Supporting Information

Development of ratiometric near-infrared fluorescent probes using analyte-specific cleavage of carbamate

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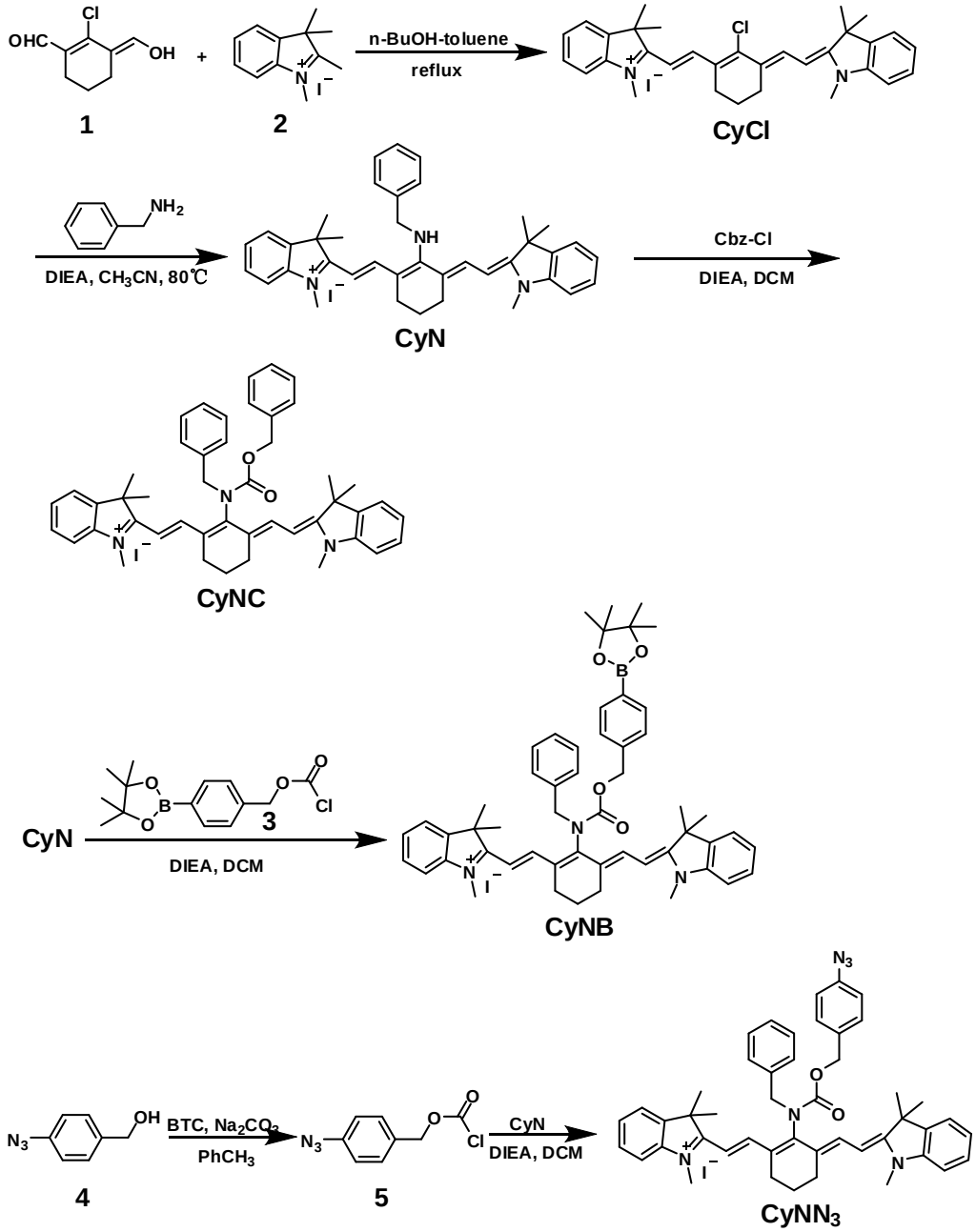
Contents

1. Synthesis of CyCl, CyN, CyNC, CyNB and $CyNN_3$

General Information. All the chemicals and solvents were purchased from Alfa Aesar, *J&K* Chemical Ltd., or Beijing Chemical Reagents, and used as received with the following exceptions. Dichloromethane(DCM) and acetonitrile were distilled from calcium hydride, toluene was distilled from sodium. ^{1}H and ^{13}C NMR spectra were measured on Bruker Avance-400 400 MHz NMR spectrometer and referenced to solvent signals. HRMS-ESI were measured on Bruker Apex IV Fourier transform mass spectrometer. 1[S1], 2[S2], 3[S3], and 4[S4] were prepared according to the literatures.



Scheme S1. Synthesis of CyCl, CyN, CyNC, CyNB and $CyNN_3$.

