Electronic Supplementary Information

Remote optically controlled hydrolase model based on supramolecular assembly and disassembly of its enzyme-like active site

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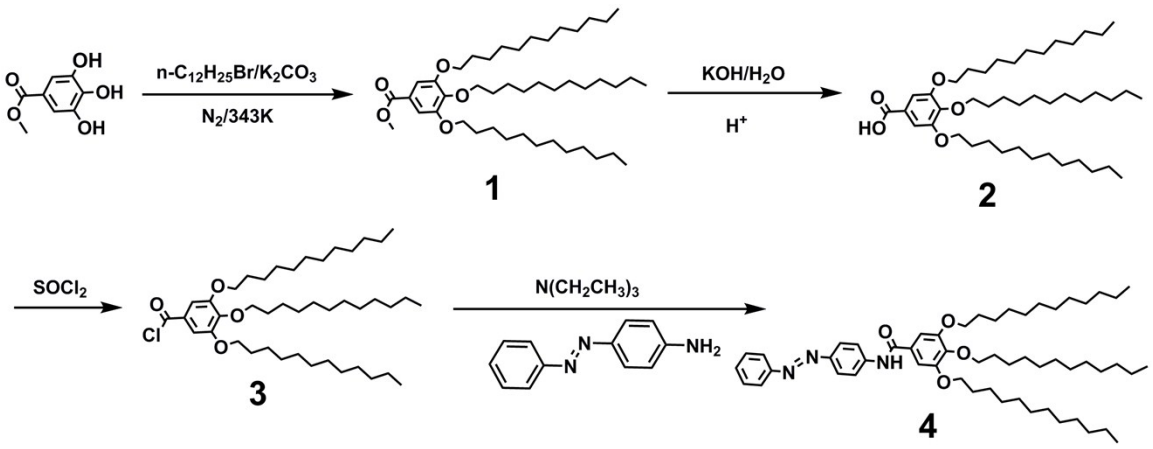
Synthesis procedure of Azo-TBA.



Methyl gallate (100 mmol) and K_2CO_3 solution (500 mmol) were dissolved in dimethylformamide (DMF, 70 mL), bromododecane (360 mmol) was added dropwise by a syringe under nitrogen (N_2) for 4 h. After DMF was removed, filtrated and recrystallized the crude product for 3 times in acetone. The resulting mixture was purified by silica gel chromatography to give 1 as white solid (90 mmol, 90%). The product 1 (50 mmol) and KOH (200 mmol) solution were added in ethanol (100 mL) to reflux at room temperature for 4 h. The reaction mixture was poured into in cold water (200 mL) and then dilute HCl was added to regulate pH into acidity cooling over night for precipitate. Compound 2 (10 mmol) and oxalyl chloride (50 mmol) were dissolved in dry dichloromethane (CH_2Cl_2, 10 mL) separately. Under N_2, the product solution was added dropwisely to oxalyl chloride solution. After reacting for 4 h at room temperature, the reaction mixture was concentrated to get compound 3 (9 mmol, 90%). A mixture of p-aminoazobenzene (5 mmol), dry triethylamine (TEA, 20 mmol) and DMF (20 mL) were added gradually to compound 3 (5 mmol) dissolved in DMF (10 mL) with stirring and

cooling under N_2 at room temperature for 12 h. The resulting mixture was purified by silica gel chromatography to give 4 as yellow solid (4 mmol, 80%).

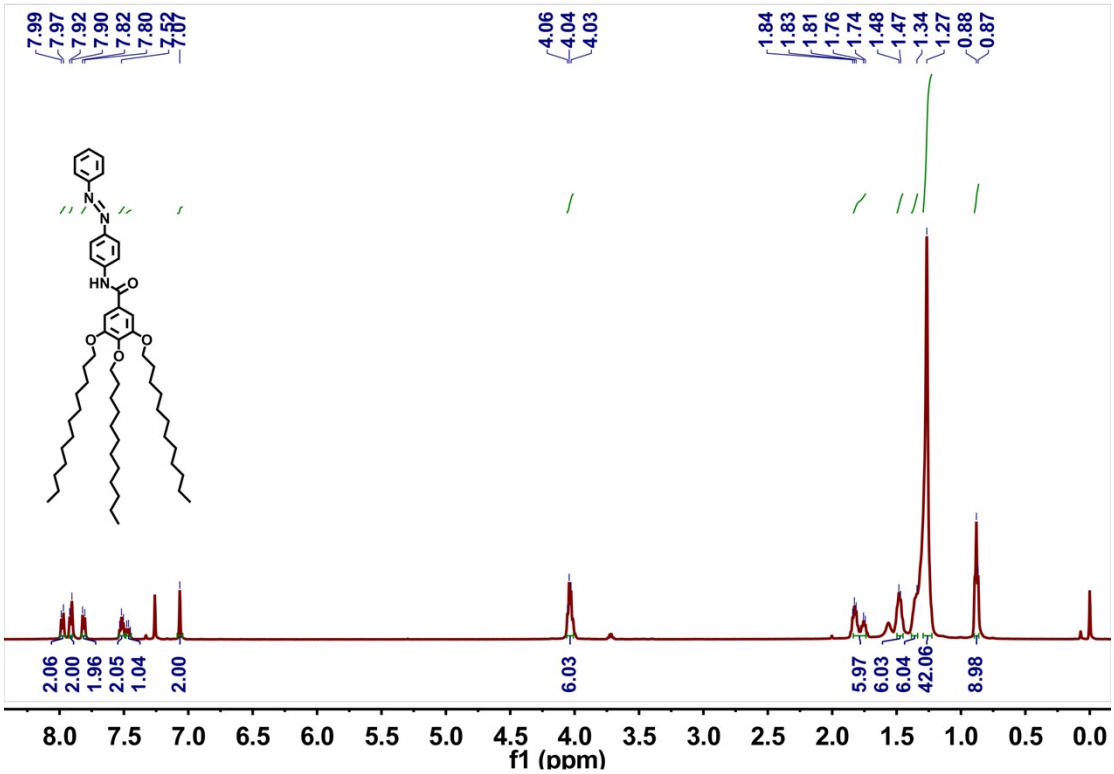


Fig. S1 ^{1}H NMR spectrum of the compound 4 in $CDCl_3$ at 25 °C.

