Aladdin Industrial Corporation (Shanghai). All the reagents and solvents used in this article are commercially available and without further purification. The polyoxometalates $Na_6V_{10}O_{28}$ (V_{10}),[2] $Na_9PW_9O_{34}$ (PW_9),[3] $K_6CoW_{12}O_{40}$ (CoW_{12}),[4] $K_6P_2W_{18}O_{62}$ (P_2W_{18}),[5] $K_9EuW_{10}O_{36}$ (EuW_{10}),[6] $K_{12.5}Na_{1.5}[NaP_5W_{30}O_{110}]$ (P_5W_{30}),[7] and $K_4PW_{11}VO_{40}$ ($PW_{11}V$),[2] were prepared according to the corresponding literatures. Tetraphenylporphyrin tetrasulfonic acid hydrate (TPPS, 85%) was purchased from Tokyo Chemical Industry (TCI). Deionized water was obtained from a Reverse Osmosis Drinking water system at a resistivity of 18.25 MΩ·cm.

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2. Synthesis of the peptides

All the peptides were synthesized using a standard 9-fluorenylmethoxycarbonyl (Fmoc) solid-state synthesis method. Briefly, the synthesis was initiated using 48 μmol of Fmoc-Rink Amide MBHA resin (using 3 mL of CH_2Cl_2 to make it fully swell), then dried piperidine/DMF solution (v/v= 20%，2 mL) was added and stirred for 2 min to deprotect the resin twice for activating and then rinsed and filtered using DMF and CH_2Cl_2 in turn. The chain extension was accomplished using an in situ neutralization/HATU activation procedure, where 2-fold excess of Fmoc-amino

acids (in relation to the resin) was pre-activated with 3 equivalents of HATU (in relation to the resin) and 6 equivalents of NMM (in relation to the resin) in DMF (1 mL) for 2 min before it was added to the resin. After the coupling reaction, a dried piperidine/DMF solution (v/v= 20%，2 mL) was added and stirred for 2 min to deprotect the resin twice. Then, the resin was rinsed using DMF and CH_2Cl_2 in turn. The cycle of Fmoc deprotection and coupling with N-terminal-protected amino acids was repeated until the designed peptide sequences were obtained. Each coupling step took 5 min, whereas each deprotection step took 2 min. The obtained peptide segments were cleaved from the resin manually by treatment with trifluoroacetic acid (TFA), anisole, water and triisopropyl silane ($V_{TFA}/V_{anisole}/V_{water}/V_{TIPS}$ = 88:5:5:2) for 5 h under constant agitation in a rotary shaker (25 ^{o}C, 480 rpm of constant agitation). The obtained mixing solution was dropped into 10 mL of ice diethyl ether, and the resulted precipitate was dissolved in a mixed solvent containing acetonitrile and water (v/v = 1:1) in the presence of 0.1% TFA. The crude peptide solutions were purified by reverse-phase High Performance Liquid Chromatography (HPLC) equipped with C18 reverse phase column (Vydac, USA) using the gradient agent with water and acetonitrile in the presence of 0.1% TFA and the column eluents were monitored by UV absorbance at 254 and 326 nm. The obtained peptide product is detected by MALDI-TOF-MS (Autoflex speed TOF/TOF, Brucker) to confirm the molecular weight.

Pep1: (MALDI-TOF-MS) m/z 2520.51 (2520.36 calcd for [M+H$^+$]), 2542.24 (2542.34 calcd for [M+Na$^+$]), 2558.63 (2558.32 calcd for [M+K$^+$]).

Pep2: (MALDI-TOF-MS) m/z 1959.96 (1960.10 calcd for [M+H$^+$]), 1981.94 (1982.08 calcd for [M+Na$^+$]), 1997.90 (1998.05 calcd for [M+K$^+$]).

3. Sample preparation of the ionic self-assembly complexes (Peptides/POMs)

The peptides and POMs solutions were prepared by dissolving the POMs or the lyophilized peptides in deionized water with sonication for 10 min, respectively. Then, the solution of POMs was added to the solution of peptides with vortex stirring, and the pH value of the mixed solution was controlled at ~ 6. The final concentration of peptides in the aqueous solution was kept at 25 μM, and the molar ratio of peptides to POMs was 1:1. The self-assembly structures were obtained after aging the mixing solution for at least 12 h at 25 ^{o}C. The photo-responsive self-assembly was performed by exposing the peptides/POMs solution to ultraviolet light (365 nm) for at least 40 min.

4. Measurements

MALDI-TOF-MS. MALDI-TOF MS was performed on an Autoflex speed TOF/TOF (Brucker) operating in positive mode within a mass range from 700 to 3500 Da.

Transmission Electron Microscopy (TEM). The TEM images were captured on a JEOL-2010 electron microscope operating at 200 kV. The solution samples were casted onto a carbon coated copper grid and then dried completely in air. The individual peptide specimens were stained with 0.5 wt % uranyl acetate aqueous solution for 2-3 min, the excess amount of staining solution was removed by filter paper. However, the peptides/POMs specimens without staining were used directly for TEM measurements.

Cryogenic transmission electron microscopy (Cryo-TEM). Cryo-TEM was carried out on a JEOL-JEM 2100 TEM instrument (about 90 K, 120 kV) equipped with a SC 1000 CCD camera (Gatan, Inc., USA). A liquid droplet of Pep1/CoW_{12} (3 μL) was transferred to an ultrathin copper grid after hydrophilic treatment under the conditions of controlled temperature and humidity (97-99 %) to prevent sample solution from evaporating. Then, the superfluous liquid droplets were removed with filter paper and the thin aqueous films were rapidly vitrified by plunging them into liquid ethane and cooled to approximately 90 K by liquid nitrogen. The excess amount of ethane was removed using blotting paper after the sample solution was frozen. Finally, the grid was inserted into a Gatan 626 cryo holder using a cryotransfer device for cryo-TEM measurements.

Scanning transmission electron microscopy (STEM). High-angle annular dark field-scanning transmission electron microscopy (HAADF-STEM) measurements were performed on Tecnai F20 G2S-Twin (FEI, USA). The HAADF-STEM specimens of the Pep1/CoW_{12} sample were prepared by using the same procedure with TEM.

Atomic force microscopy (AFM). AFM measurements were performed on a Bruker Dimension 3100 instrument (Germany) by using both the tapping and contact mode in air (25 ^{o}C). The AFM samples were prepared by casting the Pep1/CoW_{12} solution on the surface of a mica wafer, and staying for 3 min. Subsequently, the excess amount of solution was removed by filter paper, and the air-dried samples were utilized for AFM tests.

Laser scanning confocal microscopy (LSCM). LSCM measurements were performed on a FV1000 confocal microscopy. Nile Red was used as an environment-sensitive hydrophobic probe, which exhibits an increase of emission when inserting into a hydrophobic domain. The Pep1/CoW_{12}

solution was incubated with Nile Red solution for at least 5 h, the resultant solution was casted on glass for LSCM measurements with an excitation wavelength of 488 nm.

