

Supporting Information for

Near-infrared fluorescent probes for the detection of glutathione and their application in the fluorescent imaging of living cells and tumor-bearing mice

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normal (square) tissues obtained from the *in vivo* imaging
(a).....S11

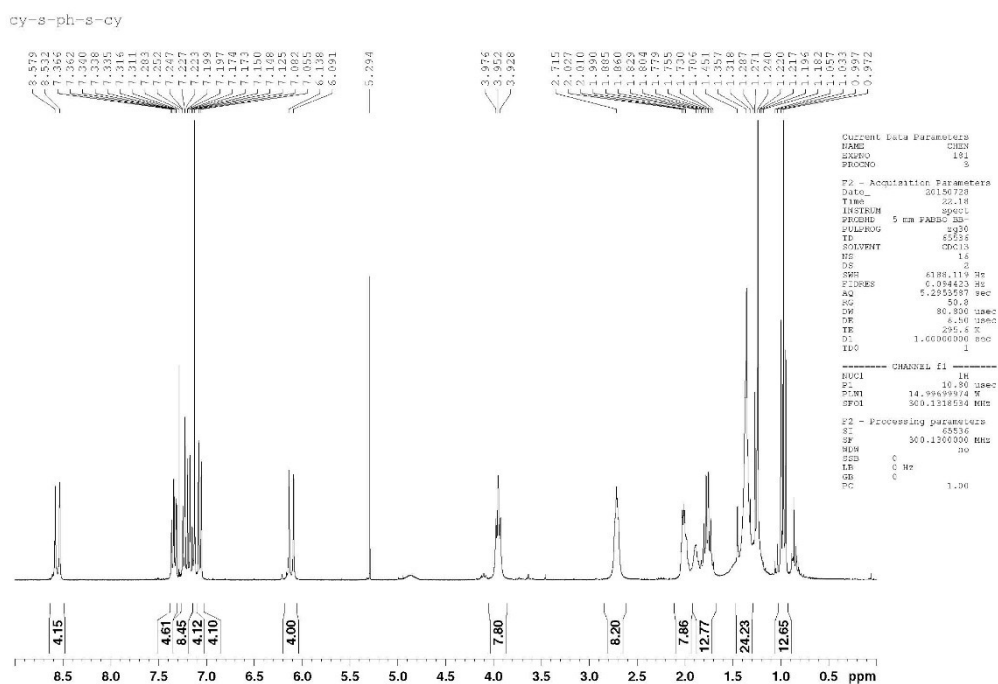


Figure S1. ¹H NMR (300 MHz) of compound 1 in CDCl₃

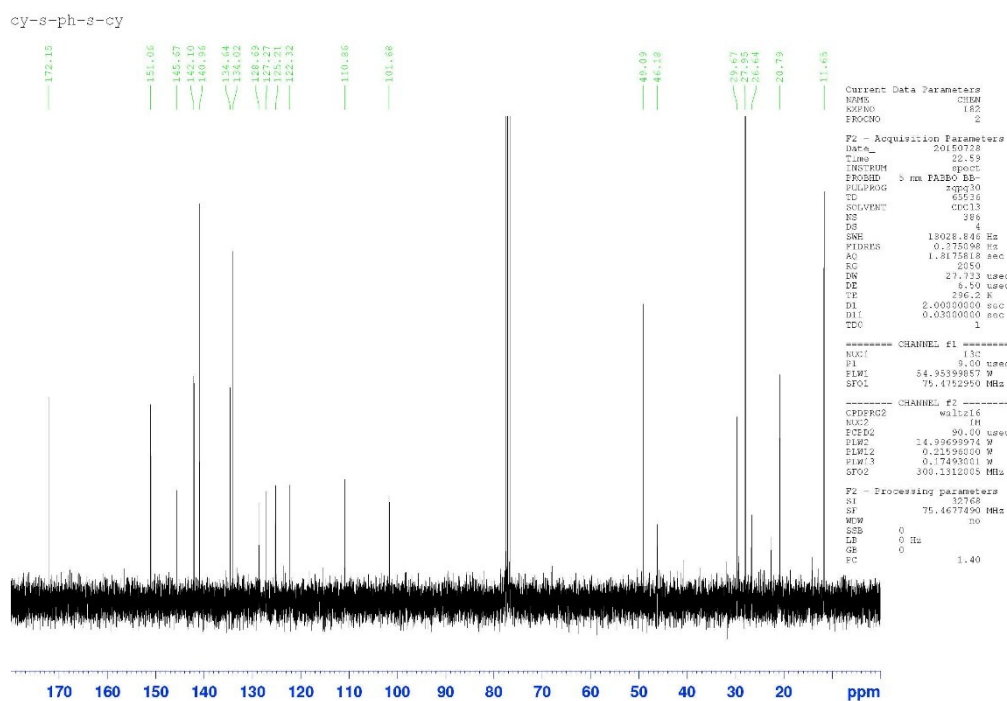


Figure S2. ¹³C NMR (75 MHz) of compound 1 in CDCl₃.

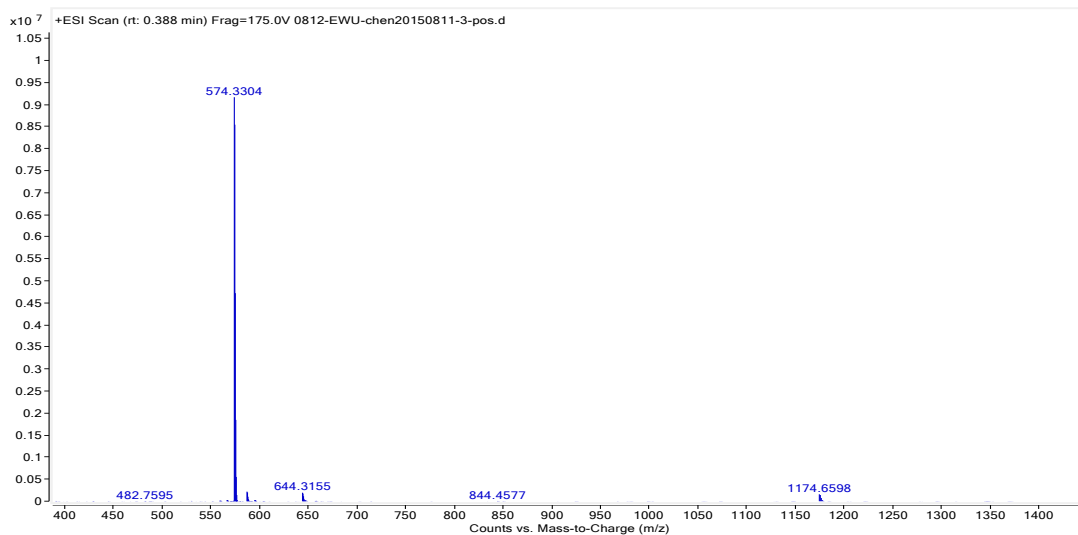


Figure S3. ESI mass spectrum of compound 1.

