Electronic Supplementary Information for

Significant emission enhancement of a bola-amphiphile with salicylaldehydeazine moiety induced by the formation of [2]pseudorotaxane with γ-cyclodextrin

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Experimental

General method and materials

Unless specifically mentioned, all chemicals are commercially available and were used as received. NMR spectra were obtained on a Bruker Avance 400 spectrometer (400 MHz and 500 MHz ^{1}H NMR) at room temperature. UV-vis spectra were obtained on a Shimadzu UV-1601PC spectrophotometer. Steady-state fluorescence measurements were carried out using a Hitachi 4500 spectrophotometer.

Cell Culture

HeLa (human cervical cancer) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS, penicillin (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}). The culture was maintained in a humidified atmosphere with 5% CO_2 at 37 ^oC.

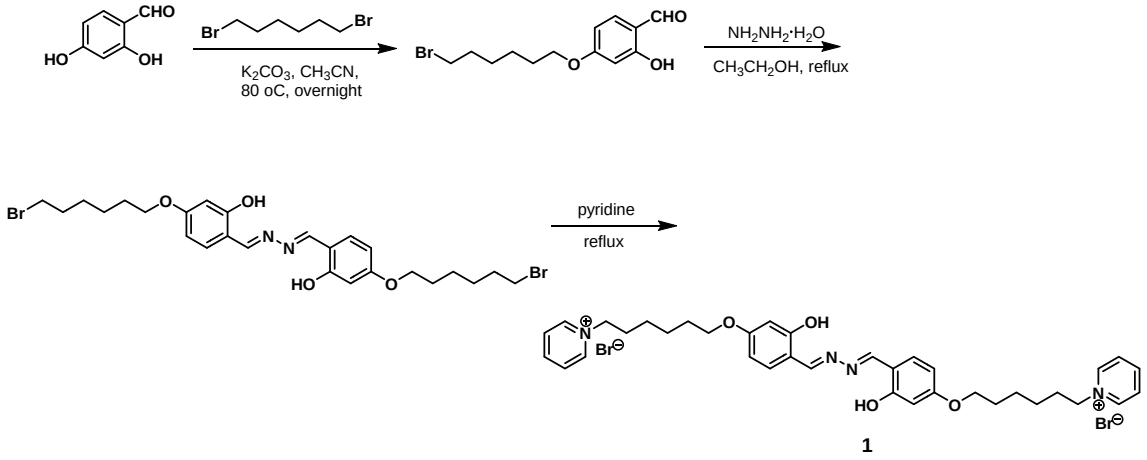
Confocal Laser Scanning Microscopy (CLSM)

HeLa cells were seeded in 35 mm plastic-bottomed Ibidi μ–dishes and allowed to grow for 24 h. After incubation with **1@γ-CD** or **1** for 20 min, the cells were washed three times with PBS (pH 7.4) and then stained with 0.05 μM of MitoTracker Deep Red FM for 20 min (by adding 1 μL of a 50 μM stock solution of MitoTracker Deep Red FM in DMSO to 1 mL culture medium). After washing with PBS, the cells were subjected to confocal microscopy observation (100× oil objective, 408/561 nm excitation).

MTT Cytotoxicity Assay

HeLa cells were seeded into a 96-well plate at a density of 1×10^4 cells per well and allowed to grow for 24 h. The culture medium was then replaced with complete DMEM medium (100 μL) containing **1@γ-CD, 1** or MitoTracker Deep Red FM at different concentrations. After incubation for 12 h, the medium in each well was replaced with 100 μL of MTT solution (0.5 mg mL^{-1} in medium). After another 4 h of incubation, the medium was removed, and DMSO (100 μL) was added into each well. The plate was gently shaken for 10 min to dissolve the formazan crystals, and the absorbance at 570 nm was recorded using a microplate reader (Synergy H4, BioTek). The spectrophotometer was calibrated to zero absorbance using culture medium without cells. The relative cell viability compared to control wells that were only treated with medium was calculated from $[A]_{test}/[A]_{control}$, where $[A]_{test}$ and $[A]_{control}$ are the average absorbance of the test and control samples, respectively.