Circular Dichroism (CD). CD spectra of individual Pep1 and the Pep1/CoW_{12} solutions were recorded on a JASCO model J-810 spectropolarimeter (25 ^{o}C, Xe lamp) under a constant flow of nitrogen gas during operation. The samples were loaded into a rectangular quartz cell with a 0.1 cm path length, the wavelength range of CD spectra was captured from 260 to 180 nm with a step of 0.5 nm, a response time of 5 s, and a scan speed of 2 nm s^{-1}. The data were repeated five times and averaged. The JASCO software was used for background subtraction.

Fourier Transform Infrared (FT-IR). FT-IR spectra of all the lyophilized powder samples (Pep1, CoW_{12}, and Pep1/CoW_{12}) were measured on a Bruker Optics Vertex 80 V FT-IR spectrometer equipped with a DTGS detector (32 scans) with a resolution of 4 cm^{-1} using KBr pellets. The FT-IR spectra of solid samples were recorded to confirm the structural stability of CoW_{12} and investigate the secondary structure of Pep1, Pep1/CoW_{12} before and after UV irradiation.

UV-visible (UV-vis): The UV-vis spectra were measured at room temperature on a Varian Cary 50 UV-vis spectrophotometer. The wavelength is in the range of 200 ~ 600 nm with a step of 1 nm.

Thioflavin T (ThT) Binding Study. The binding study was performed according to the following procedure. A ThT solution was added into the aqueous solution of Pep1/CoW_{12}, and the final concentration of ThT was controlled at 10 μM. The resultant Pep1/CoW_{12}/ThT solution (pH ~ 6) was incubated at room temperature for 5 h. The fluorescence spectra of individual ThT solution (10 μM) and the Pep1/CoW_{12}/ThT sample were recorded on a 5301PC spectrophotometer (Shimadzu, Toko, Japan) with an excitation wavelength of 420 nm.

Isothermal Titration Calorimetry (ITC). ITC experiments are performed on a MicroCal iTC200 (TA Instruments, USA) system. Experiments described in this work were performed by adding Pep1 to CoW_{12} in the cell. The Pep1 solution (0.1 mM, pH ~ 6) and CoW_{12} solution (1 mM, pH ~ 6) are placed in injection syringe and microcalorimeter cell, respectively. The Pep1 is injected in 0.9 μL aliquots in the cell containing the CoW_{12} solution. A waiting time of at least 2 min is allowed between injections. Constant stirring is applied with the paddle-shaped tip of the syringe at a rate of 750 rpm. The raw ITC data using a TA Nano Analyze instrument with origin.

Dynamic Light Scattering (DLS). DLS experiments were performed at 25 °C on a Malvern Zetasizer Nano ZS (Malvern Instruments; UK) using a detection angle of 173° and a 3 mW He-Ne

laser operating at λ = 633 nm. The temperature equilibration time was set to 120 s in all cases, and the measurements were repeated at least 3 times.

5. Characterization

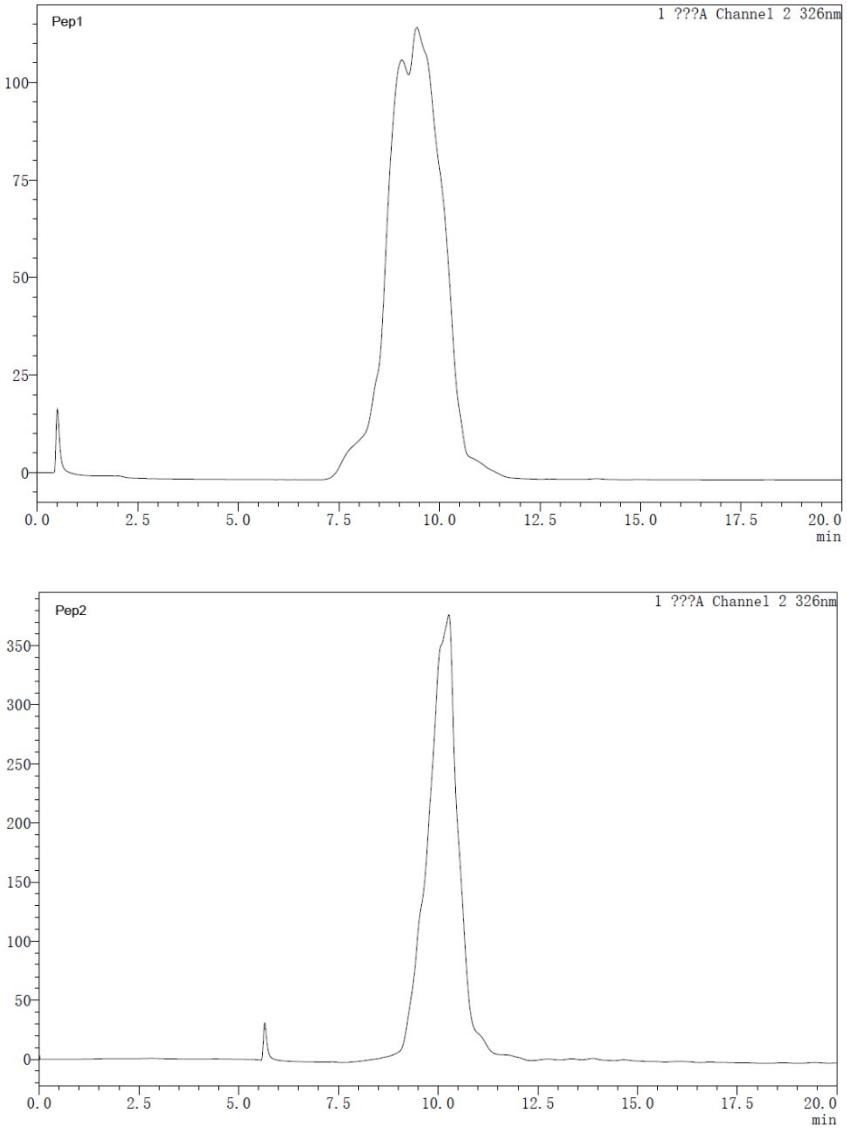

Fig. S1 HPLC profiles of Pep1 and Pep2.