Compound CyCl

1 (518 mg, 3 mmol) and 2 (1.81 g, 6 mmol) were dissolved in n-BuOH-toluene (7:3)

under argon atmosphere, and refluxed for 10 h with a Dean-Stark condenser. Afterwards, the solvent was evaporated, and the resulting green solid mixture was washed with Et_2O and purified by silica gel column chromatography using DCM/ 0-2% methanol as eluent to afford desired products. Yield: 1.75 g (75%). ^{1}H NMR (400 MHz, d_6-DMSO, ppm) δ 8.22 (d, J = 14.0 Hz, 2H), 7.61 (d, J = 7.2 Hz, 2H), 7.44 (t, J = 3.6 Hz, 4H), 7.30-7.26 (m, 2H), 6.28 (d, J = 14.4 Hz, 2H), 3.68 (s, 6H), 2.71 (t, J = 5.6 Hz, 4H), 1.84 (q, J = 5.6 Hz, 2H), 1.67 (s, 12H). ^{13}C NMR (400 MHz, d_6-DMSO, ppm) δ 172.68, 152.60, 147.70, 142.87, 142.71, 141.02, 128.58, 126.10, 125.16, 122.40, 111.44, 101.89, 48.89, 31.53, 27.34, 27.22, 25.89, 20.45. HRMS (ESI) for $C_{32}H_{36}ClN_2^+$ ([M-I]$^+$): calcd: 483.25615, found: 483.25562.

Compound CyN

CyCl (61 mg, 0.1 mmol) was mixed with benzylamine (44 µL, 0.4 mmol), *N*,*N*-diisopropylethylamine(DIEA) (33 µL, 0.2 mmol), and dissolved in dry acetonitrile (10 mL). The reaction mixture was heated at 80℃ for 40 min under argon atmosphere, quenched with 0.1 N HCl. The organic phase was separated, and the aqueous phase was extracted with dichloromethane (3 × 20 mL). The organic phases were combined and dried over anhydrous sodium sulfate. The solvents were evaporated to give crude solid, which was purified by silica gel column chromatography using DCM/ 0-2% methanol as eluent to afford desired products. Yield: 59 mg (86%). ^{1}H NMR (400 MHz, d_6-DMSO, ppm) δ 8.81 (br s, 1H), 7.57 (d, J = 12.8 Hz, 2H), 7.47 (t, J = 7.6 Hz, 2H), 7.41-7.37 (m, 5H), 7.30-7.26 (m, 2H), 7.13 (d, J = 8.0 Hz, 2H), 7.04 (t, J = 7.2 Hz, 2H), 5.72 (d, J = 13.2 Hz, 2H), 4.87 (d, J = 6.0 Hz, 2H), 3.41 (s, 6H), 2.54 (t, J = 6.0 Hz, 4H), 1.75 (q, J = 6.4 Hz, 2H), 1.38 (s, 12H). ^{13}C NMR (400 MHz, d_6-DMSO, ppm) δ 168.83, 167.76, 143.43, 139.71, 138.25, 137.72, 128.98, 128.03, 127.70, 127.01, 122.45, 121.86, 119.99, 109.00, 94.69, 52.62, 46.92, 27.69, 25.01, 21.27. HRMS (ESI) for $C_{39}H_{44}N_3^+$ ([M-I]$^+$): calcd: 554.35297, found: 554.35223.

Compound CyNC

CyN (68 mg, 0.1 mmol), benzyl carbonochloridate (21 µL, 0.2 mmol) and DIEA (66 µL, 0.4 mmol) were dissolved in dry DCM (20 mL). The resulting solution was stirred at room temperature overnight under argon atmosphere. The reaction mixture was washed with 0.1 N HCl, saturated sodium carbonate and brine, dried over anhydrous sodium sulfate. The solvents were evaporated to give crude solid, which was purified by silica gel column chromatography using DCM/ 0-5% methanol as eluent to afford desired products. Yield: 11 mg (13%). ^{1}H NMR (400 MHz, CD_2Cl_2, ppm) δ 7.43-7.37 (m, 6H), 7.32-7.15 (m, 14H), 6.04 (d, J = 14.0 Hz, 2H), 5.07 (s, 2H), 4.77 (s, 2H), 3.56 (s, 6H), 2.84-2.78 (m, 2H), 2.60-2.55 (m, 2H), 2.14-2.10 (m, 1H), 1.88-1.85 (m, 1H), 1.33 (s, 6H), 1.24 (s, 6H). ^{13}C NMR (400 MHz, CD_2Cl_2, ppm) δ 172.89, 156.03, 155.26, 143.23, 142.89, 141.24, 137.14, 136.66, 130.97, 129.24, 129.05, 128.81, 128.37, 127.96, 125.55, 122.47, 110.97, 101.39, 67.94, 55.95, 49.37, 32.02, 30.09, 29.72, 28.05, 27.77, 25.44, 23.10, 21.25, 14.27. HRMS (ESI) for $C_{47}H_{50}N_3O_2^+$ ([M-I]$^+$): calcd: 688.38975, found: 688.38949.

Compound CyNB

CyN (68 mg, 0.1 mmol), 3 (118 mg, 0.4 mmol) and DIEA (132 µL, 0.8 mmol) were dissolved in dry DCM (20 mL). The resulting solution was stirred at room temperature overnight under argon atmosphere. The reaction mixture was washed with saturated ammonium chloride and brine, dried over anhydrous sodium sulfate. The solvents were evaporated to give crude solid, which was purified by silica gel column chromatography