Synthesis and characterization



Scheme S1. Synthesis of compound**1** was followed by the literature procedure.[S1] The ^{1}H NMR of **1** in d_6-DMSO confirmed its identity.

^{1}H NMR (400 MHz, DMSO) δ 11.48 (s, 2H), 9.12 (s, 4H), 8.86 (s, 2H), 8.61 (t, *J* = 7.5 Hz, 2H), 8.17 (t, *J* = 7.0 Hz, 4H), 7.53 (d, *J* = 8.7 Hz, 2H), 6.55 (dd, *J* = 8.6, 2.3 Hz, 2H), 6.50 (d, *J* = 2.1 Hz, 2H), 4.62 (d, *J* = 3.4 Hz, 4H), 4.01 (t, *J* = 6.3 Hz, 4H), 2.03 – 1.89 (m, 4H), 1.79 – 1.65 (m, 4H), 1.46 (dt, *J* = 14.7, 7.3 Hz, 4H), 1.40 – 1.29 (m, 4H).

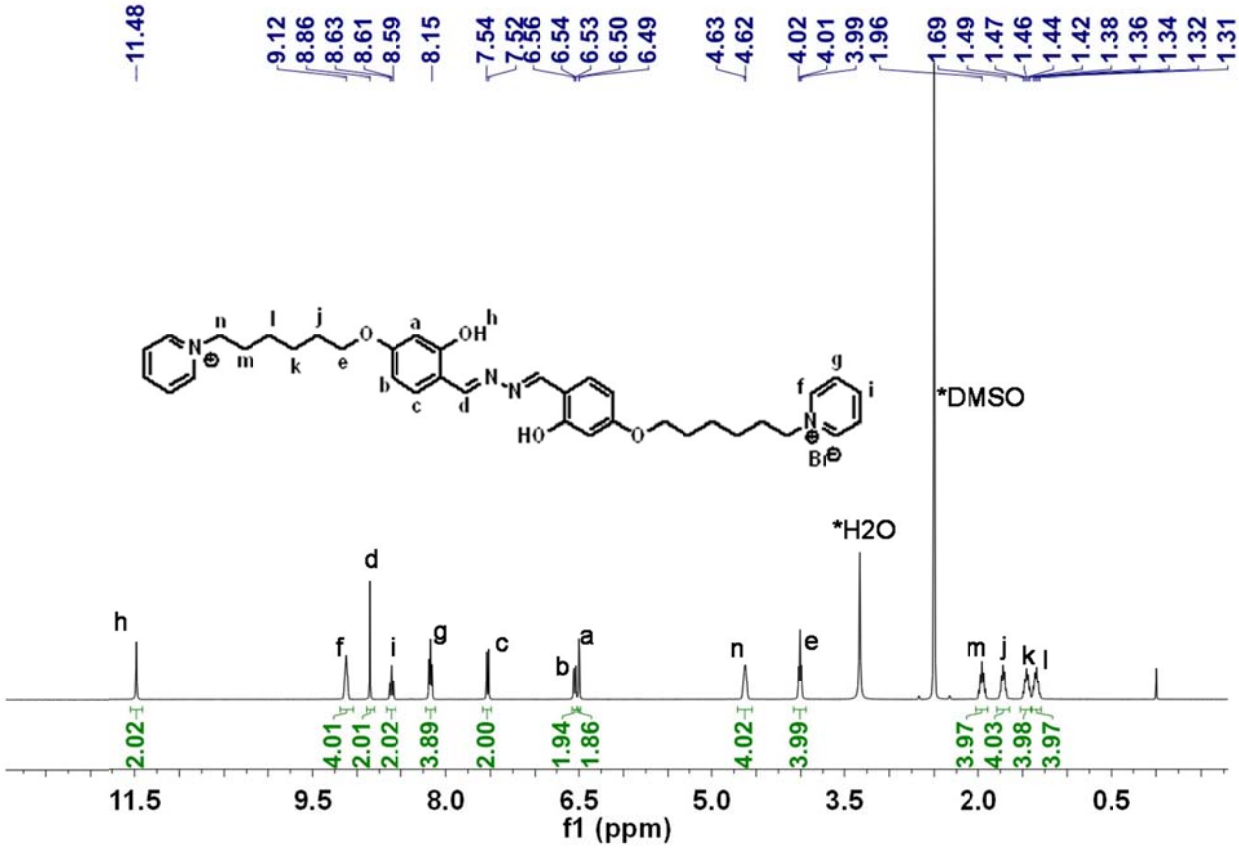


Fig.S1 ^{1}H NMR spectrum of compound **1** in d_6-DMSO.

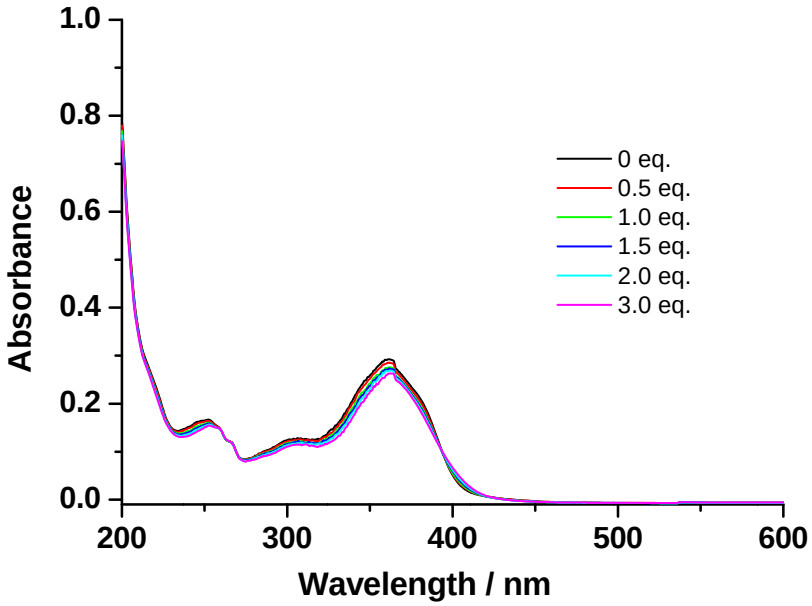


Fig. S2 Absorption spectra of **1** (10 μM) upon titration of γ-CD (0−3.0 equiv) in PB buffer solution (10 mM, pH 7.0).

