Electronic Supplementary Information

Cucurbit[8]uril Mediated Triphenylamine Coumarin Derivative Cascade Assembly for NIR Targeted Cell Imaging

Zhen-Hai Yu,[†a] Hui-Juan Wang,[†b] QingWen Cheng,[a] Heng-Yi Zhang[a] and Yu Liu*[a]*

a College of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, P. R. China

b Shandong Provincial Key Laboratory of Chemical Energy Storage and Novel Cell Technology, School of Chemistry and Chemical Engineering, Liaocheng University, Liaocheng 252000, P. R. China

† These authors contributed equally to this work.

*E-mail: hyzhang@nankai.edu.cn; yuliu@nankai.edu.cn

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Section I. Experimental section

1. Instrumentation and methods

Compound **3**, **4** and HACD were synthesized according to previous reports[1-3]. All the reagents and solvents were commercially available and used as received without further purification. Column chromatography was performed on silica gel (200-300 mesh). NMR spectra were recorded on Bruker AV400 instrument at 25 °C and chemical shifts were recorded in parts per million (ppm). High-resolution MS spectra were measured on a Fourier transform ion cyclotron resonance ultra high resolution mass spectrometer. UV/vis spectra were recorded on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller. Photoluminescence were recorded by FS5 instrument (Edinburg Instruments, Livingstone, UK). The samples for TEM measurement were prepared by dropping the solution onto copper grids. The grids were then air-dried. The samples were examined by a high-resolution TEM (Tecnai G2 F20 microscope, FEI) equipped with a CCD camera (Orius 832, Gatan) operating at an accelerating voltage with a CCD camera (Orius 832, Gatan) operating at an accelerating voltage of 200 kV. Fluorescence microscopy images were recorded by a confocal laser scanning microscope (A1R+, Nikon, Japan).

Cell culture. HeLa cell was purchased from Cell Resource Center, Chinese Academy of Medical Science Beijing. HeLa cells were incubated in DMEM nutrient medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were harvested from the cell culture medium by incubating in a trypsin solution for 2 min. After centrifugation for 2 min at 900 rpm, the supernatant was discarded. A protion of serum-supplemented medium (3 ml) was added to neutralize the residual trypsin. Then, the cells were resuspended in serum-supplemented medium and cultured at 37 °C and 5% CO_2. The cells were sub-cultured when the density reached 80%. For fluorescence confocal microscopy, the cells were incubated for 10 hours at 37 °C after treating with samples in medium. Next, Mito Tracker Green, Lyso Tracker Green and Hoechst 33342 cocultured with the cells at 37 °C for 30 min to stain the mitochondria, lysosomes and nucleus. Then, cells were washed 3 times with PBS buffer before imaging by confocal laser scanning microscopy. The excitation wavelengths for the green and red channels were all 405 nm, and the emission wavelength ranges for the green and red channels were 500-545 nm and 650-750 nm, respectively.

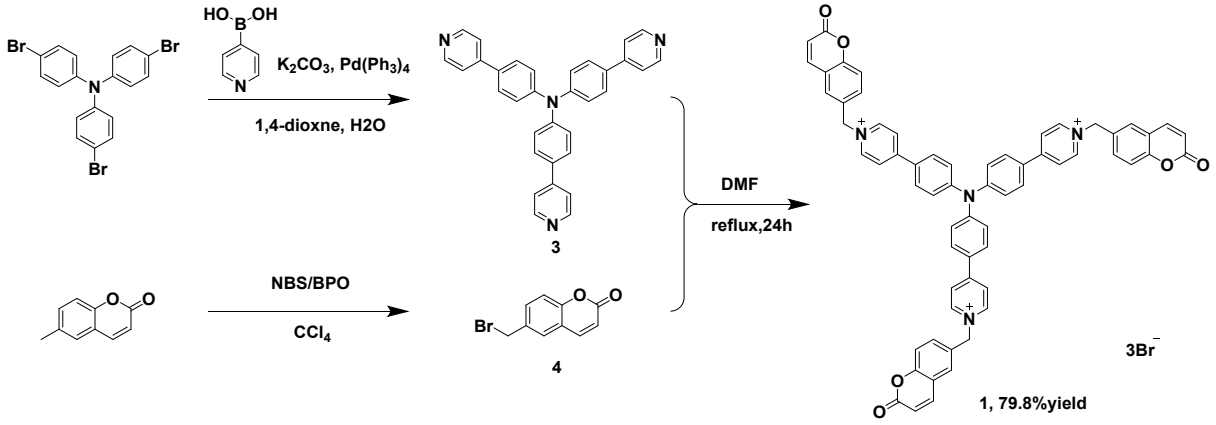
Calculations of Energy-Transfer Efficiency (Φ_{ET}). Energy-transfer efficiency, Φ_{ET}, the fraction of the absorbed energy that is transferred to the acceptor is experimentally measured as a ratio of the fluorescence intensities of the donor in the absence and presence of the acceptor (I_D and I_{DA}).

$$\Phi_{ET} = 1 - I_{DA}/ I_D \quad \text{S1}$$

Where I_D and I_{DA} represent the fluorescence intensities of **1**⊂CB[8]@HACD and **1**⊂CB[8]@HACD-Cy5 at 579 nm (λ_{ex} = 445 nm), respectively.

