Electronic Supplementary Material (ESI) for ChemComm.

Reversible Dynamic Optical Sensing Based on Coumarin Modified β-Cyclodextrin for Glutathione in Living Cells

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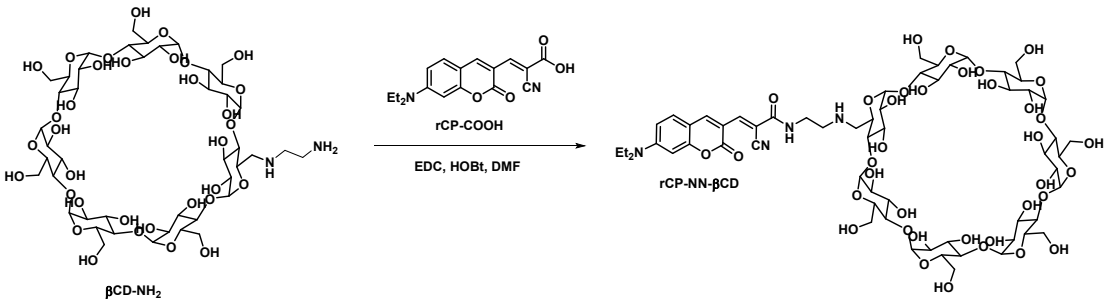
Experimental

All chemicals reagents and solvents for synthesis were purchased from commercial sources (Aladdin Industrial Corporation, Tokyo Chemical Industry and Sigma-Aldrich Chemical) and were used without further purification. Ultrapure water was used after passing through a water ultra-purification system. ^{1}H-NMR, ^{13}C-NMR spectra were recorded on an Ascend 400 MHz (BRUKER) at room temperature. High-resolution mass spectra (HRMS) were measured on 6520 Q-TOF LC/MS (Agilent). Absorption spectra was recorded on a UV-vis spectrophotometer (UV-2700, Shimadzu), and steady-state fluorescence emission spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm) at 25 °C on a Varian Cary Eclipse equipped with a Varin Cary single-cell peltier accessory to control temperature. Absolute fluorescence quantum yields were recorded on a FLS980 instrument (Edinburg Instruments Ltd., Livingstone, UK). CD spectra were recorded on Jasco J 715 CD spectrophotometers. Confocal fluorescence and bright-field imaging were recorded with FV1000 (Olympus).

Synthesis



Scheme S1. Synthesis of **rCP-NN-βCD** and the structure of **rCP-βCD.**

βCD-NH_2: βCD-NH_2 was synthesized according to the reported procedure.[1]

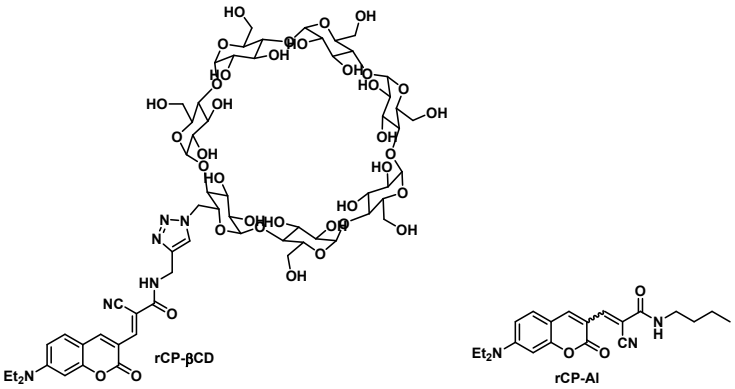
rCP-COOH: rCP-COOH was synthesized according to the reported procedure.[2]

rCP-NN-βCD: rCP-COOH (400 mg, 1.28 mmol), EDC (245.7 mg, 1.28 mmol) and HOBt (172.9 mg, 1.28 mmol) were dissolved in anhydrous DMF and the solution was stirred at room temperature for 30 min. After that, βCD-NH_2 (233.8 mg, 1.07 mmol) was added, and the reaction mixture was stirred overnight. Fianlly, the solution was evaporated, and the resultant residue was purified by silica gel chromatography to afford **rCP-NN-βCD** (160.9 mg, 66%) as a red solid.

^{1}H-NMR (400 MHz, DMSO) δ 8.68 (s, 1H), 8.20 (s, 1H), 8.15 (s, 1H), 7.60 (d, *J* = 9.1 Hz, 1H), 6.82 (d, *J* = 7.1 Hz, 1H), 6.64 (s, 1H), 5.88 – 5.49 (m, 14H), 4.85 (m, 7H), 4.47 (d, *J* = 3.9 Hz, 6H), 3.82 – 3.49 (m, 30H), 2.65 – 2.84 (m, 5H), 1.15 (t, *J* = 7.0 Hz, 6H).

^{13}C-NMR (100 MHz, DMSO) δ 160.20, 157.23, 152.98, 144.25, 143.46, 131.92, 116.69, 110.48, 110.14, 107.78, 102.30, 101.98, 96.54, 83.07 , 82.06, 81.55, 81.26, 73.01, 72.38, 71.99, 59.89, 44.53, 12.36.

HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{61}H_{90}N_4O_{37}$: 1471.5284; found: 1471.5362.



