A new cucurbit[10]uril-based AIE fluorescent supramolecular polymer for cellular imaging

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Materials

Q[10] was synthesized by our laboratory using the reported method [1]. $TiCl_4$, 4,4-dihydroxybenzophenone, K_2CO_3, 18-crown-6, 1,4-dibromobutane and 4-dimethylaminopyridine were purchased from Aladdin (Shanghai, China). All the solvents used below were purchased from Aladdin (Shanghai, China). All reagents were of analytical grade and were used without further purification. Deionzied water was used throughout.

1. Instruments

Varian Cary Eclipse Fluorescence emission spectra (Varian, Inc., Palo Alto, CA, USA). JEOL JNM-ECZ400s ^{1}H NMR spectra. 8453 UV-visible spectra from Agilent (Agilent Technologies, Santa Clara, CA, USA). Zeiss Sigma VP field emission scanning electron microscope (Germany). Brookhaven BI-200SM laser light scattering spectrometer. Confocal microscope (ZEISS, LSM710). Flow cytometry (NovoCyte 3008). Agilent G6545 A UHD Accurate-Mass Q-TOF (California, USA).

2. Experimental method

^{1}H NMR measurements:

The ^{1}H NMR spectra were recorded at 25°C on a JEOL JNM-ECZ400s spectrometer. D_2O was used as a field-frequency lock and the observed chemical shifts are reported in parts per million (ppm) relative to that for the internal tetramethylsilane (TMS) standard (0.0 ppm).

SEM measurements:

10 μL of an aqueous solution of TPE-B@Q[10] (molar ratio = 1:2, 20 μM) was added onto silicon wafers, then the wafers were placed in a cool and ventilated place to dry. The sample was coated with platinum prior to SEM characterization.

Measurement of fluorescence spectra:

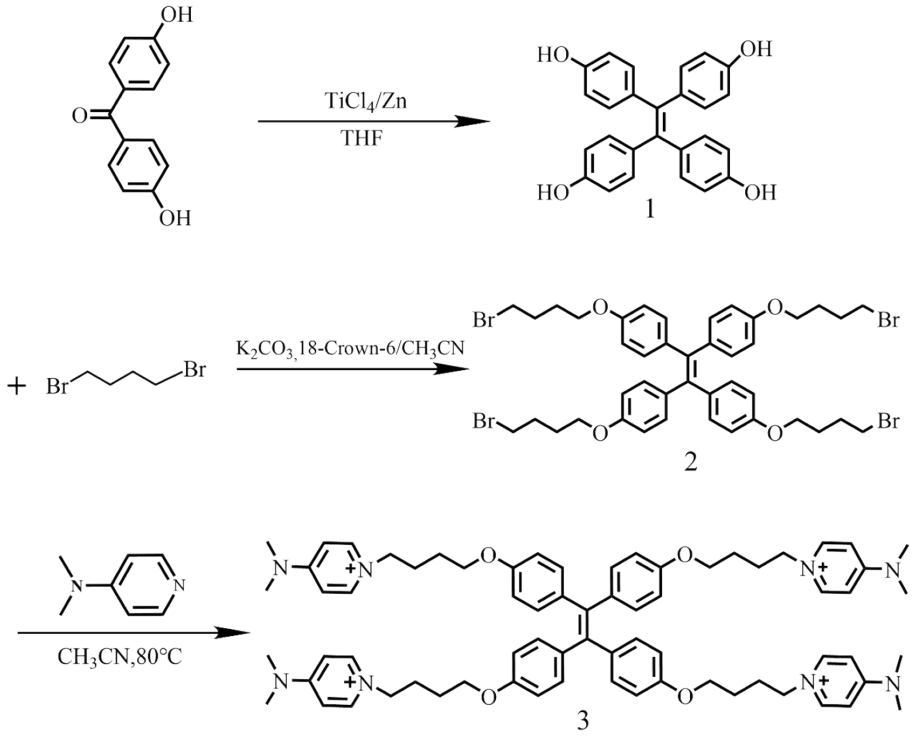
Fluorescence data were determined by a titration method. Add 0 to 5.0 times Q[10] in the fixed TPE-B (20 μM). Fluorescence spectrometry conditions: excitation wavelength of 339 nm, slit width of 10 nm, voltage of 480 V.

