Host-guest assemble of adamantyl tethered squaraine in β-cyclodextrin for monitoring pH in living cells

Wenjian Xu,[a,b] Xiaochan Zhu, [a,b] Guimei Wang, [a,b] Chuanguo Sun, [a,b] Qingfeng Zheng, [a,b]

Huanghao Yang[b,]* and Nanyan Fu [a,b,]*

[a]*Research Institute of Photocatalysis, State Key Laboratory of Photocatalysis on Energy and Environment, Fuzhou University, Fuzhou 350002, P. R. China.*

[b]*Key Laboratory of Analysis and Detection for Food Safety, Ministry of Education & Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, and Department of Chemistry, Fuzhou University, Fuzhou 350108, P. R. China.*

*E-mail: nanyan_fu@fzu.edu.cn

Supplementary Supporting Information

Contents:

1. Experimental details

1.1. Materials and general methods

The ^{1}H NMR spectra were recorded on Bruker AV-400 and JEOL JNM LA-500 spectrometer and the chemical shifts were measured relative to TMS (0.00 ppm). FTIR spectra were recorded on a Perkin Elmer Spectrum 2000 Fourier Transform Infrared Spectrophotometer. MS and HRMS were recorded on DECAX-30000 LCQ Deca XP Ion Trap Mass Spectrometer and Applied Biosystems (Sciex) QStar Mass Spectrometer by positive ESI-Q-TOF, respectively. Absorption spectra were detected on a Perkin Elmer Lambda750 UV spectrophotometer. Fluorescent emission spectra were collected on a Cary Edipse fluorescence spectrophotometer. Melting points of compounds were determined with SGW X-4 and were uncorrected. All the solvents were redistilled before use. Except for specific note, other chemicals and reagents were obtained from commercial suppliers and used without further purification. The syntheses and manipulations of squaraine dyes were carried out under dry N_2 atmosphere.

1.2. Synthesis

1.2.1. Synthesis of N-(2-adamantyl)aniline (**2**)

2-Adamantanone (**1**) (1.50 g, 10 mmol) was ground with aniline (0.93 g, 10 mmol) for 10 min in an agate mortar and a pestle at room temperature under solvent-free conditions. To the resulting mixture was added $NaBH_4$ (0.38 g, 10 mmol) and boric acid (0.62 g, 10 mmol), and then the mixture was ground under identical conditions until TLC showed complete disappearance of the starting ketone **1**. The reaction mixture was quenched with water (30 mL) and extracted with CH_2Cl_2. The combined extract was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude products obtained were further purified by column chromatography on silica gel with ethyl acetate/petroleum ether (1/30 v/v) to afford a white solid (1.41 g, 6.20 mmol). Yield: 62%. m.p. 57-58 ˚C [1]. IR (KBr): 3419, 2903, 2842, 1599, 1498, 1445, 1420, 1311, 1128, 747, 688 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58-1.61 (d, J = 12.3 Hz, 2H) , 1.75 (s, 2H), 1.81-1.84 (d, J = 10.4 Hz, 4H), 1.89-1.94 (dd, J = 14.1, 7.9 Hz, 5H), 2.03 (s, 2H), 3.54 (s, 1H), 6.60 (d, J = 8.0 Hz, 2H), 6.65 (t, J = 7.3 Hz, 1H), 7.15 (t, J = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.35, 27.49, 31.62, 31.63, 37.43, 37.74, 56.80, 113.04, 116.70, 129.27, 147.41.

1.2.2. Synthesis of **SQ**

A mixture of **2** (114 mg, 0.5 mmol), squaric acid (29 mg, 0.25 mmol), toluene (10 mL) and *n*-

butanol (10 mL) was refluxed at 120 ˚C for 24 h under nitrogen while the water formed was azeotropically removed by using a Dean–Stark trap. After cooling to room temperature, the solvent was removed under reduced pressure and then the residue was purified by column chromatography on silica gel with ethyl acetate/petroleum ether (1:4, v/v) as eluent and eluting again with methanol/chloroform (1:100 to 1:30, v/v) to afford a blue solid (47 mg, 0.088 mmol). Yield: 35%. m.p. >300 ˚C. IR (KBr): 3296, 2898, 2845, 1611, 1583, 1538, 1485, 1465, 1384, 1163, 1107, 846, 804, 750, 518 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 5:1, v/v): δ 1.67-1.70 (d, *J* = 12.6 Hz, 4H), 1.80 (s, 4H), 1.87-1.97 (dd, *J* = 29.6, 11.8 Hz, 18H), 2.08 (s, 4H), 3.76 (s, 2H), 6.73 (d, *J* = 7.7 Hz, 4H), 8.25 (d, *J* = 7.6 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 5:1, v/v) δ 27.22, 27.24, 31.52, 31.76, 37.21, 37.46, 57.04, 113.80, 119.92, 133.75, 154.49, 184.42, 184.92; ESI-MS: *m*/*z* 531.9 ($[M-H]^-$). ESI-HRMS: Calcd for $C_{36}H_{41}N_2O_2$ ($[M+H]^+$): 533.3168, Found: 533.3173.

[1] Averin, A. D. Palladium-catalyzed amination of isomeric dihalobenzenes with 1- and 2-aminoadamantanes. *Russian Journal of Organic Chemistry* **2010**, *46*, 64-72.

2. Characterization of **2** and **SQ**

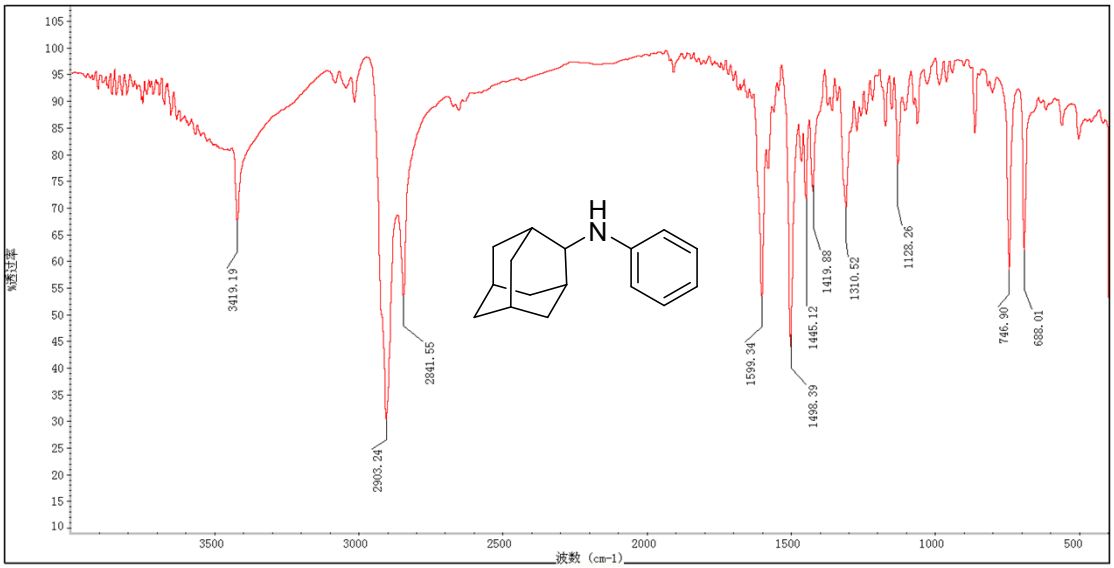


Figure S1. The IR spectrum of **2** in KBr disc.