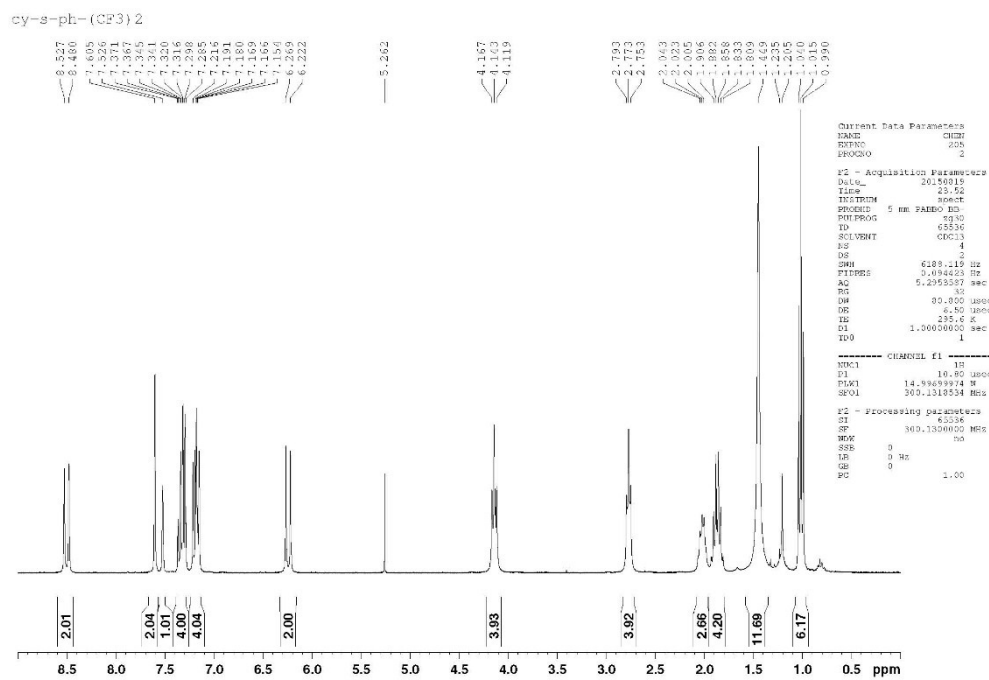


Figure S4. ¹H NMR (300 MHz) of compound 2 in CDCl₃

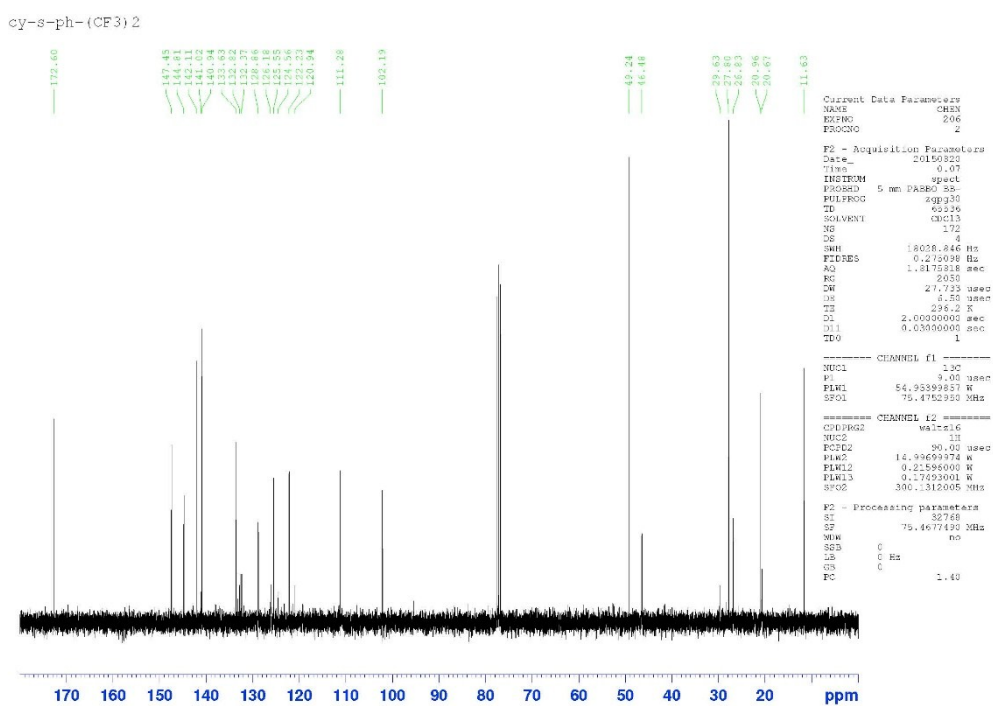


Figure S5. ¹³C NMR (75 MHz) of compound **2** in CDCl₃.