2. Synthesis and characterization

Synthetic route of **1**



Compound **3** (133 mg, 55.6 μmol) and compound **4** (60mg, 9.4 μmol) were added to DMF (4 ml) and the mixture was stirred for another 24 h at 100 °C. Then the reaction mixture was cooled, and an excess amount of acetone (200 ml) was added the solution. The precipitate was filtered and washed with petroleum ether and acetone to give rise to a yellow solid. (yield, 79.8%). ^{1}H NMR (400 MHz, D_2O-d_2): δ 9.21 (d, 6H), 8.53 (d, 6H), 8.15 (d, 6H), 8.06 (d, 6H), 7.94 (s, 3H), 7.84 (d, 3H), 7.52 (d, 3H), 7.34(d, 6H), 6.56(d, 3H), 5.91(s, 6H). ^{13}C NMR (400 MHz, D_2O): 160.08, 154.32, 154.29, 149.50, 145.14, 144.25, 132.82, 131.17, 130.60, 129.43, 129.24, 125.36, 124.57, 119.52, 117.78, 117.57, 61.80. HRMS (ESI) *m/z* for **1** ($C_{63}H_{45}N_4O_6Br_3$) calcd. [**1**-3Br]$^{3+}$/3: 317.7774, found:317.7777.

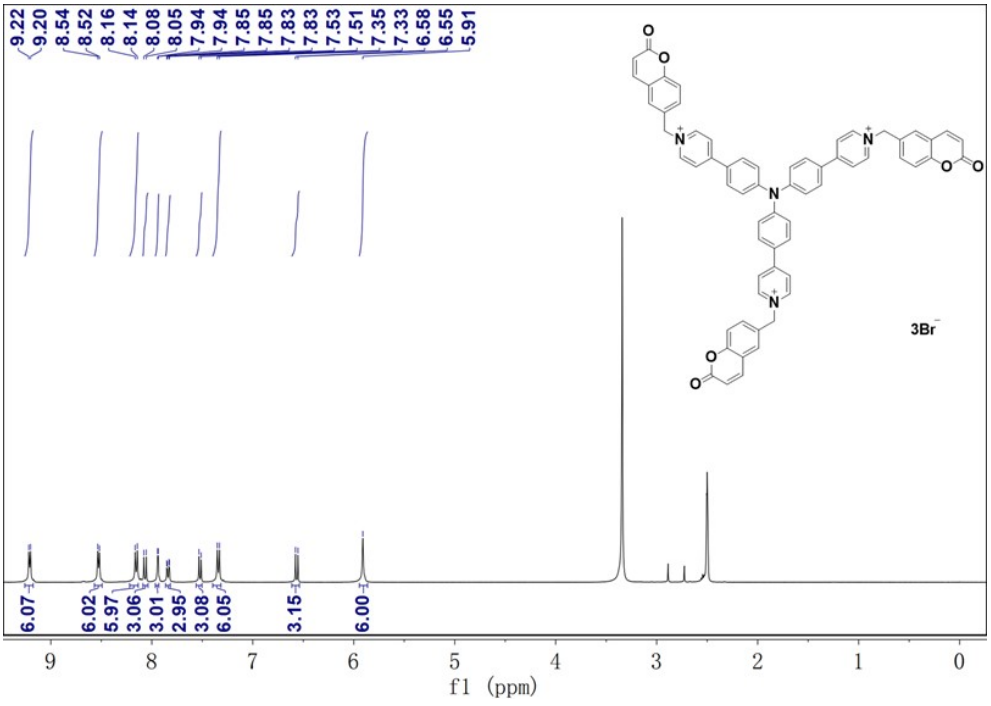


Fig. S1 ^{1}H NMR spectrum of **1** in D_2O, 400 MHz, 298K.