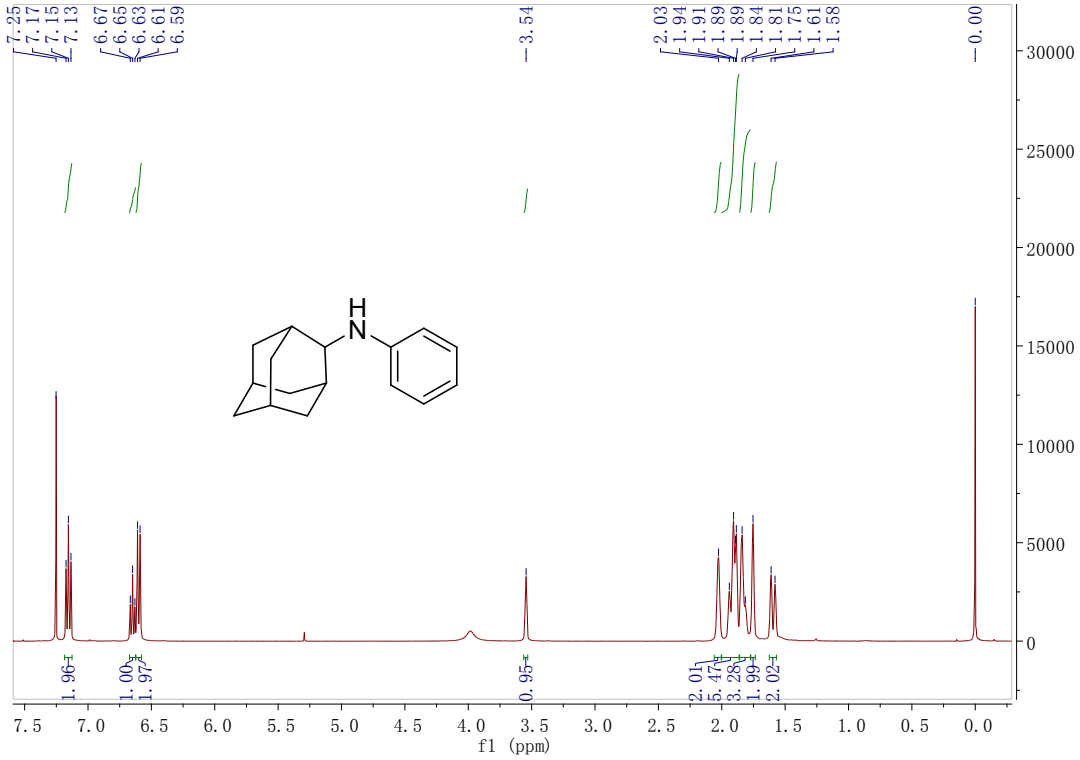


Figure S2. The ^{1}H NMR spectrum of **2** in $CDCl_3$ (400 MHz).

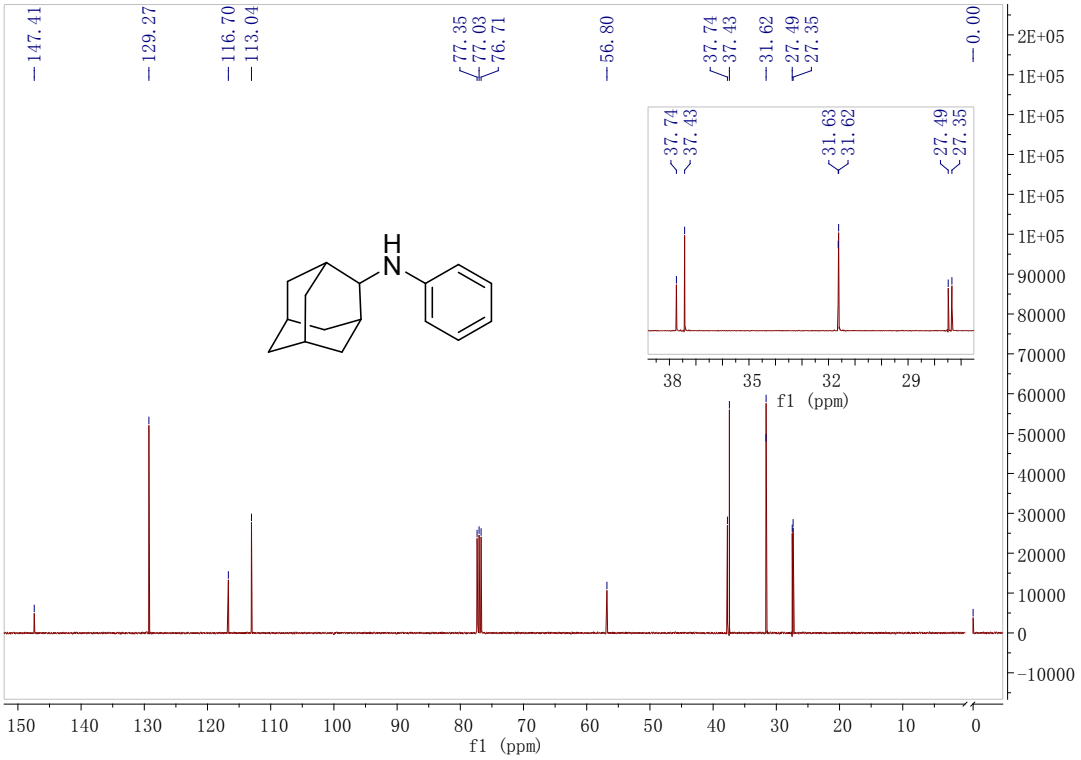


Figure S3. The ^{13}C NMR spectrum of **2** in $CDCl_3$ (100 MHz).

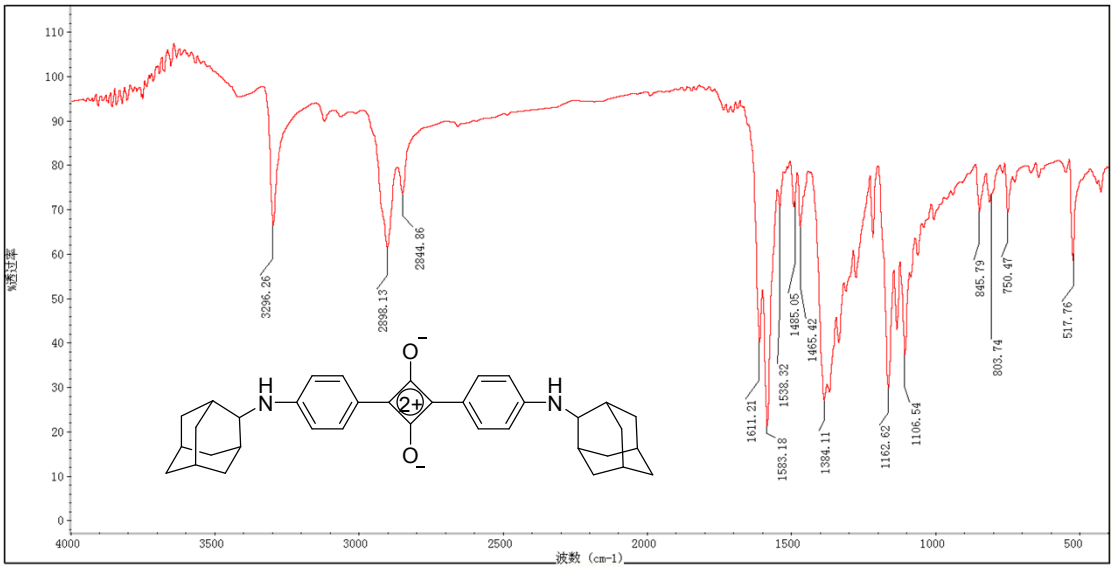


Figure S4. The IR spectrum of **SQ** in KBr disc.