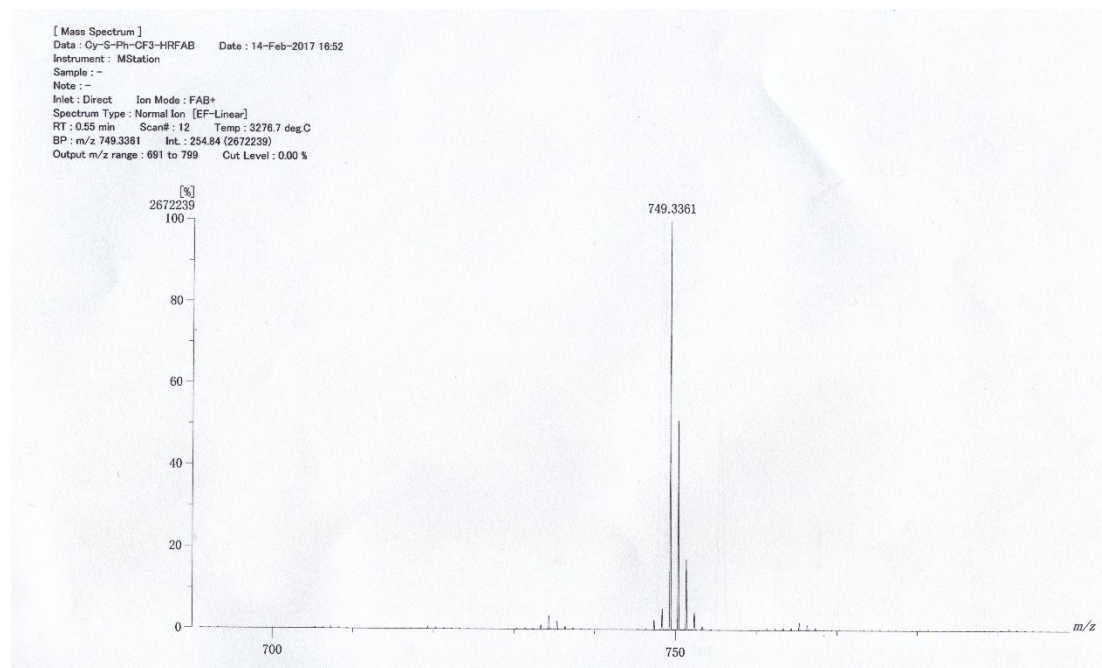


Figure S6. FAB mass spectrum of compound **2**.

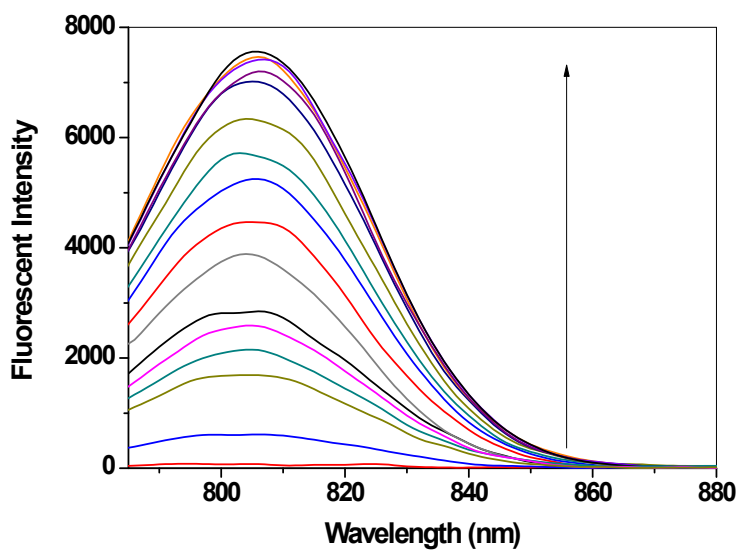


Figure S7. Fluorescence titrations of probe 1 (10 μ M) with GSH (0-50 eq.) in HEPES (0.01 M, pH 7.4) containing 1% DMSO (excitation wavelength : 780 nm). Spectrum was obtained 60 min after mixing.