Cell culture and treatment

HUVEC and HeLa cells were maintained in DMEM medium supplemented with 10% FBS. The cell culture condition is an atmosphere of 5% CO_2 and 95% humidity. Then, High Content Analysis System Operetta CLS^{TM}(Perkin Elmer, US) was used to capture the fluorescent signals produced by TPE-B and Q[10]. Briefly, HUVEC and Hela cells were seeded into 96-well plate (Perlin Elmer CellCarrier Ultra) until satisfying confluence. 2 μM TPE-B or 2 μM TPE-B + 4 μM Q[10] was added into the media of cells, then the 96-well plate was placed into the machine and every picture was captured according to the manufacturer's instruction. During the whole process, 37 ℃ and 5% CO_2 environment was set to guarantee the viability of cells.

For flow cytometry analysis, HUVEC and HeLa cells were seeded into six-well plates and separated with trypsin before TPE-B and Q[10] staining. 1 μM TPE-B or 2 μM Q[10]+ 1 μM TPE-B were added into the cell suspension and cells were incubated, then stained cells were counted by flow cytometry (NovoCyte 3008) and the fluorescence signals were captured every 10 minutes. The fluorescence peak produced by dyes indicated the distribution of relative fluorescence intensity of cells and reflected the blue shift effect of the Q[10] on the TPE-B.

3. The Synthetic Methods



Scheme S1. Synthesis of compound **3** (TPE-B)

Synthesis of compound 1. $TiCl_4$ (1.71 g, 9.0 mmol) slowly added into 4,4-dihydroxybenzophenone (1.61 g, 7.5 mmol) and Zn dust (1.18 g, 18.0 mmol) in 350 mL THF at 0 ℃. The system was then refluxed overnight under an N_2 atmosphere. After filtration and solvent evaporation, the product was purified by silica gel column (PE/EA). Compound **1** was obtained as a light brown red solid in 89% yield (2.14 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ: (TMS, ppm): 9.23 (s, 4H), 6.65 (d, J= 8.68 Hz, 8H) and 6.43 (d, J= 8.63 Hz,8H).

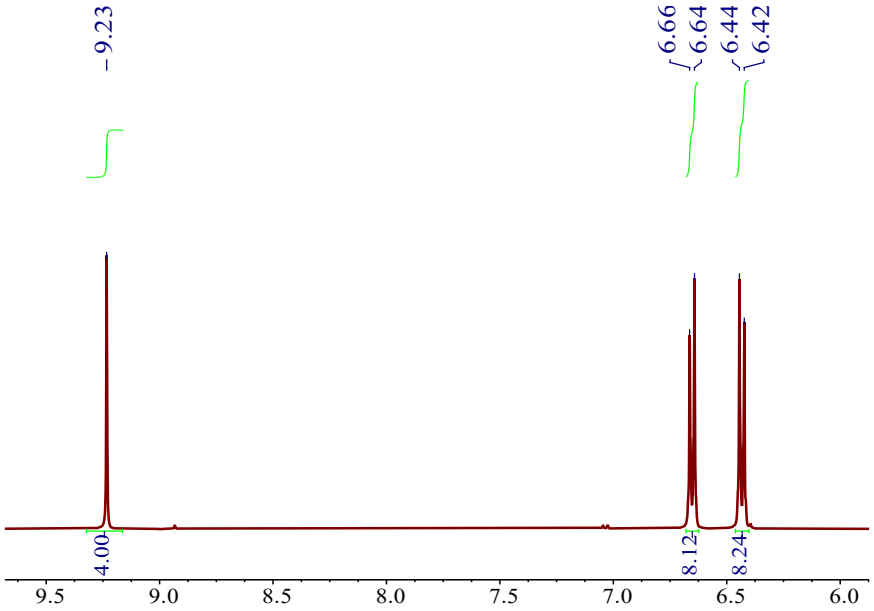


Figure S1. The ^{1}H NMR spectrum of **1** recorded in DMSO-d_6 at 25℃.