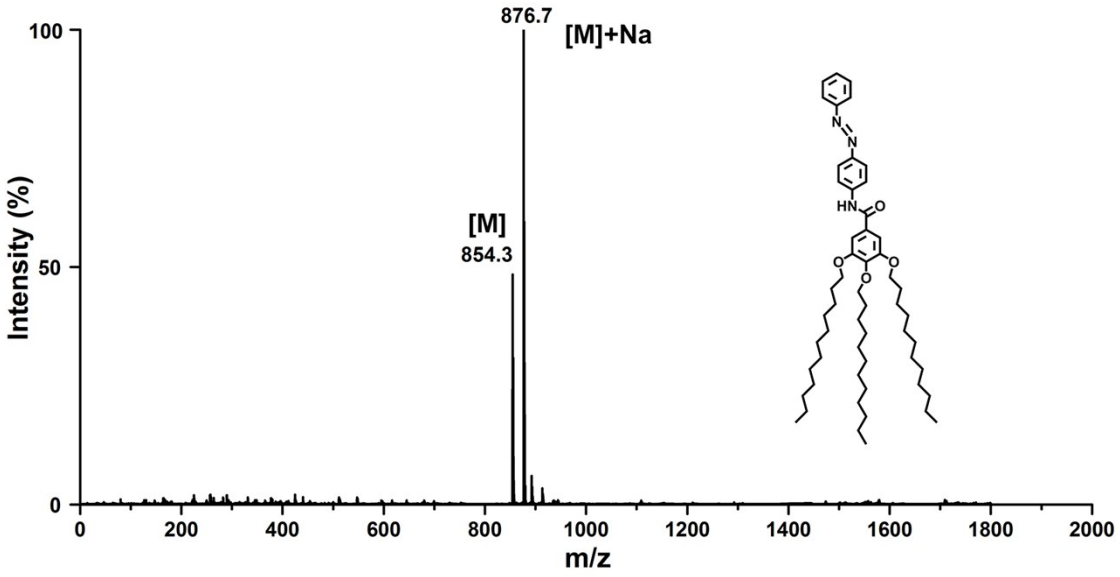
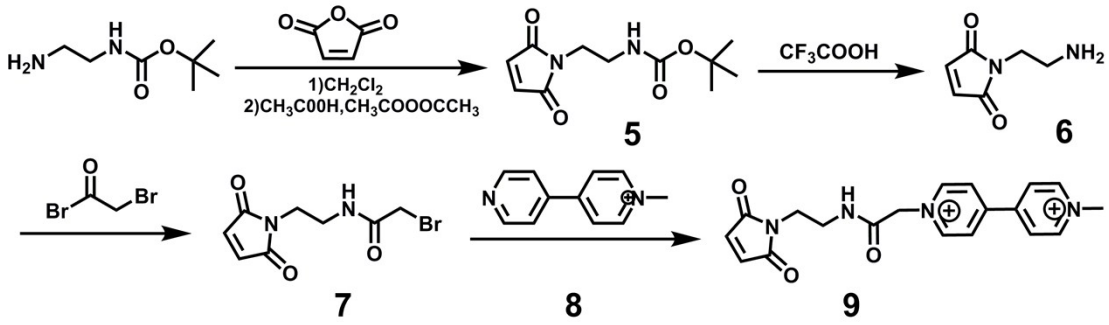


Fig. S2 ESI-MS spectrum of the compound 4 at m/z [M] 854.3 and [M] with Na in methanol at pH = 7 at 25 °C.

Synthesis procedure of MV.



N-Boc-ethylenediamine (22 mmol) and maleic anhydride (24.2 mmol) were dissolved in 50 mL dry CH_2Cl_2 and then stirred at room temperature for 4 h. The reaction mixture was concentrated and poured into in 150 mL acetone that contains acetic anhydride (28.6 mmol), sodium acetate trihydrate (11 mmol), and TEA (22 mmol). The reaction mixture was refluxed at 60°C under N_2 for 24 h. After cooling to room temperature, the reaction mixture was concentrated. Filtrated and recrystallized the crude product for 3 times in water to get a white solid 5 (21 mmol，95%). Compound 5 (2 mmol) was added to a solution of trifluoroacetic acid (10 mmol) in 10 mL CH_2Cl_2 at room temperature. After solvent evaporation under vacuum, the product 6 was obtained as white solid (1.95 mmol, 99%). A 50 mL round flask equipped with a small magnetic stirrer was charged with dry TEA (2.86 mmol) and the product 6 (1.43 mmol). The mixture was cooled to zero degree, and bromoacetyl bromide (2.15 mmol) was added dropwise by a syringe under cold water. After the solvent was removed, the resulting mixture was purified by silica gel chromatography to give 7 as a white solid (1.15 mmol, 80%). A

mixture of 4,4'-Bipyridine (10 mmol), iodomethane (10 mmol) and 10 mL DMF was stirred at room temperature stay overnight. The volatiles were removed to obtain product 8 as yellow solid (9.8 mmol, 98%). Under N_2 atmosphere, the white solid 7 (0.5 mmol) and the yellow solid 8 (0.5 mmol) was added in 5 mL DMF with 100°C stay overnight. After DMF was removed under vacuum, the mixture diluted with distilled water (5 mL). Impurity was removed by negative-pressure filtration. Water was removed and the product 9 was obtained as yellow oily liquid (0.25 mmol, 50%).

