Supporting Information

Supramolecular switching the self-assembly of cyclic peptide–polymer conjugate *via* host–guest chemistry

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S1. Materials and Characterization

Materials

Fmoc-protected amino acids and coupling agents were purchased from Iris Biotech GmbH. mPEG5k-NH_2 was purchased from Rapp Polymere. CB[7] was kindly provided by Dr. Hao Chen from Shandong University. Boc-Phe-NHS, BCN-NHS and other chemicals were purchased from Sigma-Aldrich. Solvents were purchased from several departmental suppliers, Honeywell, Fisher and Sigma-Aldrich.

Characterization

Nuclear Magnetic Resonance Spectroscopy (NMR): ^{1}H NMR spectra were measured using either a Bruker Avance III HD 400 MHz NMR spectrometer or a Bruker Avance 500 MHz NMR spectrometer. The residual solvent peaks were used as internal references. Diffusion-ordered NMR spectroscopy (DOSY) was conducted using a Bruker Avance 500 MHz NMR spectrometer at 25 °C.

Gel Permeation Chromatography (GPC): GPC was measured using an Agilent PL50 instrument with a differential refractive index detector. The instrument contained two PolarGel H columns (300 mm × 7.5 mm) and a PolarGel 5 μm guard column. DMF with 0.1% LiBr additive was used as the eluent. The system ran at 1 mL min^{-1} (50 °C), with an injection volume of 100 μL. The samples were prepared by filtering them through 0.22 μm pore size PTFE membranes, before injection. Agilent EasyVial poly(methyl methacrylate) standards were used to calibrate the instrument and output data were analyzed using Agilent GPC/SEC software.

High-Performance Liquid Chromatography (HPLC): High-performance liquid chromatograms were measured using a Shimadzu Prominence HPLC, equipped with a Phenomenex Luna C18 column (250 mm × 4.6 mm) with 5 μm micron packing (100 Å). Acetonitrile and water were used as mobile phase A and B, respectively. All solvents contained 0.04 vol% TFA. The gradient used for HPLC analysis was increased from 5% to 95% B in 30 minutes. Detection was achieved via monitoring UV absorption at 280 nm.

Mass Spectrometry (ESI-TOF): ESI-TOF mass spectra were measured using an Agilent 6130B single Quad to characterize the peptides in both positive and negative ionisation modes. Samples were dissolved in methanol.

Isothermal Titration Calorimetry (ITC): The ITC experiment was carried out on a Microcal ITC200 Malvern at 298.15 K in DI water. The guest molecule Phe_2-CP-PEG was in the sample cell, and the host molecule CB[7] in the injection syringe was prepared in the ten-fold concentration of the binding unit on the guest peptides. The concentration of CB[7] was calibrated by the titration with a standard solution of 1-adamantanamine hydrochloride. As for data analysis, the ITC data was fitted by a two sequential-binding sites model. In this way, all the thermodynamic parameters (binding constant K, dH, dS) of the host-guest complexation between CB[7] and Phe_2-CP-PEG were finally obtained.

Small Angle Neutron Scattering (SANS): SANS was carried out on Larmor at the ISIS Pulsed Neutron Source (STFC Rutherford Appleton Laboratory, Didcot, UK). Prior to measurement, each sample was dissolved in D_2O and placed in a 2 mm quartz cuvette. The scattering cross-section was measured over a Q-range of 0.004 - 0.5 $Å^{-1}$ where Q is defined as:

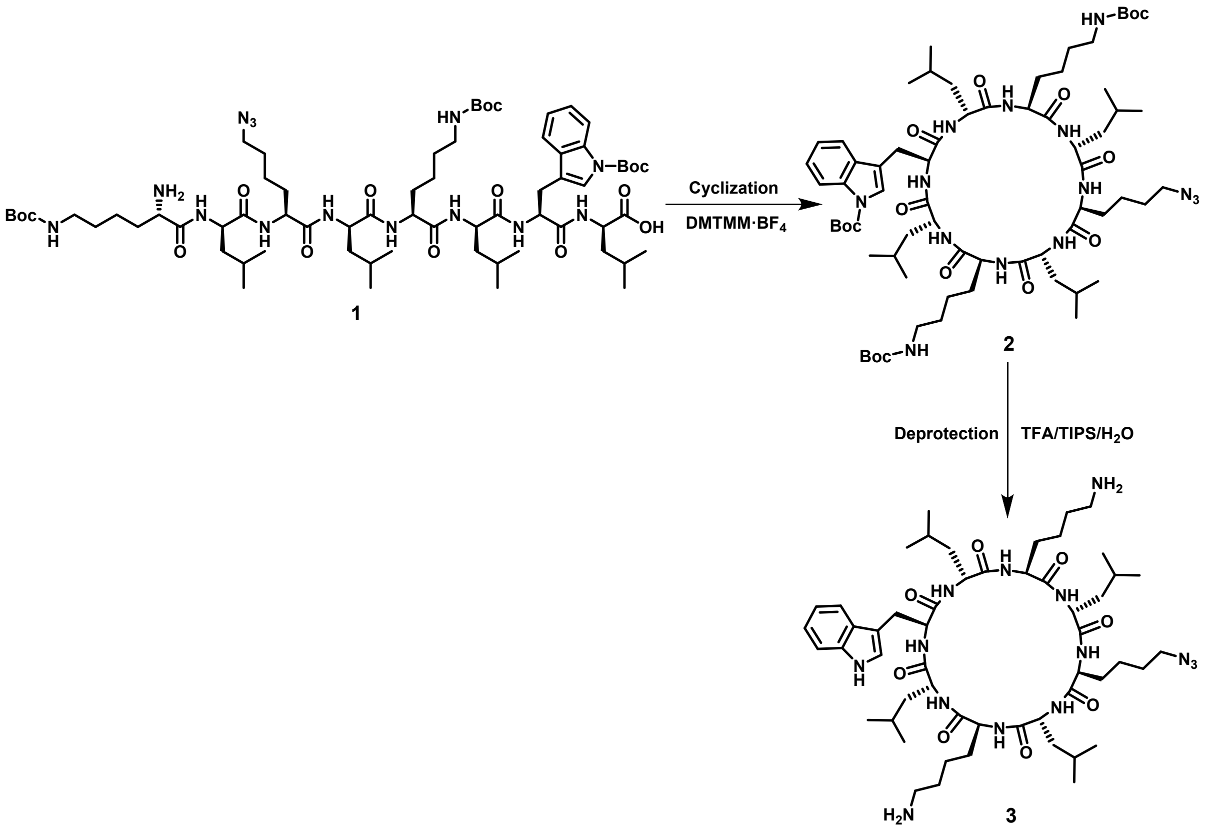
$$Q = \frac{4\pi sin\frac{\theta}{2}}{\lambda}$$

Here, θ is the scattered angle, and λ is the incident neutron wavelength.