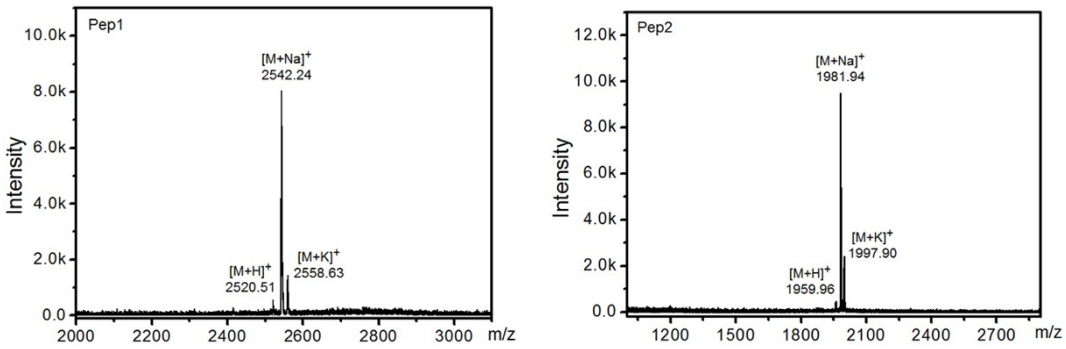

Fig. S2 MALDI-TOF-MS data of Pep1 and Pep2.

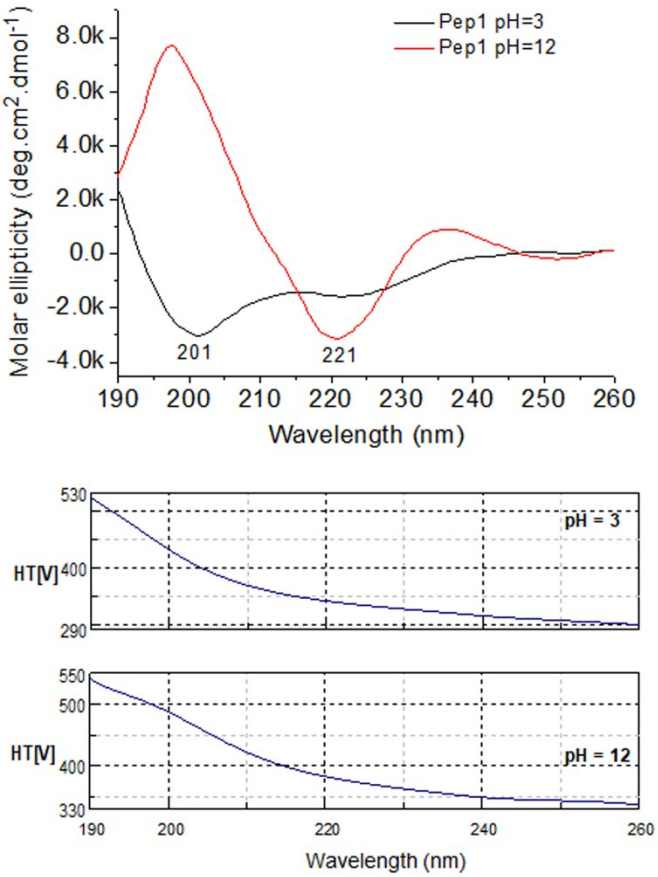

Fig. S3 CD spectra and HT signal of individual Pep1 in aqueous solution with pH = 3 and pH = 12. It is clear that the Pep1 segments at pH =3 show random-coil conformation due to the strong electrostatic repulsion among protonated lysine residues (pK ≈ 10.5). However, the Pep1 segments adopt β-sheet conformation with increasing the pH value to 12, where the intermolecular electrostatic repulsion was suppressed by the deprotonated lysine residues.

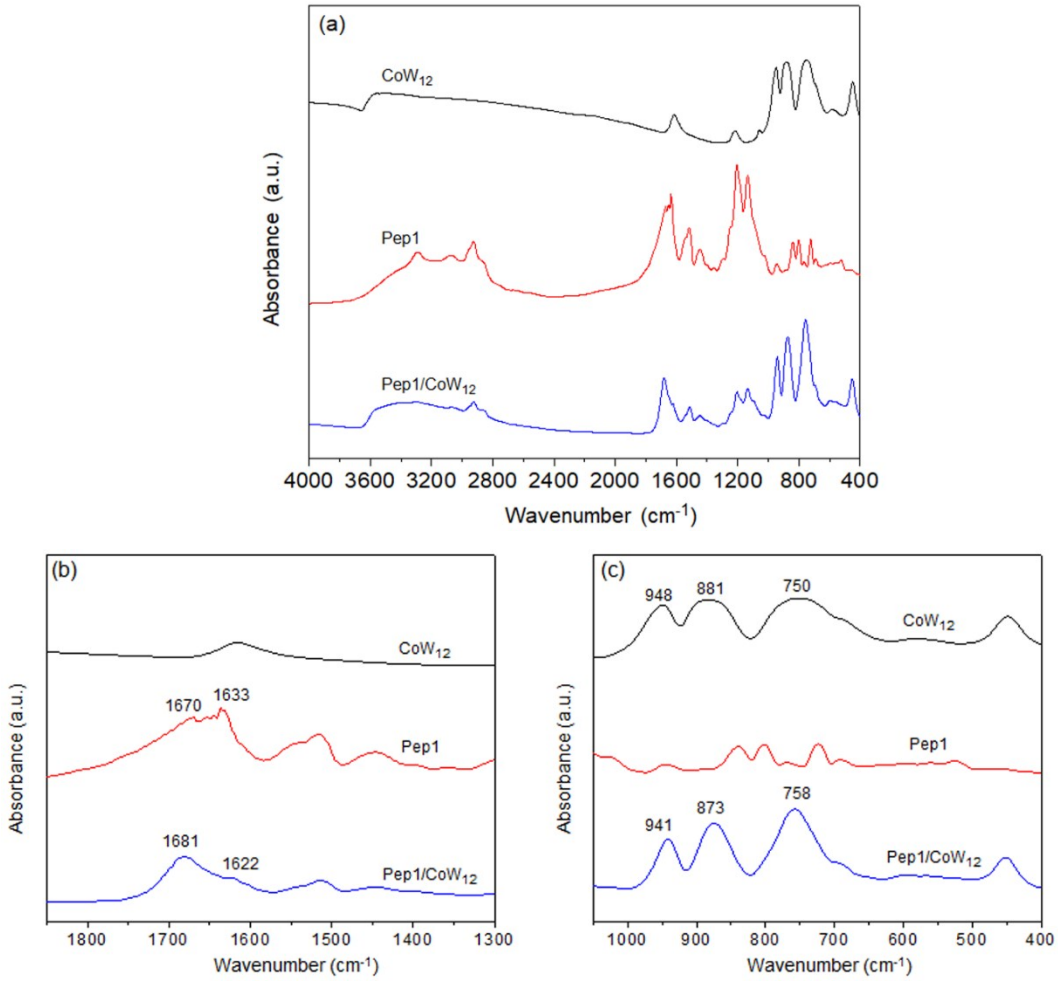

Fig. S4 FT-IR spectra of CoW_{12}, Pep1, and Pep1/CoW_{12} complex in the range of 4000-400 cm^{-1} (a), 1850-1300 cm^{-1} (b), and 1050-400 cm^{-1} (c). (Pep1 alone shows the bands at 1633 and 1670 cm^{-1}, which is attributed to the random coil together with β-sheet conformation of Pep1 during the freezing dry step. However, the Pep1/CoW_{12} complex exhibits two bands at 1622 and 1681cm^{-1}, which correspond to the β-sheet conformation. CoW_{12} alone shows several typical bands at 948, 881, and 750 cm^{-1}, corresponding to the stretching vibrations of ν_{as} (W-O_d), ν_{as} (W-O_b-W), and ν_{as} (W-O_c-W), respectively. In the case of Pep1/CoW_{12}, these vibration modes slightly shift to 941, 873, and 758 cm^{-1}, respectively, due to the non-covalent interactions between CoW_{12} and Pep1. The above results indicate that the topology structure of CoW_{12} is stable during the ionic self-assembly process).