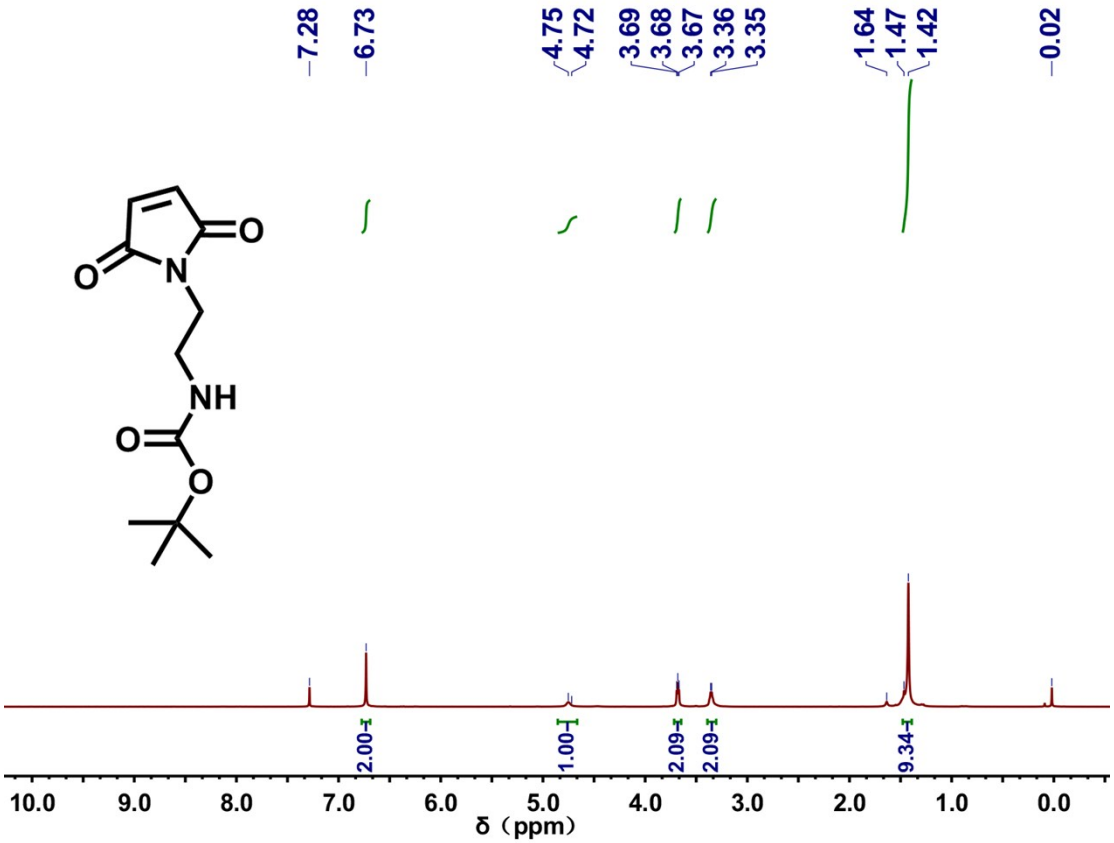


Fig. S3 ^{1}H NMR spectrum of the compound 5 in $CDCl_3$ at 25 °C.

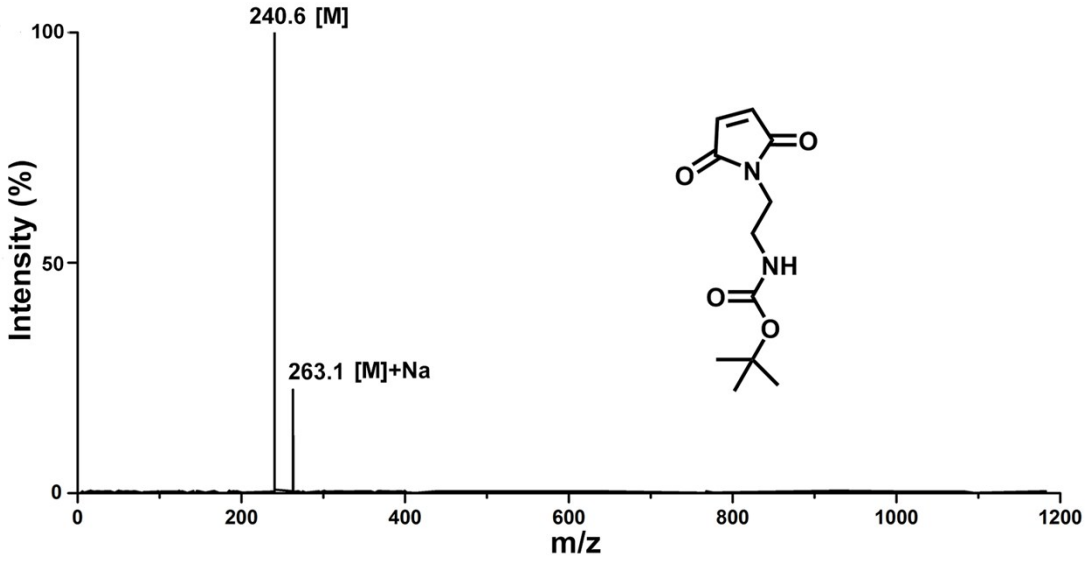


Fig. S4 ESI-MS spectrum of the compound 5 at m/z [M] 240.6 and [M] with Na in methanol at pH = 7 at 25 °C.

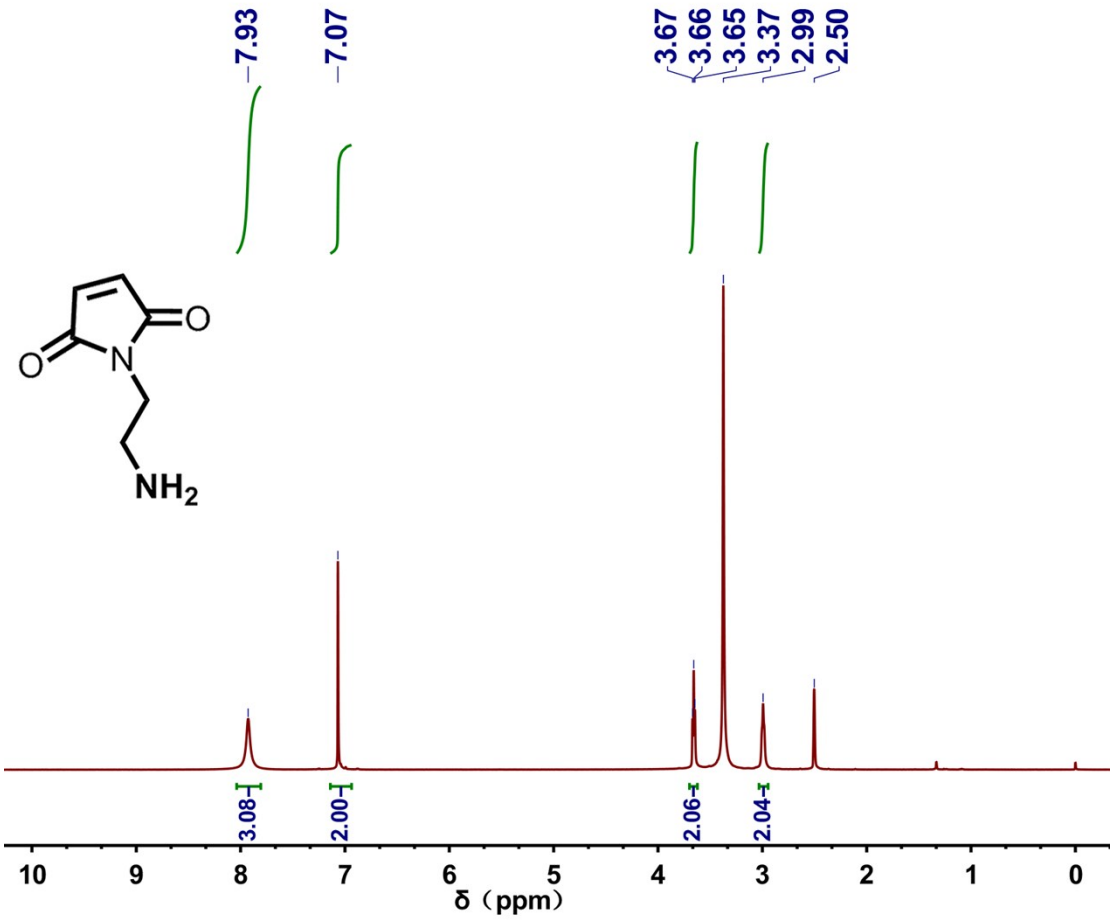


Fig. S5 ^{1}H NMR spectrum of the compound 6 in $CDCl_3$ at 25 °C.

