Electronic Supplementary Information

A lysosome-targeted fluorescent sensor for the detection of glutathione in cells with extremely fast response

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1. Materials and instruments

All regents and chemicals, unless otherwise stated, were used without further purification from the commercial resources. All glassware was oven-dried before used. NMR spectra were recorded on a Varian Mercury VX-300 spectrometer, using tetramethylsilane (TMS) as the internal standard. Mass spectra were measured with a LCMS-2010 mass spectrometer. Fluorescence spectra were measured using a Perkin-Elmer LS-55 spectrofluorophotometer. A Cary 100 spectrophotometer was used to measure absorption spectra. Confocal laser scanning microscopy was conducted with Leica SP8 and Zeiss LSM710.

2. Synthesis

2.1 Synthesis of 1a

To a 100 mL round-bottomed flask, Rhodamine B hydrochloride (1.2 g, 2.2 mmol) was dissolved in ethanol (30 mL), then 3.0 mL (excess) hydrazine hydrate (85%) was added dropwise with stirring at room temperature. After the addition, the mixture was heated to reflux for 3 h. The dark rose red solution became orange and clear. Cooled by air, the mixture was concentrated by evaporation under reduced pressure to an orange solid. The solid was dissolved in 1 M HCl (50 mL) completely to generate a clear red solution. 1 M NaOH (about 50 mL) was added to the solution dropwise with stirring until the pH of the solution reached 9-10. The suspension was filtered and washed with water, dried in vacuo, affording a pale-pink solid (as powder) of **1a** (Yield: 70.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 1H), 7.45 (s, 2H), 7.11 (s, 1H), 6.47-6.42 (t, 4H), 6.31-6.28 (d, 2H), 3.61 (s, 2H), 3.35-3.33 (d, 8H), 1.16-1.14 ppm (t, 12H). ESI-MS: *m/z* calcd. for $C_{28}H_{33}N_4O_2$ $[M+H]^+$: 457.26; found 457.20 $[M+H]^+$.

2.2 Synthesis of RhB-1

0.456 g of **1a** was dissolved in N, N-dimethylformamide (DMF, 5.0 mL) with K_2CO_3 (0.21 g), and stirred at room temperature. Acyloyl chloride (0.21 g) dissolved

in DMF (2.0 mL) was added into the solution of **1a** dropwise when stirring with an ice-bath for 5 h. The resulting solution was added with CH_2Cl_2 (50 mL) for attenuation and washed with water (30 mL×3). The organic phase was dried over $MgSO_4$ and filtered. The pink solvent was concentrated by evaporation under reduced pressure and purified by column chromatography (silica gel, ethyl acetate/petroleum ether = 4/1, v/v), affording a pale-yellow solid of **RhB-1** (Yield: 47.7%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (d, 1H), 7.69 (s, 1H), 7.41-7.43 (t, 2H), 7.12 (d, 1H), 6.49-6.52 (d, 2H), 6.37 (s, 2H), 6.27-6.29 (d, 2H), 3.29-3.36 (m, 6H), 2.70 (s, 4H), 1.13-1.8 ppm (t, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.8, 153.8, 150.4, 148.5, 131.9, 131.4, 128.3, 127.8, 123.5, 122.4, 107.8, 107.0, 97.8, 77.2, 76.9, 76.6, 44.2, 12.5 ppm. ESI-MS: *m/z* calcd. for $C_{31}H_{35}N_4O_3$ $[M+H]^+$: 511.27; found 511.25 $[M+H]^+$.

3. Preparation for solution

The stock solution of **RhB-1** was prepared at 2 mM in dimethyl sulfoxide (DMSO). Testing solutions were prepared by placing 5 μL of stock solution into 2 mL double-distilled water. 5 μL of stock solutions (50 mM) of amino acid including GSH, Cys, Hcy, Thr, Ser, Arg, Ala, Pro, Val, Gly were added into the testing solutions. The fluorescence spectra were measured after the addition of each analytes.

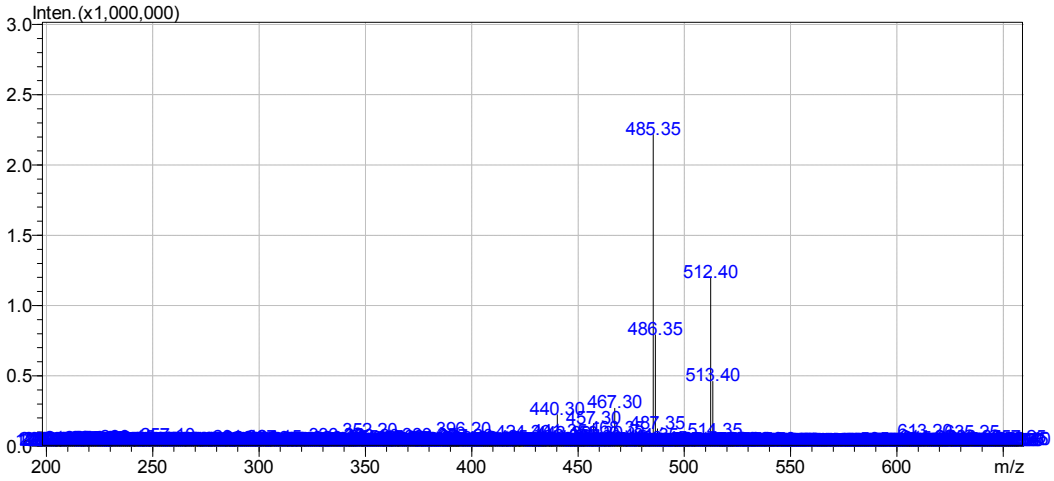


Fig. S1 Mass spectrum of **RhB-1** after addition of GSH.