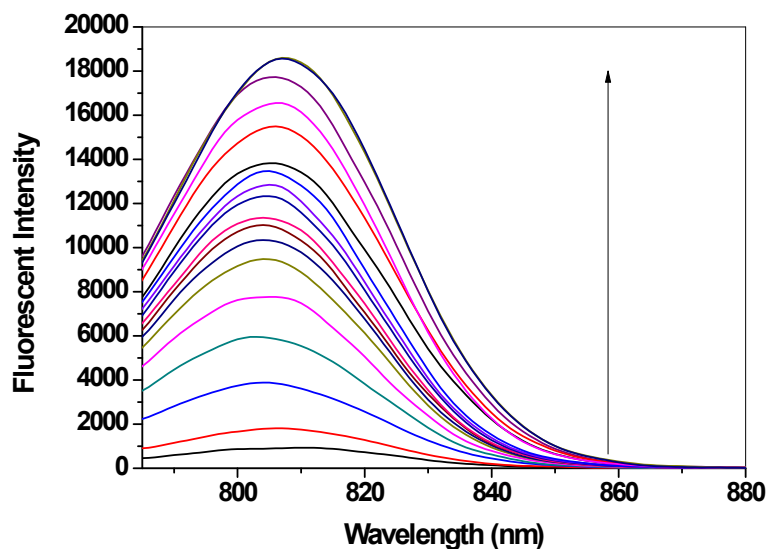


Figure S8. Fluorescence titrations of probe 2 (10 μ M) with GSH (0-50 eq.) in HEPES (0.01 M, pH 7.4) containing 1% DMSO (excitation wavelength : 780 nm). Spectrum was obtained 60 min after mixing.

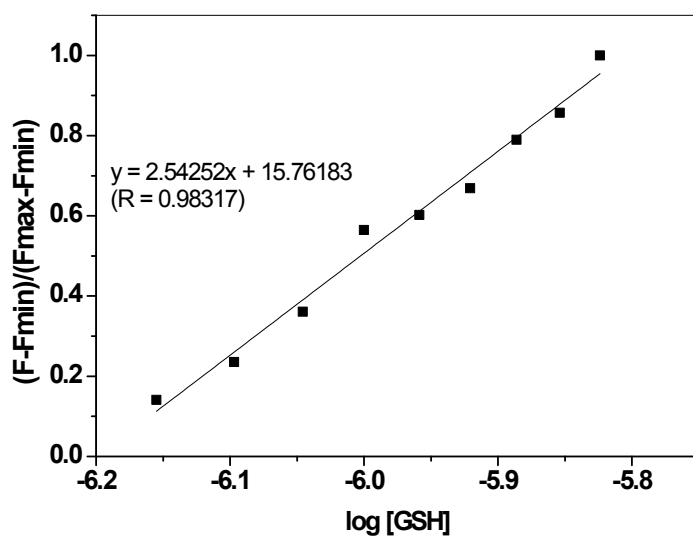


Figure S9. Normalized fluorescence responses of probe **1** (1 μM) to changing GSH concentrations in DMSO-HEPES (0.01M, pH 7.4) (1:99, v/v). (Detection limit = $6.32 \times 10^{-7} M$)

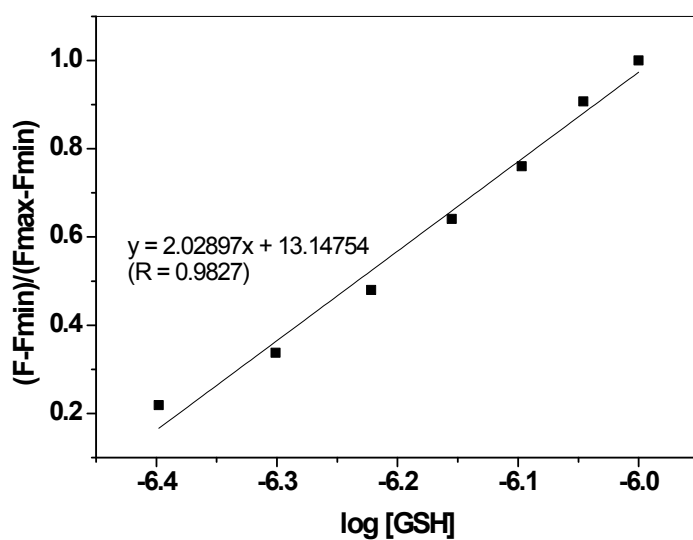


Figure S10. Normalized fluorescence responses of probe **2** (1 μM) to changing GSH concentrations in DMSO-HEPES (0.01M, pH 7.4) (1:99, v/v). (Detection limit = $3.31 \times 10^{-7} M$)