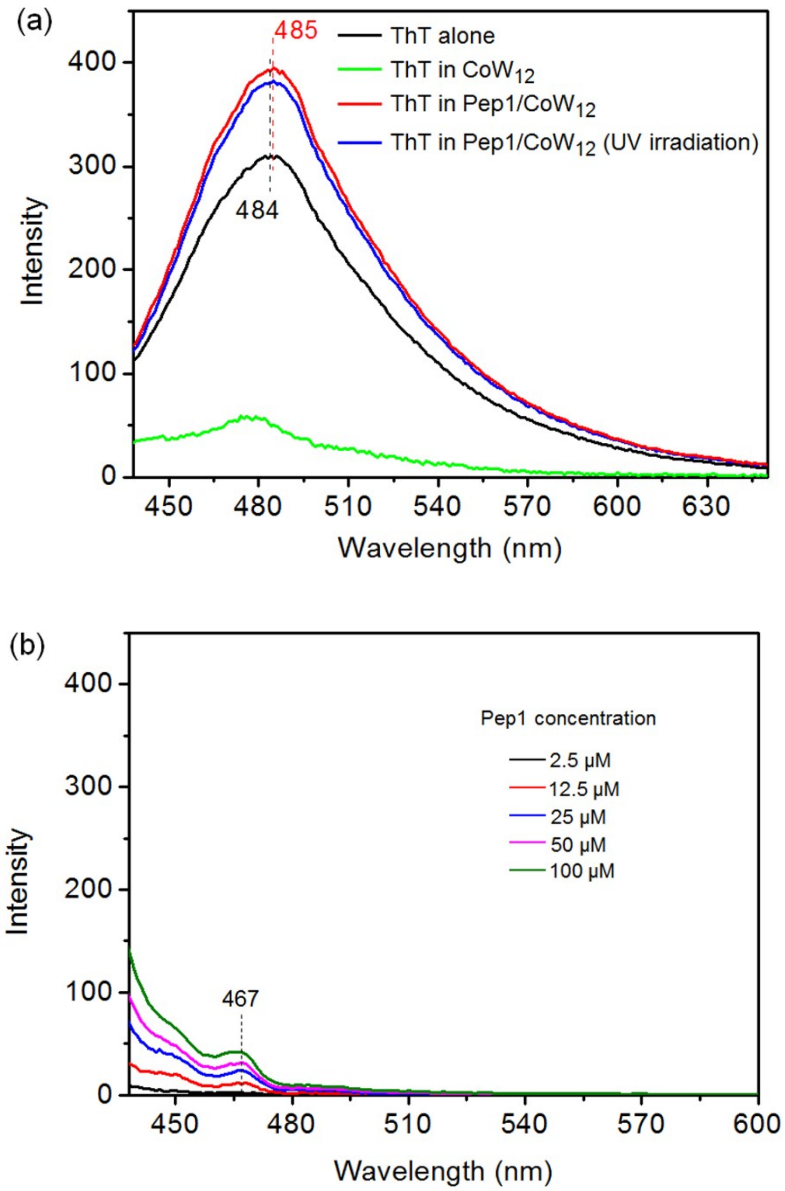

Fig. S5 (a) Fluorescence spectra of thioflavin T (ThT) dissolved in water (blank), Pep1/CoW_{12} aqueous solution (pH ~ 6.0) before and after UV irradiation. The final concentration of ThT in all samples was kept at 10 μM. (The enhancement of fluorescence intensity confirms the formation of β-sheet peptide in the Pep1/CoW_{12} aqueous solution);[8-9] (b) Fluorescence spectra of the Pep1/CoW_{12} aqueous solution without ThT. It is clear that the faint signal of the Pep1/CoW_{12} complex could be ignored comparing with the intensity of ThT in (a).

[8] P. Jana, M. Ehlers, E. Zellermann, K. Samanta, and C. Schmuck, *Angew. Chem. Int. Ed*. 2016, **55**, 15287-15291.

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2915-2918.

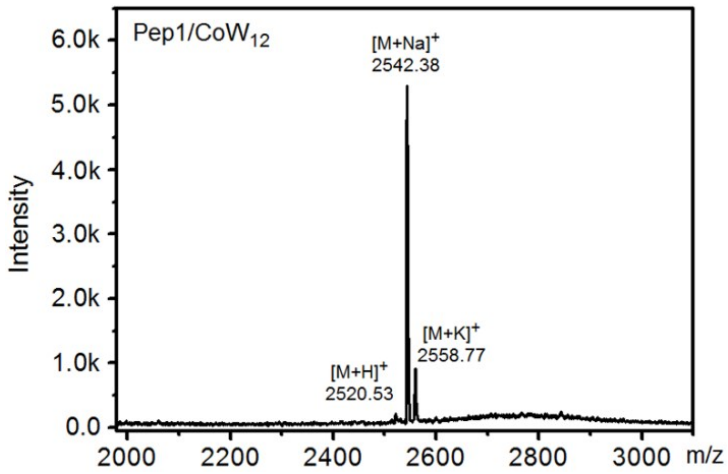

Fig. S6 MALDI-TOF-MS data of the Pep1 obtained from the aqueous solution of Pep1/CoW_{12}.