using DCM/ 0-5% methanol as eluent to afford desired products. Yield: 9.4 mg (10%). ^{1}H NMR (400 MHz, CD_2Cl_2, ppm) δ 7.56 (d, J = 7.6 Hz, 2H), 7.43-7.36 (m, 6H), 7.28 (t, J = 7.6 Hz, 4H), 7.24-7.15 (m, 7H), 6.05 (d, J = 14.0 Hz, 2H), 5.09 (s, 2H), 4.76 (s, 2H), 3.57 (s, 6H), 2.84-2.78 (m, 2H), 2.62-2.54 (m, 2H), 2.16-2.10 (m, 1H), 1.89-1.85 (m, 1H), 1.29 (s, 6H), 1.22 (s, 6H), 1.19 (s, 12H). ^{13}C NMR (400 MHz, CD_2Cl_2, ppm) δ 172.82, 155.91, 155.13, 143.19, 142.84, 141.21, 139.60, 137.10, 135.05, 130.93, 129.22, 129.00, 128.79, 128.29, 127.01, 125.48, 122.40, 110.98, 101.40, 84.18, 67.69, 55.90, 49.32, 32.02, 30.05, 27.95, 27.71, 25.43, 25.09, 24.92, 21.23. HRMS (ESI) for $C_{53}H_{61}BN_3O_4^+$ ([M-I]$^+$): calcd: 814.47496, found: 814.47401.

Compound 5

Sodium carbonate (1.06 g, 10 mmol) was flame dried in a round-bottom flack. The flask was cooled in an ice bath and triphosgene (592 mg, 2 mmol) in toluene (15 mL) was added. After stirring for 0.5 h at 0 °C, 4 (149 mg, 1 mmol) in toluene (10 mL) was added and stirred for 5 h at room temperature. The crude product after usual work-up was used without further purification.

Compound CyNN$_3$

CyN (68 mg, 0.1 mmol), 5 (84 mg, 0.4 mmol) and DIEA (132 µL, 0.8 mmol) were dissolved in dry DCM (20 mL). The resulting solution was stirred at room temperature overnight under argon atmosphere. The reaction mixture was washed with 0.1 N HCl, saturated sodium carbonate and brine, dried over anhydrous sodium sulfate. The solvents were evaporated to give crude solid, which was purified by silica gel column chromatography using DCM/ 0-5% methanol as eluent to afford desired products. Yield: 17 mg (20%). ^{1}H NMR (400 MHz, CD_2Cl_2, ppm) δ 7.42-7.35 (m, 5H), 7.32-7.17 (m, 12H), 6.85 (d, J = 8.0 Hz, 2H), 6.07 (d, J = 14.0 Hz, 2H), 5.04 (s, 2H), 4.75 (s, 2H), 3.58 (s, 6H), 2.84-2.80 (m, 2H), 2.61-2.58 (m, 2H), 2.12 (br, 1H), 1.85 (br, 1H), 1.32 (s, 6H), 1.23 (s, 6H). ^{13}C NMR (400 MHz, CD_2Cl_2, ppm) δ 172.80, 155.92, 155.03, 143.20, 142.73, 141.17, 140.24, 137.05, 133.39, 130.96, 129.79, 129.25, 129.06, 128.82, 128.35, 125.56, 122.45, 119.31, 111.01, 101.49, 67.32, 55.93, 49.32, 32.24, 30.06, 28.02, 27.77, 25.51, 21.27. HRMS (ESI) for $C_{47}H_{49}N_6O_2^+$ ([M-I]$^+$): calcd: 729.39115, Found: 729.39144.

2. UV-visible Absorbance and Fluorometric Analysis

UV-visible spectra were recorded on SHIMADZU UV-2550 UV-vis spectrometer. Fluorescence spectra were recorded using a HITACHI F-4600 spectrometer. The PMT voltage was 700 V, excitation slit and emission slit was 10 nm. The path length was 1 cm with cell volume of 3.0 mL. The stock solution of CyN, CyNC, CyNB, and CyNN$_3$ were prepared in DMSO (2 mM). H_2O_2, *tert*-butylhydroperoxide (TBHP), and hypochlorite (NaOCl) were delivered from 30%, 70%, and 14.5% aqueous solutions, respectively. Hydroxyl radical (•OH), and tert-butoxy radical (•O^{t}Bu) were generated by reaction of Fe^{2+} with H_2O_2 or TBHP, respectively.[S5] Singlet oxygen($^1O_2$) was generated from ClO^- and H_2O_2.[S6] Nitric oxide (NO) was generated from SNP.[S7] Peroxynitrite(ONOO-) was prepared according to the literature.[S8] O_2^-, NO_2^-, NO_3^-, S^{2-}, Cl^-, Br^-, I^-, AcO^-, N_3^-, SCN^-, HCO_3^-, HSO_3^-, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_4^{2-}$, and $S_2O_5^{2-}$ were prepared from KO_2,[S9] $NaNO_2$, KNO_3, Na_2S, NaCl, KBr, KI, NaOAc, NaN_3, KSCN, $NaHCO_3$, $NaHSO_3$, Na_2SO_3, $Na_2S_2O_3$, $Na_2S_2O_4$, and $Na_2S_2O_5$, respectively.