Synthesis of compound 2. Compound **1** (1.12 g, 2.7 mmol), 18-crown-6 (0.15 g, 0.55 mmol), and K_2CO_3 (3.84 g, 0.028 mol) were dissolved in 500 mL acetonitrile. Then 0.055 mol of 1,4-dibromobutane was added, and the system was stirred overnight at 65℃. The reaction mixture was filtered, and evaporated to produce the crude product. Compound **2** was further purified by silica gel column (DCM/PE) to obtained a yellow solid in 45% yield (1.13 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ: (TMS, ppm):6.78 (d, J= 8.62 Hz, 8H), 6.64 (d, J= 8.12 Hz, 8H), 3.87 (t, J= 12.27 Hz, 8H), 3.55 (t, J= 13.26 Hz, 8H), 1.89 (m, J= 28.28 Hz,8H) and 1.75 (m, J= 28.15 Hz, 8H).

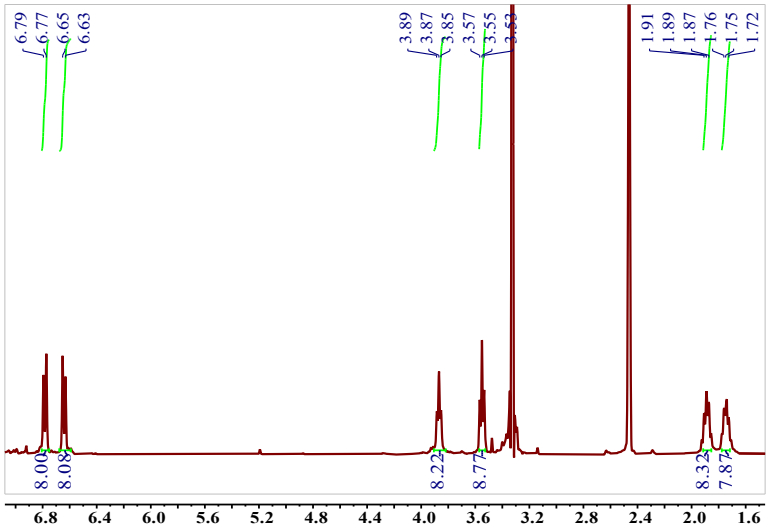


Figure S2. The ^{1}H NMR spectrum of **2** recorded in DMSO-d_6 at 25℃.

Synthesis of compound 3. Compound **2** (0.52 g, 5.31 mmol), and 4-dimethyla minopyridine (0.64 g, 53.1mmol) were dissolved in 50 mL acetonitrile and hea ted to 80℃ for 8h. The reaction mixture was filtered and washed with acetoni trile to obtain pure compound **3** (**TPE-B**) as a yellow solid in 45% yield (0.8 8 g). ^{1}H NMR (400 MHz, D_2O) δ: (TMS, ppm):7.78 (d, J= 23.08 Hz, 8H), 6. 70 (t, J= 102.20 Hz, 24H), 3.80 (m, J= 21.96 Hz, 8H), 3.74 (d, J= 52.63 Hz, 8H), 2.99 (s, 24H), 1.72 (s, 8H) and 1.52 (s, 8H). M.S.: m/z = 632.26 corresp onds to $[M+2Br^-]^{2+}$.