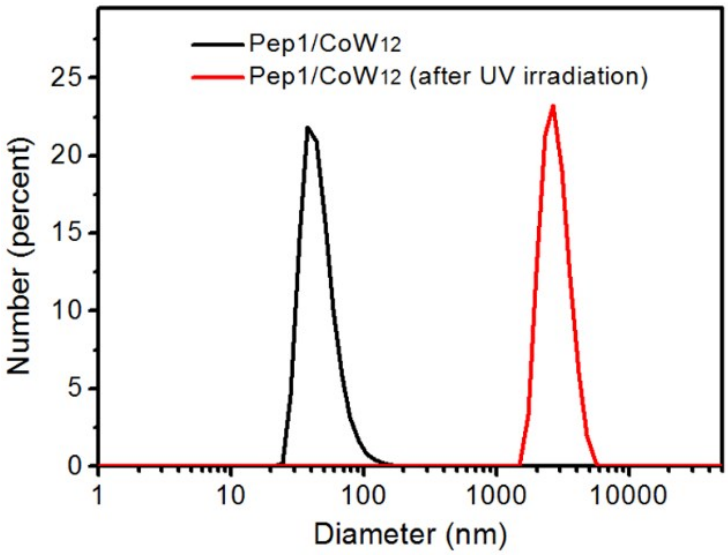

Fig. S7 DLS data of the Pep1/CoW_{12} aqueous solution before and after UV irradiation. (pH ~ 6.0, the concentration of Pep1 is 25 μM)

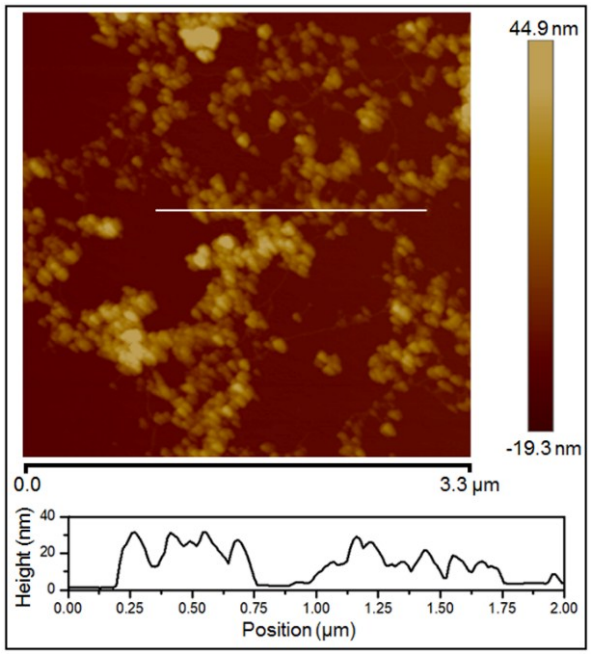

Fig. S8 Tapping-mode AFM image of the *trans*-Pep1/CoW_{12} complex on mica. (The height of nanospheres is less than 40 nm.)

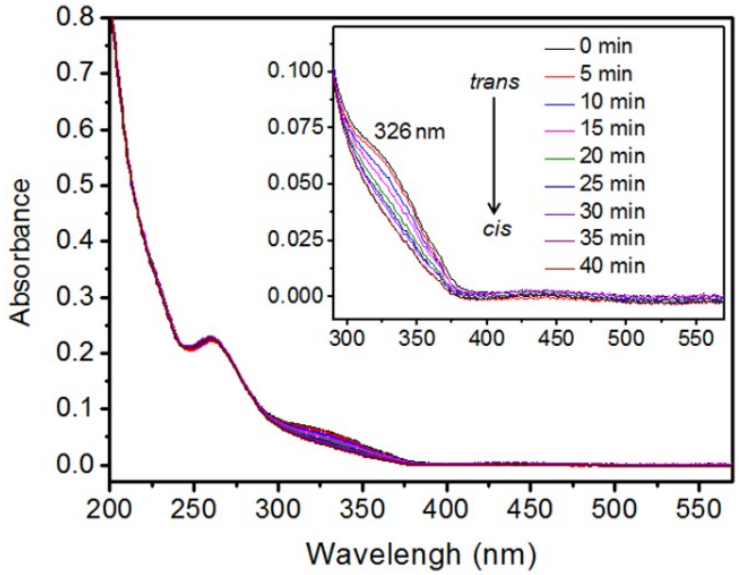

Fig. S9 UV-vis spectra of Pep1/CoW_{12} solution under UV light (365 nm) with various irradiation time. (pH ~ 6.0, the concentration of Pep1 is 25 μM)

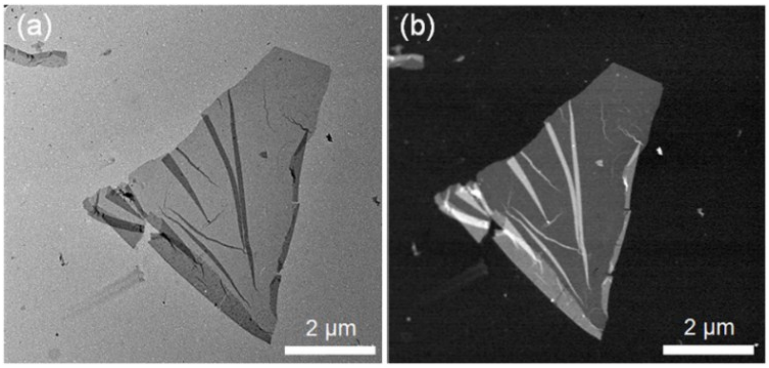

Fig. S10 TEM image (a) and the corresponding dark-field STEM image (b) prepared from the aqueous solution of *cis*-Pep1/CoW_{12}. (pH ~ 6.0, the concentration of Pep1 is 25 μM)

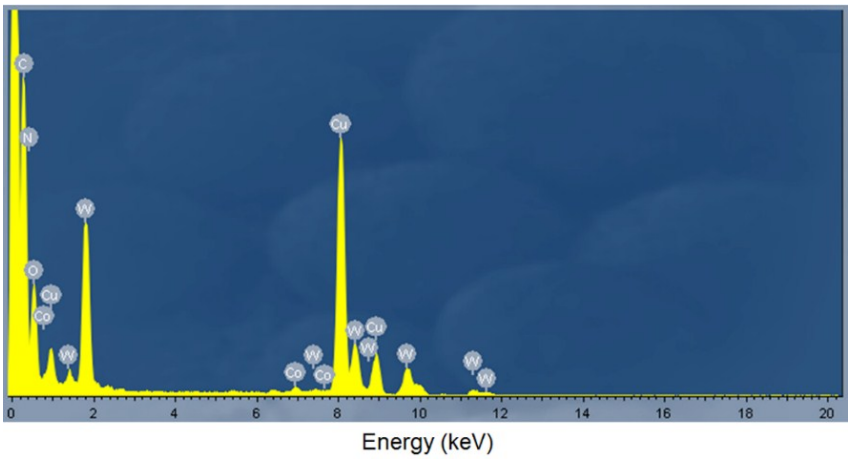

Fig. S11 EDX spectra of *cis*-Pep1/CoW_{12} nanosheets. (The presence of W and Co elements provides strong evidence that CoW_{12} clusters are located in the nanostructures).