Scheme S2. The structures of **rCP-βCD** of **rCP-AI**.

rCP-βCD and rCP-AI were synthesized according to the reported procedure.[2]

UV-vis absorption and fluorescence spectroscopy

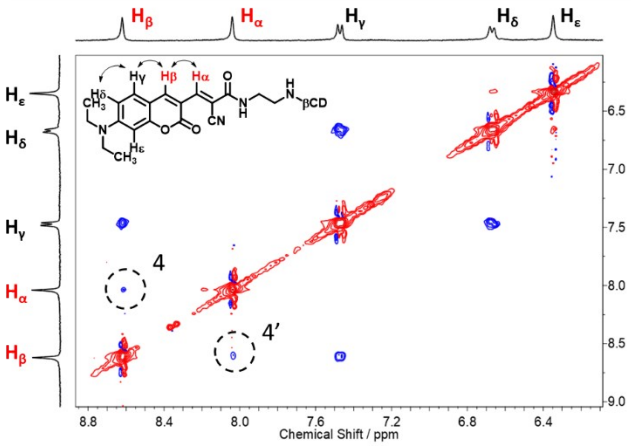


Figure S1. Enlarged NMR ROESY spectrum of the coumarin moiety on rCP-NN-βCD (4 mM) in D_2O and the configuration of coumarin (400 MHz).

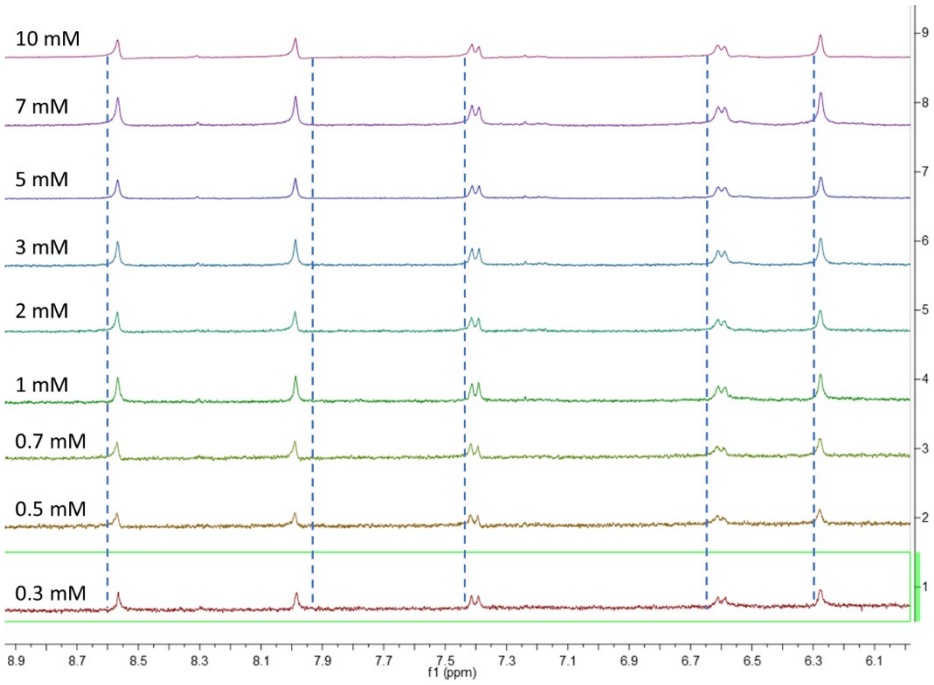


Figure S2. ^{1}H-NMR spectra of **rCP-NN-βCD** at different concentration in D_2O (400 MHz).

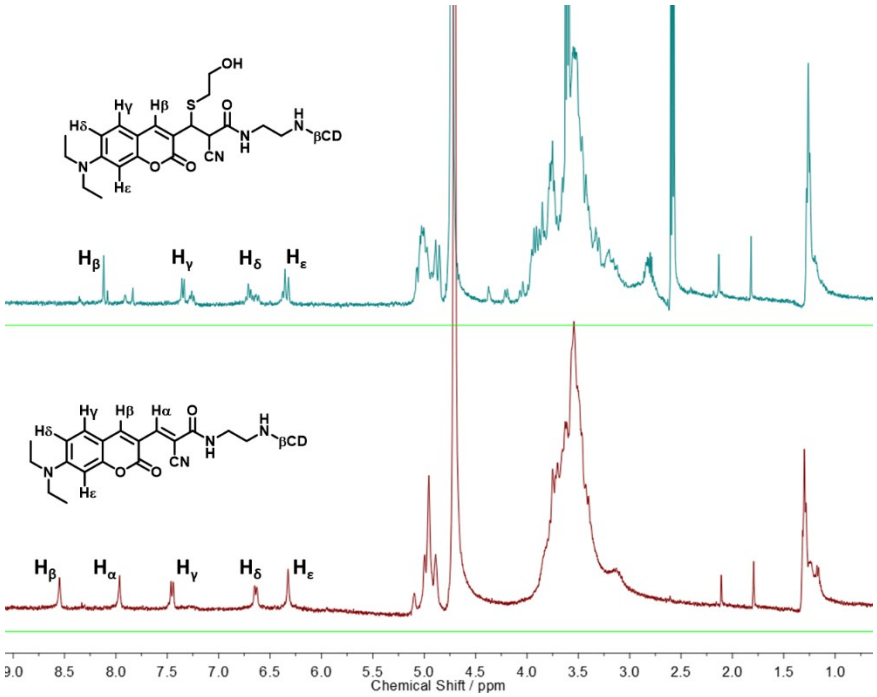


Figure S3 ^{1}H-NMR spectra of rCP-NN-βCD (2 mM) and its Michael addition adduct with 2-mercaptoethanol (4 mM) in D_2O (400 MHz).