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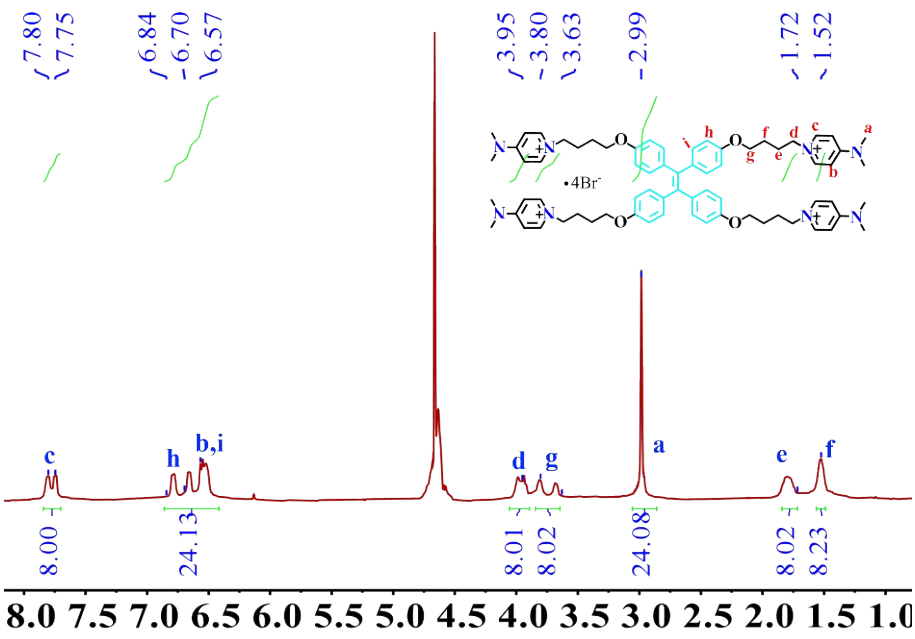

Figure S3. ^{1}H NMR spectrum of **3** (**TPE-B**) recorded in D_2O at 25℃.

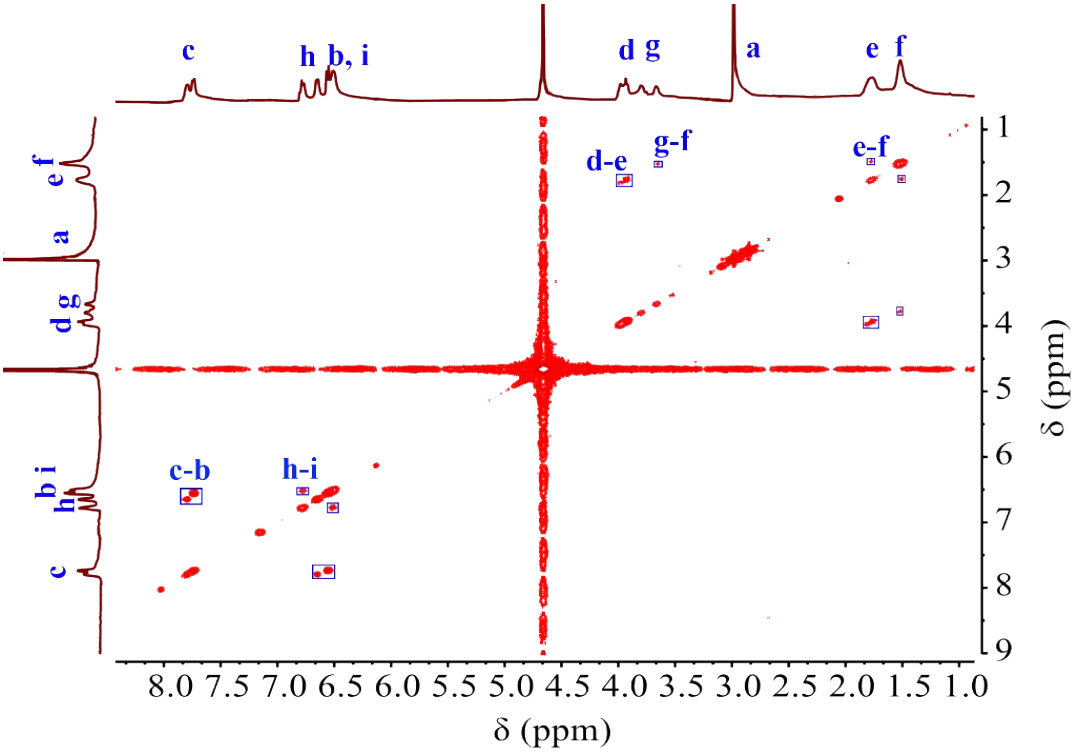

Figure S4. Partial COSY spectrum of **TPE-B** recorded in D_2O at 25℃.