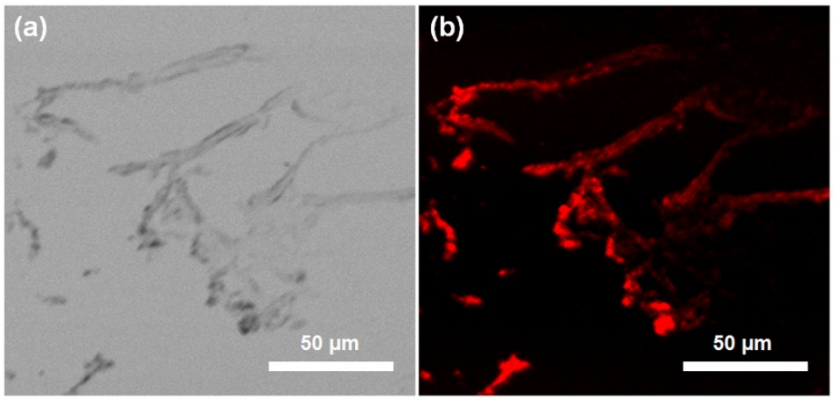

Fig. S12 LSCM images of *cis*-Pep1/CoW_{12} solution sample (pH ~ 6.0) stained with Nile Red (2.0 μM): (a) bright field, and (b) dark field. (the concentration of Pep1 is 25 μM).

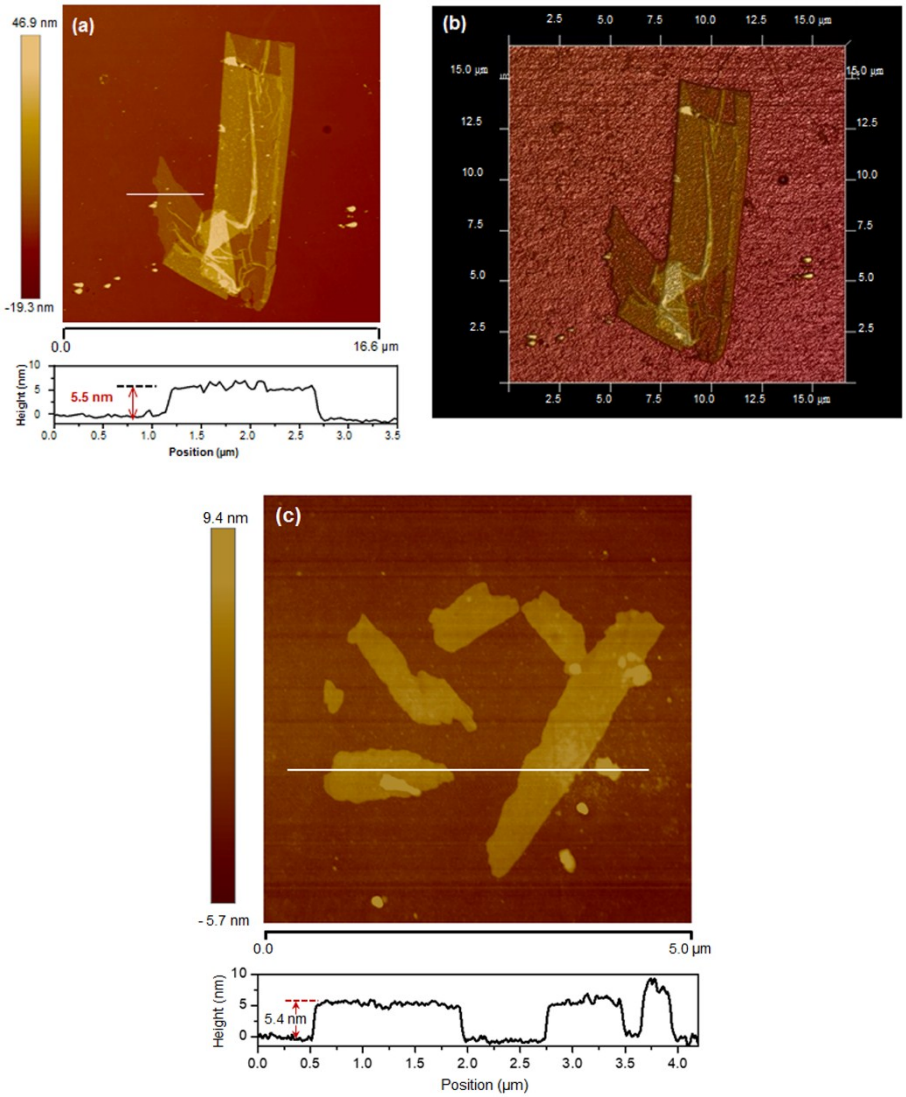

Fig. S13 (a) AFM image and cross-section analysis of *cis*-Pep1/CoW_{12} nanosheet measured by tapping mode; (b) the corresponding 3D image of (a); (c) AFM image and cross-section analysis of *cis*-Pep1/CoW_{12} nanosheet measured by contact mode.

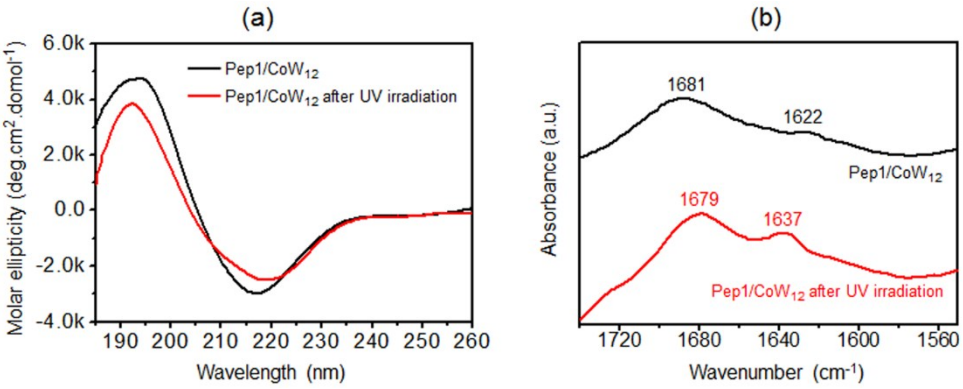

Fig. S14 (a) CD spectra of Pep1/CoW_{12} aqueous solution (pH ~ 6.0, the concentration of Pep1 is 25 μM) before and after UV irradiation, and (b) FT-IR spectra of the Pep1/CoW_{12} sample before and after UV irradiation.