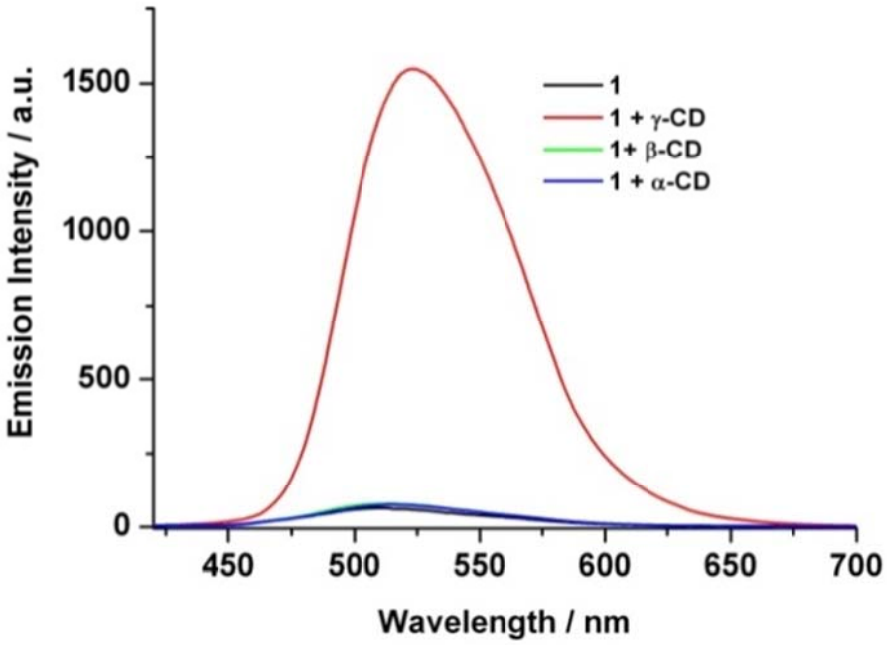


Fig. S3 Fluorescence spectra (λ_{ex} = 400 nm) of **1** (50 μM) in the presence of α-, β-, and γ-CDs (1.0 equiv) in PB buffer solution (10 mM, pH 7.0).

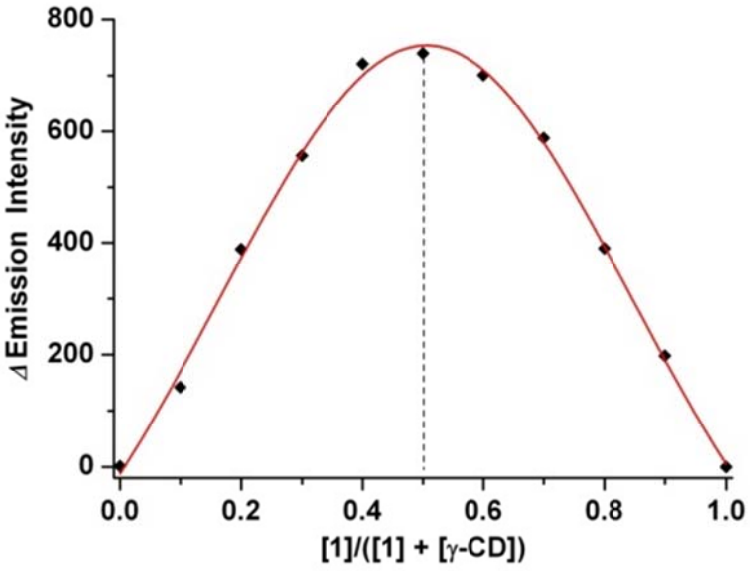


Fig. S4 Job's plot of the change in emission spectra at 523 nm, [**1**] + [**γ-CD**] = 50 μM.

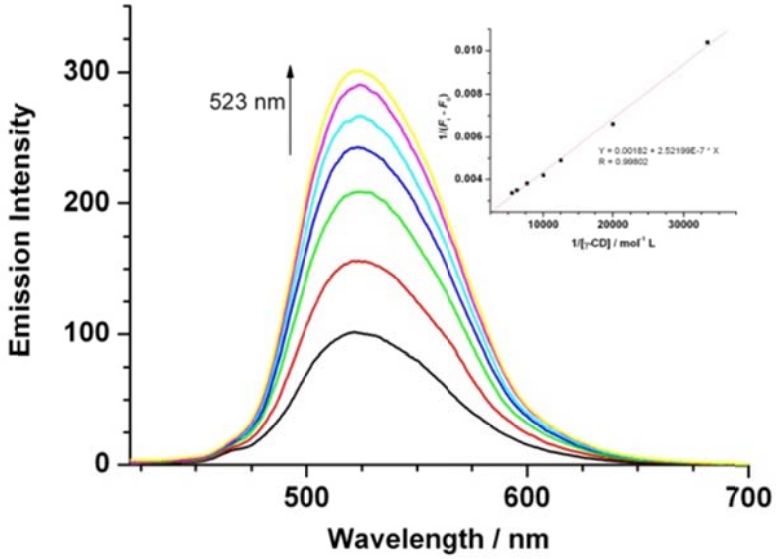


Fig. S5 The family of fluorescence spectra of **1** (10μM) at different concentrations of γ-CD (30 μM-180 μM). Inset: plot of the $1/(F_i-F_0)$ vs. 1/[γ-CD].