A Q-range of 0.004 - 0.5 $Å^{-1}$ was achieved utilizing an incident wavelength range of 0.9 - 13.3 Å. The detector is located 4.1 m from the sample and is 664 mm wide * 664 mm high with the beam in the centre of the detector. The beam size is 6 mm wide and 8 mm high. Each raw scattering data set was corrected for the detector efficiencies, sample transmission and background scattering and converted to scattering cross-section data ($\partial\Sigma/\partial\Omega$ vs. Q) using the instrument-specific software. These data were placed on an absolute scale (cm^{-1}) using the scattering from a standard sample (a solid blend of hydrogenous and perdeuterated polystyrene) in accordance with established procedures.

S2. Synthesis

a. Linear peptide (1), protected cyclic peptide (2), and cyclic peptide $(H_2N)_2$-CP-N_3 (3).



Linear peptide (1)

H$_2$N-L-Lys(Boc)-D-Leu-L-Lys(N$_3$)-D-Leu-L-Lys(Boc)-D-Leu-L-Trp(Boc)-D-Leu-COOH

Fully protected linear octapeptide was prepared *via* solid phase peptide synthesis (SPPS) on a Prelude Automated Peptide SynthesizerTM (Protein Technologies Inc.) using 2-chlorotrityl chloride resin as the solid support. The first Fmoc protected amino acid was coupled to the resin using DIPEA (4 eq.) in DCM, followed by capping of unreacted resin sites using a solution of MeOH:DIPEA:DCM (7:1:2, *v*/*v*/*v*). Deprotection of the Fmoc group of the amino acids was done using 20% piperidine in DMF. Subsequent amino acids were coupled using Fmoc-amino acids (5 eq.), HCTU (5 eq.) and NMM (10 eq.) in DMF. In the last step, the linear octapeptide was cleaved from the resin (while keeping protecting groups on) by a solution of 20 vol % 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in DCM.

^{1}H-NMR (400 MHz, TFA-*d*, ppm): δ = 8.19 (d, 1H), 7.67-7.33 (m, 4H), 4.79-4.62 (m, 7H), 4.58 (m, 1H), 3.49 (d, 2H), 3.34 (m, 2H), 3.20 (m, 4H), 2.04-1.18 (m, 57H), 1.14- 0.68 (m, 24H)

MS (ESI-ToF) (m/z): [M+H]$^+$ 1367.8 (calculated: 1367.9), [M+Na]$^+$ 1389.8 (calculated: 1389.8).

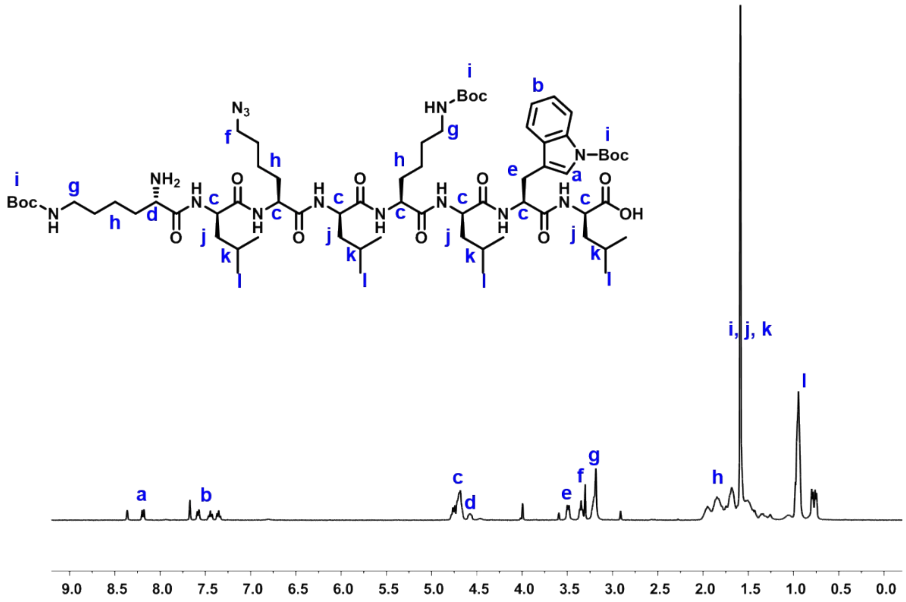


Figure S1 ^{1}H NMR spectrum of linear peptide (1) (400 MHz, CDCl$_3$)

Protected cyclic peptide (2)

Linear peptide (827 mg, 0.511 mmol) was cyclized by stirring at room temperature for 5 days in the presence of 1.2 equivalents of DMTMM·BF$_4$ (201 mg, 0.614 mmol) in 100 mL DMF. The solution was then concentrated to 10 mL under vacuum and then precipitated with cold methanol/water=1/1 to obtain a white powder as protected cyclic peptide **2** (yield: 450 mg).

^{1}H-NMR (400 MHz, TFA-*d*, ppm): δ = 8.12 (d, 1H), 7.65-7.30 (m, 4H), 5.24 (t, 1H), 4.90-4.63 (m, 7H), 3.37-2.99 (m, 8H), 1.94-1.14 (m, 57H), 1.10-0.69 (m, 24H).

MS (ESI-ToF) (m/z): [M+Na]$^+$ 1371.8 (calculated: 1371.8).

