SUPPORTING INFORMATION

Extremely Selective and Fluorescent Detection of Cysteine Or Superoxide: with Aliphatic Ester Hydrolysis

Dhiraj P. Murale, a Hwajin Kim, b Wan Sung Choi, b Youngsam Kim, a David G. Churchill* a

a Molecular Logic Gate Laboratory, Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), 373-1 Guseong-dong, Yuseong-gu, Daejeon, 305-701, Republic of Korea. Fax: (+82) 42-350-2810; Tel: (+82) 42-350-2845; E-mail: dchurchill@kaist.ac.kr

b Department of Anatomy and Neurobiology, Medical Research Center for Neural Dysfunction, Institute of Health Science, School of Medicine, Gyeongsang National University, 92 Chilamdong, Jinju, Gyeongnam 660-751, Republic of Korea.
Experimental Section

**General Remarks.** All reagents used herein were used as received from commercial suppliers (Aldrich, Acros, and Junsei companies). $^1$H and $^{13}$C NMR spectra were acquired using a Bruker Avance 400 MHz spectrometer. TMS was used as an internal standard. $^1$H and $^{13}$C NMR spectral signals were calibrated internally by the respective protio impurity or carbon resonance of the NMR spectroscopic solvent, e.g., DMSO-$d_6$. ESI-mass spectrometry was performed on a VG AUTOSPEC ULTIMA by the research support staff at KAIST. This instrument possesses a trisector double focusing magnetic sector analyzer and was operated at a resolution of 80,000. Absorption spectra were measured using a JASCO V–530 UV/Vis spectrophotometer. Fluorescence measurements were carried out with a Shimadzu RF–5301pc spectrofluorophotometer.
**Synthesis of probe 1.** In a 100 mL round bottom flask, Fluorescein (0.50 g, 1.5 mmol) and vinylacetic acid (0.25 mL, 3.0 mmol) were dissolved up in 30 mL of tetrahydrofuran. Then 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.69 mg, 3.61 mmol) and 4-dimethylaminopyridine (DMAP) (0.367 mg, 3.01 mmol) were added at room temperature. The reaction mixture was allowed to react at room temperature overnight; after this time, the solvent was removed under pressure. The obtained crude material was subjected to column chromatography and purified by using hexane: ethyl acetate (8:2) as an eluent. Probe 1 was obtained as a white solid (0.47 g, 67 % yield).

**1H NMR spectroscopy:** (400 MHz, CDCl$_3$): 8.10 (d, $^3J_{(H,H)}$ = 7.24 Hz, 1H), 7.69-7.60 (2H, m), 7.22-7.12 (5H, m), 6.84 (d, $^3J_{(H,H)}$ = 1.52 Hz, 4H), 6.03 (d, $^3J_{(H,H)}$ = 15.6 Hz, 2H), 1.95 (dd, $^4J_{(H,H)}/^3J_{(H,H)}$ = 1.64, 6.92 Hz, 6H).

**13C NMR spectroscopy:** (100 MHz, CDCl$_3$): 169.11, 164.10, 152.87, 152.20, 151.57, 147.93, 135.30, 130.06, 128.86, 126.13, 125.15, 124.07, 121.59, 117.83, 116.30, 110.41, 81.76, 18.27.

**ESI-MS:** [Fig. S3] probe-1 [M+Na]$^+$ = 491.1107 (calc.), 491.1101(exp.)

**ESI-MS:** [Fig. S4] of probe 1 + L-cys [M+H]$^+$ = 333.068 (calc.), 333.075 (exp.) [Fluorescein]
**Analysis of Cys in living cells.** A human neuroblastoma cell line, SH-SY5Y, was grown in a 1:1 mixture of Eagle’s Minimum Essential Medium, and Ham’s F12 Medium supplemented with 10 % FBS, 100 U/mL penicillin and 100 μg/mL streptomycin and incubated in a humidified 5 % CO₂ incubator at 37°C. Cells were seeded in a 24-well plate in ~80 % confluence for one day before the experiments. Cells were incubated with probe 1 (1~20 μM) or vehicle (DMSO) for 1 h, washed with PBS. For the N-ethyl maleimide (NEM) treated samples, cells were pre-incubated with NEM (0.25 mM) for 30 min. Cells were washed with PBS before fluorescence imaging. Fluorescence images of the cells were obtained by an epifluorescence microscope (Olympus, JP IX-71). The excitation source was a 490 nm laser and all images were taken under the same experimental conditions to minimize variations in fluorescence intensity.
Fig. S1. $^1$H NMR spectrum of Probe 1.
Fig. S2a. $^{13}$C NMR spectrum of Probe 1.
Fig. S2b. $^{13}$C NMR spectrum of Probe 1 (EXPANDED VIEW).
Fig. S3. ESI–mass spectrum of Probe 1.
**Fig. S4.** ESI–mass spectrum of Probe 1 + dissolved cysteine. This data aided in identifying fluorescein as a final product.
**Fig. S5.** $^1$H NMR spectrum of probe 1 and probe 1a with 0.2 equiv of DMAP (4-dimethylaminopyridine).
Fig. S6. Relative fluorescence intensity of compound 1 (4.0 × 10⁻⁶ M, buffered H₂O:DMSO 80:20; pH 7.4 PBS) with Cys (~10 equiv) with other amino acids (from left to right: A–compound, B–comp + cys, C–comp + cys + GSH, D–comp + cys + GSH, E–comp + cys + N–acetyl–L–Cys (~10 equiv). $\lambda_{exc} = 490$ nm. Slit width = 1.5.
Fig. S7. Detection limit determinations for cysteine calculated from the linear fit (33.3 – 266 μM in water).
**Fig. S8.** Proposed sensing mechanism with probe 1a.

**Fig. S9.** Time–dependent emission spectra of probe (2.0 × 10⁻⁶ M) with Cys (200 μM in water) λ<sub>exc</sub> = 490 nm) slit width Ex. & Em. = 1.5 nm.
**Fig. S10.** Emission spectra of probe (4.0 × 10^{-6} M, buffered H_{2}O: DMSO 80:20; pH 7.4 PBS) with amino acids L–Cys, Hcy, N–acetyl–L–Cys and GSH (66 μM in water) incubated for 1 h at RT. Slit width for Ex. & Em. = 1.5 nm.

**Fig. S11.** Relative fluorescence intensity of probe-1 (4 × 10^{-6} M, H_{2}O: DMSO 80:20;) with KO_{2} and the presence of other interfering ROS (A) comp, (B) KO_{2}, (C) KO_{2} + NaOCl, (D) KO_{2} + H_{2}O_{2}, (E) KO_{2} + \cdotOH, (F) KO_{2} + tBuOOH, (G) KO_{2} + O'Bu, (~10 equiv) incubated for 30 min at RT. λ_{exci} = 490 nm.

**END**