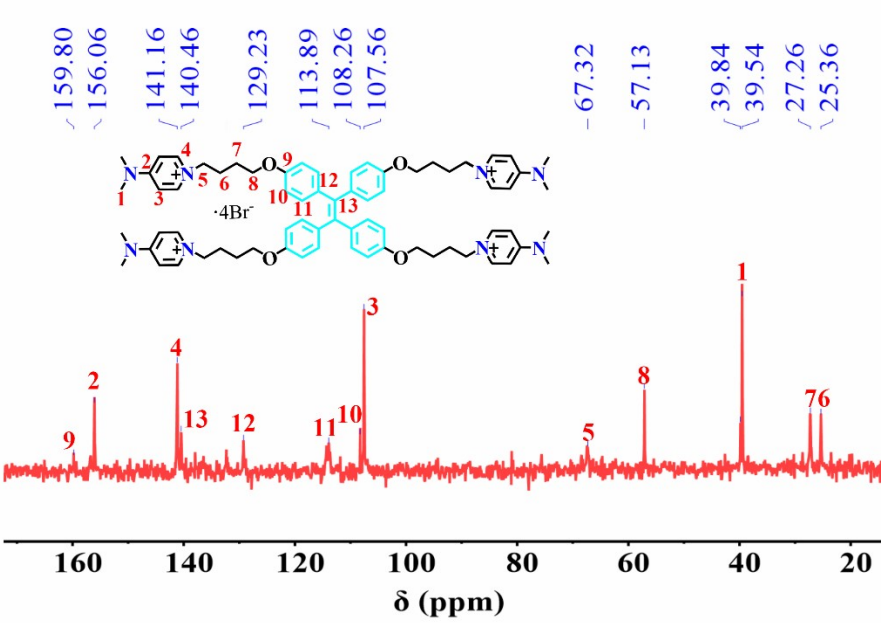

Figure S5. ^{13}C NMR (100 MHz) spectra of **TPE-B** recorded in D_2O at 25℃.