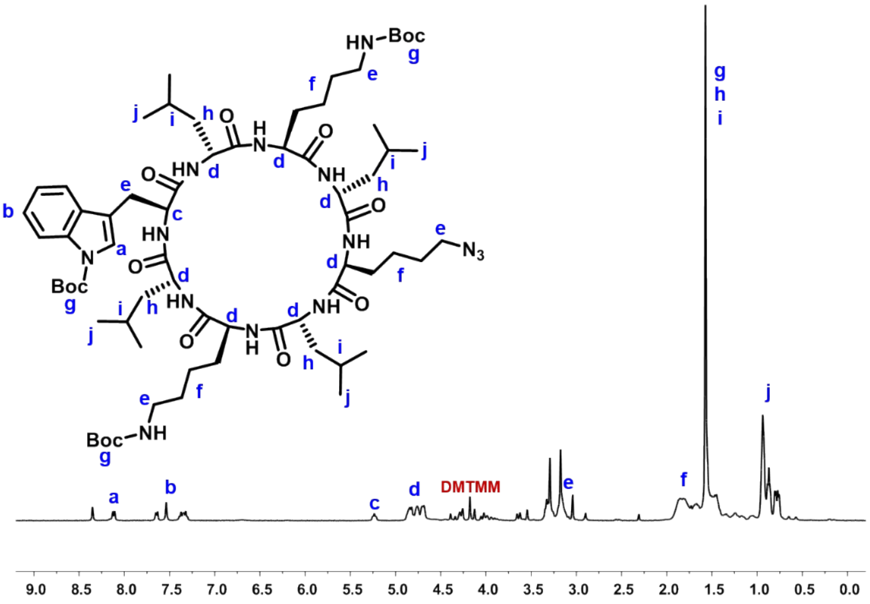


Figure S2 ^{1}H NMR spectrum of protected cyclic peptide (2) (400 MHz, TFA-*d*)

Deprotected cyclic peptide $(H_2N)_2$-CP-N_3 (3)

Removal of the -Boc protecting groups was achieved by adding a mixture of trifluoroacetic acid (TFA, 5 mL), triisopropylsilane (TIPS, 0.28 mL) and water (0.28 mL) to the protected cyclic peptide **2** (200 mg) and stirring for 3 hours. The resulting solution was then precipitated in ice cold diethyl ether and washed twice with ice cold diethyl ether to give an off-white powder as the deprotected cyclic peptide $(H_2N)_2$-CP-N_3 **3** (yield: 140 mg).

^{1}H-NMR (400 MHz, TFA-*d*, ppm): δ = 7.56 (s, 1H), 7.39-6.86 (m, 4H), 5.14 (t, 1H), 4.89-4.56 (m, 7H), 3.38-3.03 (m, 8H), 2.05-1.07 (m, 30H), 1.01-0.67 (m, 24H).

MS (ESI-ToF) (m/z): $[M+H]^+$ 1049.7 (calculated: 1049.7), $[M+Na]^+$ 1071.7 (calculated: 1071.7).

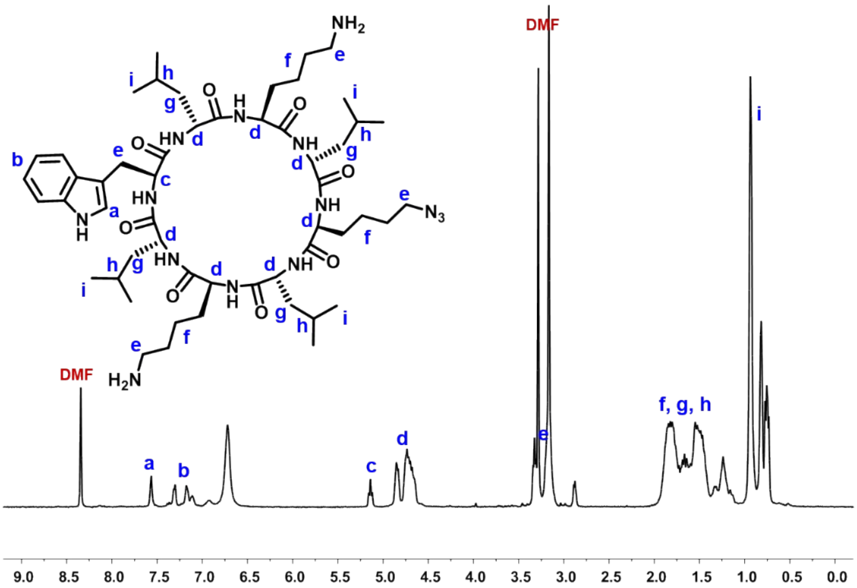
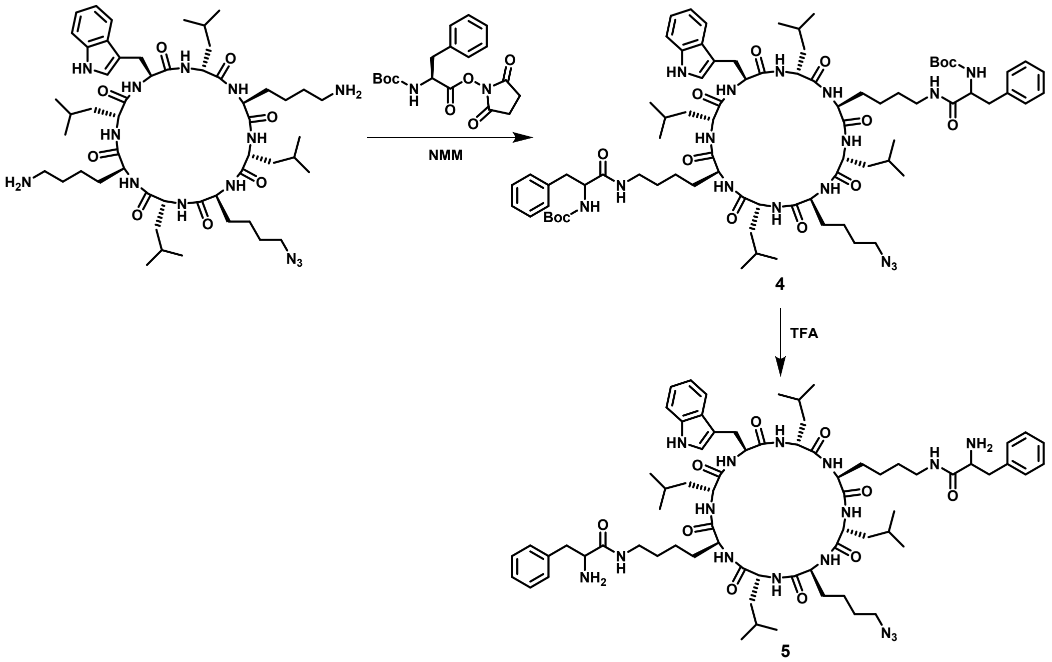