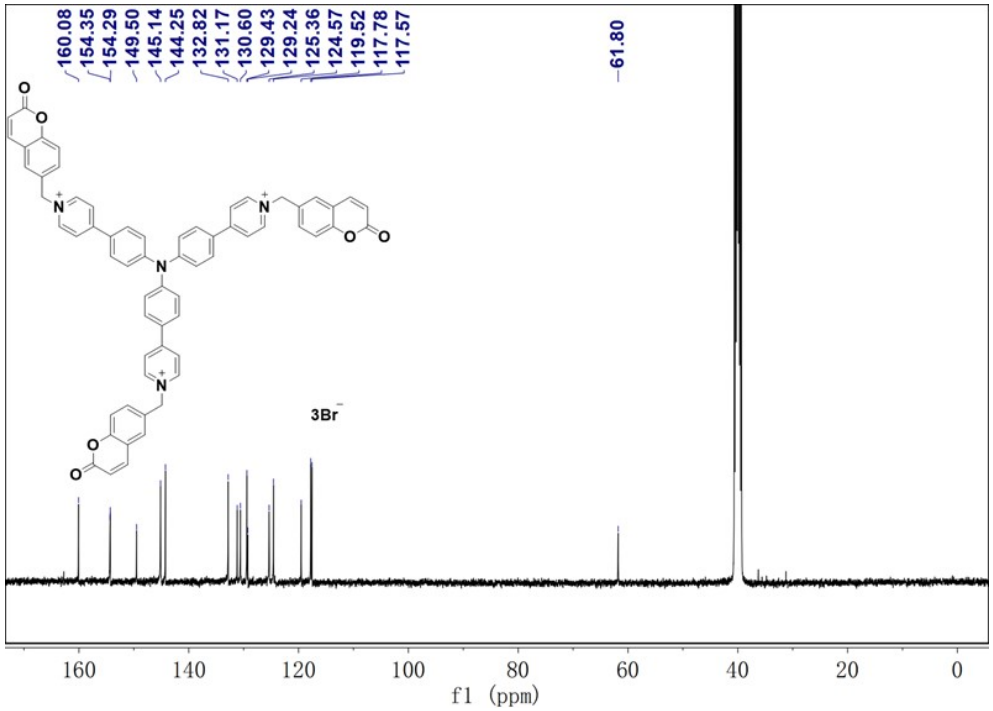


Fig. S2 ^{13}C NMR spectrum of **1** in D_2O, 400 MHz, 298K.

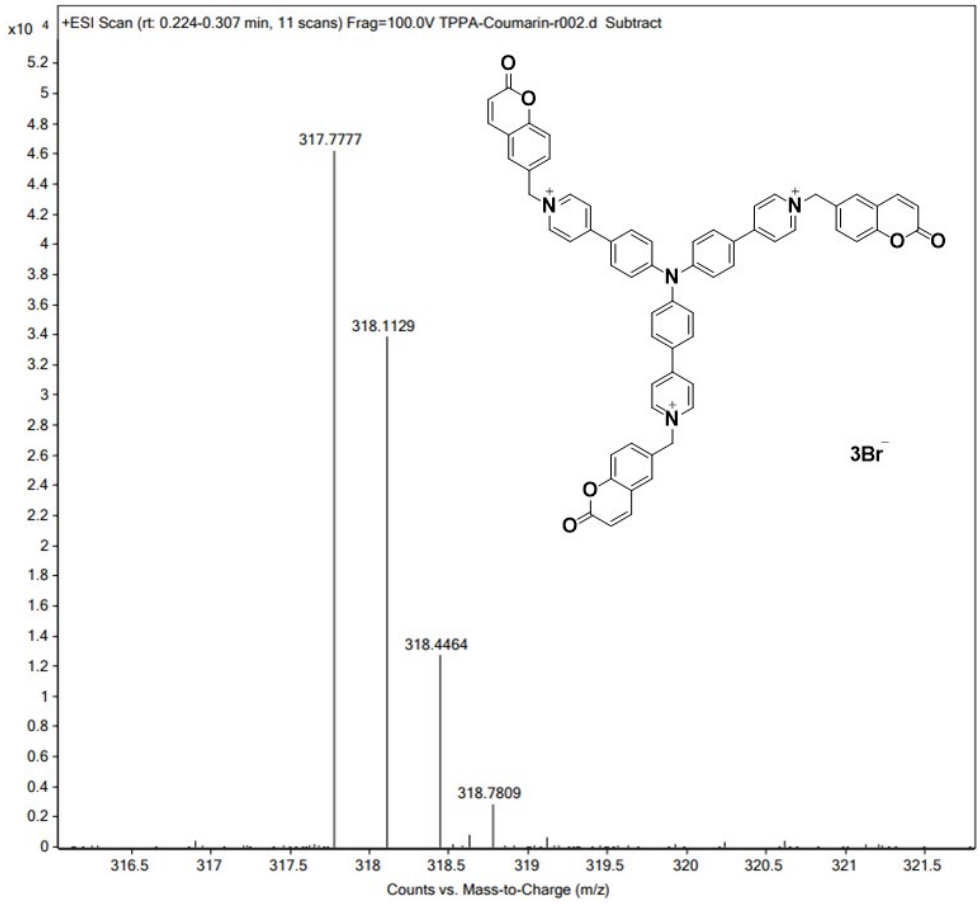


Fig. S3 HRMS (ESI) *m/z* for **1** ($C_{63}H_{45}N_4O_6Br_3$) calcd. [**1**-3Br]$^{3+}$/3: 317.7774, found: 317.7777.