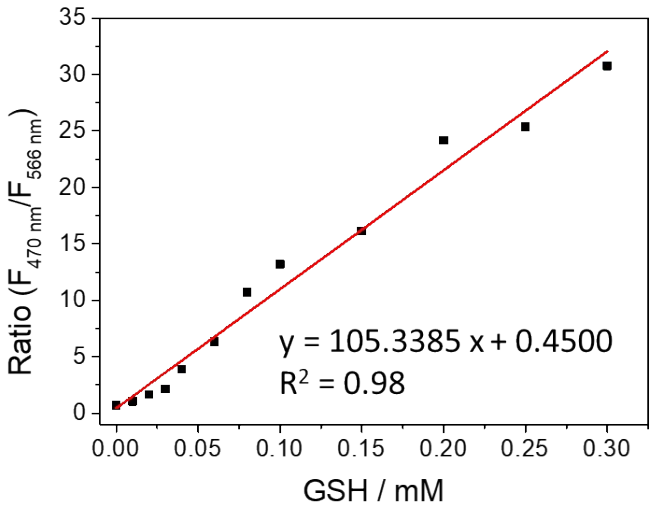


Figure S4. The line relationship between the fluorescent intensity ratio of the **rCP-NN-βCD** (10 μM at 470 nm and 566 nm) and the concentration of the GSH in PBS buffer (pH 7.4, 10 mM). Each spectrum was recorded after 5 min. (Ex = 409 nm. Slits: 5/5 nm; Ex = 506 nm. Slits: 5/5 nm)

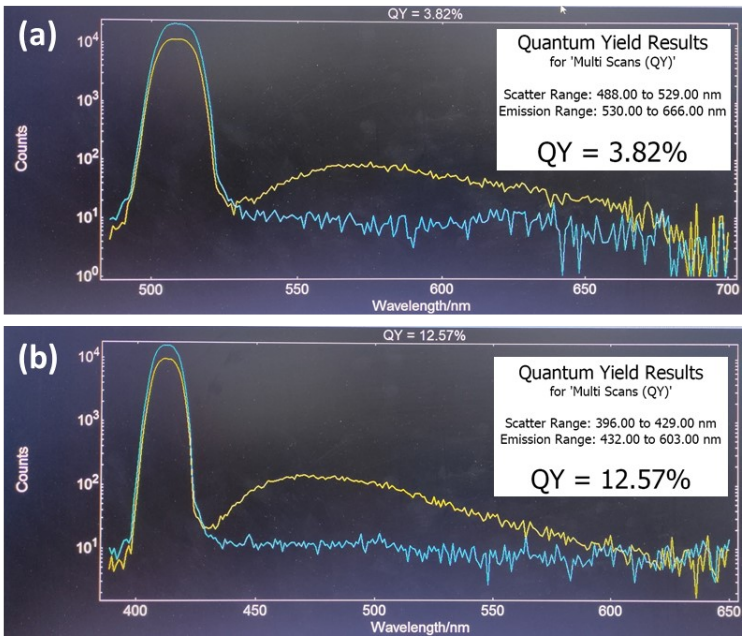


Figure S5. Quantum yields of **rCP-NN-βCD** (a) at 566 nm and **rCP-NN-βCD**+GSH (b) at 470 nm in PBS buffer solution.