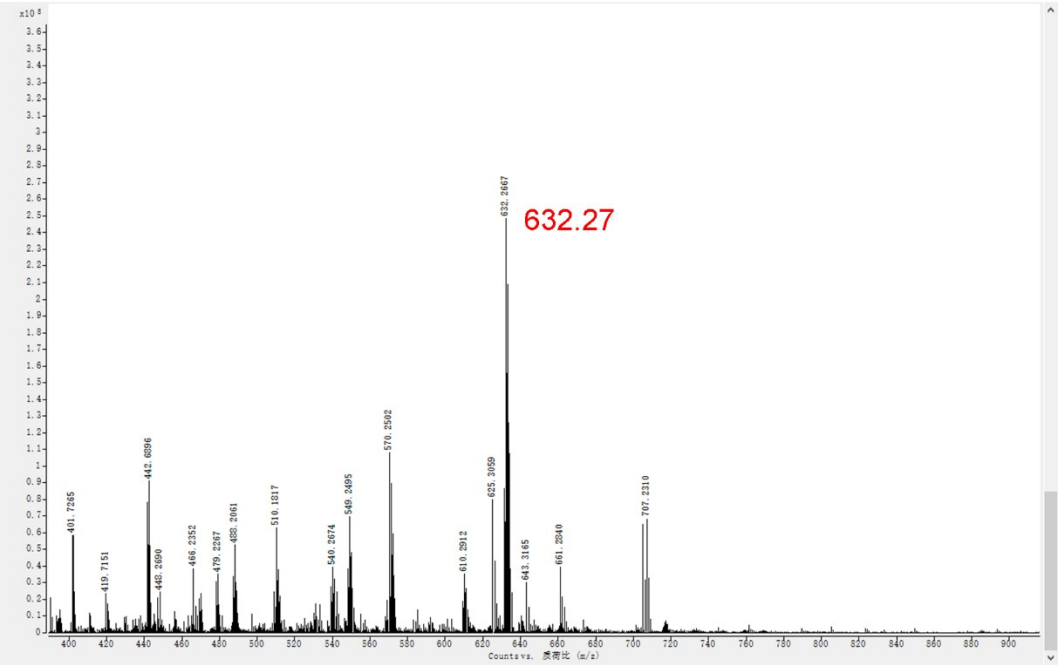

Figure S6. The MASS spectrum of **TPE-B**.

The strongest peak found at m/z = 632.27 corresponds to $[M+2Br^-]^{2+}$ (theoretical m/z is 632.24)

4. The Interaction between Q[10] and TPE-B

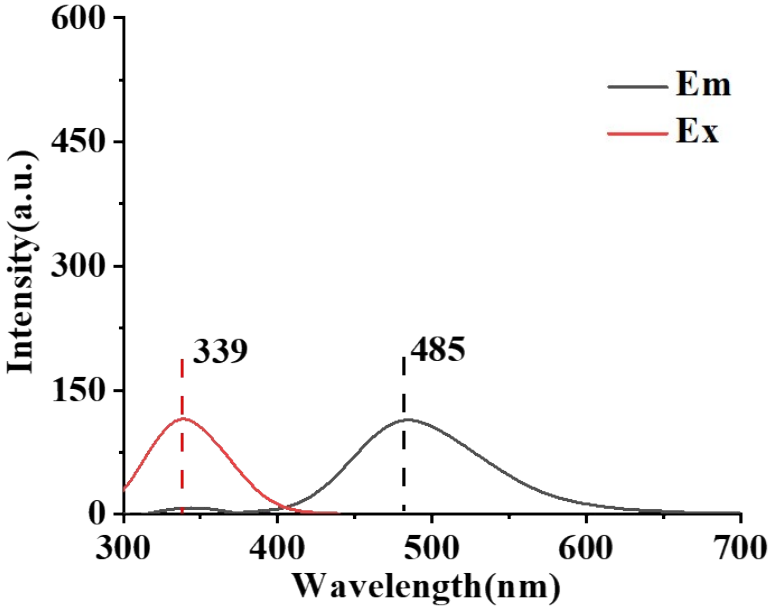

Figure S7. Excitation (Ex) and emission (Em) spectra of **TPE-B**

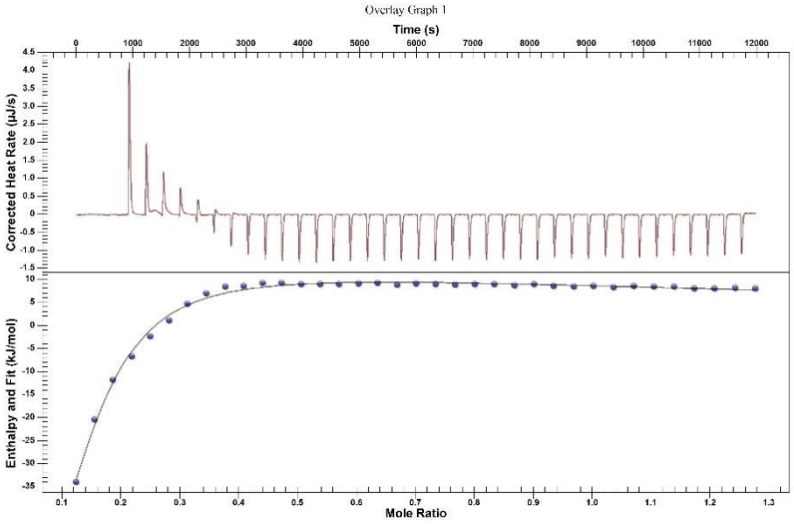

Figure S8. ITC data for the binding of Q[10] with **TPE-B** in H_2O at 25 ℃.

