Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information

A near-infrared fluorescent sensor for selective detection of cysteine and its application in live cells imaging

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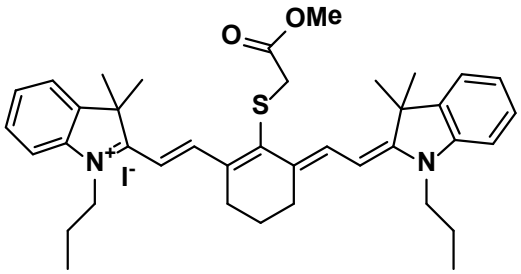
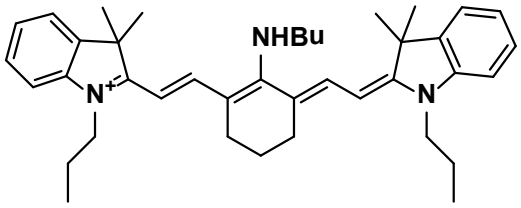
Synthesis



Synthesis of Cy-S. Cy-NO$_2$ (31 mg, 0.04 mmol) and methyl mercaptoacetate (9 μL, 0.1 mmol) was dissolved in acetonitrile (10 mL), and one drop of triethylamine was added. The reaction mixture was stirring at room temperature for 30 min, and then evaporated. The crude product was purified through column chromatography over silica (dichloromethane / methanol = 50/1 as eluent) to give **Cy-S** (26 mg, 89%) as green solid. ^{1}H NMR (400 MHz, Acetone-d_6): δ 8.76 (d, 2H, J = 14.4 Hz), 7.49 (d, 2H, J = 7.6 Hz), 7.32 (d, 4H, J = 4.0 Hz), 7.18 (m, 2H), 6.34 (d, 2H, J = 14.4 Hz), 4.16 (t, 4H, J = 7.2 Hz), 3.54 (s, 2H), 3.49 (s, 3H), 2.58 (t, 4H, J = 6.4 Hz), 1.78 (m, 4H), 1.68 (s, 6H), 0.94 (t, 6H, J = 7.6 Hz) .^{13}C NMR (100 MHz, Acetone-d_6): 173.7, 170.0, 154.9, 146.3, 143.6, 142.2, 134.1, 129.5, 126.0, 123.3, 112.1, 102.5, 52.8, 50.2, 46.4, 39.1, 28.1, 27.0, 21.7, 21.6, 11.6. ESI-HRMS: calculated for $[C_{39}H_{49}N_2O_2S]^+$ 609.35093, found 609.35069.



Synthesis of Cy-N. Cy-NO$_2$ (39 mg, 0.05 mmol) was dissolved in 10 mL acetonitrile, and the solution was added 50 μL n-butylamine (37 mg, 0.5 mmol). The reaction mixture was stirred at room temperature for 2 h, and then evaporated. The crude product was purified through column chromatography over silica (dichloromethane / methanol = 50/1 as eluent) to give **Cy-N** (30 mg, 86%) as an green solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.21 (d, 1H, J = 5.2 Hz), 7.73 (d, 2H, J = 12.8 Hz), 7.26 (m, 4H), 7.04 (m, 2H), 6.83 (m, 2H), 5.56 (d, 2H, J = 12.8 Hz), 3.75 (m, 4H), 3.61 (d, 3H, J = 5.2 Hz), 2.52 (t, 4H, J = 6.0 Hz), 1.83 (m, 6H), 1.74 (s, 12H), 1.04 (t, 6H, J = 7.2 Hz). ESI-HRMS: calculated for $[C_{40}H_{54}N_3]^+$ 576.43123, found 576.43294.

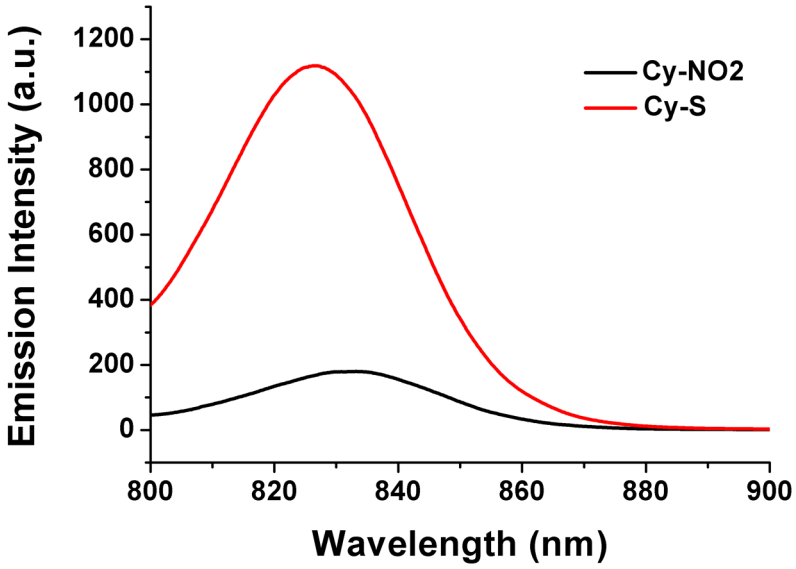


Fig. S1. Fluorescent spectra changes of **Cy-NO$_2$** (10 μM) before and after the addition of methyl mercaptoacetate in DMSO / HEPES buffer (1:4, v/v, 20 mM, pH 7.4) at 37 °C. λ_{ex} = 785 nm.