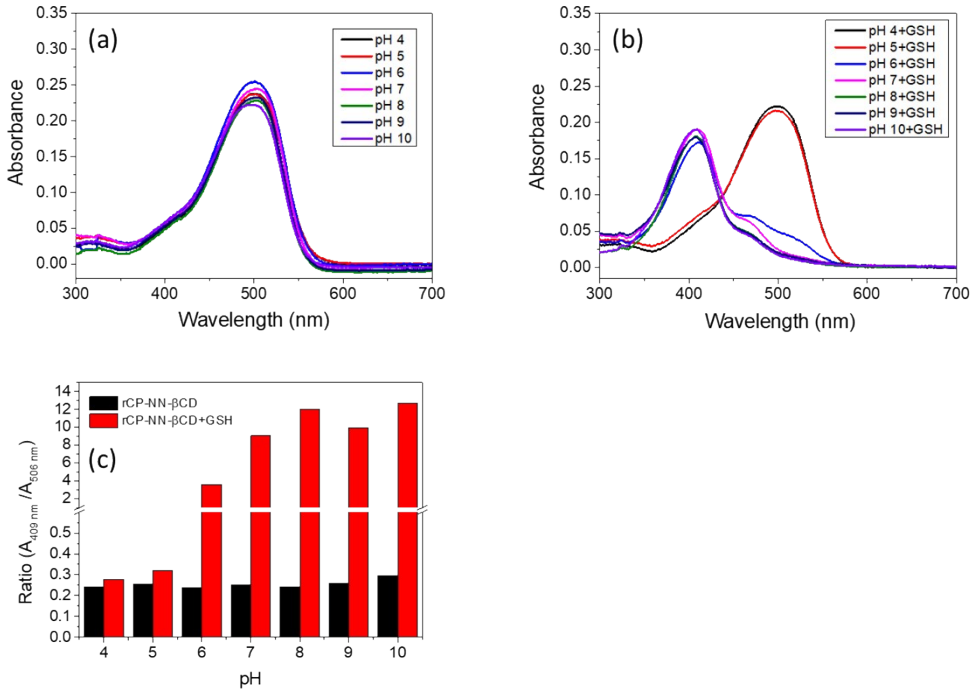


Figure S6. UV-vis absorption changes of **rCP-NN-βCD** (10 μM) in the absence or prescence of GSH (0.5 mM) in PBS buffer (10 mM) at varied pH values (4 - 10). (a) **rCP-NN-βCD**; (b) **rCP-NN-βCD**+GSH; (c) the ratio (A409nm/A506nm) of (a) and (b).

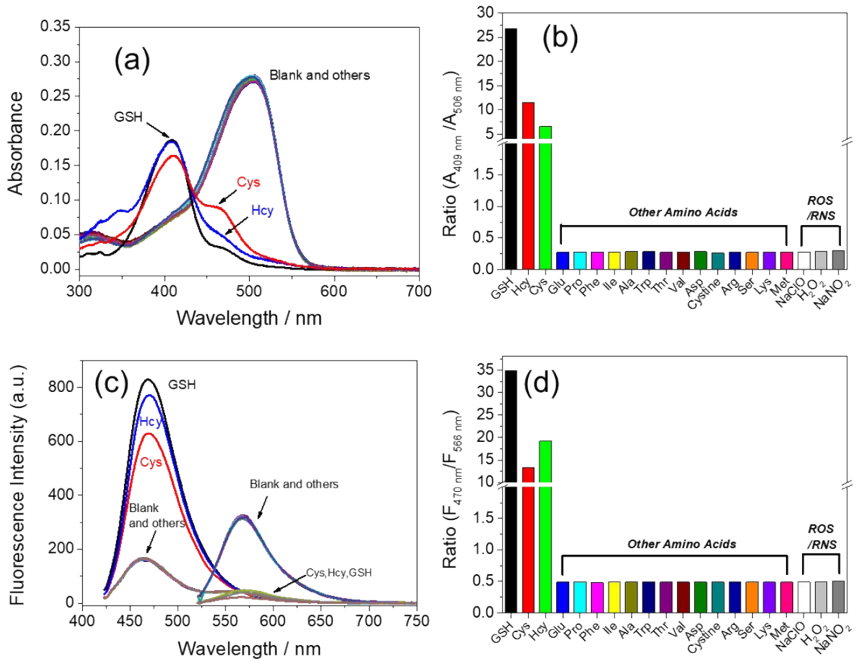


Figure S7. UV-Vis absorption and fluorescence response of **rCP-NN-βCD** to intracellular amino acids (0.5 mM), (Cys = Hcy = GSH = 0.5 mM), NaClO (0.1 mM), H_2O_2 (0.1 mM), $NaNO_2$ (0.1 mM). (Ex= 409 nm. Slits: 5/5 nm; Ex= 506 nm. Slits: 5/5 nm)

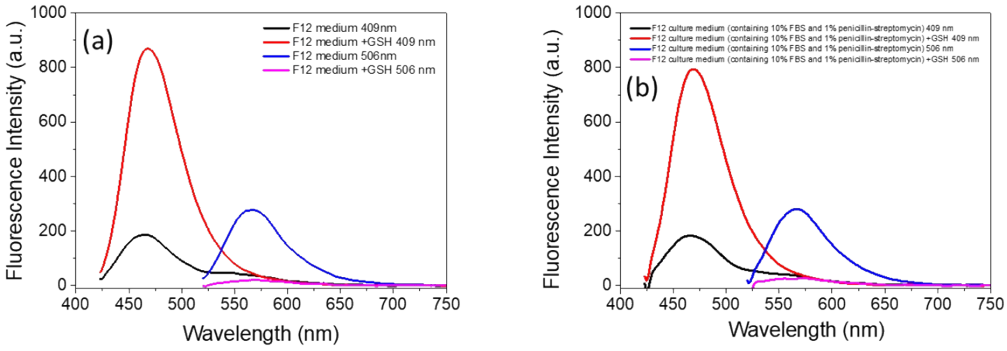


Figure S8. The detection performance of **rCP-NN-βCD** toward GSH in F12 culture medium (a) and FBS (b) (containing 10% FBS and 1% penicillin-streptomycin). Fluorescence responses of **rCP-NN-βCD** (10 μM) upon addition of GSH (0.5 mM). (Ex = 409 nm; slits: 5/ 5 nm; Ex = 506 nm; slits: 5/ 5 nm).