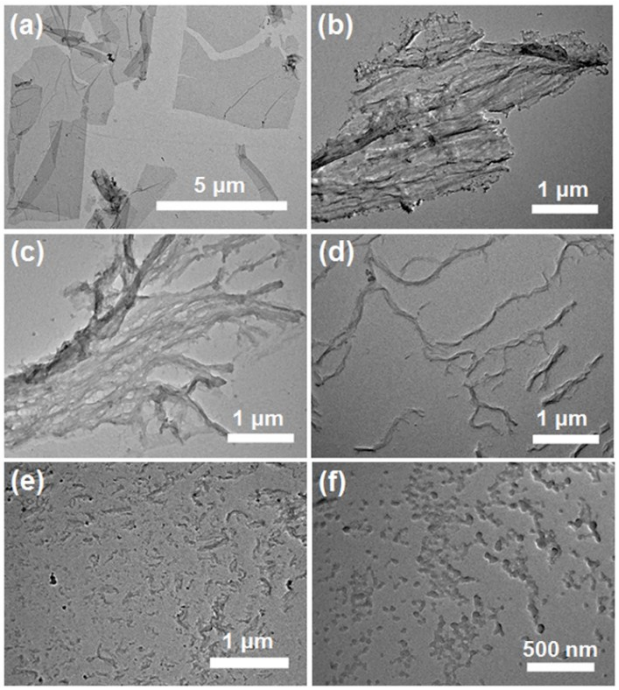

Fig. S15 TEM images of the *cis*-Pep1/CoW_{12} complex with different aging time in dark: (a) 0 h, (b) 2 h, (c) 6 h, (d) 12 h, (e) 16 h, (f) 20 h.

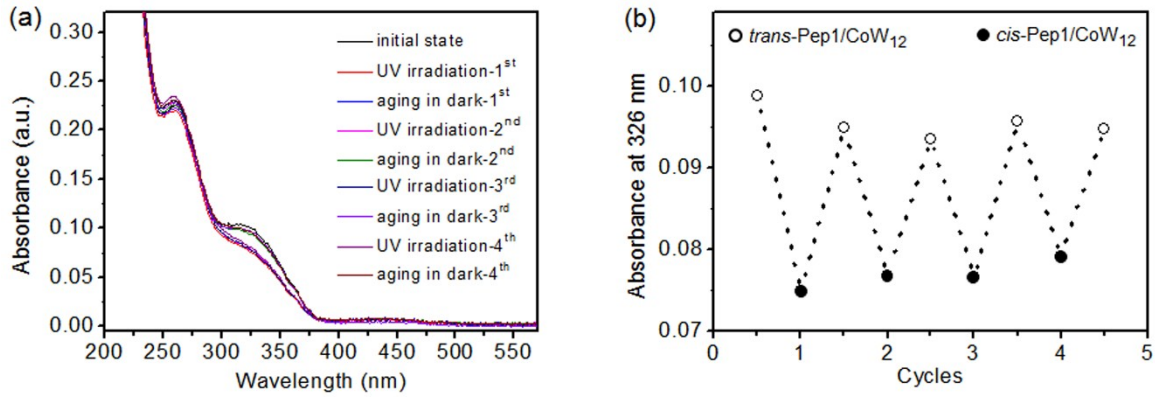

Fig. S16 (a) UV-vis spectra of Pep1/CoW_{12} solution (pH ~ 6.0) with alternating UV irradiation and aging in dark for consecutive cycles; (b) the maximum absorbance at 326 nm of Pep1/CoW_{12} as a function of cycles. (the concentration of Pep1 is 25 μM)

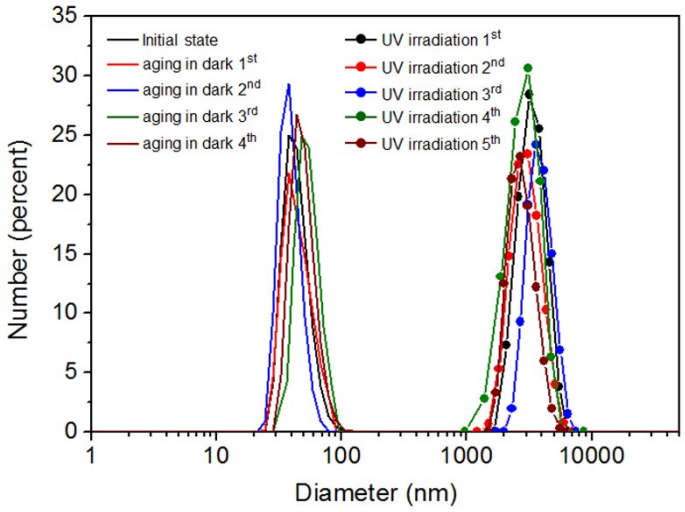

Fig. S17 DLS data of the Pep1/CoW_{12} aqueous solution samples upon alternating irradiation with UV and aging in dark for five consecutive cycles. (the concentration of Pep1 is 25 μM, pH ~ 6.0)

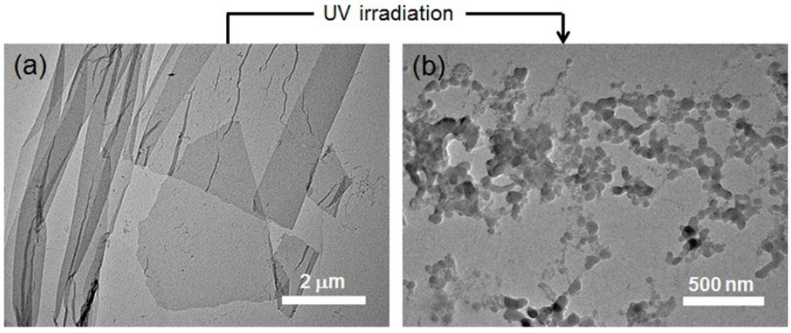

Fig. S18 TEM images of Pep1/TPPS complex before (a) and after (b) UV irradiation. (The molar ratio is 1:1, and the concentration of Pep1 is 25 μM)

Table S1. The bulky volume of POMs from their CIF data

POMs	Size (a×b×c nm^3)	POMs	Size (a×b×c nm^3)
V_{10}	0.83×0.77×0.53	EuW_{10}	1.43×0.80×0.80
PW_9	1.00×1.00×0.60	P_2W_{18}	1.20×1.00×1.00
$PW_{11}V$	1.00×1.00×1.00	P_5W_{30}	1.49×1.49×1.00
CoW_{12}	1.00×1.00×1.00		