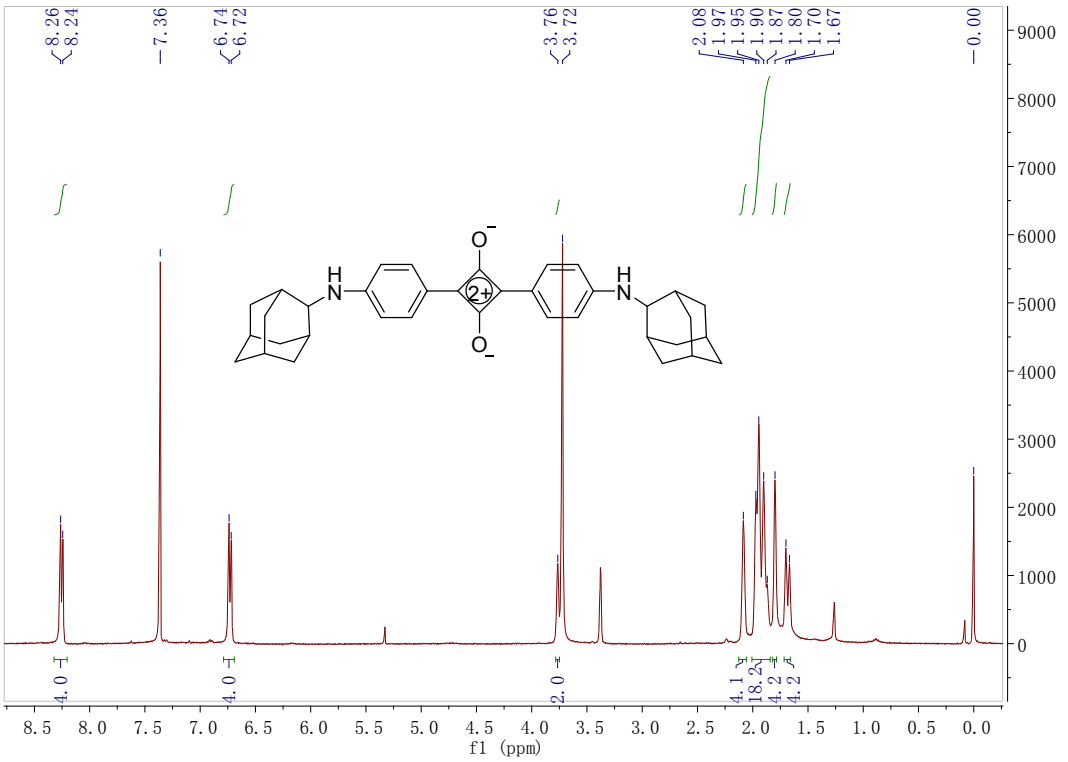


Figure S5. The ^{1}H NMR spectrum of **SQ** in $CDCl_3/CD_3OD$ (5:1, v/v) (400 MHz).

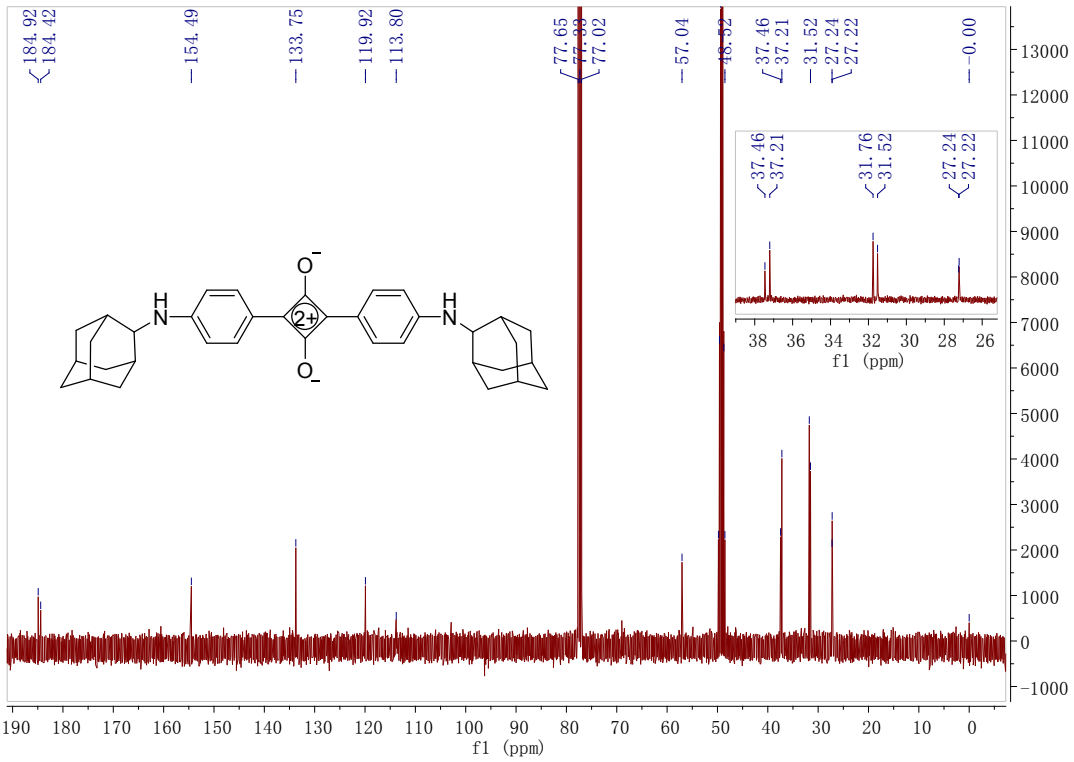


Figure S6. The ^{13}C NMR spectrum of **SQ** in $CDCl_3/CD_3OD$ (5:1, v/v) (100 MHz).

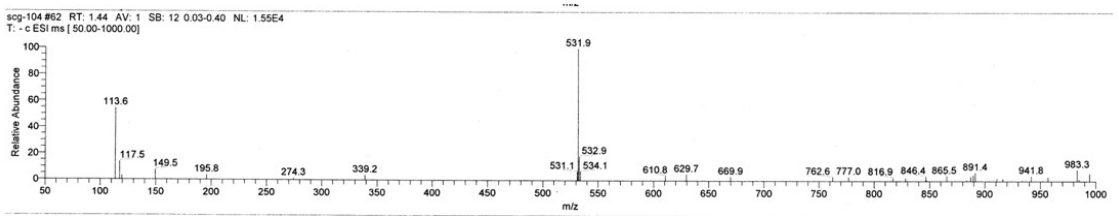


Figure S7. The ESI mass spectrum of **SQ**.

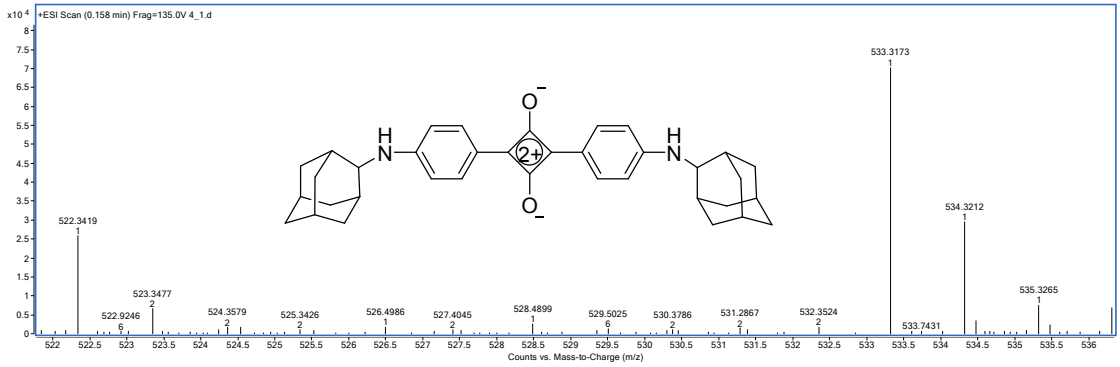
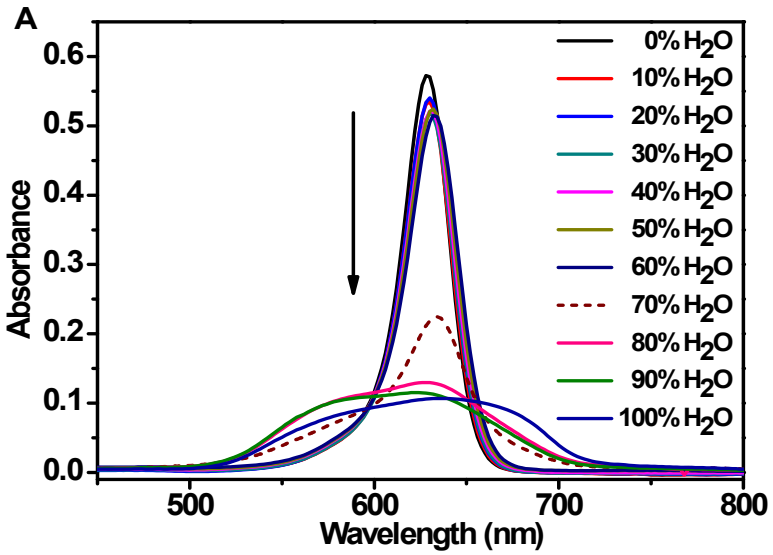


Figure S8. The ESI-HR mass spectrum of **SQ**.