Figure S3 ^{1}H NMR spectrum of $(H_2N)_2$-CP-N_3 (3) (400 MHz, TFA-*d*)

b. Synthesis of Phe_2-CP-N_3



(Boc-Phe)$_2$-CP-N$_3$ (4)

(H$_2$N)$_2$-CP-N$_3$ (50.0 mg, 0.039 mmol) and Boc-Phe-NHS (56.7 mg, 0.157 mmol) were dissolved in 3 mL DMF, and NMM (30.4 mg, 0.235 mmol) was added afterwards. The reaction was left overnight and purified by precipitation in ice cold diethyl ether twice to give an off-white powder as the product (Boc-Phe)$_2$-CP-N$_3$ **4** (yield: 50 mg).

MS (ESI-ToF) (m/z): $[M+Na]^+$ 1565.8 (calculated: 1565.9).

Phe$_2$-CP-N$_3$ (5)

Removal of the -Boc protecting groups was achieved by adding a mixture of trifluoroacetic acid (TFA, 1 mL), triisopropylsilane (TIPS, 66 μL) and water (66 μL) to the (Boc-Phe)$_2$-CP-N$_3$ 4 (40 mg) and stirring for 1 hours. The resulting solution was then precipitated in ice cold diethyl ether and washed twice to give a light yellow powder as the product Phe$_2$-CP-N$_3$ **5** (yield: 36 mg).

^{1}H-NMR (400 MHz, TFA-*d*, ppm): δ = 7.61 (s, 1H), 7.34 (m, 6H), 7.51-6.86 (m, 4H), 5.17 (t, 1H), 4.94-4.59 (m, 7H), 4.50 (m, 2H), 3.48-2.99 (m, 12H), 2.02-1.12 (m, 30H), 1.08-0.69 (m, 24H).

MS (ESI-ToF) (m/z): $[M+H]^+$ 1343.8 (calculated: 1343.8), $[M+Na]^+$ 1365.8 (calculated: 1365.8).

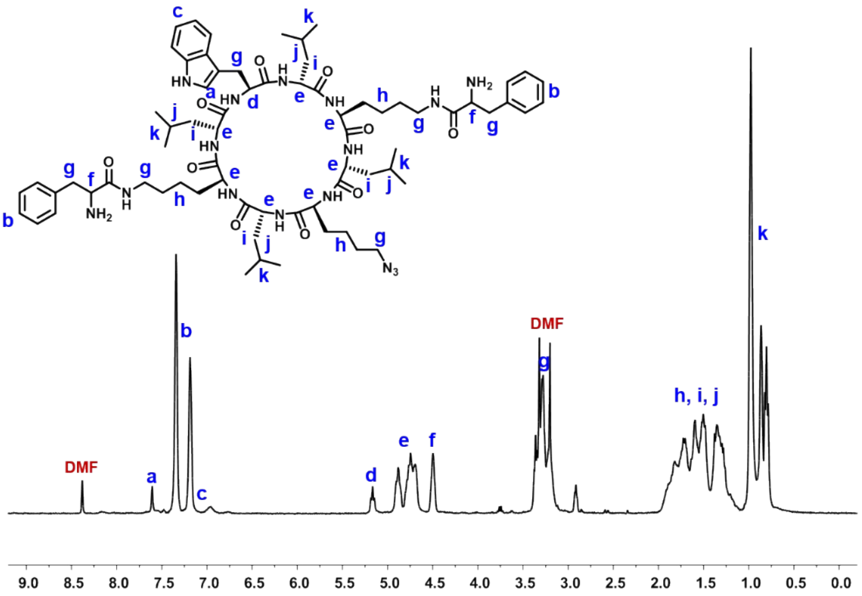
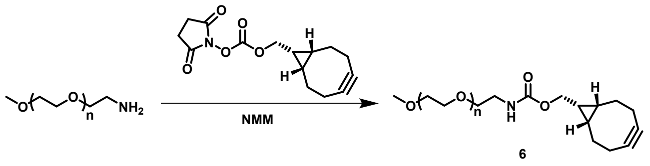


Figure S4 ^{1}H NMR spectrum of Phe$_2$-CP-N$_3$ (5) (400 MHz, TFA-*d*)

c. Synthesis of mPEG-BCN



mPEG5k-NH$_2$ (200 mg, 0.04 mmol) and BCN-NHS (17.5 mg, 0.06 mmol) were dissolved in 2 mL DMF, and NMM (10.3 mg, 0.08 mmol) was added afterwards. The reaction was left overnight. Then the DMF solution was precipitated in diethyl ether and washed once to give a white powder as the product mPEG-BCN **6** (yield: 210 mg).

^{1}H-NMR (500 MHz, D$_2$O, ppm): δ = 4.23 (d, 2H), 3.88-3.56 (br, PEG backbone), 3.41 (s, 3H), 3.36 (m, 2H), 2.38-2.20 (m, 6H), 1.64 (m, 2H), 1.44 (m, 1H), 1.02 (m, 2H).