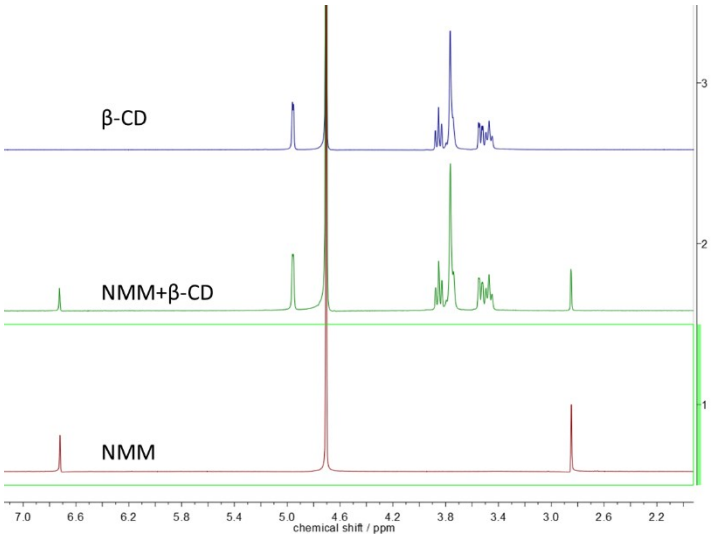


Figure S9. ^{1}H-NMR spectra of NMM (2.5 mM), β-CD (2.5 mM), and NMM+β-CD (1:1) in D_2O (400 MHz).

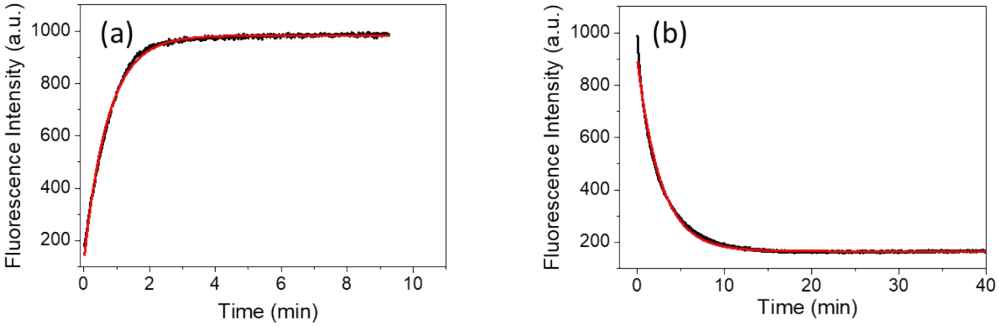


Figure S10. Time-dependent fluorescent emission of **rCP-NN-βCD** toward GSH (a) and NMM (b). (Ex = 409 nm; slits: 5/ 5 nm; Em = 470 nm; GSH = 0.5 mM; NMM = 0.5 mM)

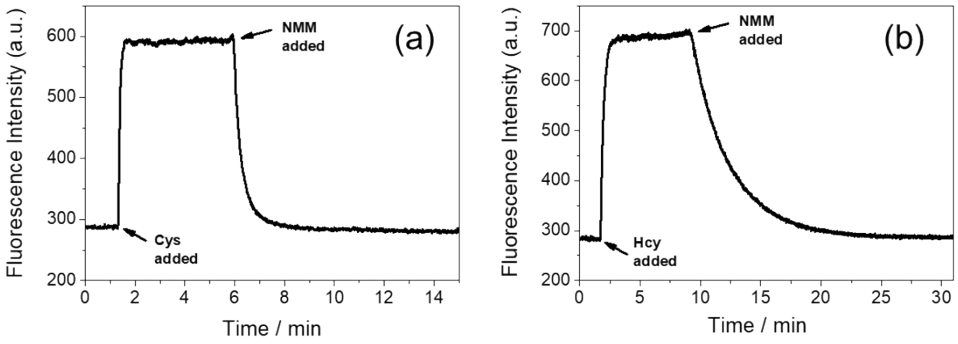


Figure S11. Time-dependence reversible fluorescence emission at 470 nm by **rCP-NN-βCD** upon reaction with (a) Cys (0.5 mM) and (b) Hcy (0.5 mM), and then upon addition of NMM (0.5 mM). (Ex = 409 nm. Slits: 5/5 nm)

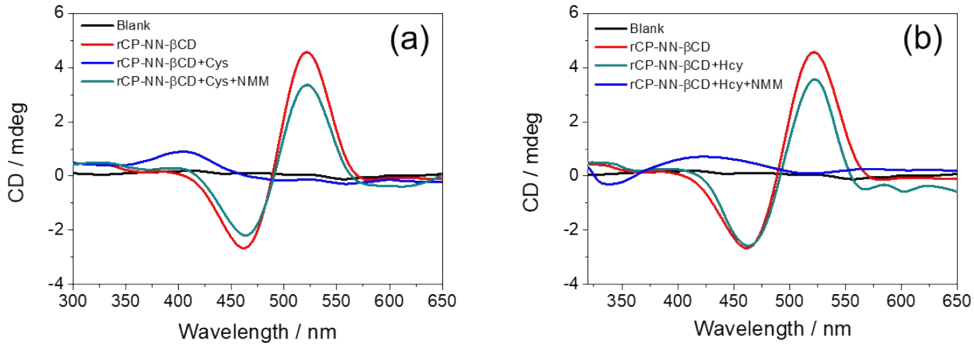


Figure S12. Circular dichroism spectra of **rCP-NN-βCD** (10 μM) toward Cys (0.5 mM) and Hcy (0.5 mM), and then upon addition of NMM (0.5 mM).