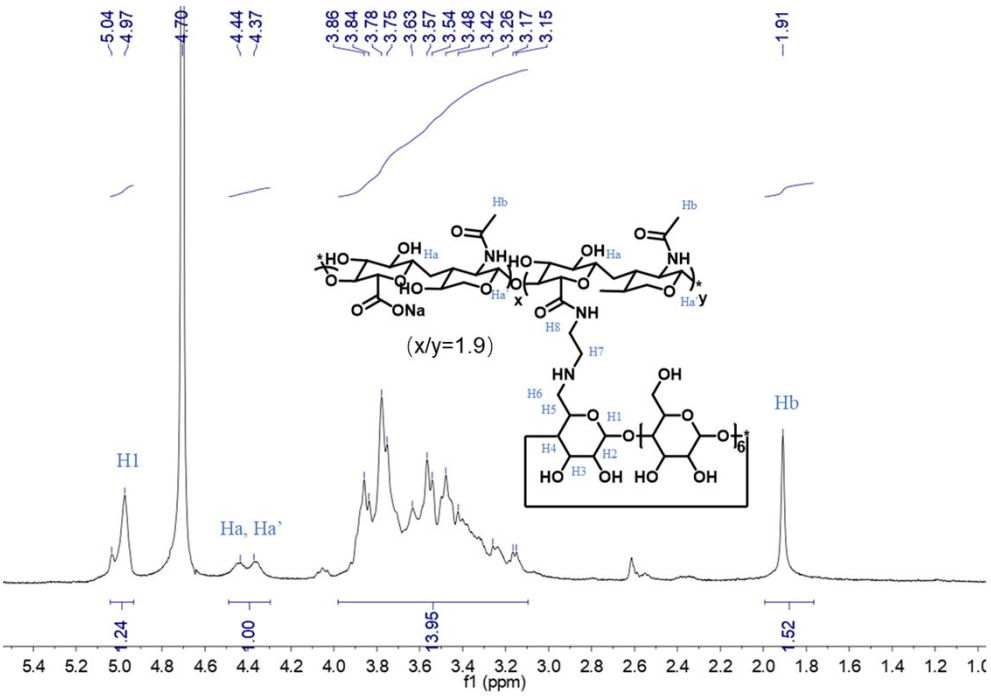


Fig. S4 ^{1}H NMR spectra of HACD (400 MHz, D_2O, 298K).

3. Characterization

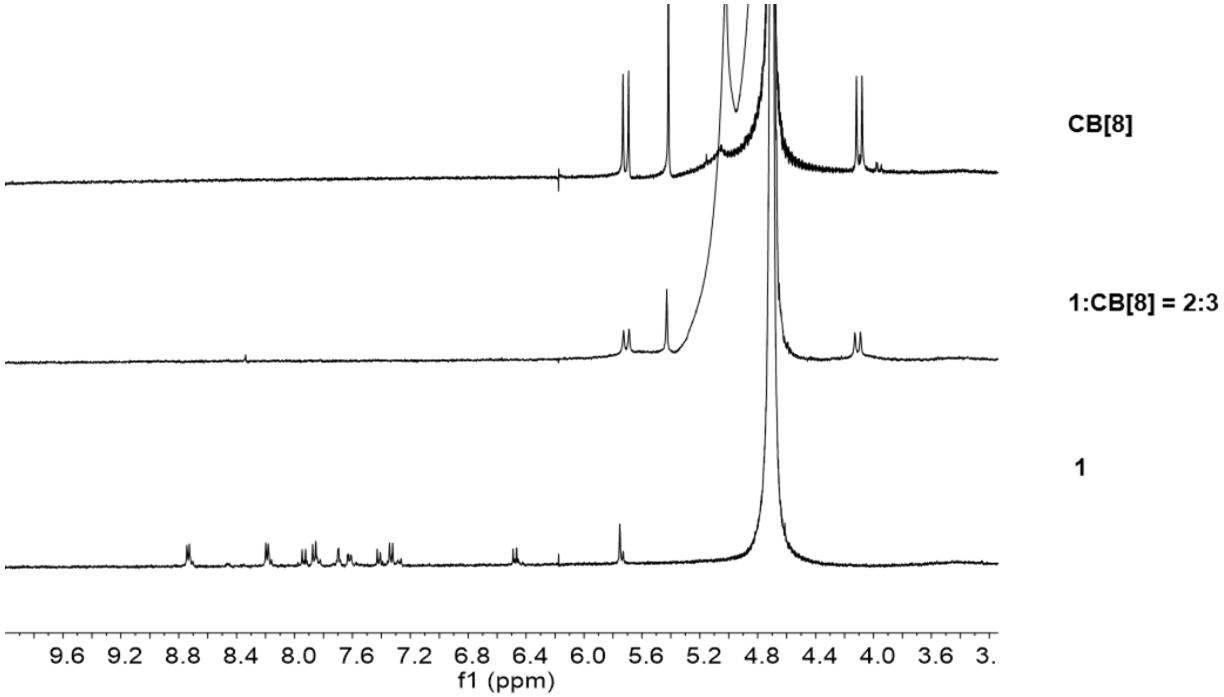


Fig. S5 ^{1}H NMR spectral changes of **1** (2×10^{-4} M), **1**:CB[8] = 2:3 and CB[8] (400 MHz, D_2O, 298 K).