Table S1. Thermodynamic data of ITC in H_2O at 25 ℃

Model	K(M^{-1})	ΔH(KJ/mol)	ΔS(J/mol)	ΔG(KJ/mol)
Multiple Sites	$K_{a1}=2.77\times10^5$	$\Delta H_1=-119.0$	$\Delta S_1=-2.95\times10^2$	$\Delta G_1=-31.1$
	$K_{a2}=1.00\times10^3$	$\Delta H_2=147.4$	$\Delta S_2=5.52\times10^2$	$\Delta G_2=-17.1$

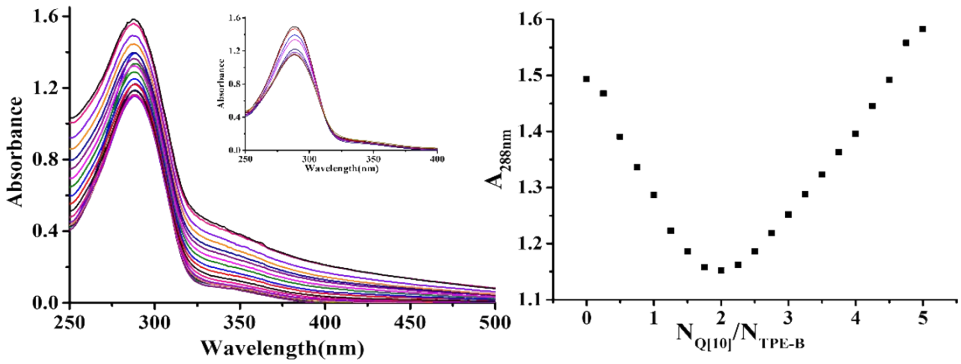

Figure S9. The plot UV absorption spectra of **TPE-B** with an increasing amount of Q[10] (left), and UV-vis plot of $N_{Q[10]}/N_{TPE-B}$ at 288 nm (right).

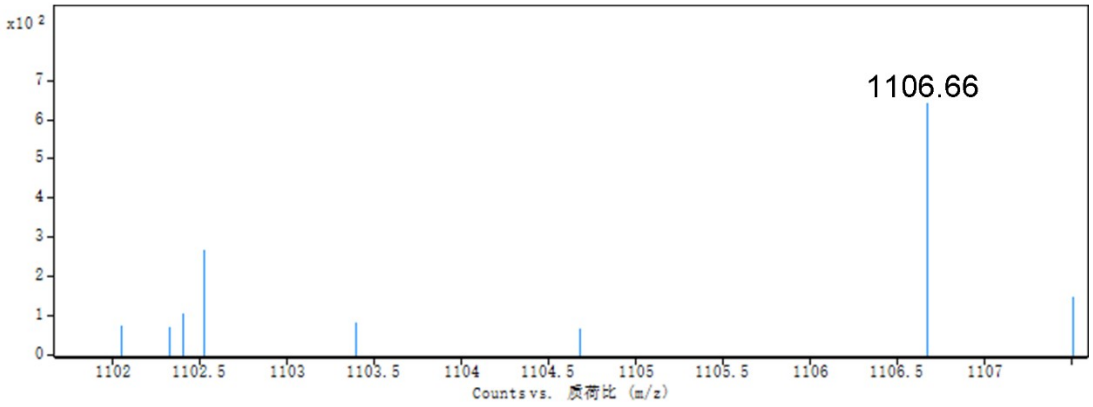

Figure S10. The MASS spectrum of **TPE-B@Q[10]**.