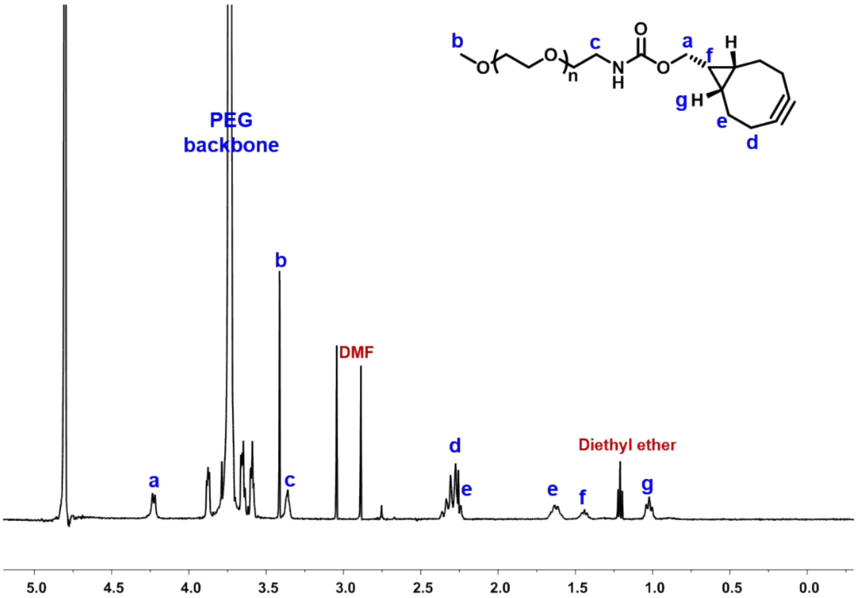
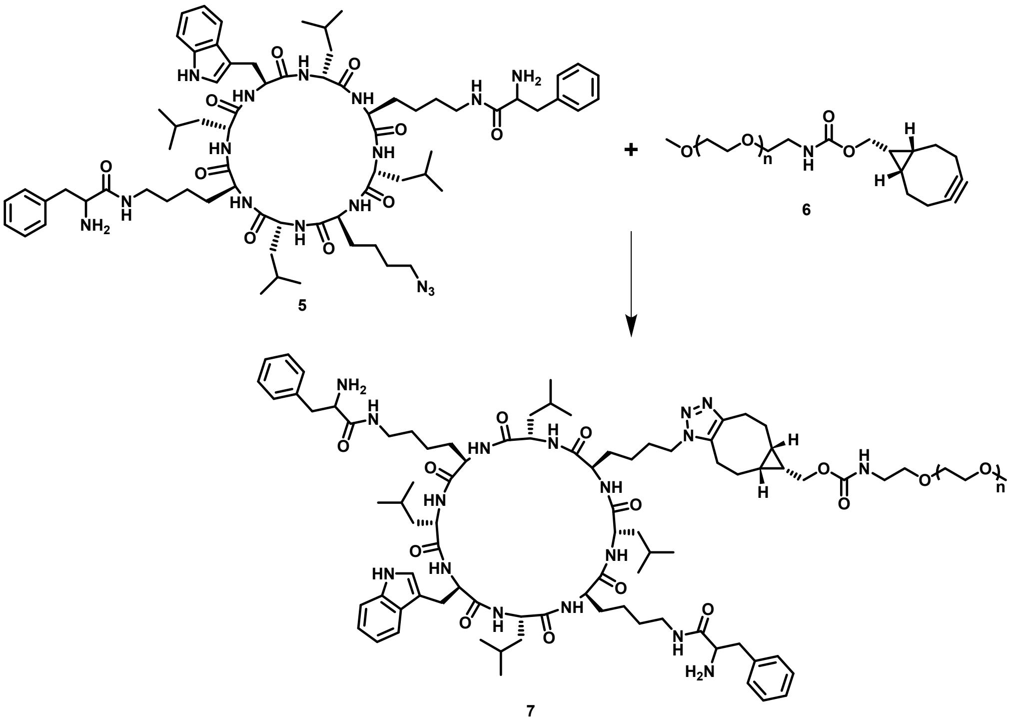


Figure S5 ^{1}H NMR spectrum of mPEG-BCN (6) (500 MHz, D_2O)

d. Synthesis of (Phe)$_2$-CP-PEG



Phe$_2$-CP-N$_3$ (10 mg, 0.0074 mmol) and mPEG-BCN (58 mg, 0.0117 mmol) were dissolved in 1 mL DMF. The reaction was left for 2 days. Then the DMF solution was precipitated in diethyl ether. The precipitate was collected using centrifugation and dried under vacuum. The resulting solid was then dissolved in 2 mL DCM and 8 mL diethyl ether was added dropwise to obtain white precipitate. Finally, the Phe$_2$-CP-N$_3$ conjugate **7** was obtained by centrifugation and dried under vacuum as an off-white solid (yield: 46 mg).

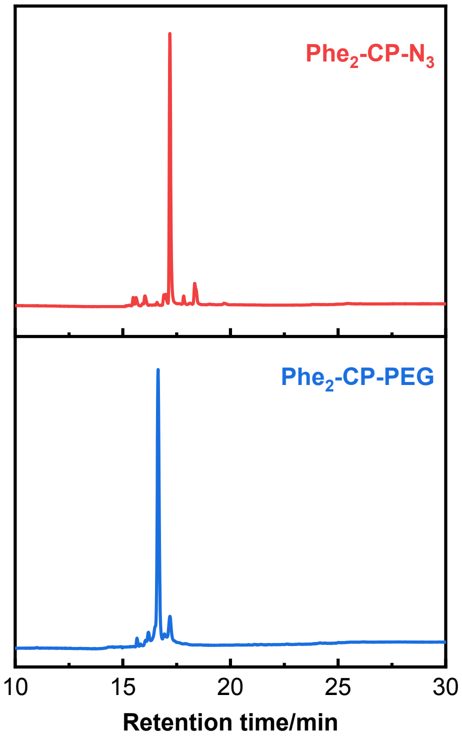


Figure S6 HPLC spectra for Phe$_2$-CP-N$_3$ and Phe$_2$-CP-PEG monitored by UV detector at 280 nm.