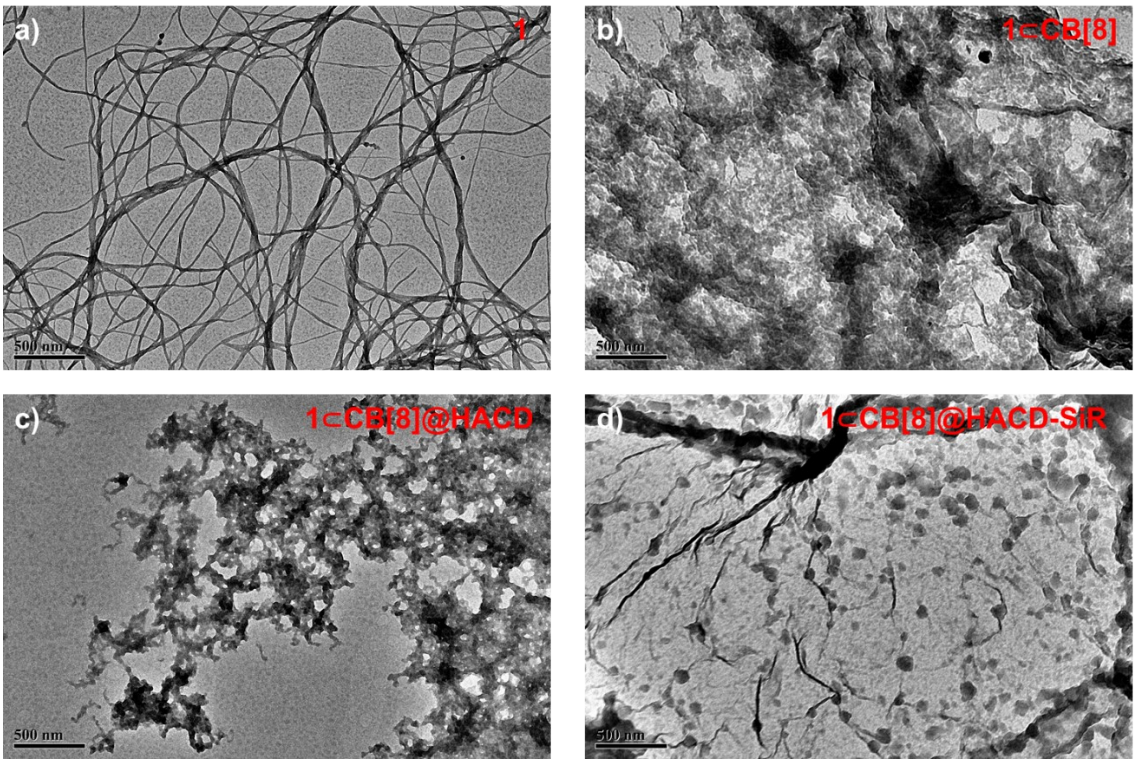


Fig. S6 TEM images of (a) **1**, (b) **1**⊂CB[8], (c) **1**⊂CB[8]@HACD, (d) **1**⊂CB[8]@HACD-SiR ([**1**] = 3.0×10^{-5} M, CB[8] = 4.5×10^{-5} M, [HACD] = 7.5×10^{-2} mg/ml, [SiR] = 6.0×10^{-6} M).

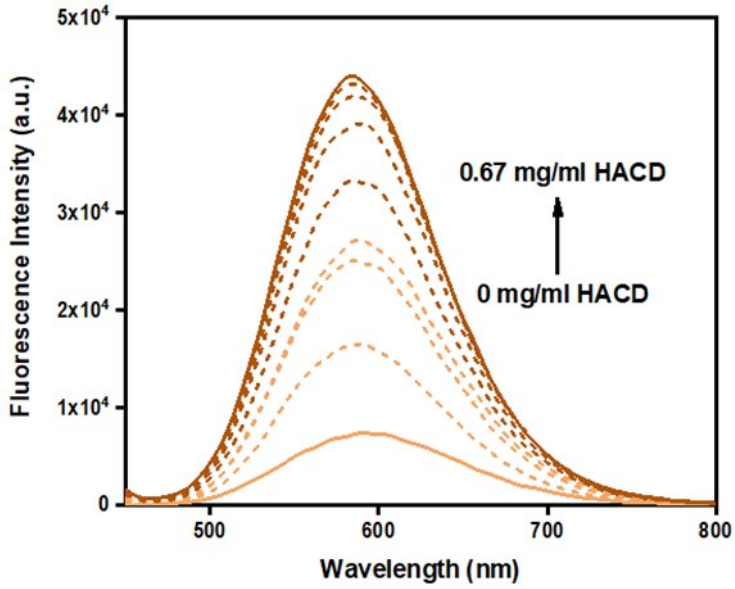


Fig. S7 Fluorescence emission spectra of **1** with increasing the doping concentration of HACD in water. ([**1**] = 1.0 × 10^{-5} M, λ_{ex} = 445 nm, E_x/E_m slit = 3 nm).