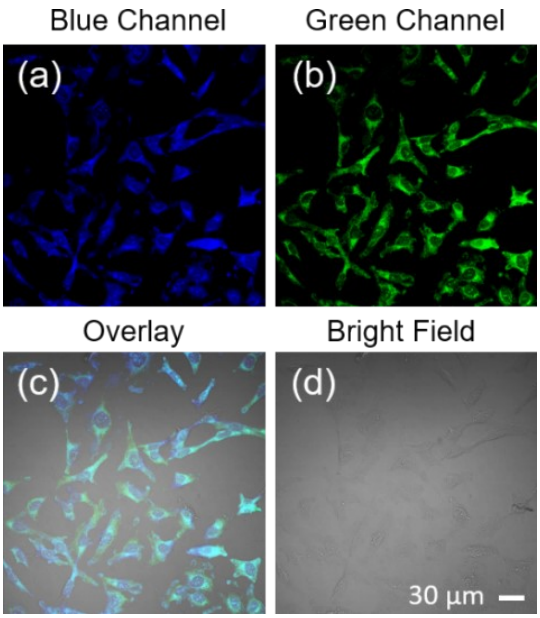


Figure S13. Confocal fluorescence images of living HeLa cells incubated with rCP-NN-βCD (10 μM). (Blue channel: Excited at 405 nm, Emission was collected at 425-525 nm; Green channel: Excited at 488 nm, Emission was collected at 525-650 nm).

Cell imaging experiment

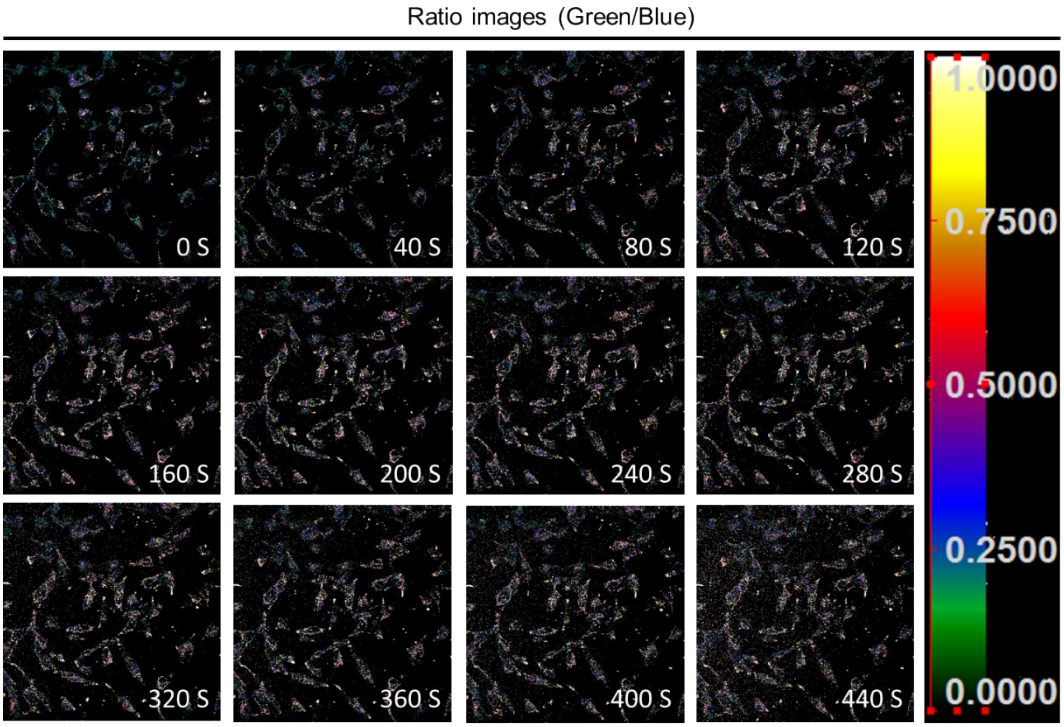


Figure S14. Time-dependent ratio (Green/Blue) imaging with **rCP-NN-βCD** (10 μM) in HeLa cells upon addition of NMM (5 mM) within 440 s.