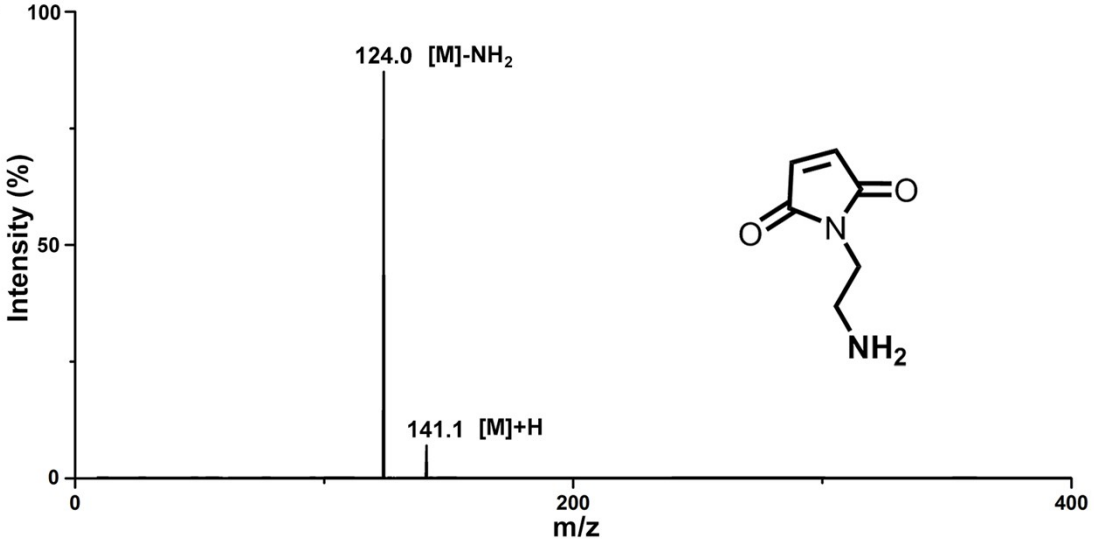


Fig. S6 ESI-MS spectrum of the compound 6 at m/z [M] without NH_2 124.0 and [M] with H in methanol at pH = 7 at 25 °C.

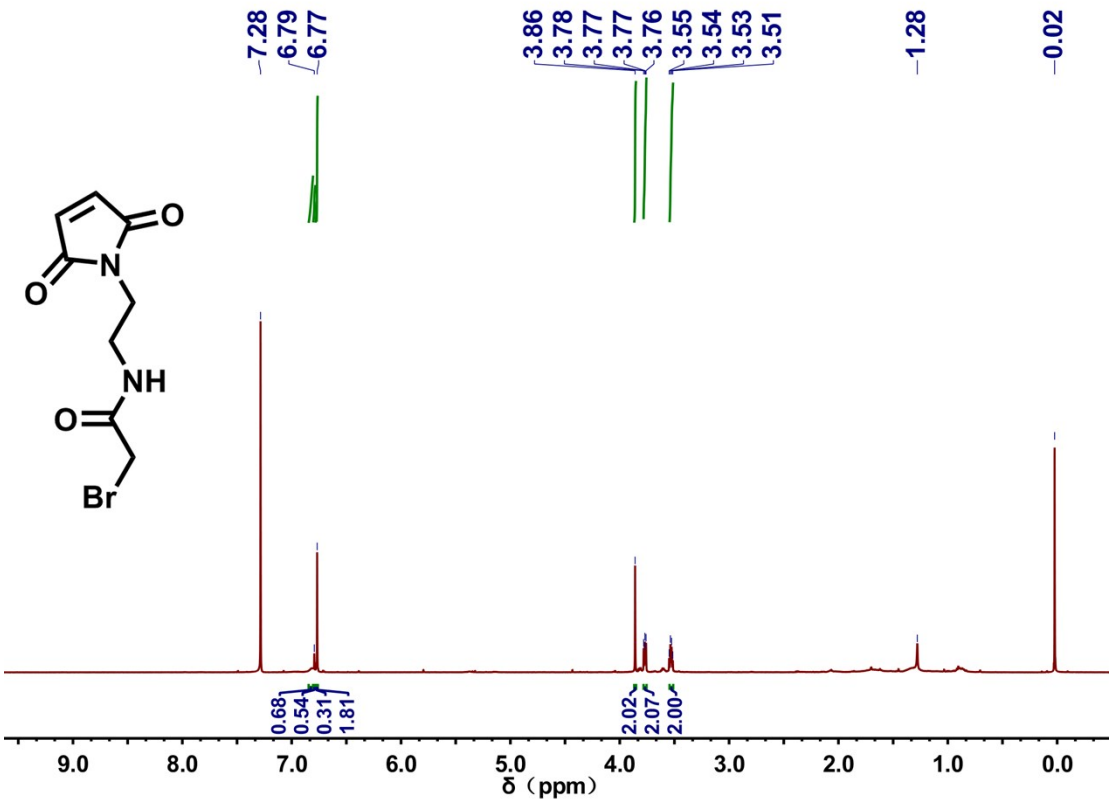


Fig. S7 ^{1}H NMR spectrum of the compound 7 in $CDCl_3$ at 25 °C.

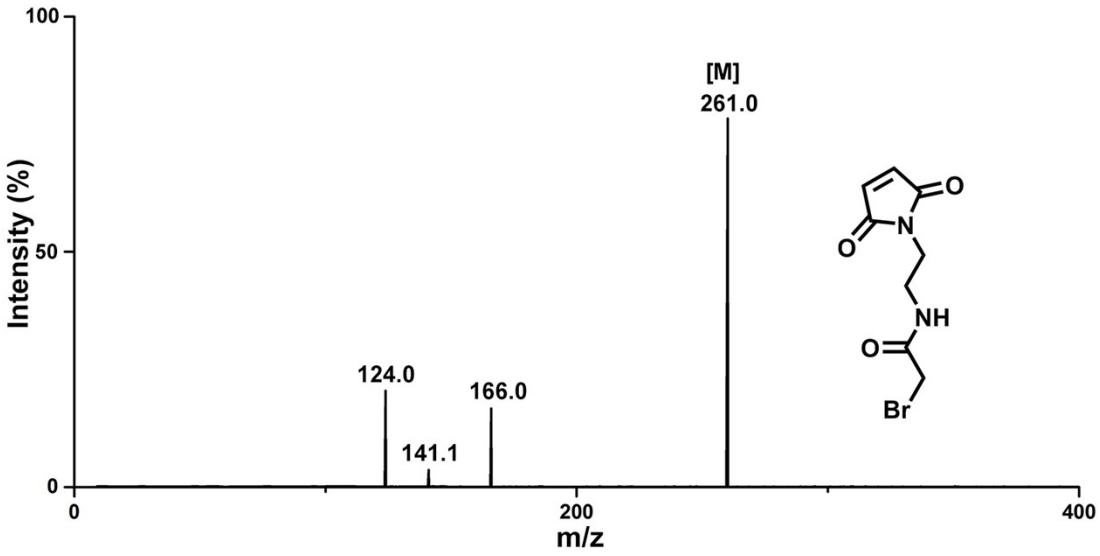


Fig. S8 ESI-MS spectrum of the compound 7 at m/z [M] 261.0, [M] without CH_3Br, [M] without C_2H_3BrO and [M] without C_2H_4BrNO in methanol at pH = 7 at 25 °C.