3. Change in absorbance and fluorescence of **SQ** with increase of H_2O percentage in EtOH solution



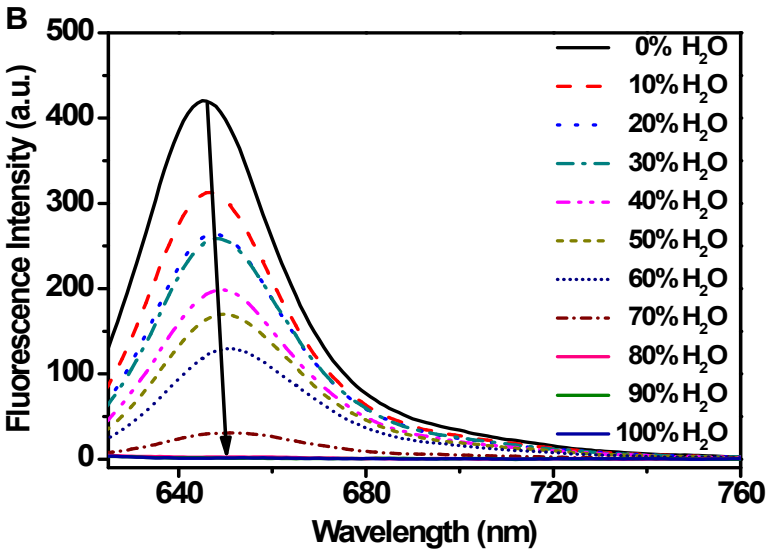
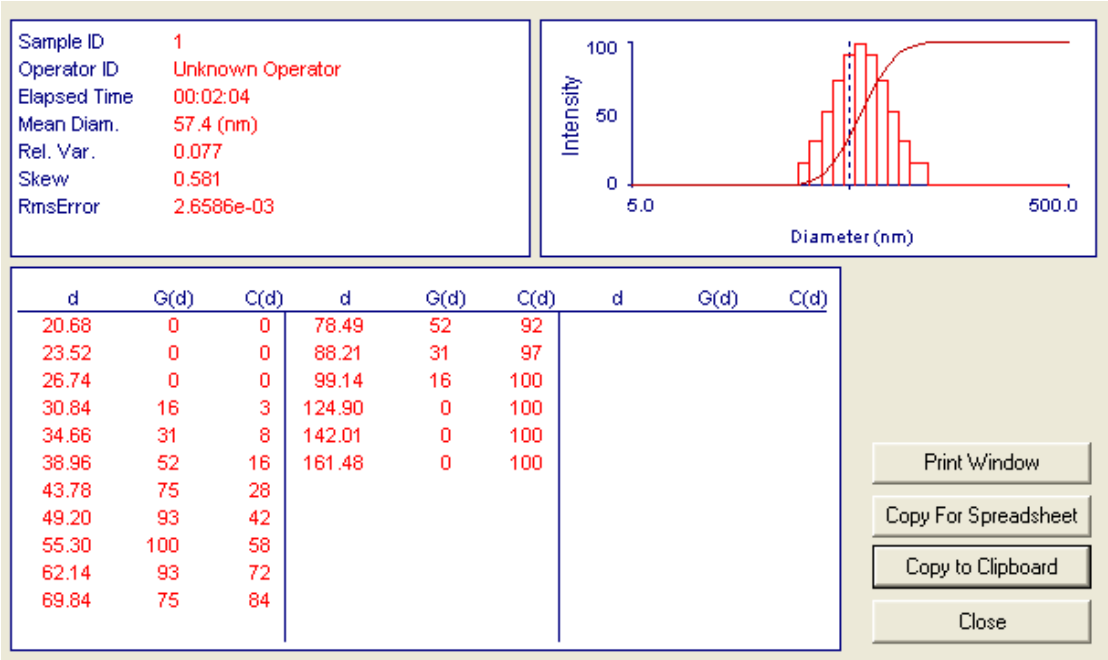


Figure S9. Change in absorbance **(A)** and fluorescence **(B)** spectra of **SQ** (2.0 μM) with increase of H_2O percentage in EtOH solution. (λ_{ex}=610 nm, slit: 5 nm/5 nm, PMT Volts: 650 V.).

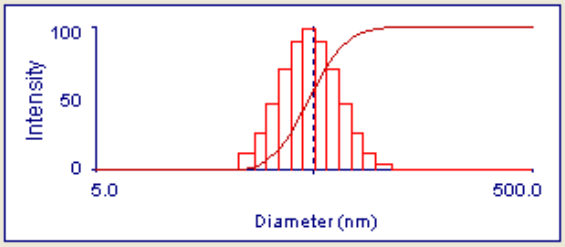
4. Dynamic light scattering experiments

A: **SQ** (1.0 μM) in pure water



B: **SQ** (1.0 μM) with β-CD (0.1 mM) in pure water

Sample ID	2
Operator ID	Unknown Operator
Elapsed Time	00:02:13
Mean Diam.	49.5 (nm)
Rel. Var.	0.091
Skew	0.814
RmsError	4.1517e-03

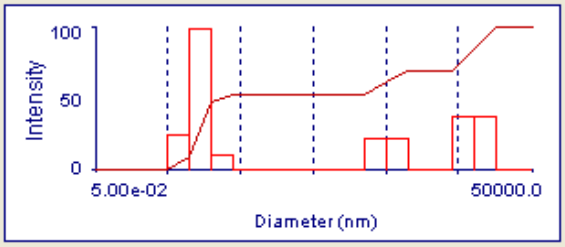


d	G(d)	C(d)	d	G(d)	C(d)	d	G(d)	C(d)
17.85	0	0	78.40	26	97			
20.51	0	0	89.05	12	99			
24.92	12	2	101.14	5	100			
28.30	26	6	124.85	0	100			
32.14	47	14	143.46	0	100			
36.51	72	26	164.84	0	100			
41.47	92	41						
47.10	100	58						
53.50	92	73						
60.77	72	85						
69.02	47	93						

Print Window
Copy For Spreadsheet
Copy to Clipboard
Close

C: **SQ** (1.0 μM) with β-CD (1.0 mM) in pure water

Sample ID	3
Operator ID	Unknown Operator
Elapsed Time	00:09:40
Mean Diam.	2315.5 (nm)
Rel. Var.	2.477
Skew	1.295
RmsError	8.0828e-03



d	G(d)	C(d)	d	G(d)	C(d)	d	G(d)	C(d)
0.17	0	0	321.24	23	62			
0.33	0	0	638.94	23	70			
0.66	25	10	1270.86	0	70			
1.31	100	48	2527.73	0	70			
2.61	11	53	5027.66	38	85			
5.19	0	53	10000.00	38	100			
10.32	0	53						
20.53	0	53						
40.82	0	53						
81.20	0	53						
161.51	0	53						