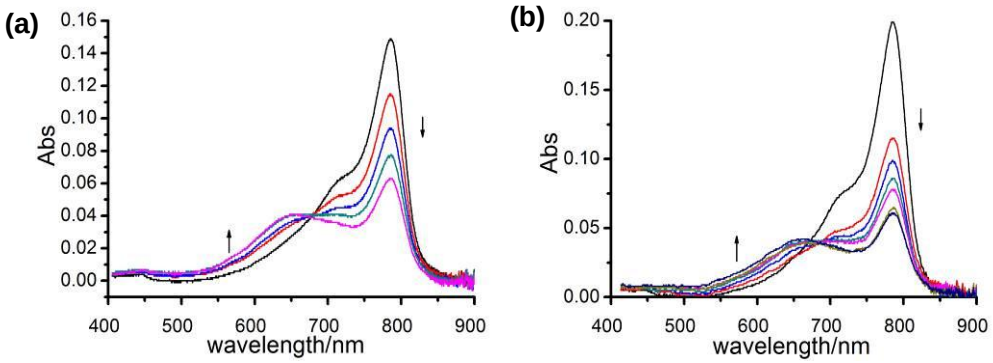


Fig. S1 (a) The change in the absorption spectra of CyNB (1 μM) every 30 min within 2 h after addition of 300 μM H_2O_2 in a mixture of H_2O-DMSO (9:1, v/v, 10 mM HEPES, 100 mM NaCl, pH = 7.4). (b) The change in the absorption spectra of $CyNN_3$ (1 μM) every 10 min within 1 h after addition of 2 mM H_2S in a mixture of H_2O-CH_3CN (8:2, v/v, 40 mM HEPES, pH = 7.4).

3. Solubility

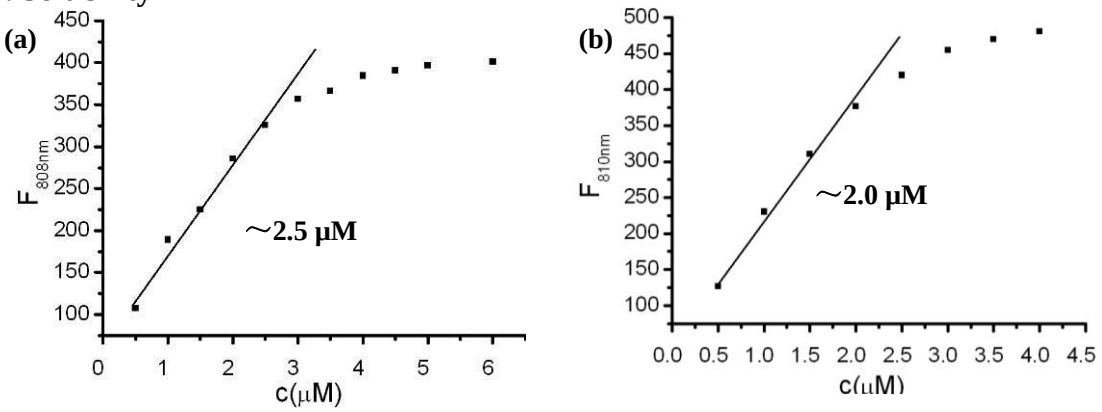


Fig. S2 (a) Plot of fluorescence intensity at 808nm against CyNB concentration in a mixture of H_2O-DMSO (9:1, v/v, 10 mM HEPES, 100 mM NaCl, pH = 7.4). (b) Plot of fluorescence intensity at 810 nm against $CyNN_3$ concentration in a mixture of H_2O-CH_3CN (8:2, v/v, 40 mM HEPES, pH = 7.4).

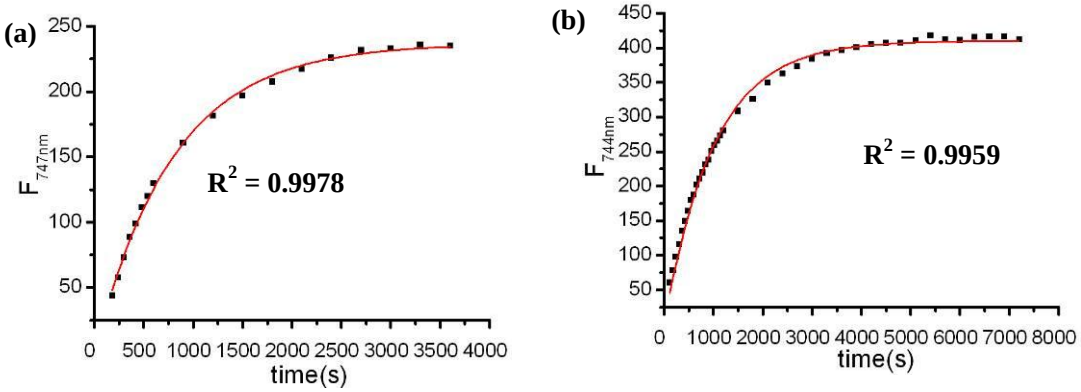


Fig. S3 (a) Kinetic plot of fluorescence emission intensity at 747 nm of the pseudo-first order reaction of 1 μM CyNB to 1 mM H_2O_2, using excitation wavelength at 689 nm. The slope of the plot corresponds to the observed reaction rate of $\mathbf{1.26 \times 10^{-3}\ s^{-1}}$. (b) Kinetic plot of fluorescence emission intensity at 744 nm of the pseudo-first order reaction of 2

μM $CyNN_3$ to 4 mM H_2S, using excitation wavelength at 698 nm. The slope of the plot corresponds to the observed reaction rate of $\mathbf{9.91 \times 10^{-4}\ s^{-1}}$.