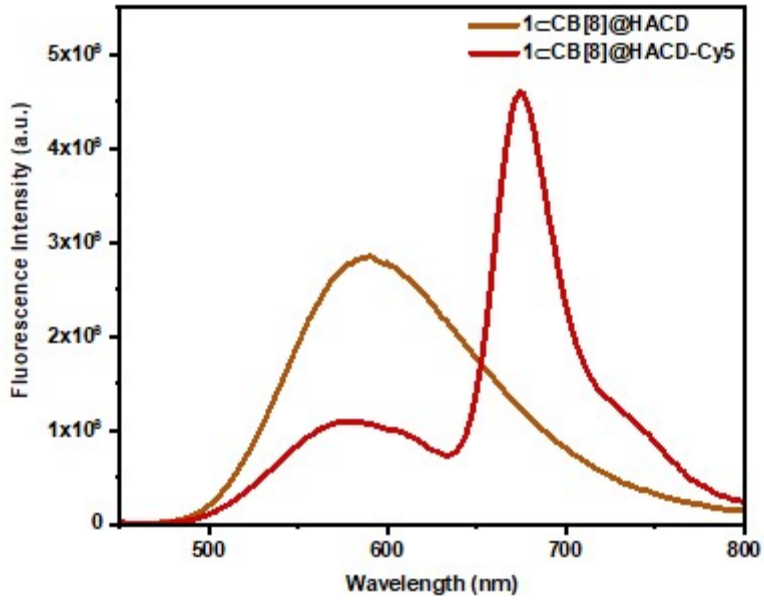


Fig. S8 Fluorescence emission spectra of **1**⊂CB[8]@HACD and **1**⊂CB[8]@HACD-Cy5 in water. ([**1**] = 1.0 × 10^{-5} M, [CB[8]] = 1.5 × 10^{-5} M, [HACD] = 2.5 × 10^{-2} mg/ml, [Cy5] = 1.0 × 10^{-5} M, λ_{ex} = 445 nm, E_x/E_m slit = 3 nm). The Φ_{ET} value was calculated as 62.6%.

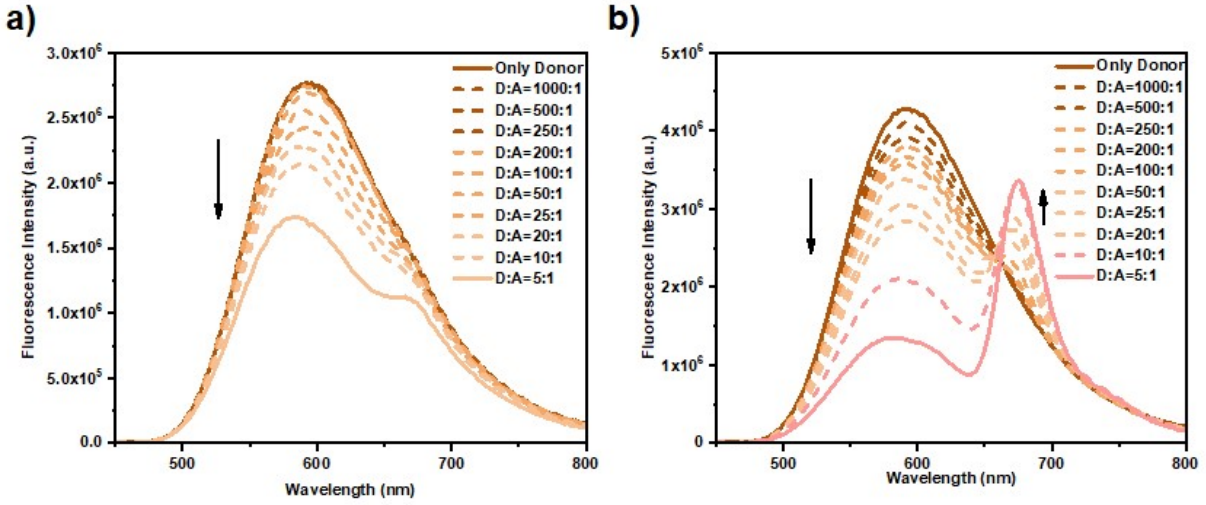


Fig. S9 Fluorescence emission spectra of **1**⊂CB[8]@HACD with increasing the doping concentration of a) NiB; b) SiR in water ([**1**] = 1.0 × 10^{-5} M, [CB[8]] = 1.5 × 10^{-5} M, [HACD] = 2.5 × 10^{-2} mg/ml, λ_{ex} = 445 nm, E_x/E_m slit = 3 nm).

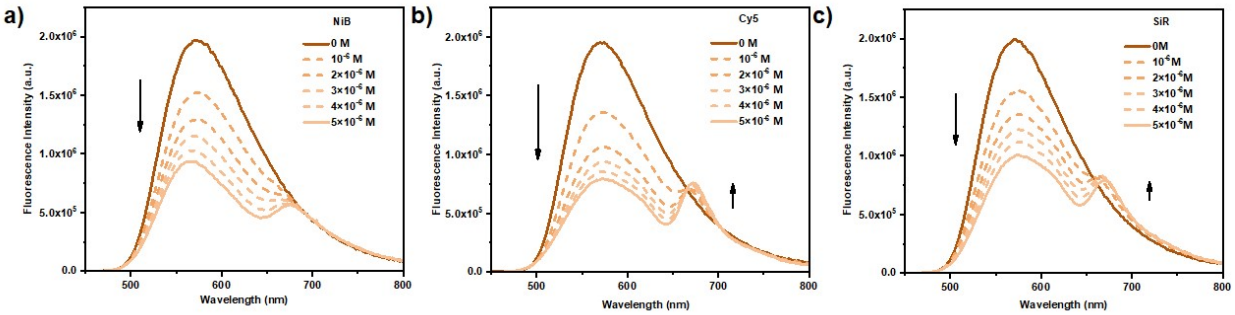


Fig. S10 Fluorescence emission spectra of **1**⊂CB[8] with increasing the doping concentration of a) NiB; b) Cy5; c) SiR in water. ([**1**] = 1.0 × 10^{-5} M, [CB[8]] = 1.5 × 10^{-5} M, λ_{ex} = 445 nm, E_x/E_m slit = 3 nm).