Print Window
Copy For Spreadsheet
Copy to Clipboard
Close

D: **SQ** (1.0 μM) with β-CD (5.0 mM) in pure water

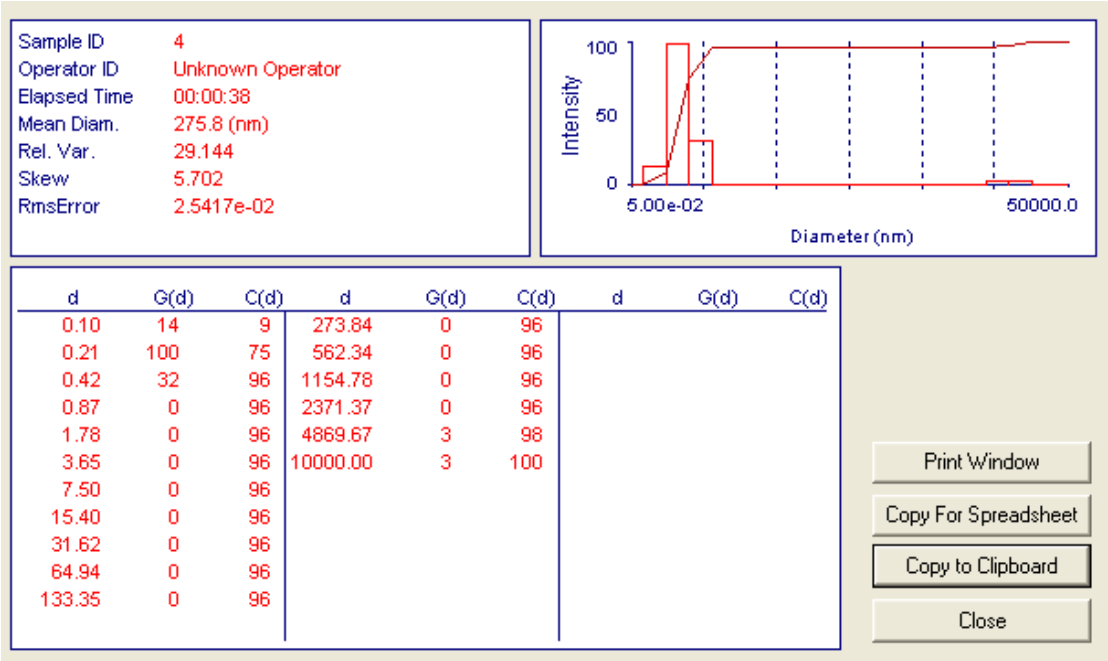


Figure S10. Solvodynamic diameters of **SQ** (1.0 μM) **(A)**, **SQ** (1.0 μM) with β-CD (0.1 mM) **(B)**, **SQ** (1.0 μM) with β-CD (1.0 mM) **(C)**, and **SQ** (1.0 μM) with β-CD (5.0 mM) **(D)** in pure water determined by dynamic light scattering.

5. Change in absorbance of **SQ** in water solution containing varied concentration of β-CD

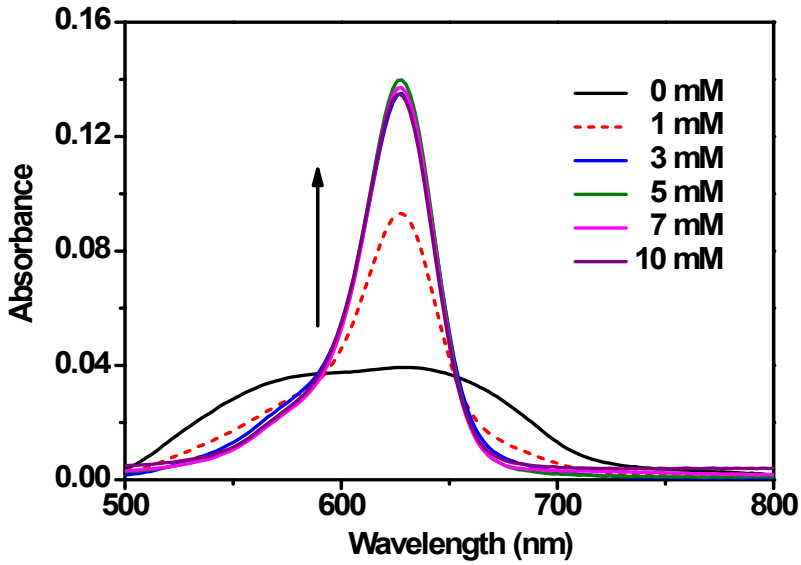


Figure S11. Absorbance spectra of **SQ** (2.0 μM) in different concentrations of β-CD solution.

6. ESI mass spectrum of **SQ**⊂β-CD inclusion complex

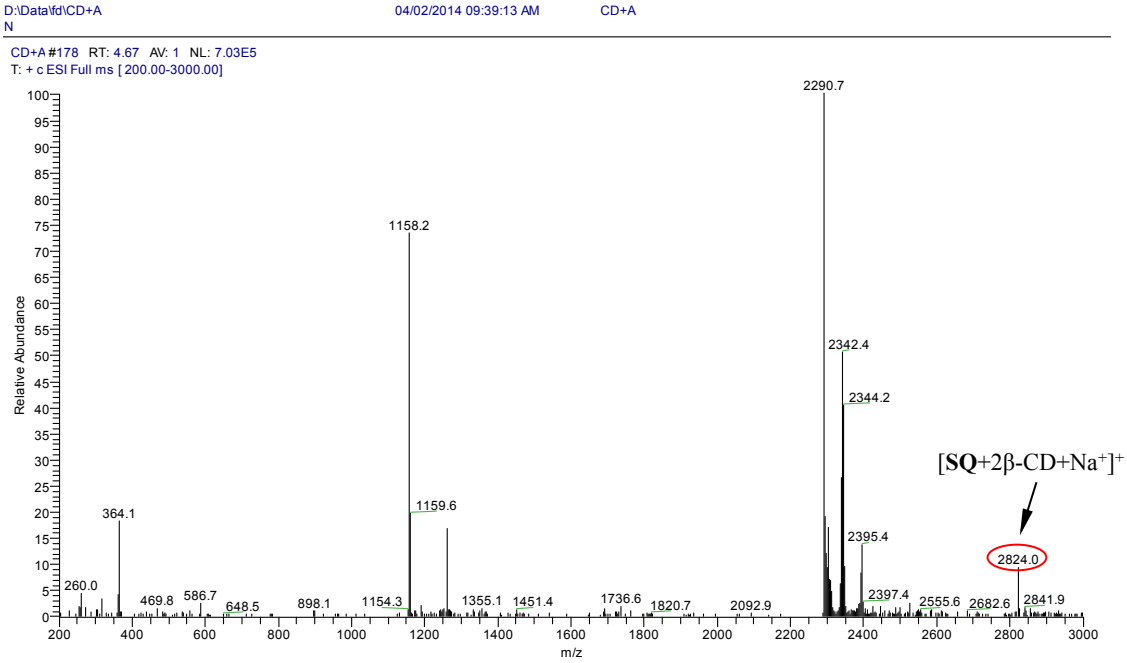


Figure S12. The ESI mass spectrum of **SQ**⊂β-CD inclusion complex $[SQ⊂β\text{-}CD+Na^+]^+$. (Calcd for $C_{120}H_{180}N_2NaO_{72}$ ($[\mathbf{SQ}+2β\text{-}CD+Na^+]^+$): 2824.0383, Found: 2824.0.).