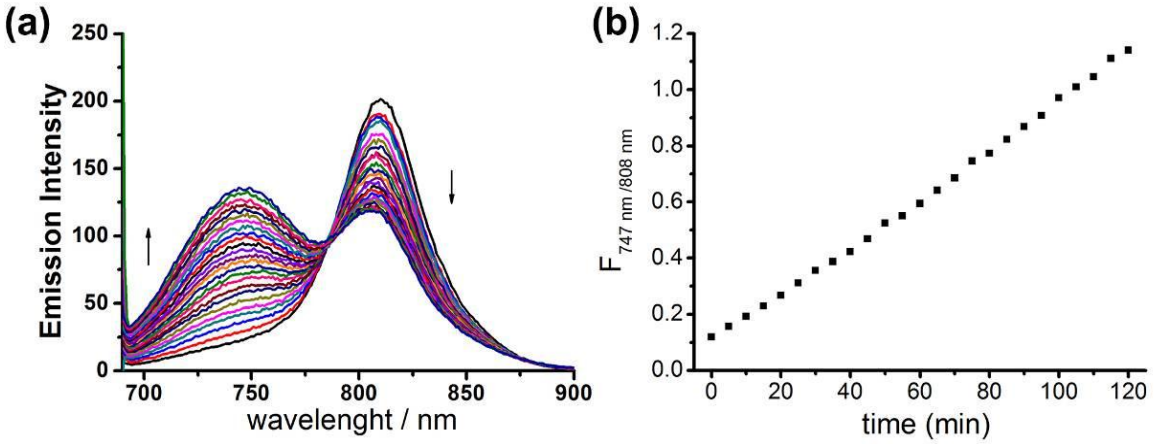


Fig. S4 (a) Fluorescence response of 2 μM CyNB to 30 μM H_2O_2. Spectra were acquired every 5 min after H_2O_2 was added. (b) The emission ratio (F_{747nm}/F_{808nm}) changes as a function of time.

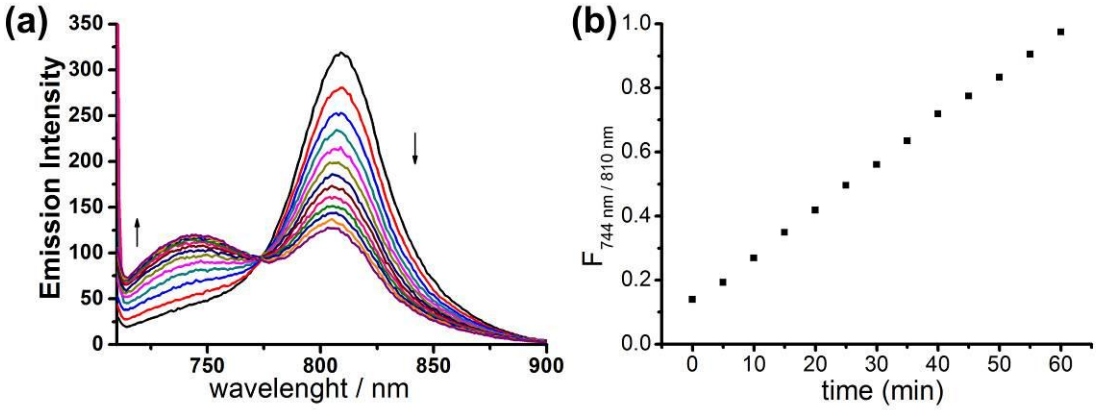


Fig. S5 (a) Fluorescence response of 2 μM $CyNN_3$ to 0.4 mM H_2S. Spectra were acquired every 5 min after H_2S was added. (b) The emission ratio (F_{747nm}/F_{808nm}) changes as a function of time.

4. Determination of quantum yields

Fluorescence quantum yields were determined in the reference of Cardiogreen ($\Phi = 0.13$) in DMSO.[S10] The quantum yields of CyN, CyNC, CyNB and $CyNN_3$ are calculated according to following equation.

$\Phi_x=\Phi_s(A_sS_x)/(A_xS_s)(n_x/n_s)^2$

A_x and A_s are the absorbance of CyN, CyNC, CyNB, $CyNN_3$ and the standard. S_x and S_s are integrated fluorescence emission corresponding to CyN, CyNC, CyNB, $CyNN_3$ and the standard. n is the refractive index of the solvent.