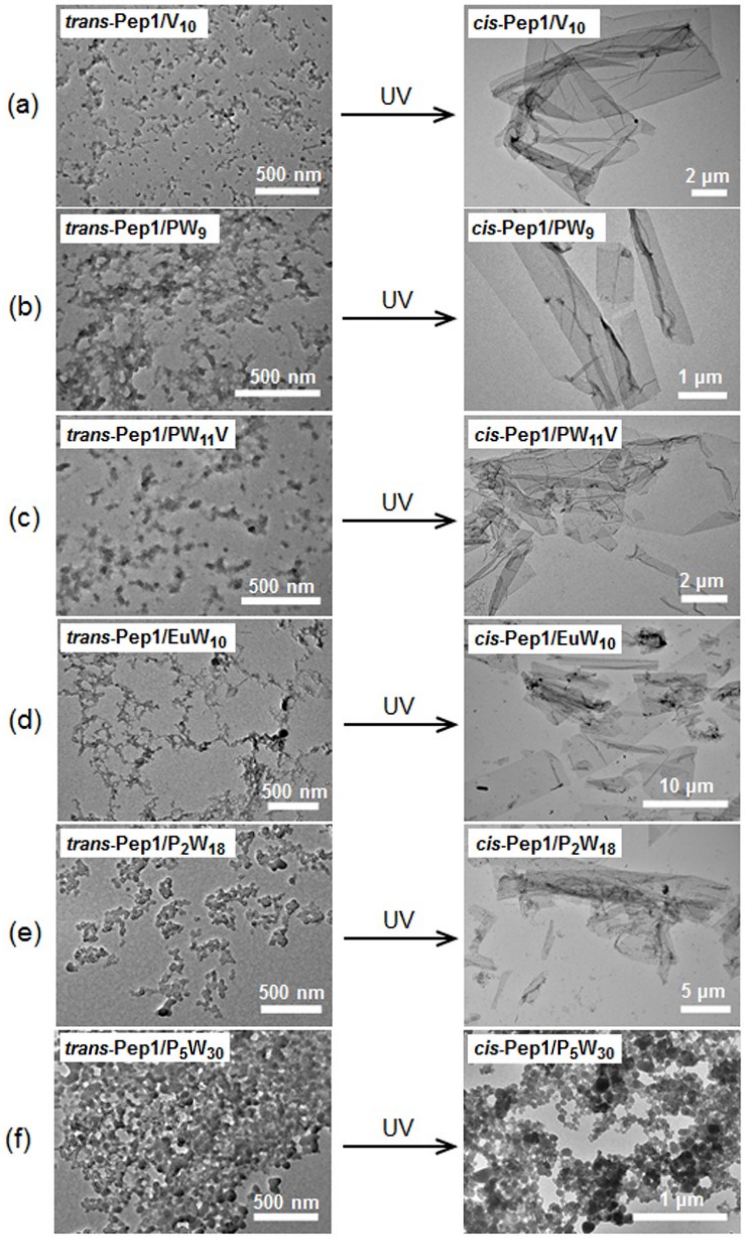

Fig. S19 TEM images prepared from the aqueous samples of Pep1/POMs complexes before and after UV irradiation: (a) Pep1/V_{10}, (b) Pep1/PW_9, (c) Pep1/$PW_{11}V$, (d) Pep1/EuW_{10}, (e) Pep1/P_2W_{18}, (f) Pep1/P_5W_{30}. (The concentration of Pep1 is 25 μM for all the samples, and the molar ratio is 1:1)

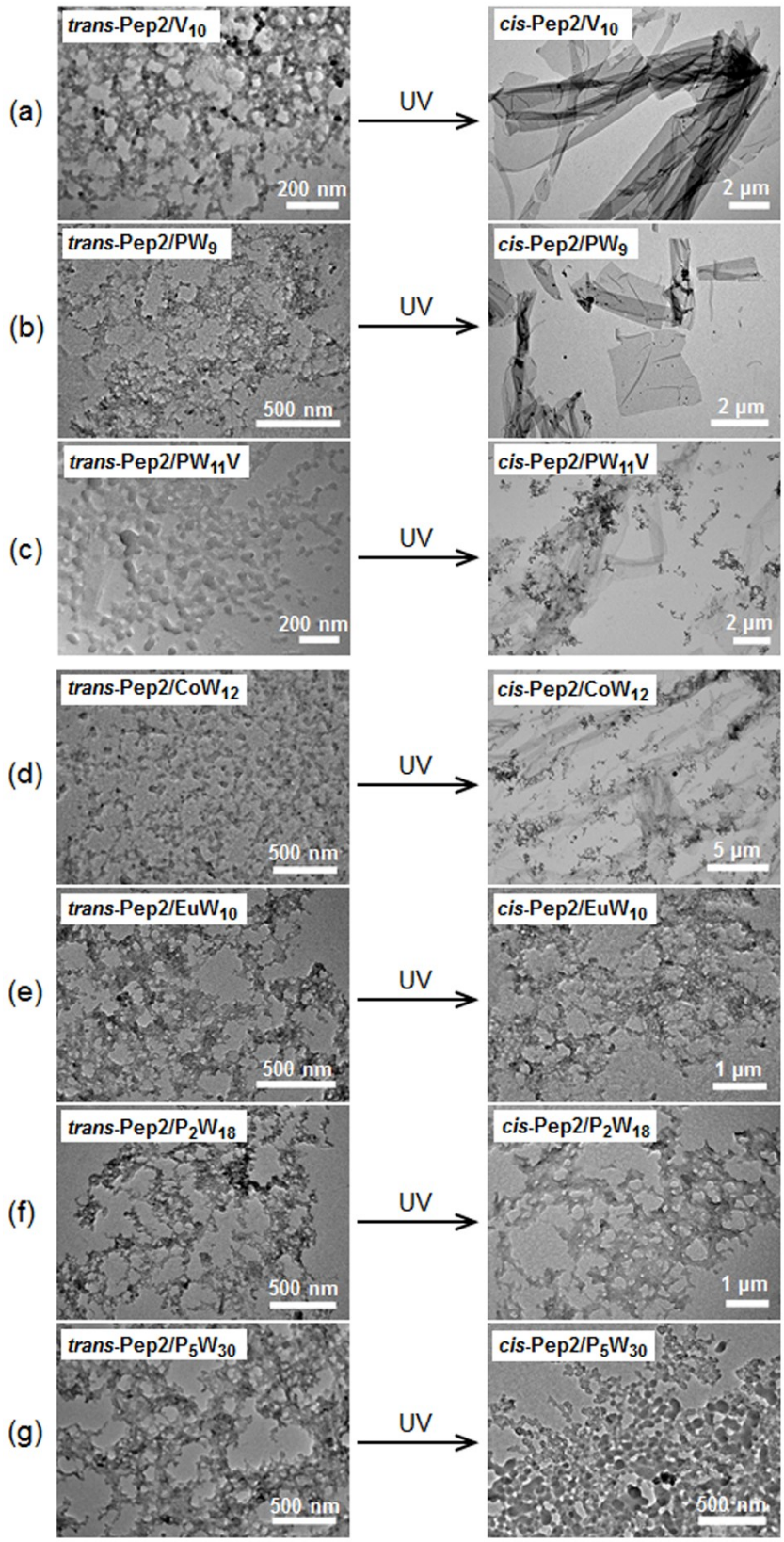

Fig. S20 TEM images of Pep2/POMs complexes before and after UV irradiation: (a) Pep2/V_{10}, (b) Pep2/PW_9, (c) Pep2/$PW_{11}V$, (d) Pep2/CoW_{12}, (e) Pep2/EuW_{10}, (f) Pep2/P_2W_{18}, (g) Pep2/P_5W_{30}. (The concentration of the Pep2 is 25 μM for all the samples, and the molar ratio is 1:1)