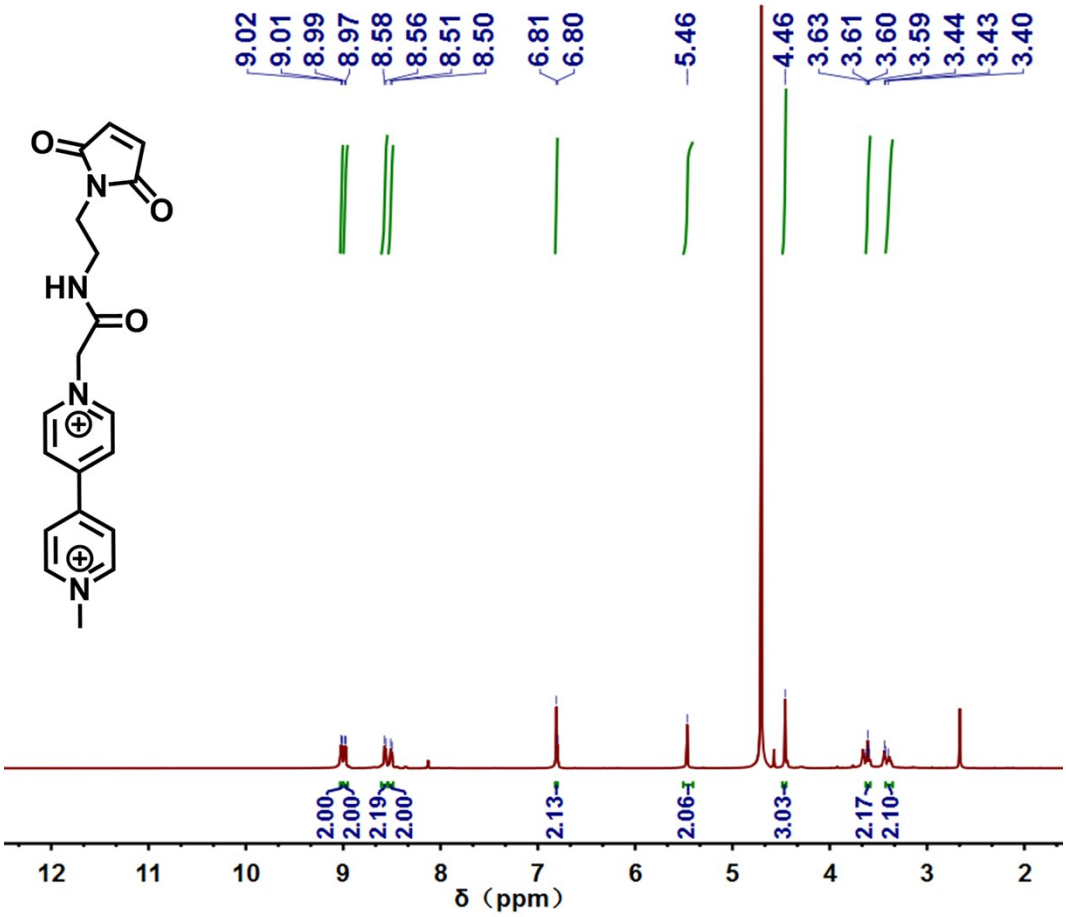


Fig. S9 ^{1}H NMR spectrum of the compound 9 in D_2O at 25 °C.

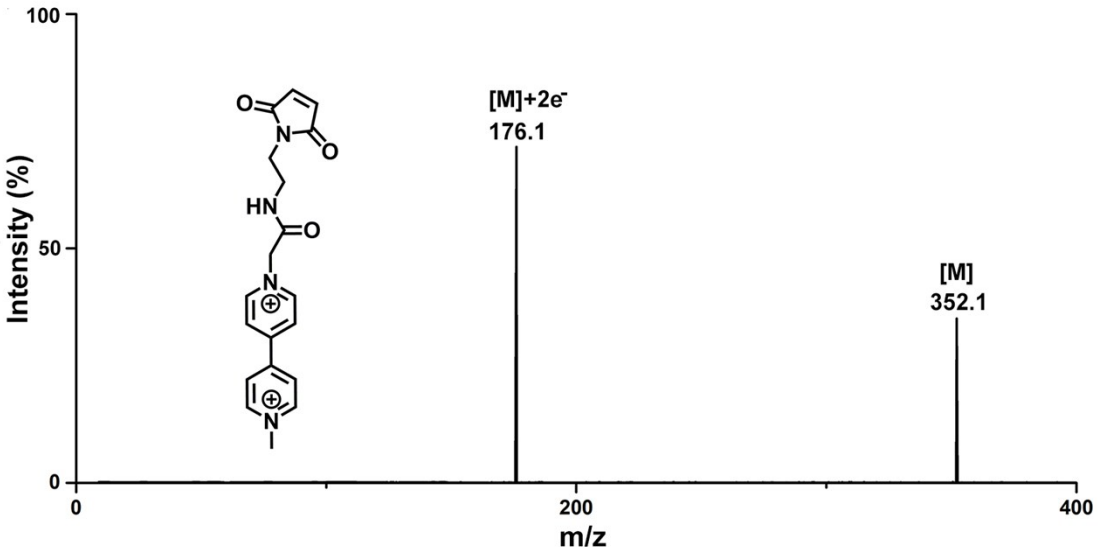


Fig. S10 ESI-MS spectrum of the compound 9 at m/z [M] with $2e^-$ and [M] 352.1 in water at pH = 7 at 25 °C.

Peptide-based supra-amphiphiles assembly and TEM characterization

To form heteroternary supramolecular vesicles, CB[8] (0.1 mM, Milli-Q), MV-HGC (or MV-RGC, 2.84 mM, Milli-Q) and Azo-TBA (3.62 mM, DMSO) stock solution were prepared and mixed to obtain a 1:1:1 molar ratio at final concentrations of 100 μM. After mixing CB[8] with MV-HGC (or MV-RGC) for sonication (0.5 h, 25 °C), Azo-TBA was added to the mixtures for another 0.5 h sonication. The sample solution (5 μL) was dropped onto a carbon-coated copper grid, and then freeze-dried using liquid nitrogen and freeze dryer (LyoLab 3000) for ~4 h. The morphology of peptide-based supra-amphiphiles was characterized by transmission electron microscopy (TEM, JEOL JEM-2100F).

HGC/RGC peptide Characterization and Modification of Vesicles Surface.