The strongest peak found at m/z = 1106.66 corresponds to $[M]^{4+}$ (theoretical m/z is 1106.86)

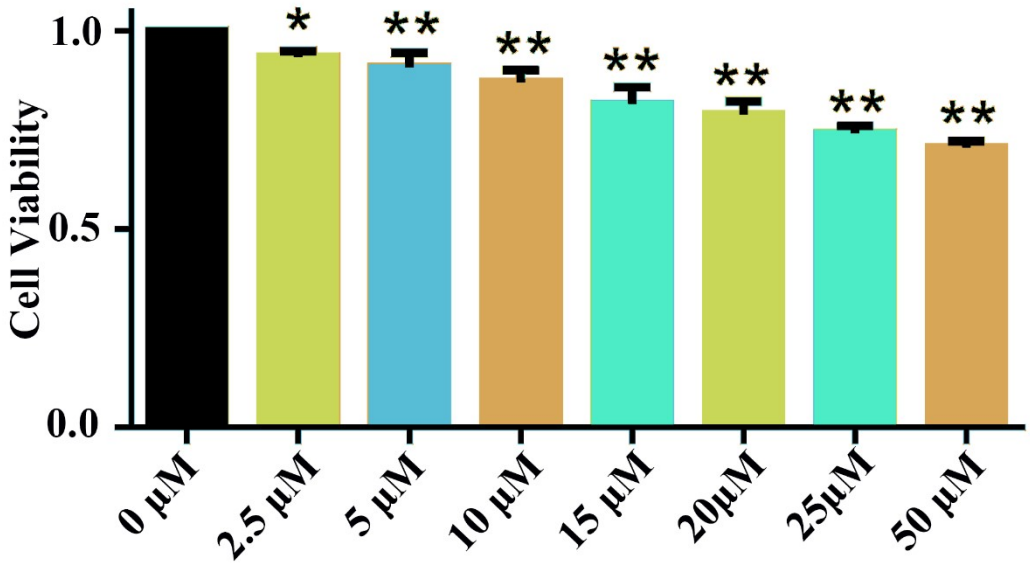

Figure S11. Data were represented as the cell viability under the joint intervention of **TPE-B** and Q[10] (the molar ratio of TPE-B: Q[10] = 1:2), and the abscissa indicates the concentration of **TPE-B**. Data were presented as mean ± SD and normalized to the 0μM group (n = 6). *P < 0.05, **P < 0.01 vs. 0μM group.

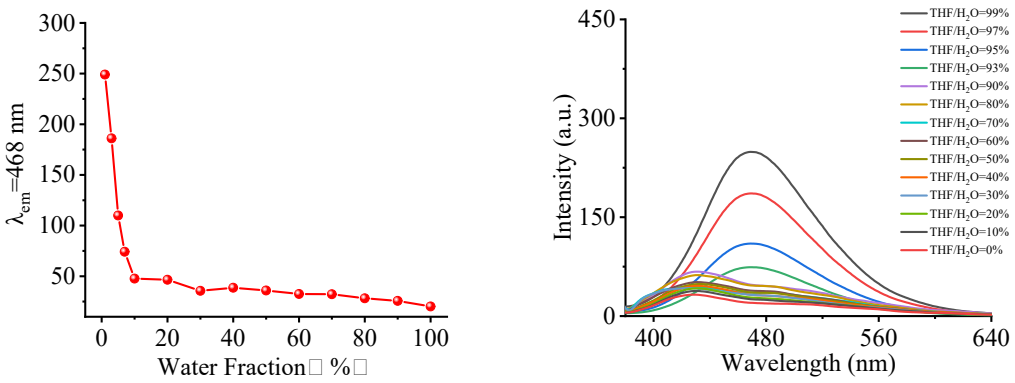

Figure S12. The solvent-dependent experiment of **TPE-B** in THF with different water contents.

Reference

[1] Simin Liu, Peter Y. Zavalij, and Lyle Isaacs, Cucurbit[10]uril, J. Am. Chem. Soc. 2005, 127, 48, 16798-16799.