7. Trifloroacetic acid titrations of **SQ** in MeCN

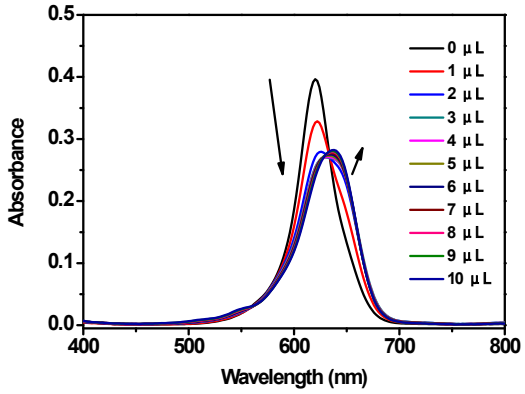


Figure S13. Change in absorbance of **SQ** (2.0 μM) with the increase of TFA (0-10 μL) in CH_3CN.

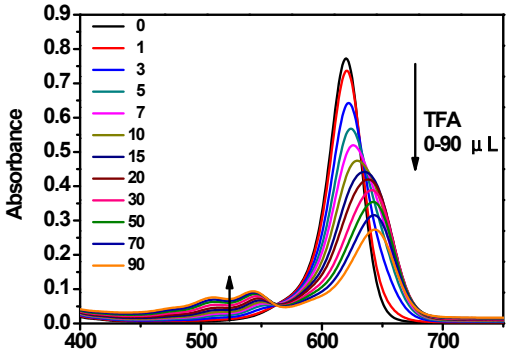


Figure S14. Change in absorbance of **SQ** (3.75 μM) with the increase of TFA (0-90 μL) in CH_3CN.

8. ^{1}H NMR titration experiment

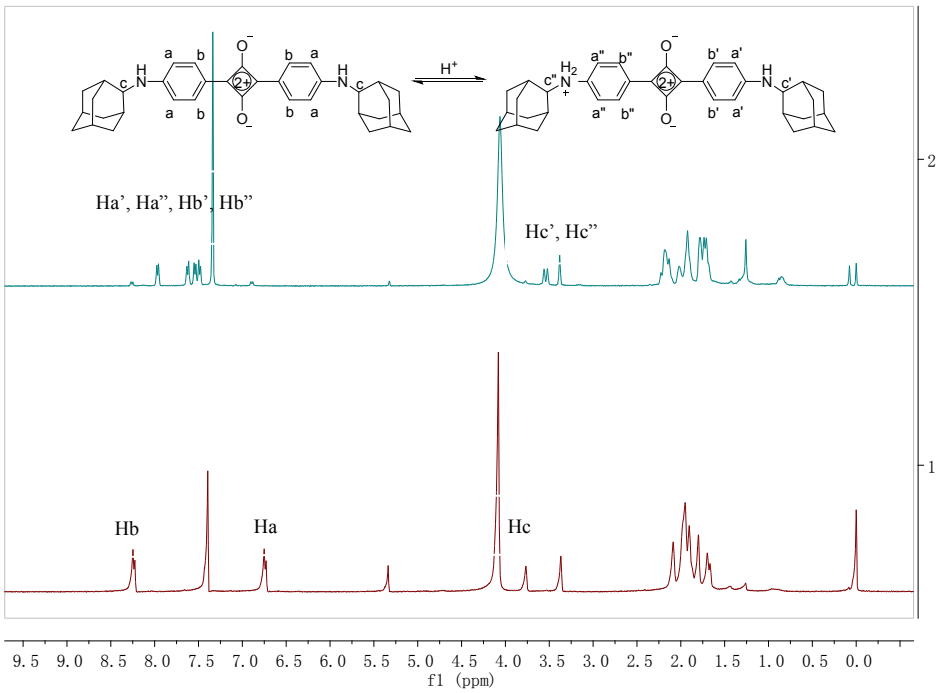


Figure S15. The ^{1}H NMR spectra of **SQ** (3.0 mg) **(1)**, and **SQ** (3.0 mg) and D_2SO_4 (1.0 μL) **(2)** in 0.6 mL $CDCl_3$/CD_3OD (5:1, v/v) (400 MHz).

9. ESI mass spectrum of **SQ**&OH adduct⊂β-CD

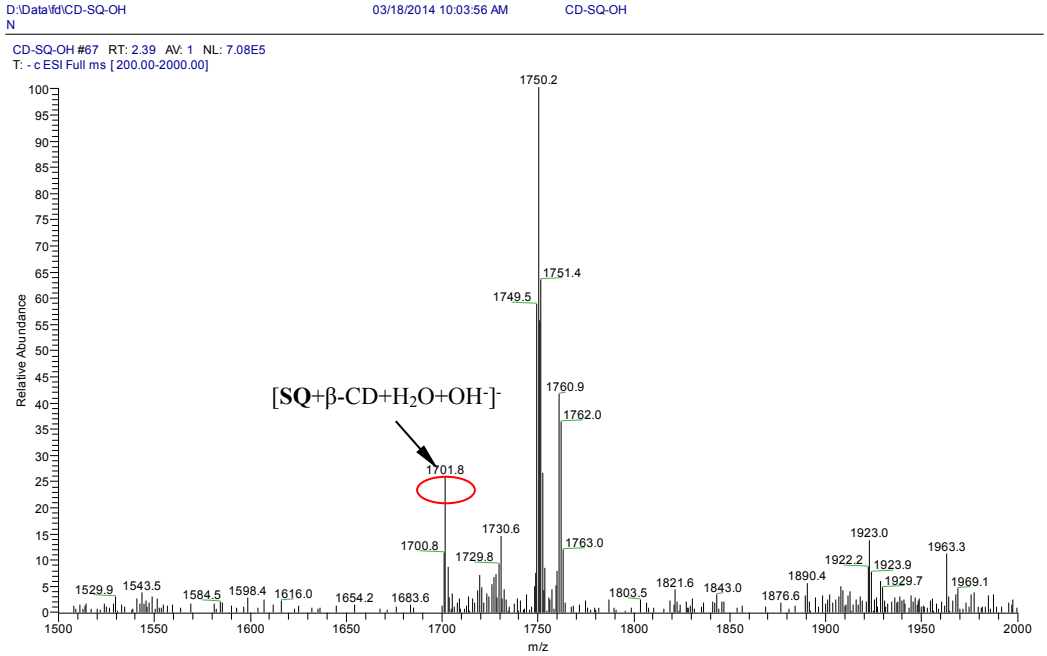


Figure S16. The ESI mass spectrum of **SQ**&OH adduct⊂β-CD. (Calcd for $C_{78}H_{113}N_2O_{39}$ ([**SQ**+β-CD+H_2O+OH^-]$^-$): 1701.6920, Found: 1701.8.).