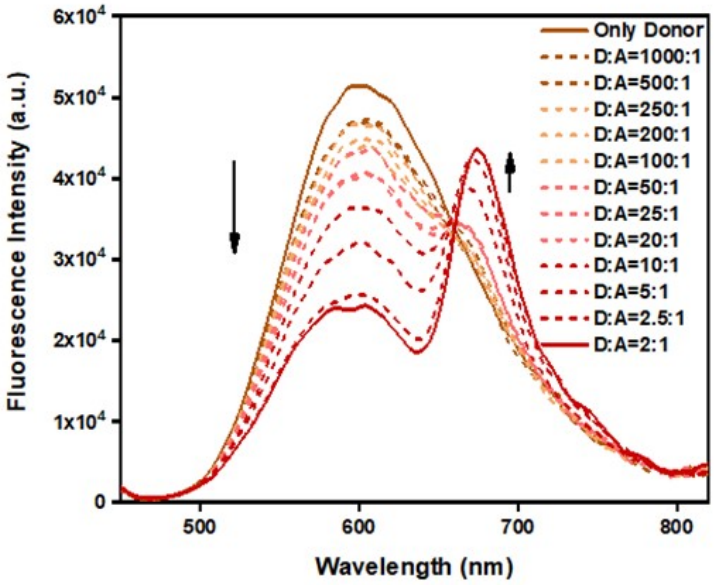


Fig. S11 Fluorescence emission spectra of **1**@HACD with increasing the doping concentration of Cy5 in water ([**1**] = 1.0 × 10^{-5} M, [HACD] = 2.5 × 10^{-2} mg/ml, λ_{ex} = 445 nm, E_x/E_m slit = 3 nm).

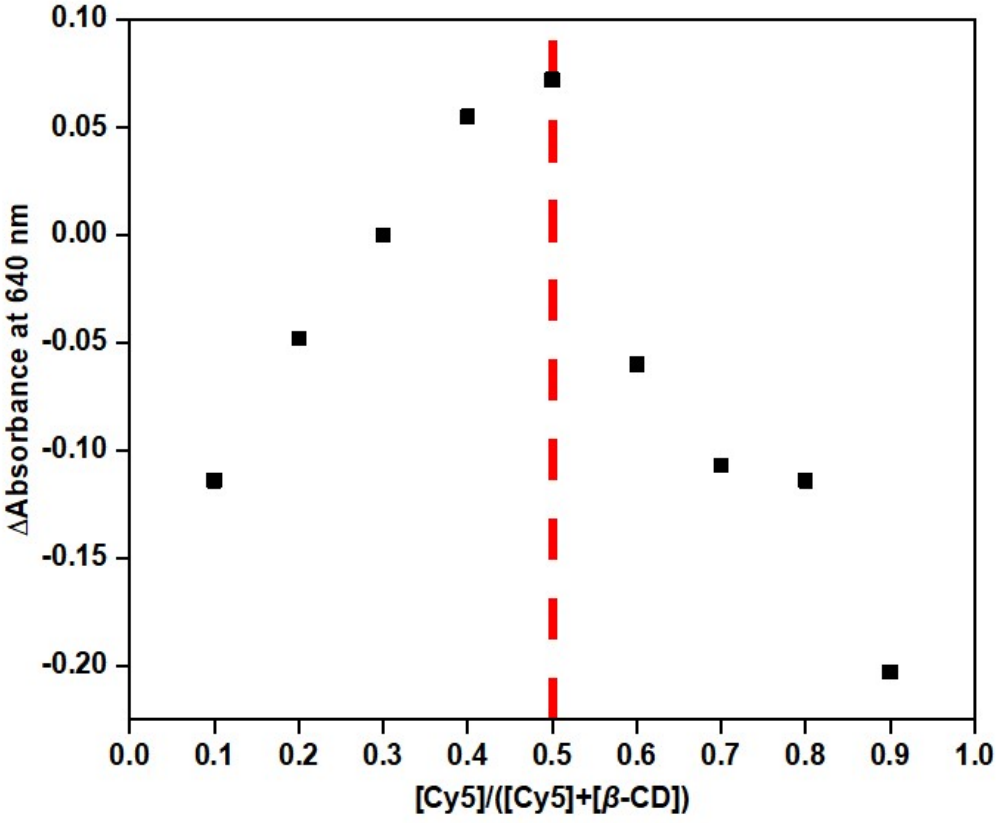


Fig. S12 Job's plot obtained by recording the absorbance for the solution of Cy5 and *β*-CD in water at 298K, confirming the 1:1 stoichiometry of their complex.