5. pH Effect on the Fluorescence Properties

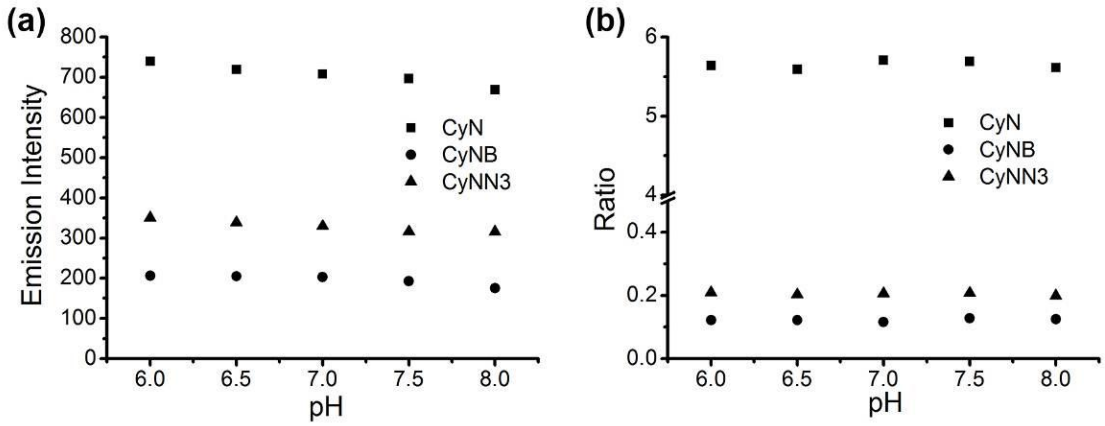


Fig. S6 (a) Effect of pH on fluorescence intensity at 747 nm for CyN, 808 nm for CyNB, 810 nm for $CyNN_3$ in HEPES buffer. (b) Effect of pH on fluorescence intensity ratio of $F_{747\ nm}/F_{808\ nm}$ for CyN, $F_{747\ nm}/F_{808\ nm}$ for CyNB, $F_{744\ nm}/F_{810\ nm}$ for $CyNN_3$ in HEPES buffer. λ_{ex} = 676 nm.

7. Confocal Fluorescence Microscopy

Confocal fluorescence imaging experiments were performed on an Olympus FV-1000 laser scanning microscopy system, based on an IX81 (Olympus, Japan) inverted microscope. The microscope was equipped with multiple visible laser lines (405, 458, 488, 515, 543, 635 nm, CW) and UPLSAPO 60×/N.A 1.42 objective.

8. Cell Culture Methods

NIH 3T3 cells were obtained from Cell Culture Center, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences; School of Basic Medicine Peking Union Medical College.

NIH 3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal calf serum (FCS, Gibco), 50 μg/mL penicillin/streptomycin (Hyclone) at 37°C in a 5/95 CO_2/air incubator. The cells were cultured 3 days before dye loading on a 35 mm diameter glass-bottomed coverslips. Then the cells were incubated with 5 μM probe CyNB in serum-free DMEM (0.5 % DMSO, v/v) for 30 min at 37°C under 5% CO_2, and then washed with PBS three times and bathed in PBS (1 mL) before imaging. Stock solution of H_2O_2 (100 mM) was diluted 500-fold with PBS prior to addition. Excitation wavelength of laser was 635 nm. Emissions were collected at 700-770 nm and 780-800 nm. The cells were incubated with 5 μM probe $CyNN_3$ in serum-free DMEM (0.5 % DMSO, v/v) for 30 min at 37 °C, and then washed with PBS three times. The $CyNN_3$-loaded cells were incubated in serum-free DMEM containing H_2S (1 mM) for another 30 min at 37 °C, the cells were then washed with PBS three times and bathed in PBS (1 mL) before imaging. As a control, the $CyNN_3$-loaded cells incubated in serum-free DMEM without H_2S for 30 min at 37 °C were also imaged. To further prove that the ratio change of $CyNN_3$ in the cells arises from H_2S, another control experiment was carried out. The cells were pretreated with 2 mM $ZnCl_2$ (an efficient eliminator of H_2S)[S11] for 30 min, then incubated with 5 μM $CyNN_3$ for 30 min, and further treated with 1 mM H_2S for 30 min at 37 °C. Excitation wavelength of laser was 635 nm. Emissions were collected at 700-770 nm and 780-800 nm. Images were gathered and processed with Olympus FV10-ASW software (Ver. 2.1c)

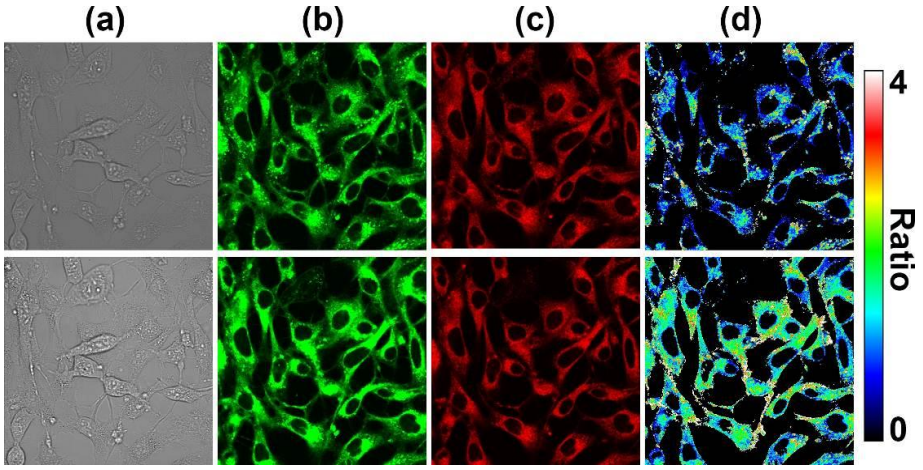


Fig. S7 Confocal fluorescence images of intracellular H_2O_2 in NIH 3T3 cells with CyNB. NIH 3T3 cells incubated with CyNB (5 μM) at 37 °C for 30 min (top); CyNB stained cells were exposed to 100 μM H_2O_2 for 30 min at 25 °C (bottom). (a) Bright-field transmission images. (b) Fluorescence images with emission collected at 700-770 nm. (c) Fluorescence images with emission collected at 780-800 nm. (d) Ratio images of $Em_{700\text{-}770}/Em_{780\text{-}800nm}$ generated from (b) and (c). Raito bar: 0 - 4.