HGC/RGC (NH_2-His-Gly-Cys-COOH/ NH_2-Arg-Gly-Cys-COOH) was synthesized on a Rink amide-AM resin using standard Fmoc solid peptide phase synthesis (SPPS) method. Piperidine solution (20%, DMF, V/V) was used to cut off Fmoc protection group. Then HATU and DIEA were used as coupling reagents. The objective peptide was cleaved by cutting reagent (TFA/TIS/H_2O=95/2.5/2.5). The crude peptide was obtained by washing and precipitating with ice ether 3 times. The binding efficiency was detected by the method of three ketones. HPLC and ESI were used for separation and characterization. Owing to HGC/RGC is a peptide with a disulfide bridge in Cys and MV is binding with a maleimide group, “click” reaction can be achieved by the sulfhydryl group on cysteine (0.1 mol) and maleimide group (0.1 mol) on MV dissolved in DMF.

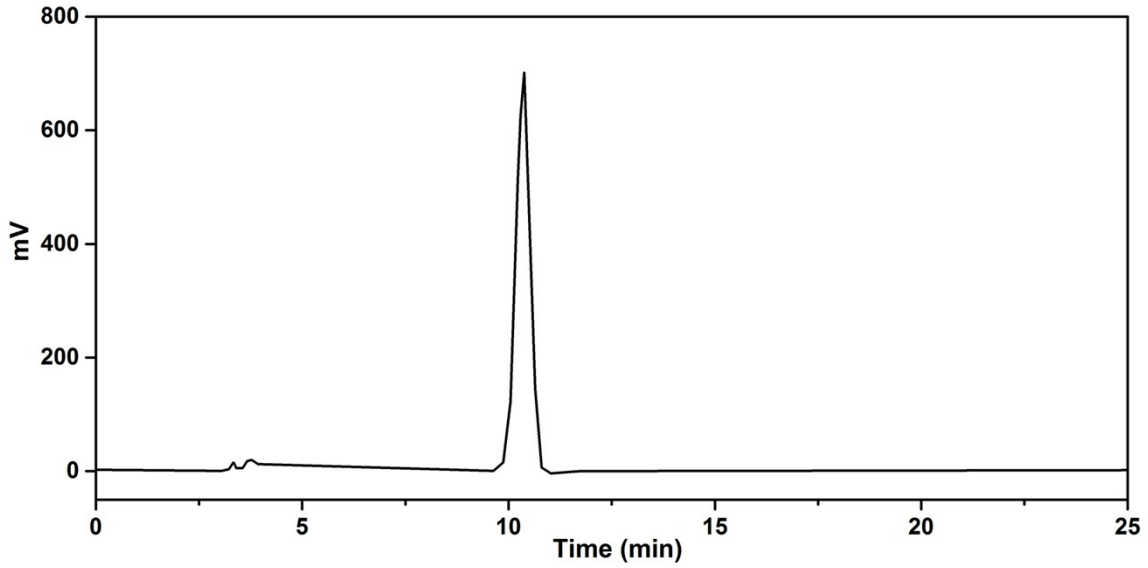


Fig. S11 HPLC chromatogram of the purified cyclic HGC peptide.

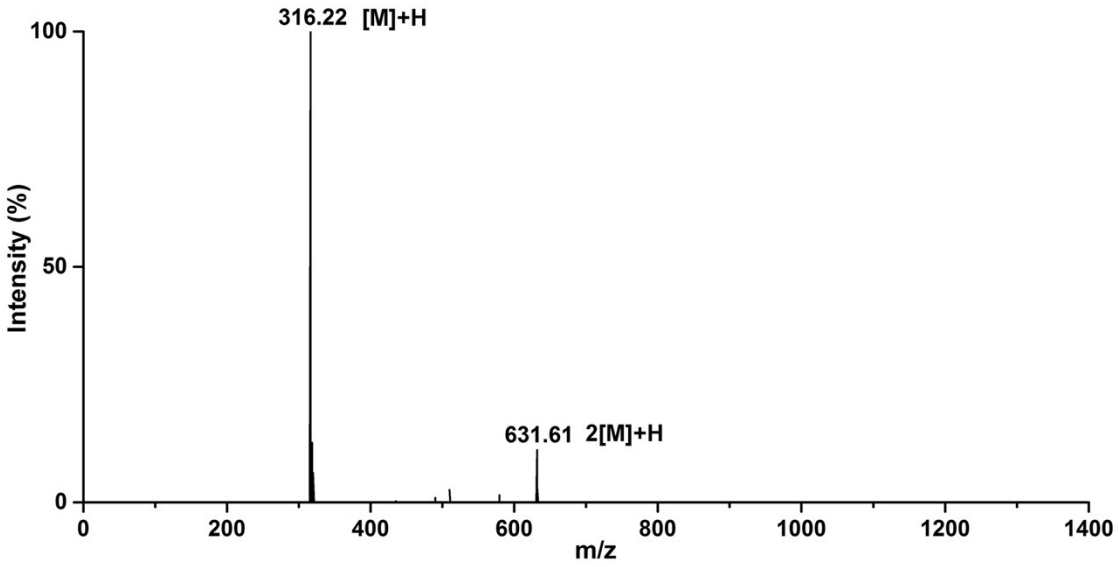


Fig. S12 ESI-MS spectrum of HGC at m/z [M]+H 316.22 and 2[M]+H 631.61 in acetonitrile at pH = 7 at 25 °C.

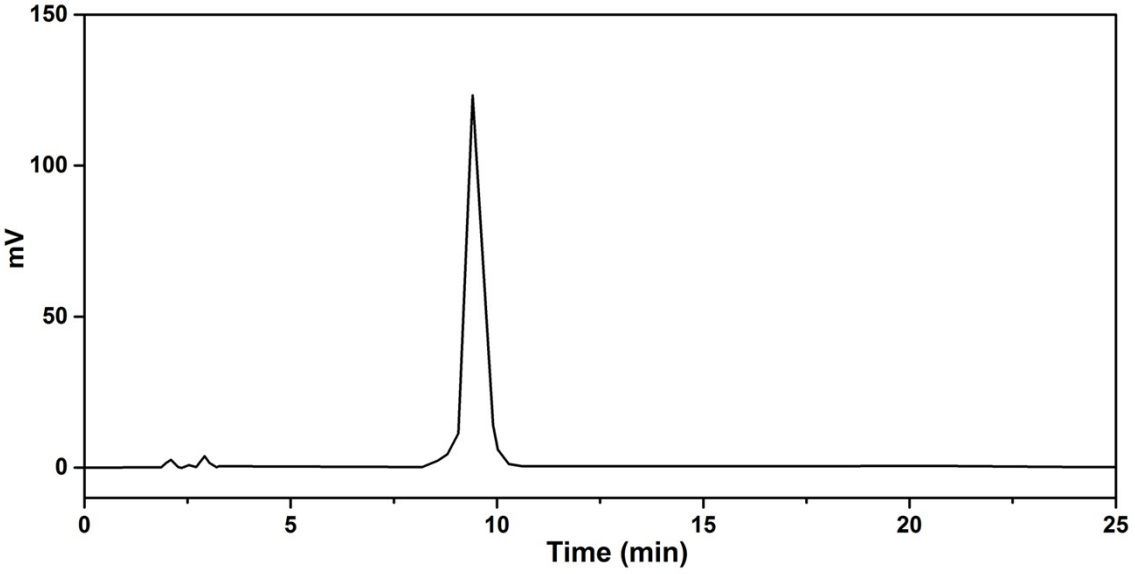


Fig. S13 HPLC chromatogram of the purified cyclic RGC peptide.