+ESI Scan (rt: 0.153 min) Frag=380.0V 170520-EWU-Cy-GS1

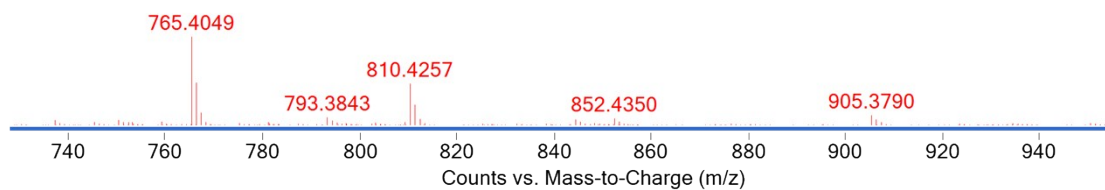


Figure S11. LC/MS characterization of probe **1**+GSH.

+ESI Scan (rt: 0.190 min) Frag=380.0V 170530-EWU-Cy-GS2

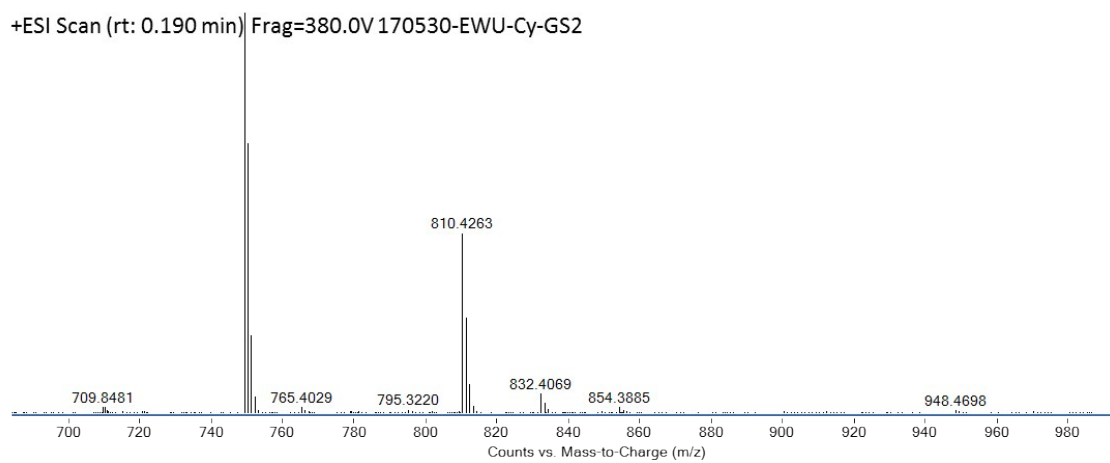


Figure S12. LC/MS characterization of probe **2**+GSH.