10. Change in absorbance of **SQ**⊂β-CD in varied pH buffers

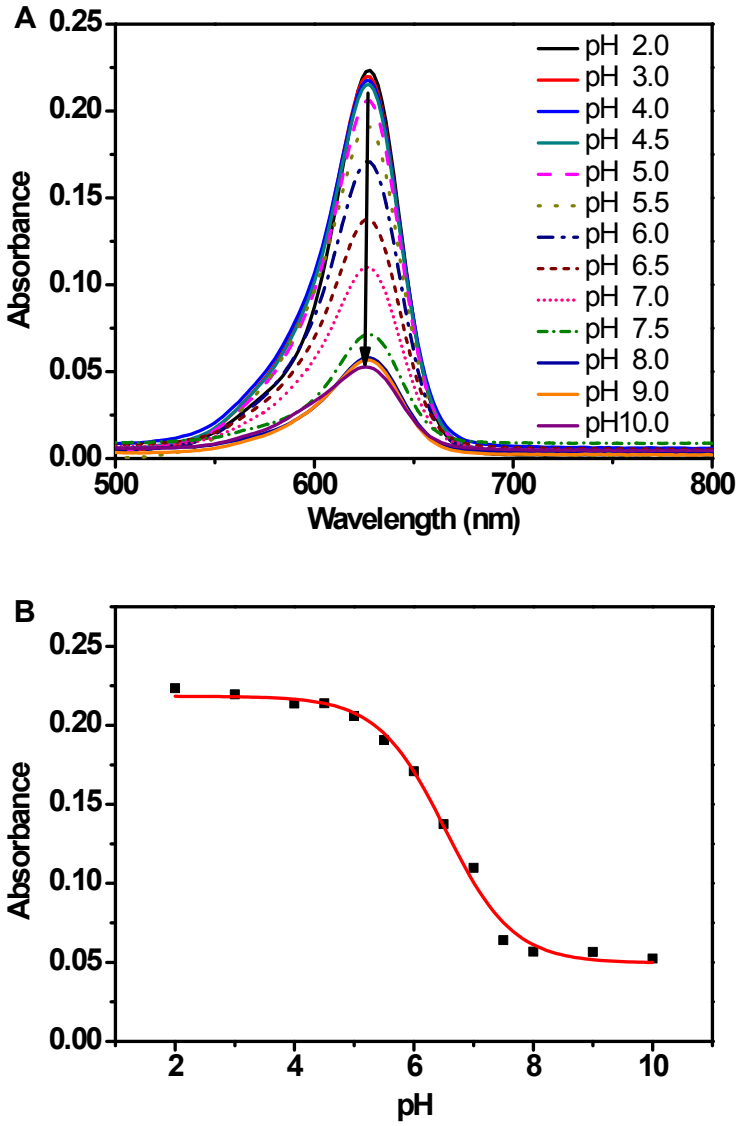


Figure S17. (A) pH-Dependence of absorption spectra of **SQ** (2.0 μM) in PB (20 mM) buffer solution with β-CD (2.0 mM). **(B)** pH-Dependence absorbance changes in PB (20 mM) buffer solution at 628 nm.

11. Reversibility of pH titrations

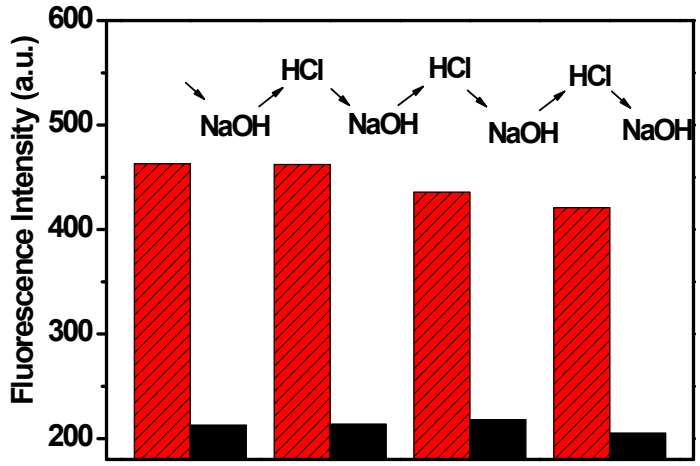


Figure S18. Fluorescence titration spectra of **SQ**⊂β-CD with NaOH and HCl. (λ_{ex}=610 nm, λ_{em}=644 nm, slit: 5 nm/5 nm, PMT Volts: 650 V.).

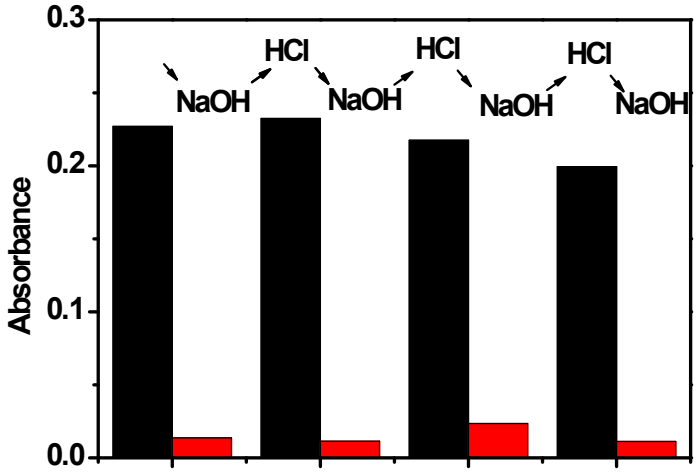


Figure S19. The absorbance response of **SQ**⊂β-CD upon the alter addition of NaOH and HCl at 628 nm.

12. Photobleaching experiments of **SQ**

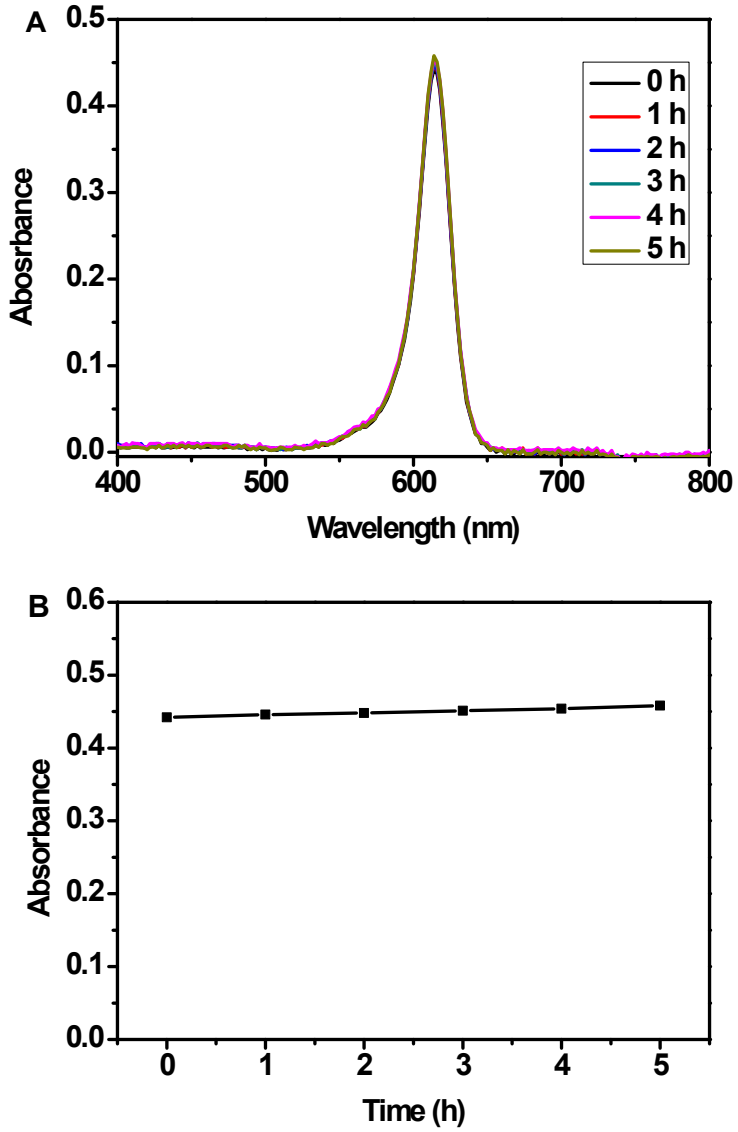


Figure S20. (A) Time dependence of absorption spectra of **SQ** (2.0 μM) in $CHCl_3$ irradiated under tungsten lamp (500 W) at 40 cm distance. **(B)** The absorption decay of **SQ** (2.0 μM) at 615 nm in $CHCl_3$ irradiated under tungsten lamp (500 W) at 40 cm distance.