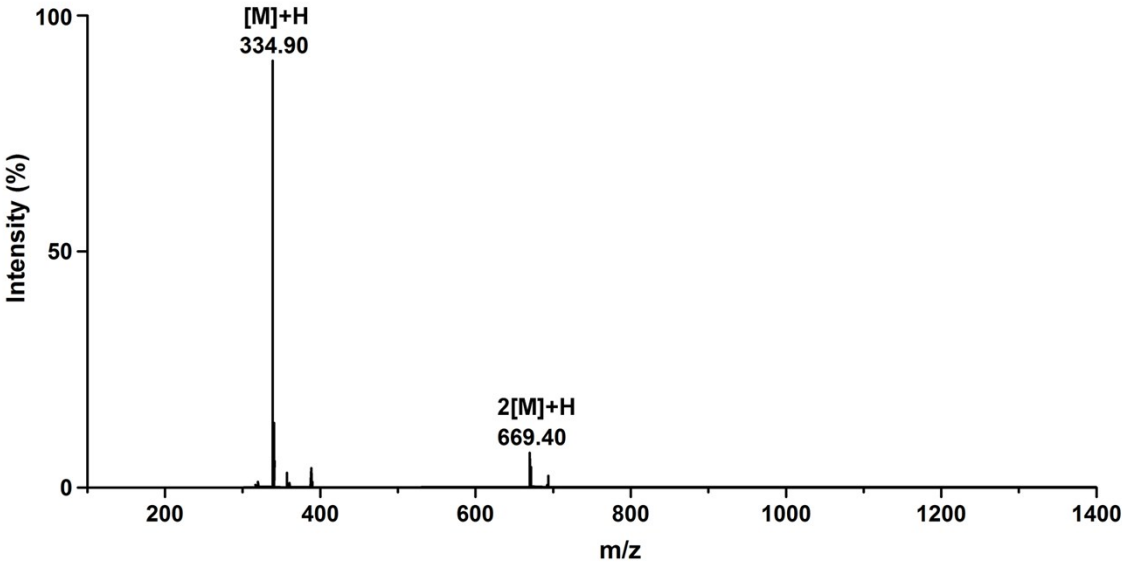


Fig. S14 ESI-MS spectrum of RGC at m/z [M]+H 334.90 and 2[M]+H 669.40 in acetonitrile at pH = 7 at 25 °C.

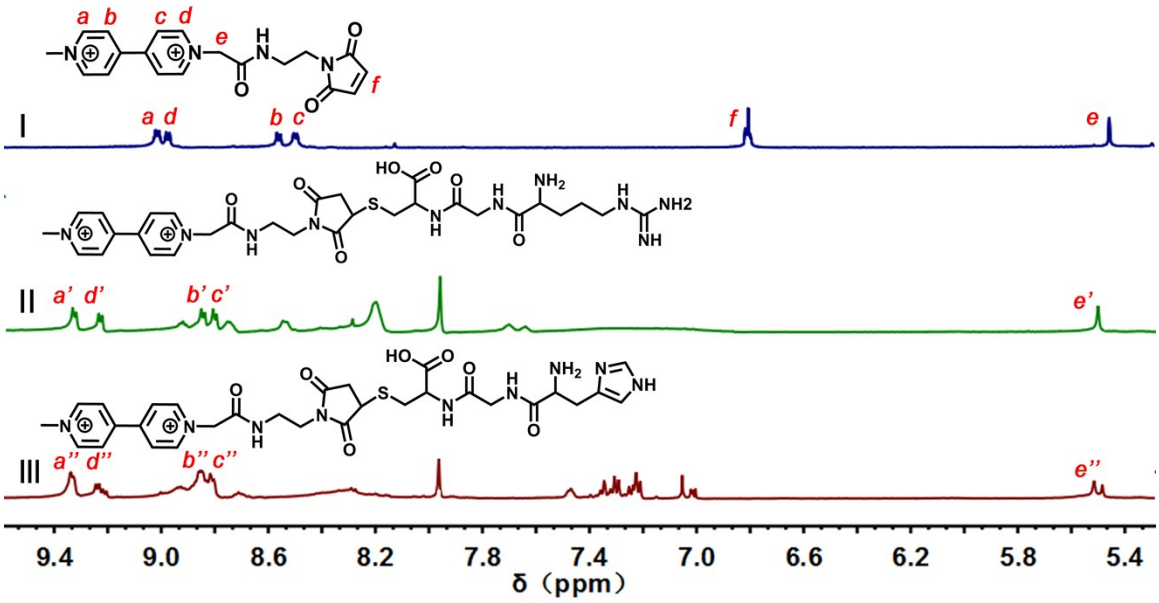


Fig. S15 ^{1}H NMR spectrum of MV before (I) and after (II, III) the click reaction in D_2O.

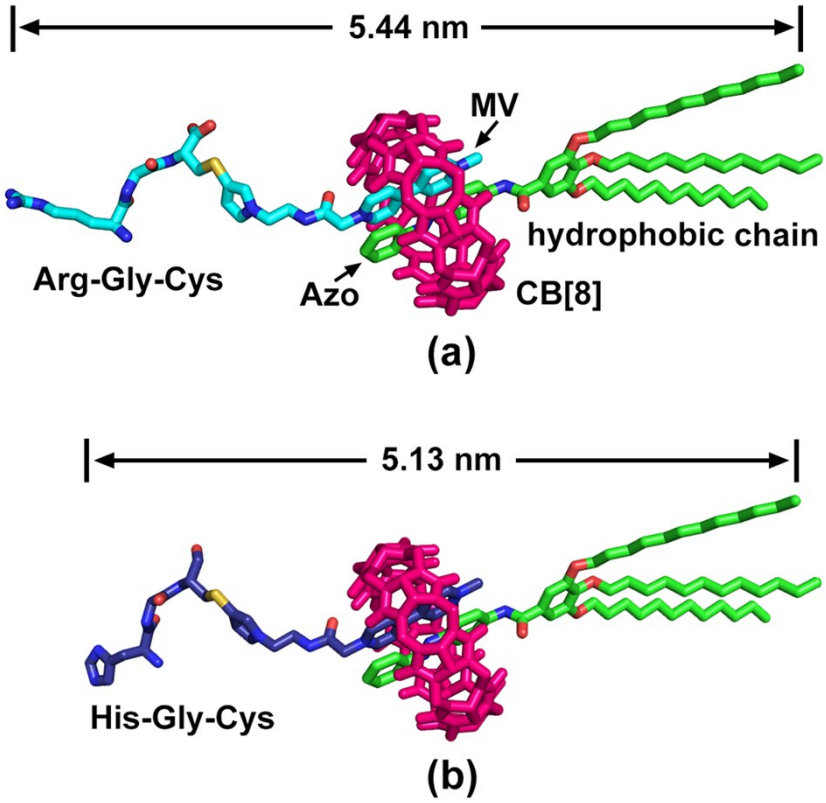


Fig. S16 The length of two peptide supra-amphiphile models created by GaussView 5.0.