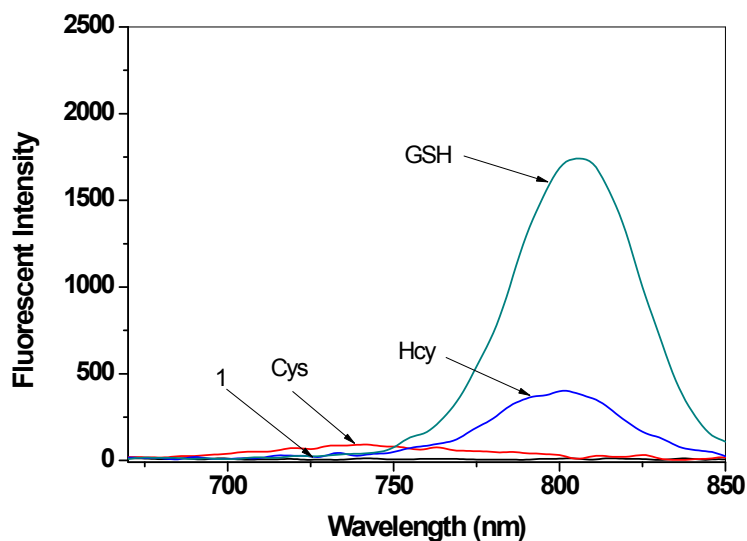


Figure S13. Fluorescent changes of probe **1** (10 μM) upon the addition of Cys, Hcy, and GSH (10 equiv.) in HEPES (0.01 M, pH 7.4) containing 1% DMSO (Excitation wavelength: 650 nm). Each spectrum was obtained 30 min after mixing.

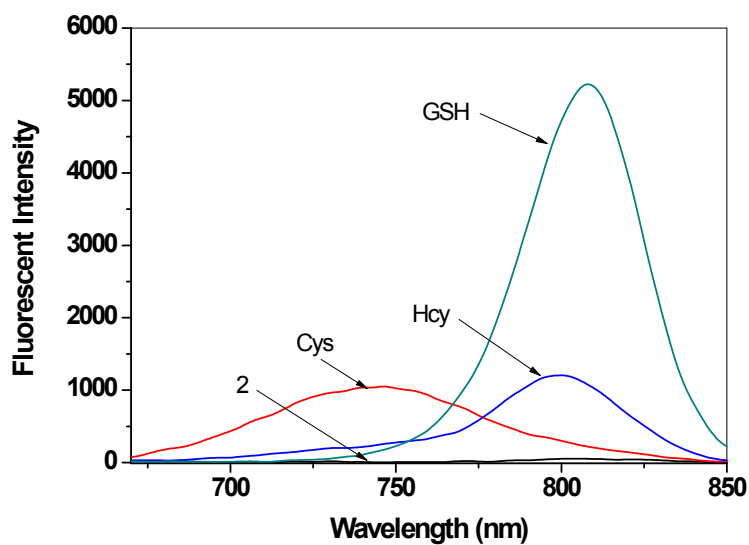


Figure S14. Fluorescent changes of probe **2** (10 μM) upon the addition of Cys, Hcy, and GSH (10 equiv.) in HEPES (0.01 M, pH 7.4) containing 1% DMSO (Excitation wavelength: 650 nm). Each spectrum was obtained 30 min after mixing.

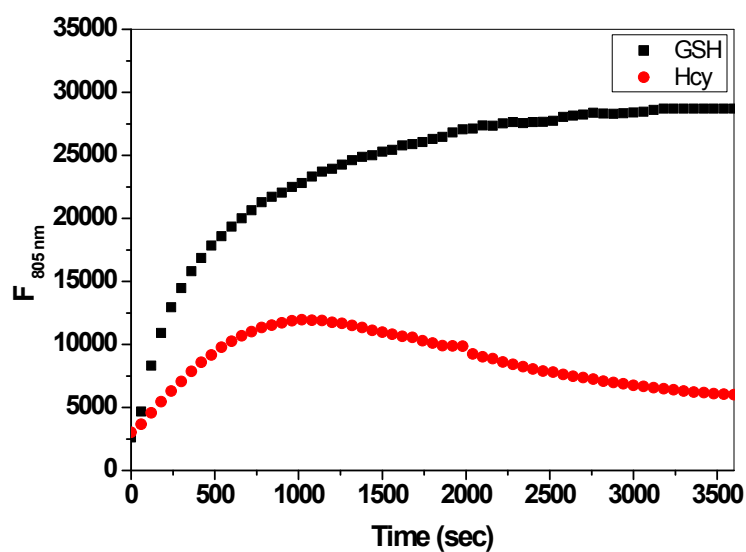


Figure S15. Time-dependent change of probe 1 (10 μ M) with the addition of 10 equiv. of GSH or Hcy in DMSO-HEPES (0.01M, pH 7.4) (1:99, v/v) (Excitation wavelength: 780 nm).