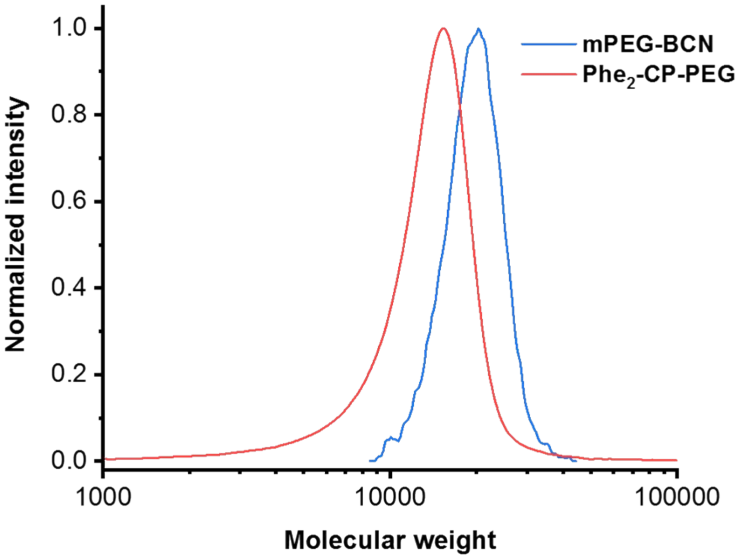


Figure S7 GPC traces (DMF + 0.1% LiBr) of mPEG-BCN and Phe$_2$-CP-PEG. The GPC was calibrated with poly(methyl methacrylate) standards.

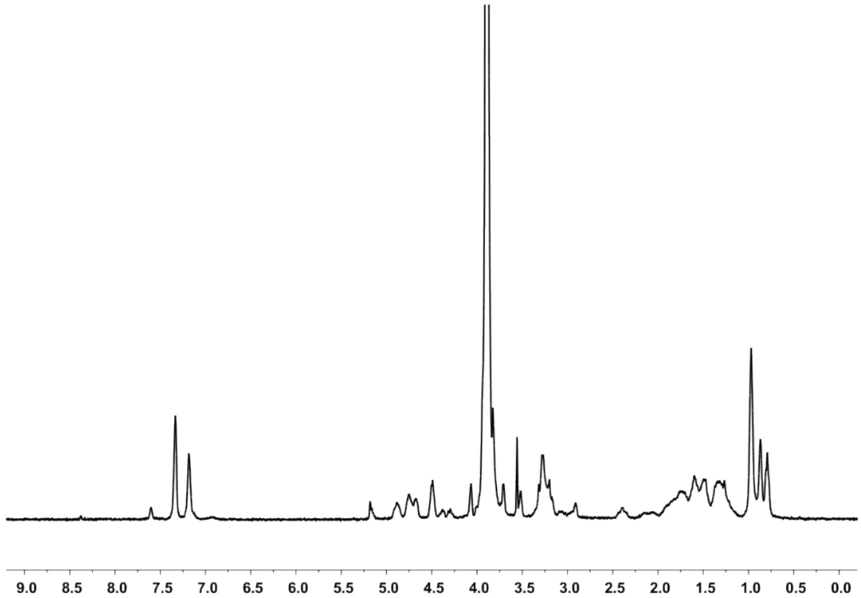
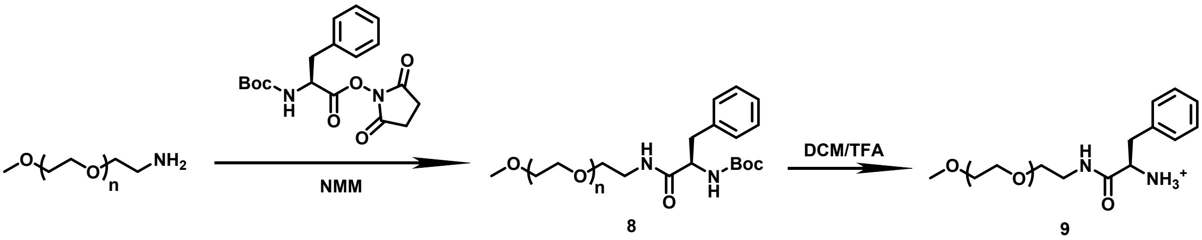


Figure S8 ^{1}H NMR spectrum of Phe$_2$-CP-PEG (7) (400 MHz, TFA-*d*)

e. Synthesis of mPEG-Phe



mPEG-Phe-Boc (8)

mPEG5k-NH$_2$ (100 mg, 0.02 mmol) and Boc-Phe-NHS (14.5 mg, 0.04 mmol) were dissolved in 1 mL DMF, and NMM (10.3 mg, 0.08 mmol) was added afterwards. The reaction was left overnight and purified by precipitation in ice cold diethyl ether twice to give a white powder as the product mPEG-Phe-Boc **8** (yield: 100 mg).

mPEG-Phe (9)

Removal of the -Boc protecting groups was achieved by adding a mixture of trifluoroacetic acid (TFA, 0.5 mL), and DCM (1 mL) to mPEG-Phe-Boc (70 mg) and stirring for 1 hour. The resulting solution was then precipitated in ice cold diethyl ether and washed twice to give a white powder as the product mPEG-Phe **9** (yield: 58 mg).