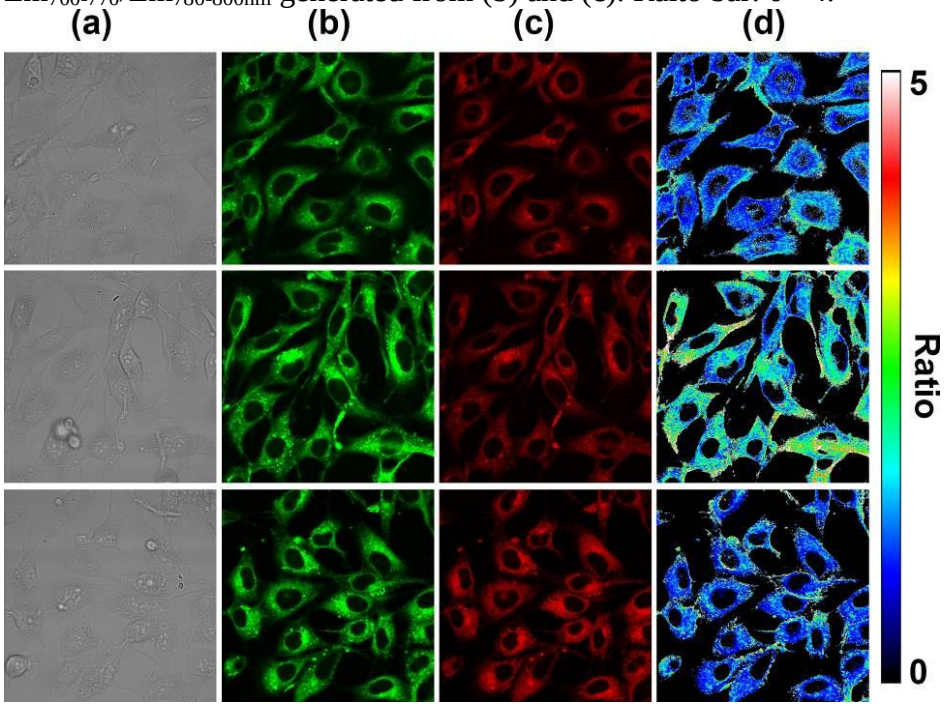


Fig. S8 Confocal fluorescence images of intracellular H_2S in NIH 3T3 cells with $CyNN_3$. Top: Cells were stained with 5 μM CyNN3 at 37 °C for 30 min and further incubated in serum-free DMEM for 30 min. Middle: the cells were pretreated with $CyNN_3$ (5 μM) at 37 °C for 30 min and then incubated with 1 mM H_2S at 37 °C for 30 min, Bottom: the cells were pretreated with 2 mM $ZnCl_2$ at 37 °C for 30 min, incubated with $CyNN_3$ (5 μM) at 37 °C for 30 min and then incubated with 1 mM H_2S at 37 °C for 30 min. (a) Bright-field transmission images. (b) Fluorescence images with emission collected at 700-770 nm. (c) Fluorescence images with emission collected at 780-800 nm. (d) Ratio images of $Em_{700\text{-}770}/Em_{780\text{-}800nm}$ generated from (b) and (c). Ratio bar: 0 - 5.