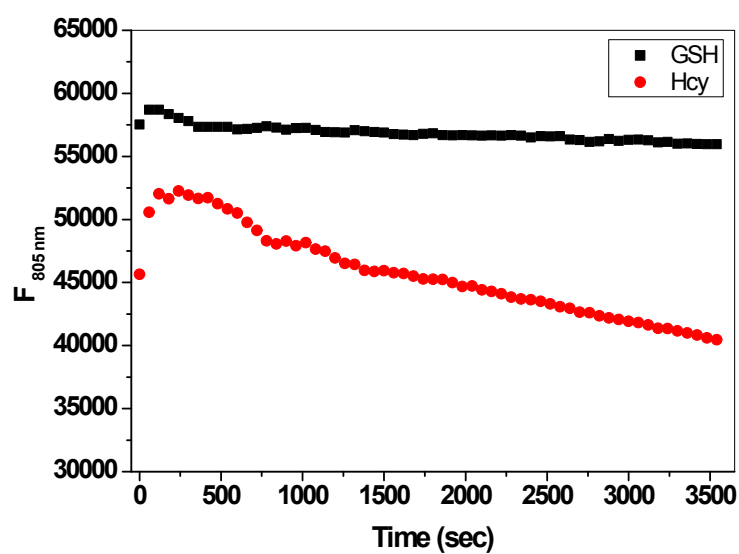


Figure S16. Time-dependent change of probe 2 (10 μ M) with the addition of 10 equiv. of GSH or Hcy in DMSO-HEPES (0.01M, pH 7.4) (1:99, v/v) (Excitation wavelength: 780 nm).

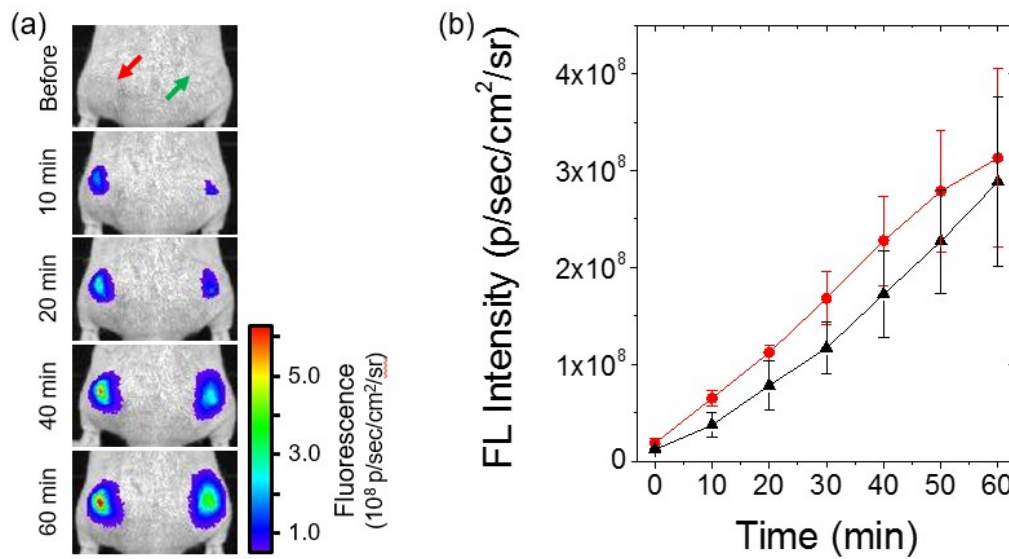


Figure S17. *In vivo* fluorescence responses of probe **2** in SCC7 tumor-bearing mice (n=4). a) Temporal fluorescence images of mice before and after the probe injection ($\lambda_{\text{ex}} = 745 \text{ nm}$, $\lambda_{\text{em}} = 820 \text{ nm}$), where probe **2** ($40 \mu\text{L}$, $10 \mu\text{M}$ in PBS, pH 7.4) was injected peritumorally (tumor side, red arrow) or subcutaneously (normal side, green arrow). b) Temporal intensity changes of fluorescence from tumor (circle) and normal (triangle) tissues obtained from the *in vivo* imaging (a).