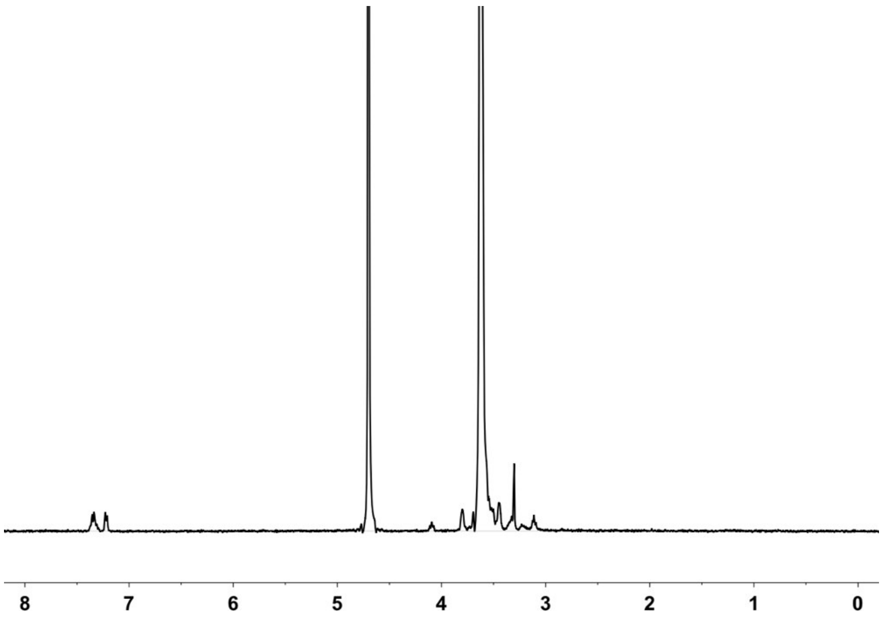


Figure S9 ^{1}H NMR spectrum of mPEG-Phe (9) (400 MHz, D_2O)

S3. Self-assembly of Phe_2-CP-PEG conjugate

The self-assembly of the conjugate was realized simply by dissolving Phe_2-CP-PEG into either H_2O or D_2O at different concentrations. Specifically, for ^{1}H NMR and DOSY measurement, 5 mg/mL D_2O solution was used, while for SANS measurement, 2 mg/mL D_2O solution was used.

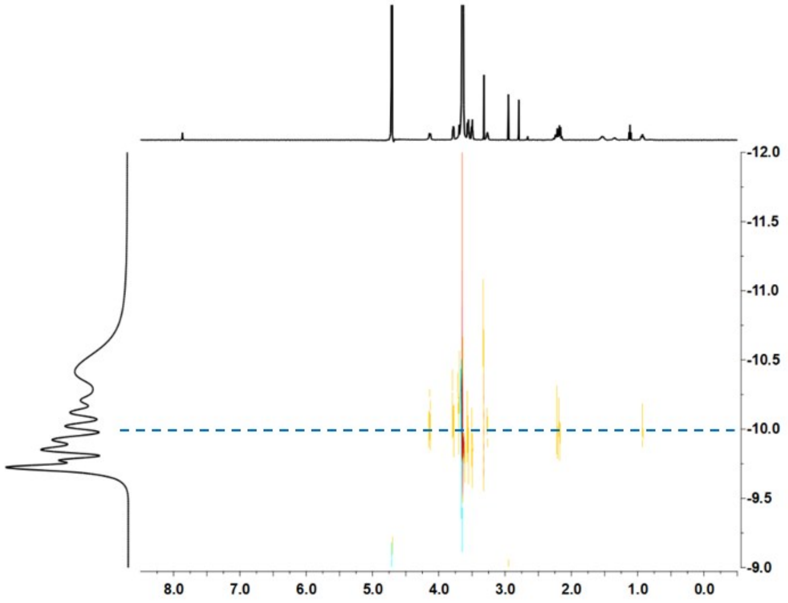


Figure S10 DOSY spectrum of mPEG-BCN (500 MHz, D_2O)

SASfit software was used to fit the data, using a cylindrical micelle (CYL+CHAINS) model. SLD values were calculated using based on the molecular structure of the conjugate and solvent, and the V_{brush} value was calculated by dividing the molecular weight of the polymer by Avogadro's number multiplied by the density. The R_{core} value was fixed at 5 Å, representing the radius of the cyclic peptide itself. To determine the N_{agg}, the length of the nanotube calculated by the CYL+CHAINS model was divided by 4.7 Å (the distance between two cyclic peptides).

Length of nanotube/Å	n_{agg}	R_g/Å	R_{core}/Å	SLD_{core} /cm^{-1}	SLD_{brush} /cm^{-1}	$SLD_{solvent}$ /cm^{-1}	V_{brush} /cm^{-3}
168±5	0.00787±0.00031	27.0±0.6	5	8.33e-7	5.18e-7	6.37e-6	7374

S4. Host-guest interaction between mPEG-Phe and CB[7]

For ^{1}H NMR measurement, a 5 mg/mL D_2O solution of mPEG-Phe was used to obtain ^{1}H NMR spectrum of mPEG-Phe in D_2O. Afterwards, 1 equivalent CB[7] was added into the mPEG-Phe solution and mixed thoroughly before measuring ^{1}H NMR spectrum of mPEG-Phe/CB[7].

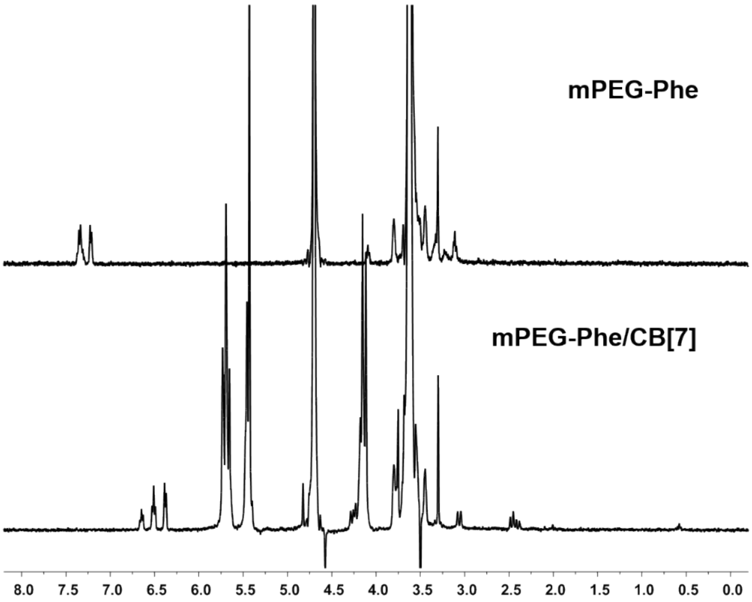


Figure S11 ^{1}H NMR spectra of mPEG-Phe and mPEG-Phe/CB[7] (400 MHz, D_2O).