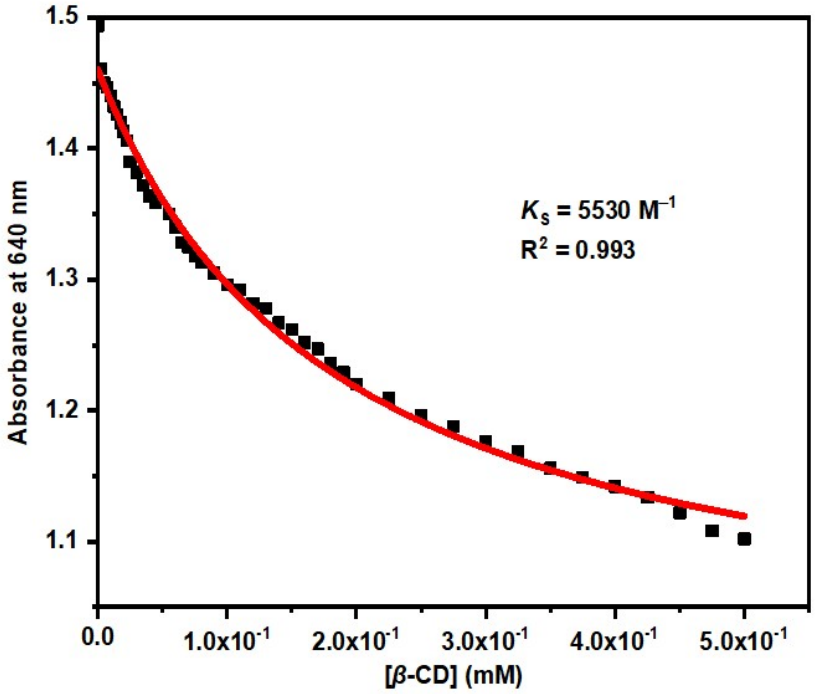


Fig. S13 Nonlinear least-squares analysis of the absorbance changes for Cy5 with *β*-CD in water in the UV-Vis titration experiments to calculate the association constant.

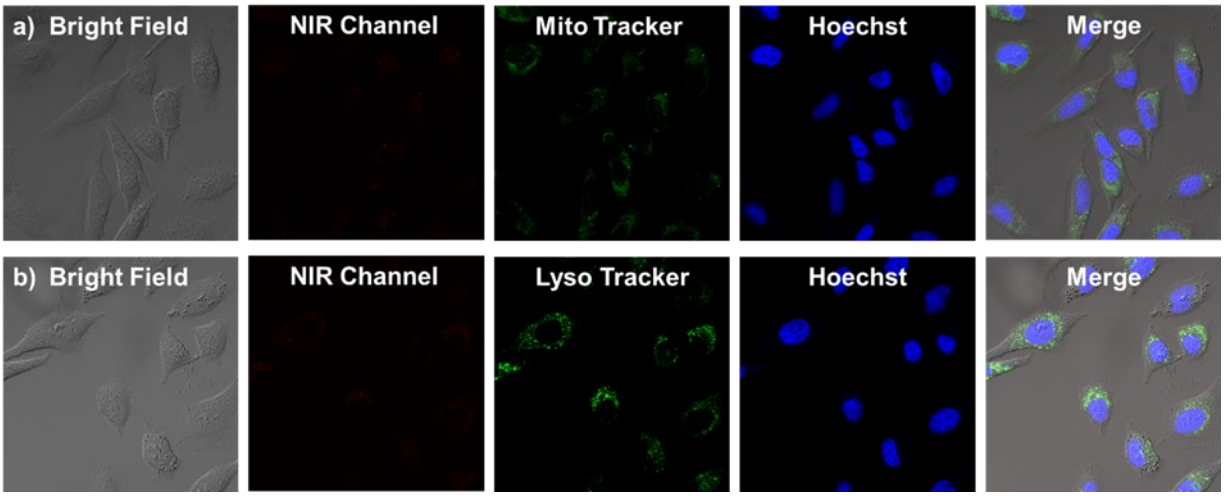


Fig. S14 The confocal fluorescence images of living HeLa cells incubated with **1**⊂CB[8]@HACD-SiR for Hoechst and a) Mito Tracker, b) Lyso Tracker ([**1**] = 4×10^{-7} M, [CB[8]] = 6×10^{-7} M, [HACD] = 10^{-3} mg/ml, [SiR] = 8×10^{-8} M).

Section II. References

[1] Z.-Q. Yao, J. Xu, B. Zou, Z. Hu, K. Wang, Y.-J. Yuan, Y.-P. Chen, R. Feng, J.-B. Xiong, J. Hao and X.-H. Bu, *Angew. Chem. Int. Ed.*, 2019, **58**, 5614-5618.

[2] Y. Li, Q. Li, X. Miao, C. Qin, D. Chu and L. Cao, *Angew. Chem. Int. Ed.*, 2021, **60**, 6744-6751.

[3] X.-Y. Dai, M. Huo, X. Dong, Y.-Y. Hu and Y. Liu, *Adv. Mater*., 2022, 34, 2203534.