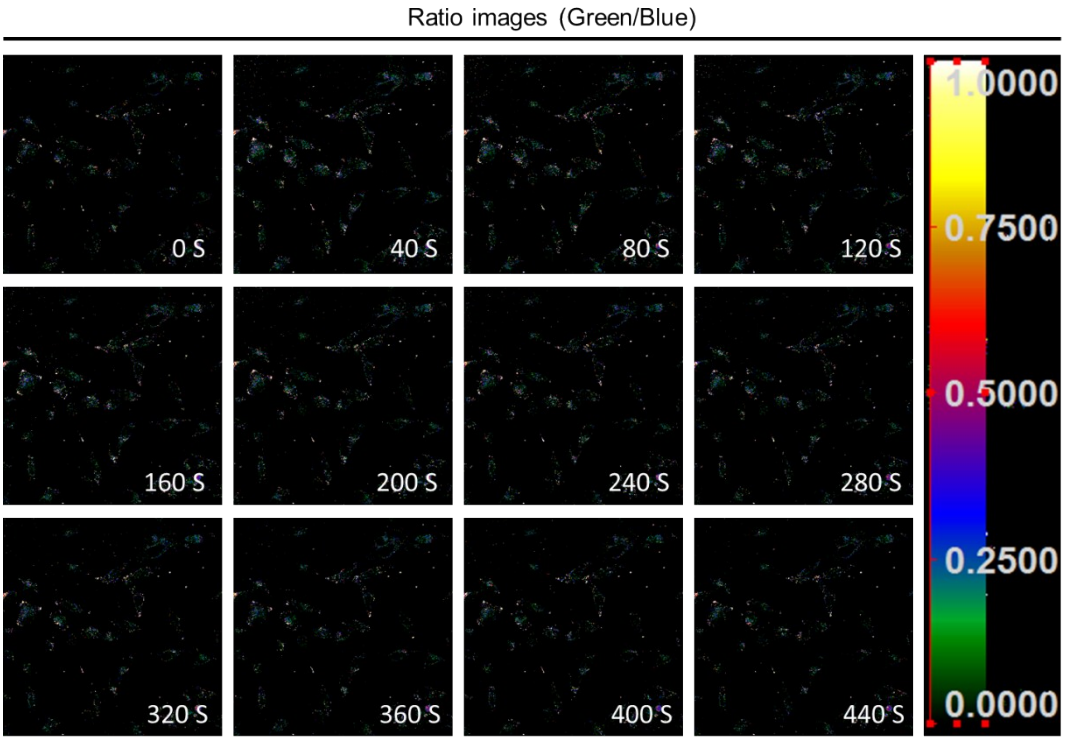


Figure S15. Time-dependent ratio (Green/Blue) imaging with **rCP-NN-βCD** (10 μM) in HeLa cells without addition of NMM within 440 s.

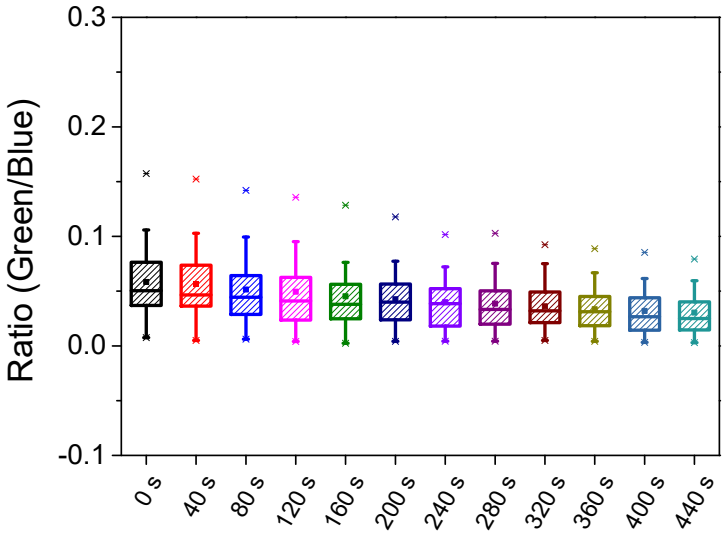


Figure S16. Fluorescence quantitative analysis of GSH change in HeLa cells without NMM in individual cells (n = 30) for 440 s.

Characterization data

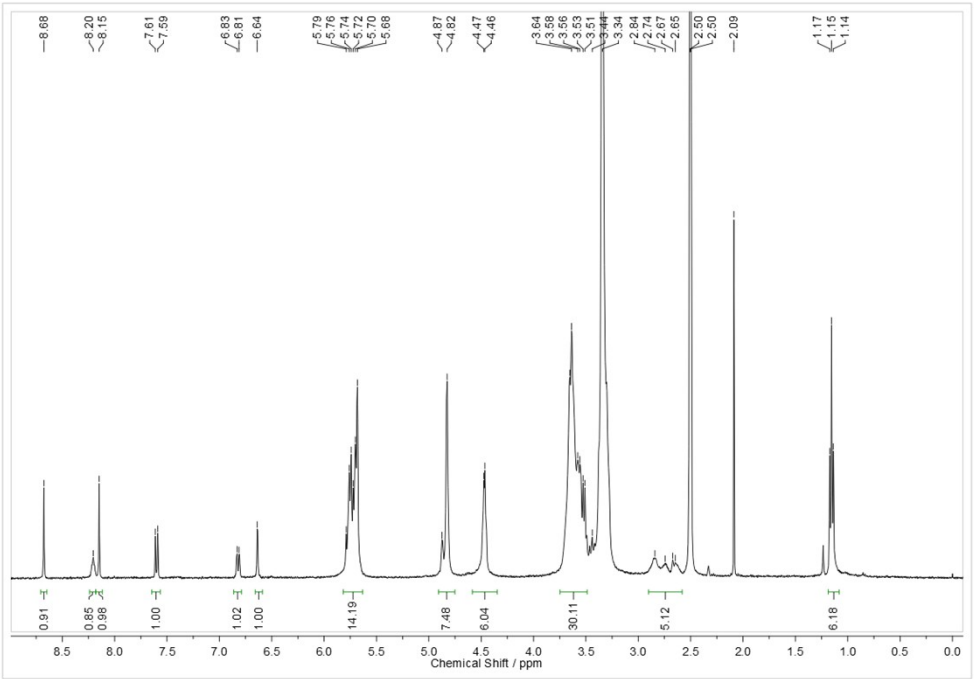


Figure S17. ^{1}H-NMR spectrum of **rCP-NN-βCD** (400 MHz, DMSO-d_6).

