## Supplementary Materials: A Simple and Effective Ratiometric Fluorescent Probe for the Selective Detection of Cysteine and Homocysteine in Aqueous Media

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## 1. Synthesis of the NL probe



Scheme S1. Synthesis routes for the NL fluorescent probe.

4-Hydroxybenzothioamide 1 (20 mmol), 2-bromo-3-oxobutyric acid ethyl ester 2 (24 mmol) and absolute ethanol (30 mL) were added to a round-bottomed flask. The mixture was refluxed for 3 h. After cooling to room temperature, the green solid was filtered off, and washed with ethanol. The crude product recrystallized from ethanol to give compound 3 in 91% yield. Yellow solid, m.p. 187–190 °C.

Compound **3** (24 mmol) and HMTA (1 mL) and trifluoroacetic acid (30 mL) were added to a round-bottomed flask. The mixture was refluxed for 20 h. After completion of the reaction, the solvent was removed by distillation. The residue was poured into ice water and stirred vigorously, the solid product was filtered and washed with water, then dried in a vacuum to give compound NL, recrystallized in toluene in 80% yield. Yellow solid; m.p. 97.0–99.0 °C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  10.32 (s, 1H), 8.22 (d, *J* = 2.4 Hz, 1H), 8.09 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.13 (d, *J* = 8.7 Hz, 1H), 4.29 (q, *J* = 7.0 Hz, 2H), 2.67 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H), <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>):  $\delta$  190.62, 168.51, 163.52, 161.80, 160.71, 134.50, 127.17, 124.04, 123.05, 120.83, 118.86, 61.59, 17.65, 14.58. HRMS (ESI) calcd for C<sub>14</sub>H<sub>12</sub>NO<sub>4</sub>S<sup>-</sup> m/z [M – H]<sup>-</sup> 290.0493, found 290.0487.

## 2. Supplementary Figures









Figure S1. NMR Spectra for NL and NL + Hcy.



## UPLC-MS chromatogram and spectrum of NL



Figure S2. UPLC-MS chromatograms and spectra of NL, NL + Cys, and NL + Hcy.



**Figure S3.** Fluorescence response of **NL** ( $1 \times 10^{-5}$  M) without (black) and with (gray) Cys ( $2 \times 10^{-4}$  M) in HEPES buffer solution with different ratios of DMSO and HEPES. (pH 7.4,  $\lambda_{ex}$  = 327 nm, slit: 2.0 nm/2.0 nm).



**Figure S4.** Fluorescence response of **NL** ( $1 \times 10^{-5}$  M) toward Cys ( $2 \times 10^{-4}$  M) without and with other analytes ( $2 \times 10^{-4}$  M) in HEPES buffer solution (DMSO/HEPES = 8:2, pH 7.4) at 501 nm ( $\lambda_{ex}$  = 327 nm, slit: 2.0 nm/2.0 nm).



**Figure S5.** Fluorescence response of **NL** ( $1 \times 10^{-5}$  M) toward Hcy ( $2 \times 10^{-4}$  M) without and with other analytes ( $2 \times 10^{-4}$  M) in HEPES buffer solution (DMSO/HEPES = 8:2, pH 7.4) at 501 nm ( $\lambda_{ex}$  = 327 nm, slit: 2.0 nm/2.0 nm).



**Figure S6.** Time-dependent fluorescence intensity at 501 nm of **NL** ( $1 \times 10^{-5}$  M) without and with Cys ( $2 \times 10^{-4}$  M), Hcy ( $2 \times 10^{-4}$  M), and GSH ( $2 \times 10^{-4}$  M) in HEPES buffer solution (DMSO/HEPES = 8:2, pH 7.4,  $\lambda_{ex}$  = 327 nm, slit: 2.0 nm/2.0 nm).



**Figure S7.** Fluorescence intensity ratio at 426 nm and 501 nm with various concentrations of Cys, Hcy, and GSH. **NL** (1 × 10<sup>-5</sup> M) in HEPES buffer solution (DMSO/HEPES = 8:2, pH 7.4,  $\lambda_{ex}$  = 327 nm, slit: 2.0 nm/2.0 nm). Data are means ± SE (bars) (n = 3).



**Figure S8.** Fluorescence response of **NL** ( $1 \times 10^{-5}$  M) without or with Cys ( $2 \times 10^{-4}$  M) in HEPES buffer solution (DMSO/HEPES = 8:2,  $\lambda_{ex}$  = 327 nm, slit: 2.0 nm/2.0 nm) at different pH.