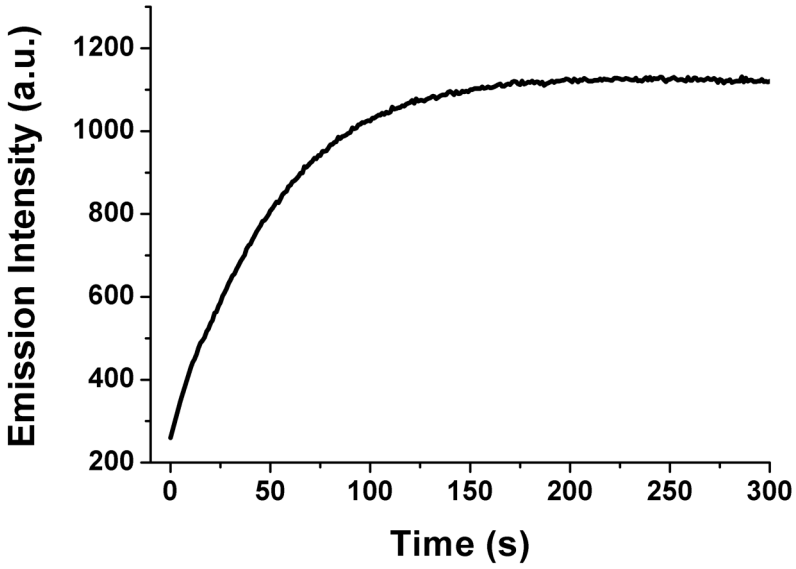


Fig. S2. Time course of the response at 827 nm of **Cy-NO$_2$** (10 μM) to 100 equiv of methyl mercaptoacetate in DMSO / HEPES buffer (1:4, v/v, 20 mM, pH 7.4) at 37 °C. λ_{ex} = 785 nm.

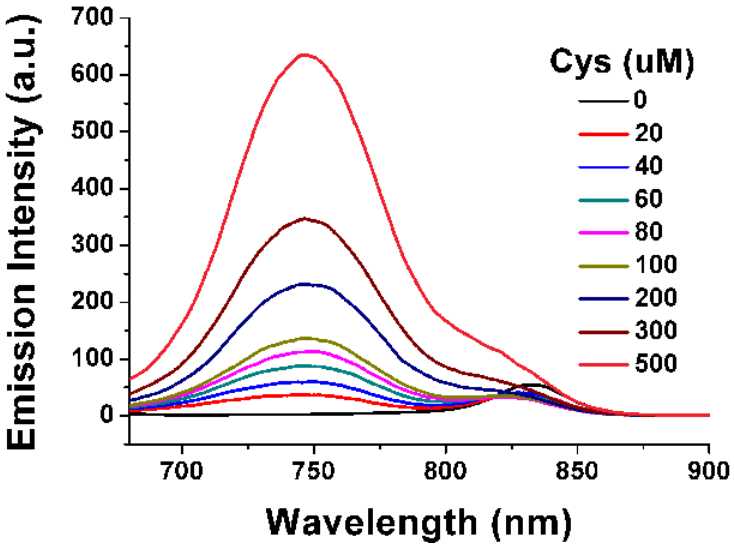


Fig. S3. Emission responses of **Cy-NO$_2$** (10 μM) to different concentrations of Cys in DMSO/HEPES buffer (1: 4, v/v, 20 mM, pH 7.4) at 37 °C. Each spectrum was recorded 60 min after addition. λ_{ex} = 650 nm.

Determination of the detection limit

The detection limit was determined based on the fluorescence titration to different concentrations of Cys (S/N = 3). The detection limit was calculated with the following equation:

Detection limit = $3\sigma/k$

Where σ is the standard deviation of blank measurement, k is the slop between the emission intensity as a function of the concentration of Cys. The fluorescence emission spectrum of **Cy-NO$_2$** was measured by five times and the standard deviation (σ) was calculated. The emission intensity at 750 nm was plotted as a concentration of Cys, and the slop (k) was achieved.