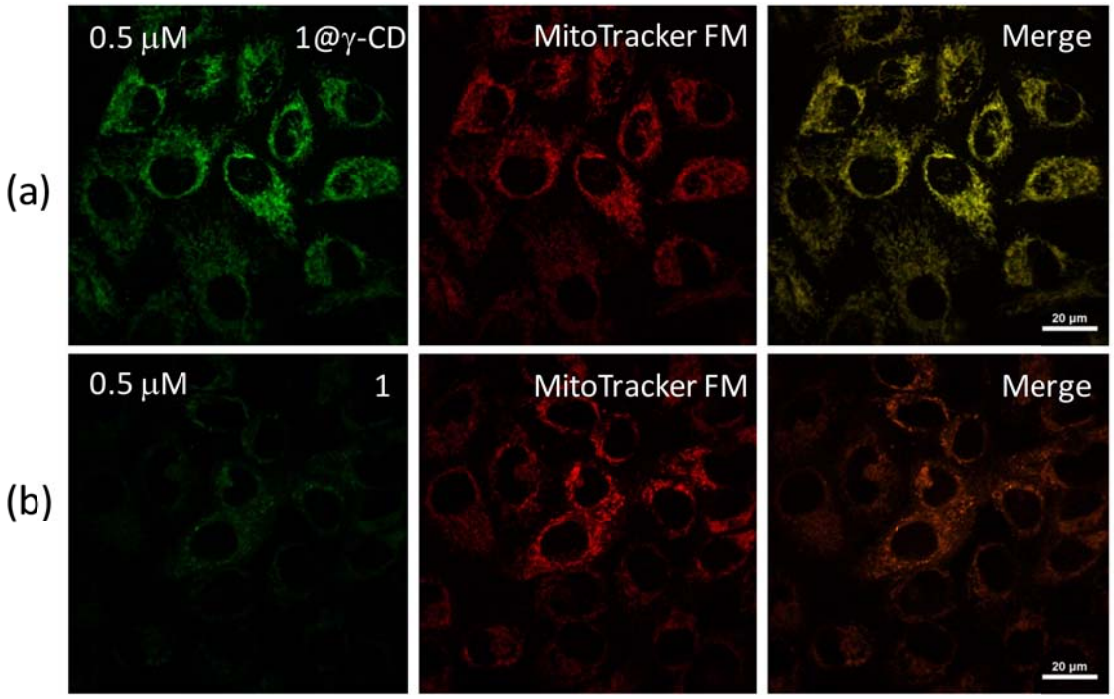


Fig. S6 CLSM images of HeLa cells incubated with (a) **1@γ-CD** and (b) **1** at the concentration of 0.5 μM. Scale bar: 20 μm. Excitation and emission wavelength: 408 nm and 500–550 nm for **1@γ-CD** and **1**; 561 nm and 570-1000 nm for MitoTracker Deep Red FM.