13. Competition experiments

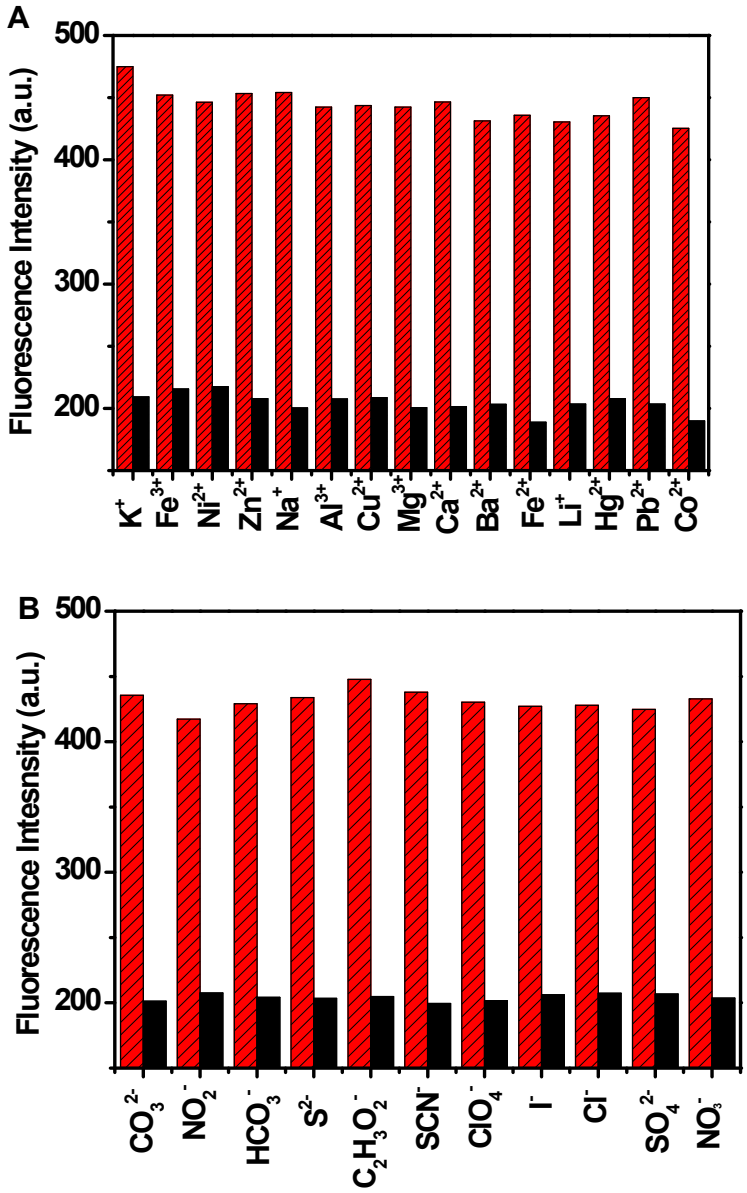


Figure S21. The fluorescence response of **SQ** (2.0 μM) toward 100 μM different metal ions **(A)** and anions **(B)** in pH=5.0 (red bar) and 8.0 (black bar) PB buffer solution with β-CD (2.0 mM). (λ_{ex}=610 nm, λ_{em}=644 nm, slit: 5 nm/5 nm, PMT Volts: 650 V.).

14. The study of **SQ** in different concentrations in pure water

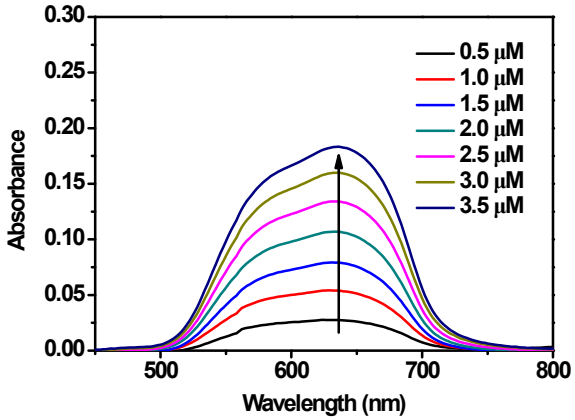


Figure S22. Change in absorbance spectra with increase the concentration of **SQ** (0.5-3.5 μM) in pure water.

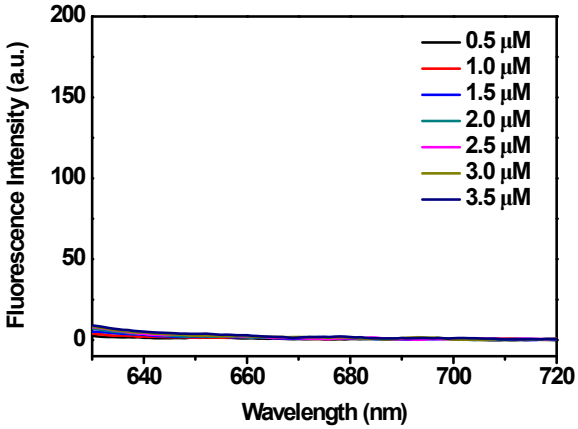


Figure S23. Change in flourescence spectra with increase the concentration of **SQ** (0.5-3.5 μM) in pure water. (λ_{ex}=610 nm, slit: 5 nm/5 nm, PMT Volts: 650 V.).

15. The control experiment of pH-dependence of **SQ** fluorescence spectra in PB buffer solution without β-CD

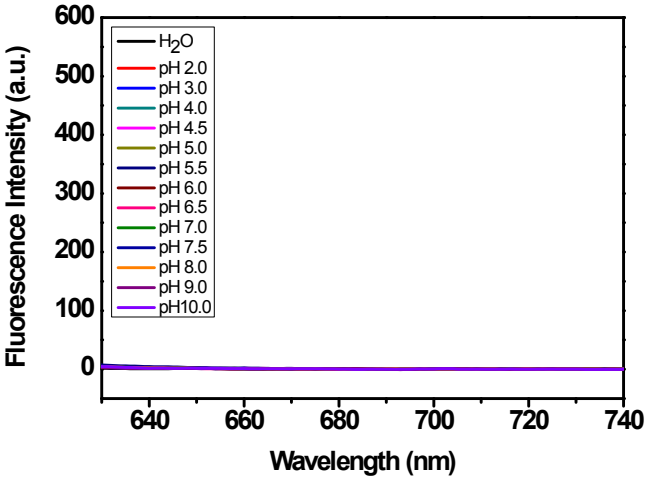


Figure S24. The control experiment of pH-dependence of **SQ** (2.0 μM) fluorescence spectra in PB (20 mM) buffer solution without β-CD. (λ_{ex}=610 nm, slit: 5 nm/5 nm, PMT Volts: 650 V.).

16. The control experiment of fluorescence images of **SQ** without β-CD in pH 4.5

and 8.0

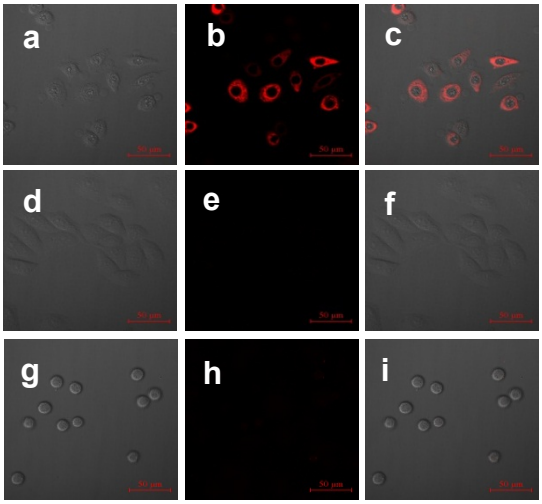


Figure 25. Fluorescence images of living HeLa cells. (a) Bright-field image of HeLa cells incubated with **SQ**⊂β-CD in PBS medium at pH 4.5; (b) Fluorescence image of (a); (c) the overlay image of (a) and (b); (d) Bright-field image of HeLa cells incubated with **SQ** in the absence of β-CD in PBS medium at pH 4.5; (e) Fluorescence image of (d); (f) the overlay image of (d) and (e); (g) Bright-field image of HeLa cells incubated with **SQ** in the absence of β-CD in PBS medium at pH 8.0; (h) Fluorescence image of (g); (i) the overlay image of (g) and (h).