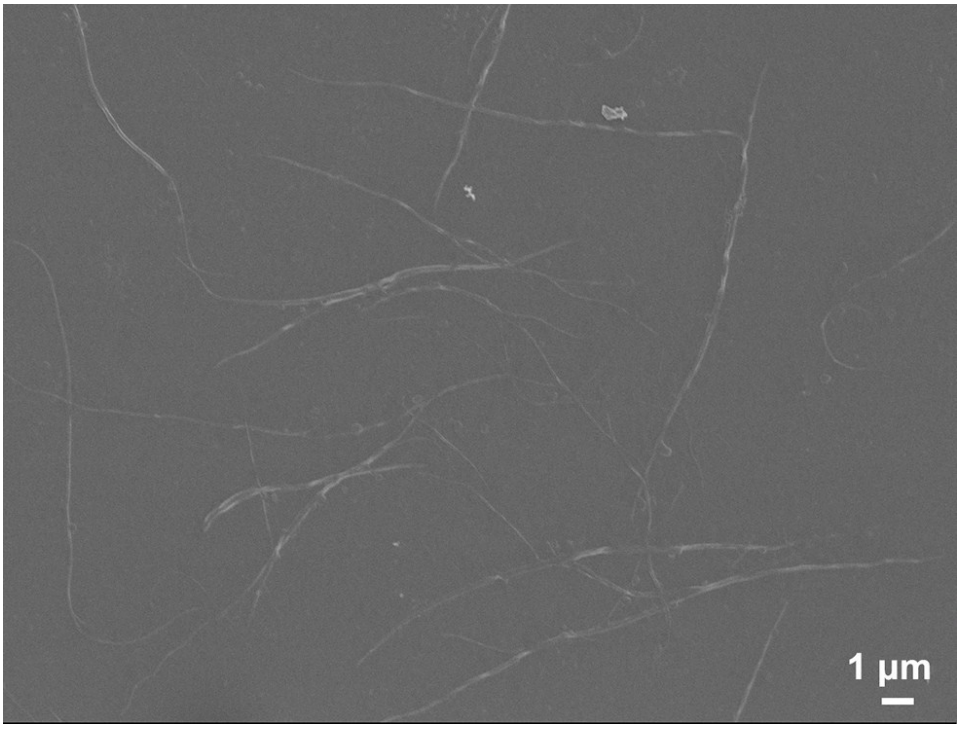


Fig. S17 The fibrillar aggregates of Azo-linked hydrophobic chains characterized by SEM.

Preparation of different vesicles hydrolase model.

MV-HGC (2 mM) and MV-RGC (2 mM) with different molar ratio (1:0, 40:1, 20:1, 15:0, 10:1, 2:1, 1:1, 1:2, 1:10, 1:20, 1:40, and 0:1) were added to 500 μL CB[8] (0.1 mM) in Milli-Q for 0.5 h sonication at 50 °C. Azo-TBA (1 mM) was mixed to obtain a 1:1:1 mixing ratio at final concentrations of 0.1 μM and then sonicated for another 2 h under the same condition. Vesicles stayed overnight at room temperature after sonication.

Catalytic activity and kinetics of p-NPA hydrolysis.

The hydrolysis activity was determined by a UV-2450 spectrophotometer (SHIMADZU) using p-nitrophenyl acetate (p-NPA) as the substrate at 400 nm. Hydrolysis reaction was performed in a quartz cuvette containing 400 μL of PB buffer (50 mM, pH=8), 50 μL p-NPA (5 mM, freshly prepared), and a certain amount of catalyst vesicles at 37 °C. Blank control was done for reference. The p-nitrophenol was monitored at catalytic time of 180 s. The kinetics experiments were performed at the same experimental conditions described above. The hydrolysis reactions were carried out at 37 °C in a quartz cuvette containing 400 μL PB buffer (50 mM, pH=8) and 50 μL catalyst vesicles (0.1 mM). Subsequently, a series of concentrations p-NPA (50 μL, freshly prepared) was added. The kinetic constants were calculated by fitting the Michaelis–Menten equation. The experimental data was obtained by three-time averaging independent measurements.

Switchable hydrolase catalysis of light-response vesicle.

The preparation of hydrolase vesicles was performed at the same experimental conditions described above. The samples were placed into a 1.5 transparent glass bottle with ice, and exposed to UV light irradiation with ultraviolet lamp (300 W) at 365 nm for 15 minutes. Subsequently, irradiation by visible light was performed using high-power fluorescent lamp (40 W). After UV irradiation or visible light, 50 μL hydrolase vesicles was taken out to measure the activity of hydrolysis. Switchable hydrolase catalysis studies of light-response vesicle were conducted on a 500 μL scale using the same procedure described in the catalytic activity and kinetics of p-NPA hydrolysis.

Cell culture and cell viability

A549 cells in the Dulbecco's modified Eagle medium (DMEM, Gibco, USA) were added to each well of a 96-well plate supplemented with fetal bovine serum (FBS, 10% (v/v)), penicillin (100 U/mL) and streptomycin (100 U/mL). Then, the cells were grown in a humid atmosphere with 5% CO_2 at 37 °C. MTT assay was chosen to evaluate the cytotoxicity of V-$H_{10}R_1$. After 24 h of incubation in triplicate at 5000 cells per well at 37 °C, the complete medium was replaced by different concentrations of vesicles (from 0 to 50 μM) for another 24 h. Then the cells were added with MTT (20 μL), which was reduced to an insoluble formazan crystal. After a further 4 h incubation, DMSO (150 μL) solution was added to each well to dissolve the formazan product. The absorbance of formazan was measured at 492 nm by using a microplate reader.

Cellular uptake experiment

To test the cellular uptake of V-$H_{10}R_1$, A549 cells were cultured in 96-well plate for 24 h and then vesicles, encapsulation of CF, were added to the cells. After incubation for 12 h, the cells were stained with Hoechst 33258 for 30 min at 37 °C. In order to observe V-$H_{10}R_1$ in actual cellular environments, the images were taken at 7 s intervals by CLSM.