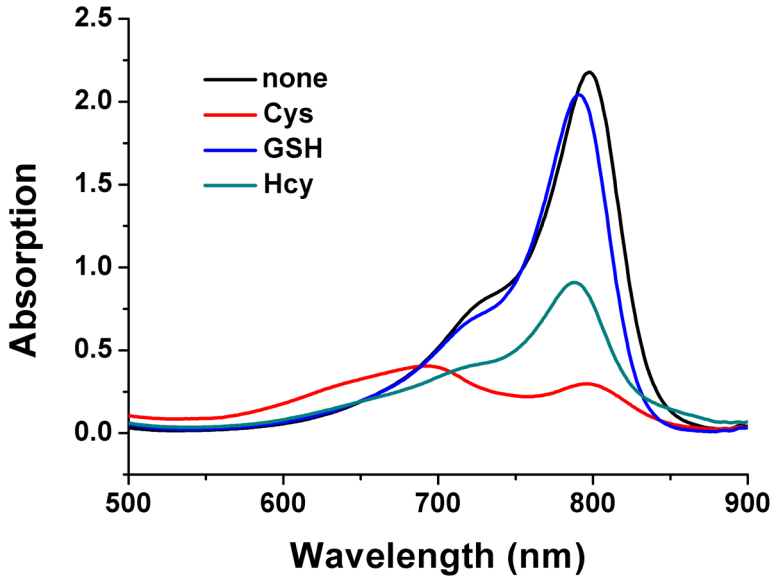


Fig. S4. Absorption spectra of **Cy-NO$_2$** in the prescence of 100 equiv of Cys, Hcy and GSH in DMSO / HEPES buffer (1:4, v/v, 20 mM, pH 7.4) at 37 °C. Each spectrum was recorded 60 min after addition.

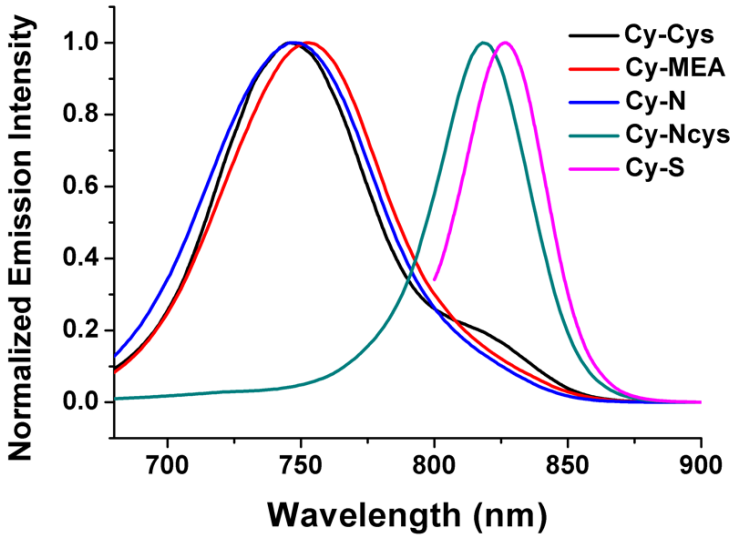


Fig. S5. Normalized emission spectra of **Cy-Cys, Cy-MEA, Cy-N, Cy-Ncys** and **Cy-S,** which correspond to the reaction products of **Cy-NO$_2$** with Cys, MEA, n-butylamine, N-acetylcysteine and methyl mercaptoacetate, respectively.

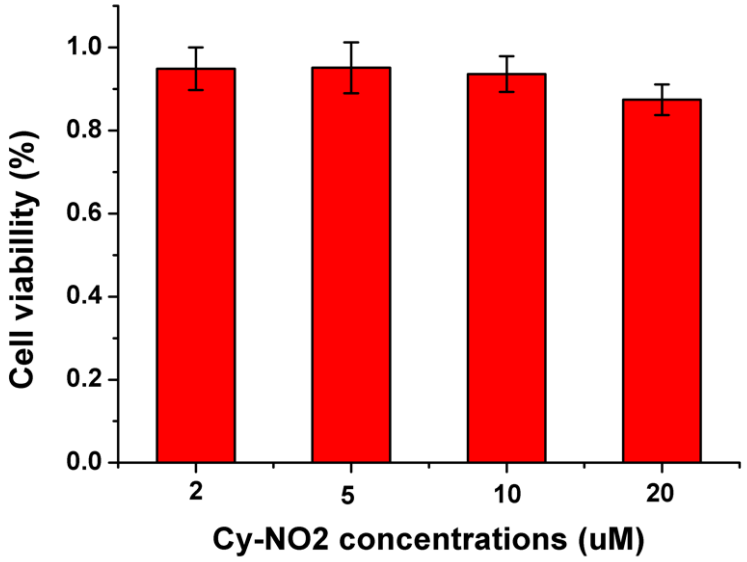


Fig. S6. Cell viability of HeLa cells to different concentrations of **Cy-NO_2** for 24 h incubation at 37 °C in a humidified atmosphere of 5% CO_2.

^{1}H NMR, ^{13}C NMR of Cy-NO_2