S5. Host-guest interaction between Phe_2-CP-PEG and CB[7]

For ^{1}H NMR and DOSY measurement, a 5 mg/mL D_2O solution of Phe_2-CP-PEG was used to obtain ^{1}H NMR spectrum of Phe_2-CP-PEG in D_2O. Afterwards, 2 equivalents, and 3 equivalents of CB[7] was added into the Phe_2-CP-PEG solution and mixed thoroughly before measuring ^{1}H NMR and DOSY spectra of Phe_2-CP-PEG/CB[7], respectively.

For SANS measurement, 2 equivalent CB[7] was added into 2 mg/mL D_2O solution of Phe_2-CP-PEG and balanced overnight before measurement. SASfit software was used to fit the data, using a polymer chain model (DozierStar).

I_0	R_g/Å	*alpha*	*nu*	*N*
0.108±0.004	75±2	0.210±0.015	0.6	2.31±0.16

S6. Reformation of tubular supramolecular polymers by adding ADA

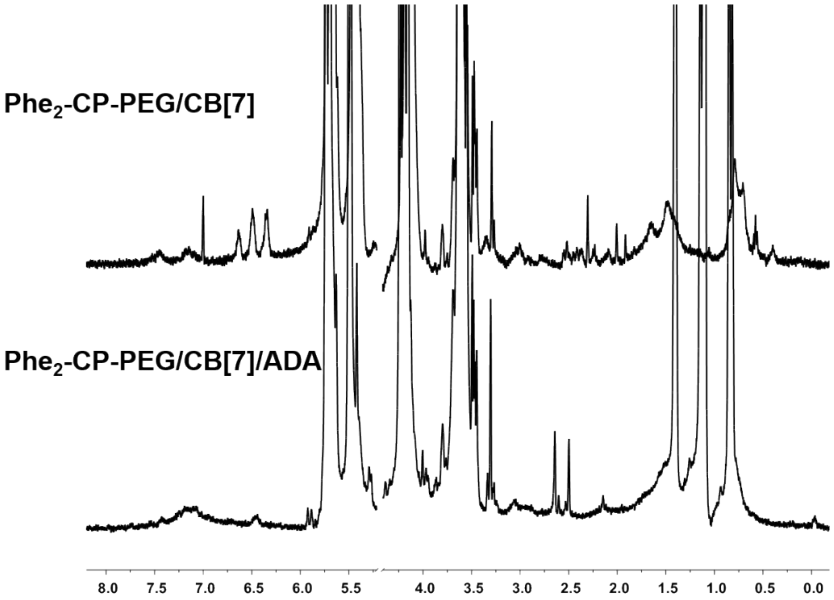


Figure S12 ^{1}H NMR spectra of Phe$_2$-CP-PEG/CB[7] and Phe$_2$-CP-PEG/CB[7]/ADA (400 MHz, D_2O).

S7. DOSY

The DOSY spectra were processed according to Stejskal-Tanner formula, as shown below:

$$\frac{I}{I_0} = exp\left[-(G\gamma\delta)^2\left(\Delta-\frac{\delta}{3}\right)D\right] = exp[-A\cdot D]\ ,\quad A = (G\gamma\delta)^2\left(\Delta-\frac{\delta}{3}\right)$$

Where I/I_0 is the intensity decay at certain gradient strength, γ is the gyromagnetic ratio of the nucleus, δ and Δ are the duration and separation of the gradient pulses, and D is the diffusion coefficient of the molecule.

The intensity I was obtained by integrating the peak of PEG backbone for mPEG-BCN, Phe$_2$-CP-PEG, and Phe$_2$-CP-PEG/CB[7], A was calculated based on gradient strength. Then *ln(I)* was plotted against A, and linear fitting gave the diffusion coefficient as the slope, as shown in Figure S13.

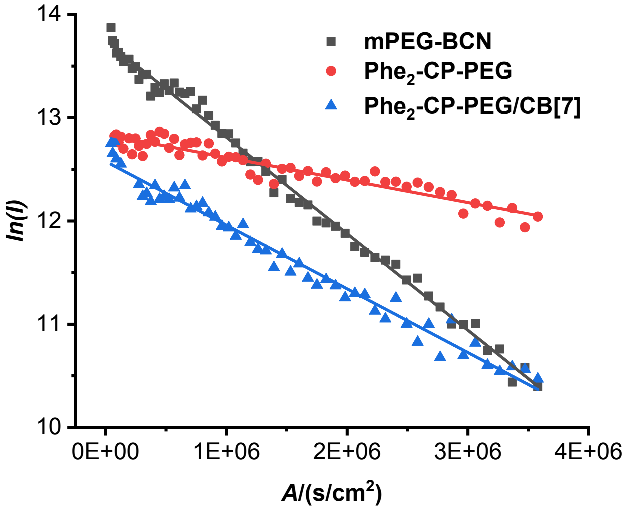


Figure S13 Relationship of *ln(I)* and *A* for mPEG-BCN, Phe_2-CP-PEG, and Phe_2-CP-PEG/CB[7] and the corresponding linear fitting.

The results are summarized as below:

Table S1 Summary of results from DOSY experiment

	Solvent	Temperature	D/(m^2/s)	r^2	R_h
mPEG-BCN	D_2O	25 °C	$(1.0\pm0.1)\times10^{-10}$	0.993	2.5 nm
Phe_2-CP-PEG	D_2O	25 °C	$(2.2\pm0.1)\times10^{-11}$	0.904	11.2 nm
Phe_2-CP-PEG/CB[7]	D_2O	25 °C	$(6.5\pm0.2)\times10^{-11}$	0.975	3.8 nm

Here, the hydrodynamic radius is estimated according to the Stokes−Einstein Equation, given the assumption that all the aggregations are hydrodynamically spherical.

$$D = \frac{k_B T}{6\pi\eta R_h}$$

Where D is the diffusion constant, k_B is Boltzmann's constant, T is temperature, η is the dynamic viscosity, and R_h is the hydrodynamic radius of the spherical particle ($k_B = 1.38\times10^{-23}$ J K^{-1}, T = 298.15 K, $\eta = 8.89\times10^{-4}$ N m s^{-2}).