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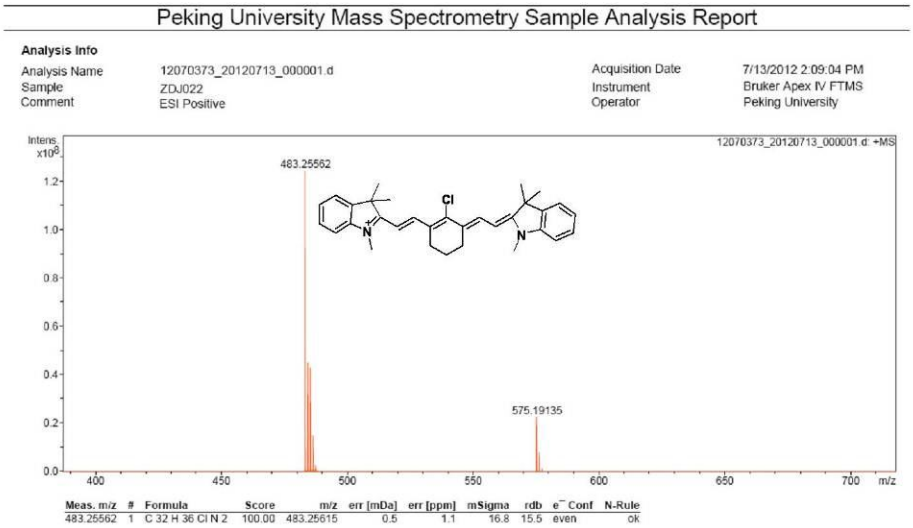
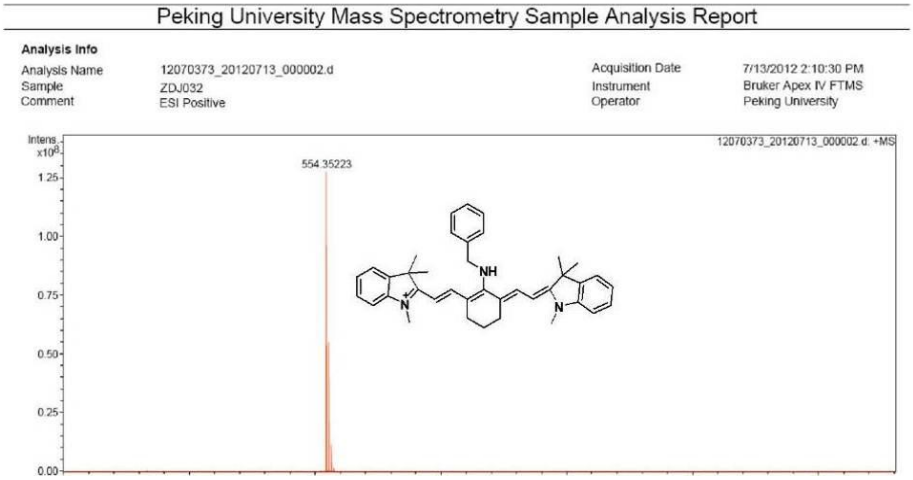
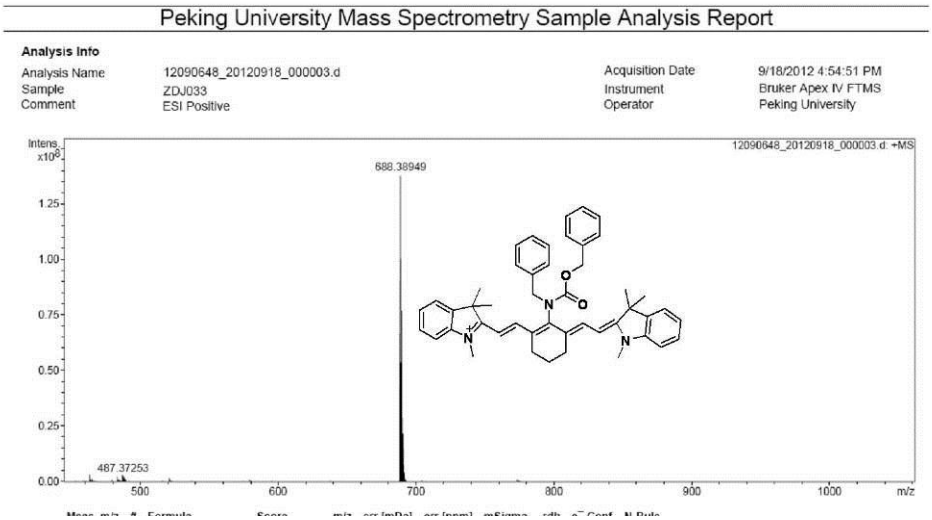
9. HRMS-ESI Spectra







Peking University Mass Spectrometry Sample Analysis Report

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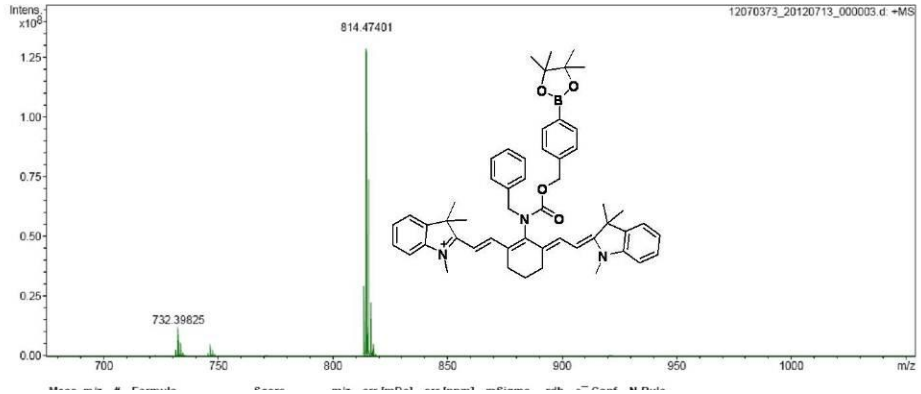

Peking University Mass Spectrometry Sample Analysis Report

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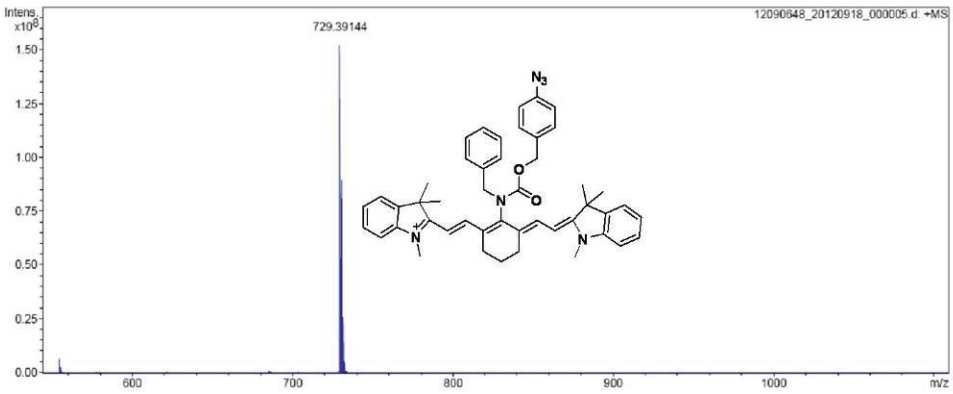

10. NMR Data