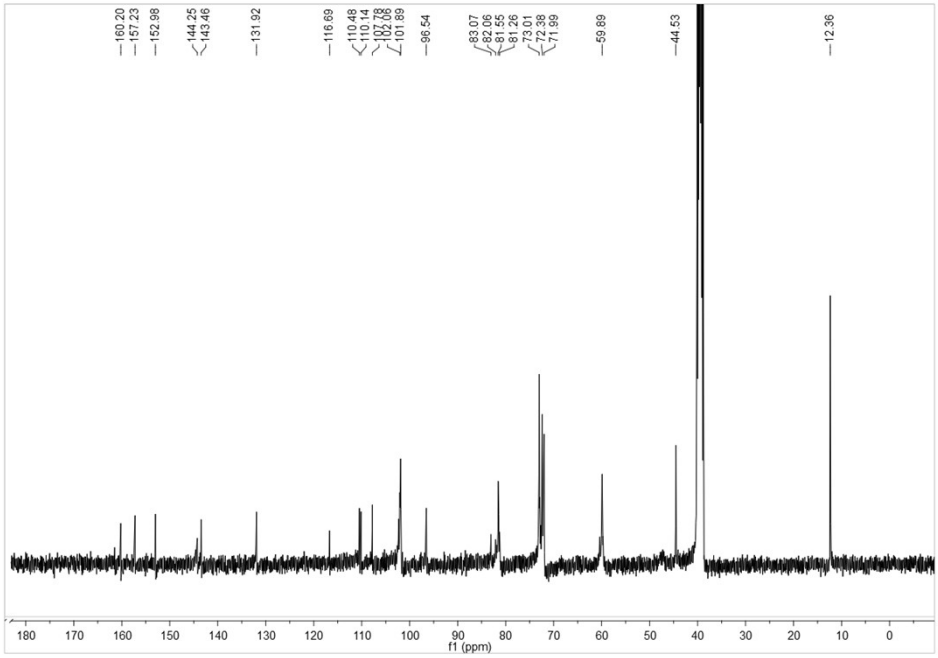


Figure S18. ^{13}C-NMR spectrum of **rCP-NN-βCD** (400 MHz, DMSO-d_6).

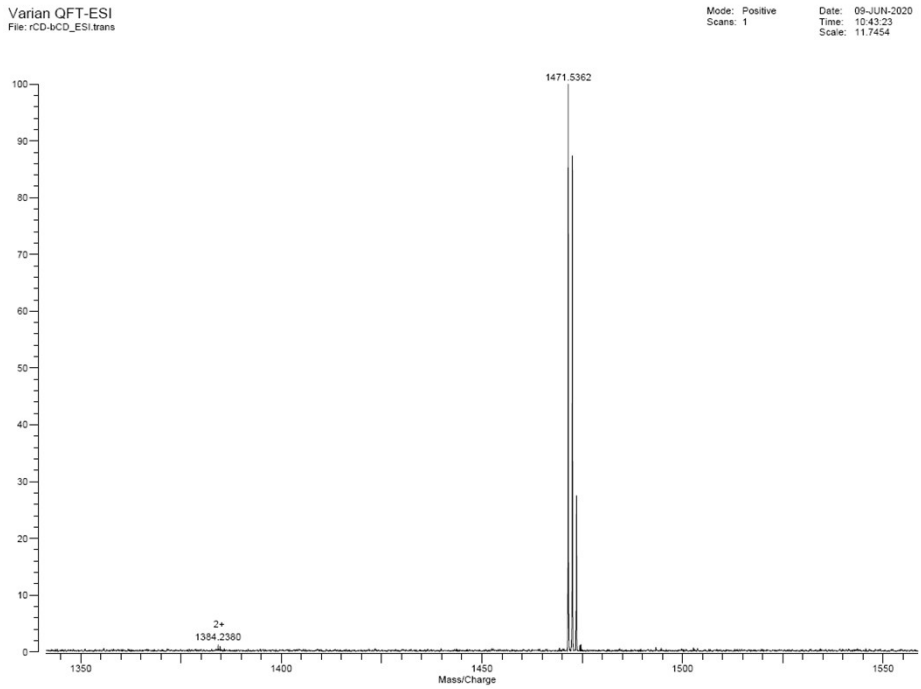


Figure S19. HRMS spectrum of **rCP-NN-βCD**.

References:

1. Chen, L.; Chen, Y.; Fu, H. G.; Liu, Y., Reversible Emitting Anti-Counterfeiting Ink Prepared by Anthraquinone-Modified beta-Cyclodextrin Supramolecular Polymer. *Adv. Sci.* **2020,** *7*, 2000803.

2. Liu, Z.; Zhou, W.; Li, J.; Zhang, H.; Dai, X.; Liu, Y.; Liu, Y., High-efficiency dynamic sensing of biothiols in cancer cells with a fluorescent beta-cyclodextrin supramolecular assembly. *Chem. Sci.* **2020,** *11*, 4791-4800.