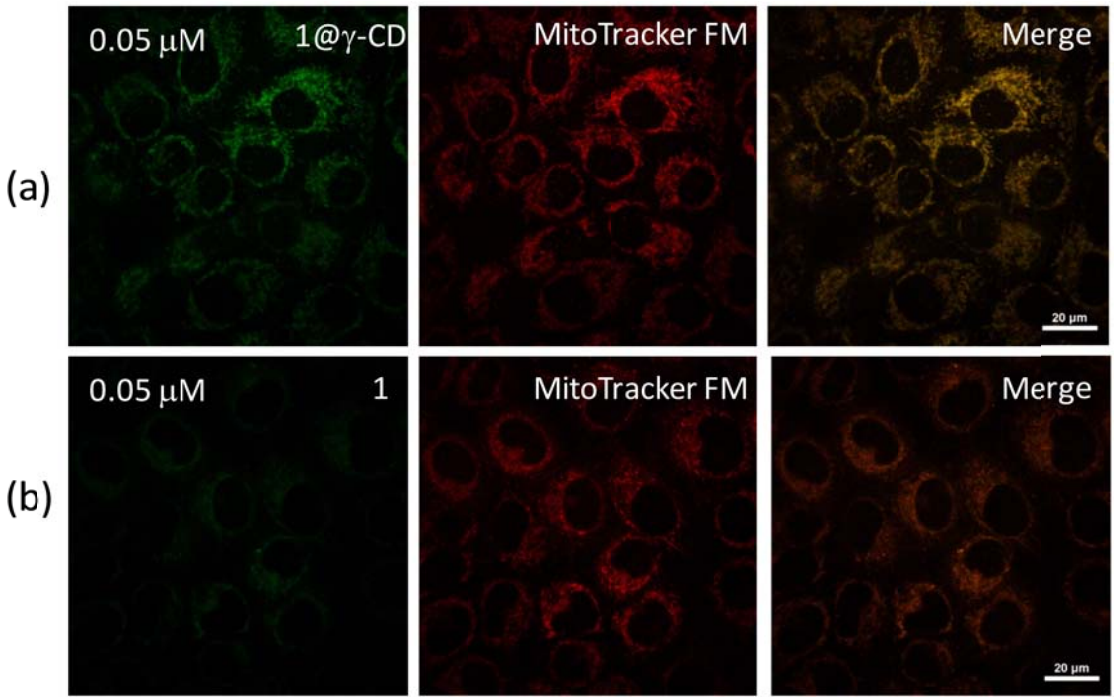


Fig. S7 CLSM images of HeLa cells incubated with (a) **1@γ-CD** and (b) **1** at the concentration of 0.05 μM. Scale bar: 20 μm. Excitation and emission wavelength: 408 nm and 500–550 nm for **1@γ-CD** and **1**; 561 nm and 570-1000 nm for MitoTracker Deep Red FM.

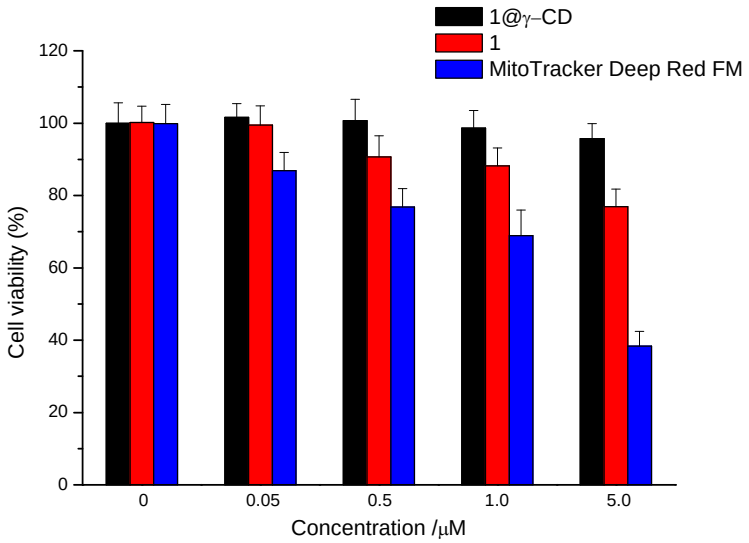


Fig. S8 Viability of HeLa cells incubated with **1@γ-CD, 1** or MitoTracker Deep Red FM at different concentrations (0, 0.05, 0.5, 1.0, 5.0 μM) for 12 h. Data are expressed as mean values ± SD (n = 6).

References:

S1. M. Gao, C. K. Sim, C. W. T. Leung, Q. Hu, G. Feng, F. Xu, B. Z. Tang and B. Liu, *Chem. Commun.*, 2014, **50**, 8312.