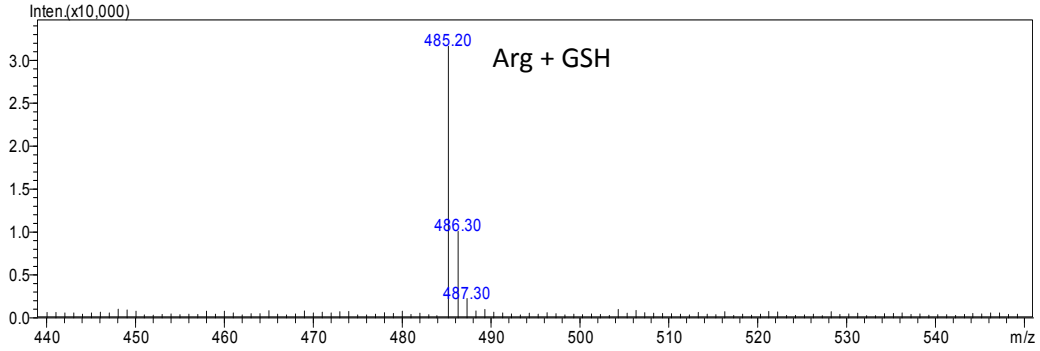


Fig. S2 Mass spectrum of Arg after addition of GSH.

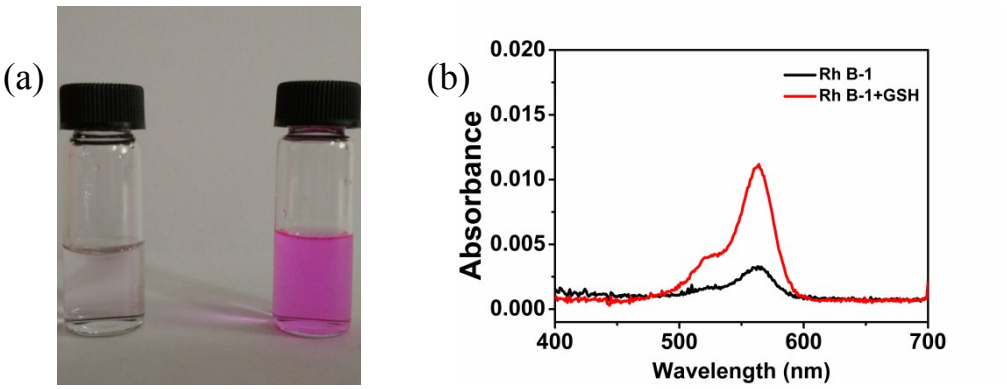


Fig. S3 (a) Color change of **RhB-1** before and after addition of GSH. (b) Absorption spectra of **RhB-1** before and after addition of GSH.

(a)

(b)

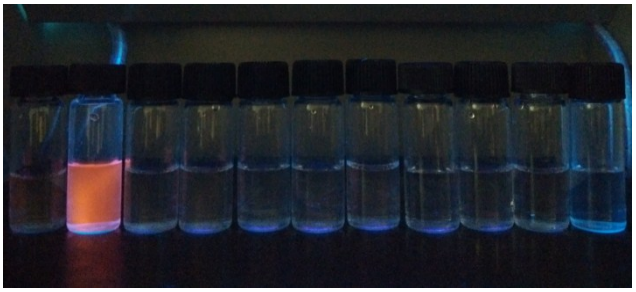


Fig. S4 (a) Color changes of **RhB-1** with different amino acids visible to naked eyes, (b) fluorescence photos of **RhB-1** with different amino acids under a UV lamp (365 nm). From left to right: control, GSH, Gly, Ala, Pro, Val, Arg, Ser, Thr, Hcy, Cys.

4. MTT Assay

An MTT assay was employed to investigate the relative cytotoxicity of the sensor *in vitro*. Firstly, the Hela cells were seeded into 96-well culture plates with a density of approximately 1×10^5 per well and cultured at 37 °C with 5% CO_2 for 24 h. Then different concentrations of sensor were added into each well. The cells in the presence of probe were further incubated for 24 h. Subsequently, the entire medium was replaced by 200 μL MTT with a concentration of 5 mg/mL. Cells were grown at 37 °C with 5% CO_2 for another 4 h until a purple precipitate was visible by naked eyes. The MTT medium was then discarded and 150 μL DMSO was added followed by vibrating for 15 min to dissolve the crystals. The absorption at 490 nm was recorded by Elx800 Micropalte reader (BioTek, USA).

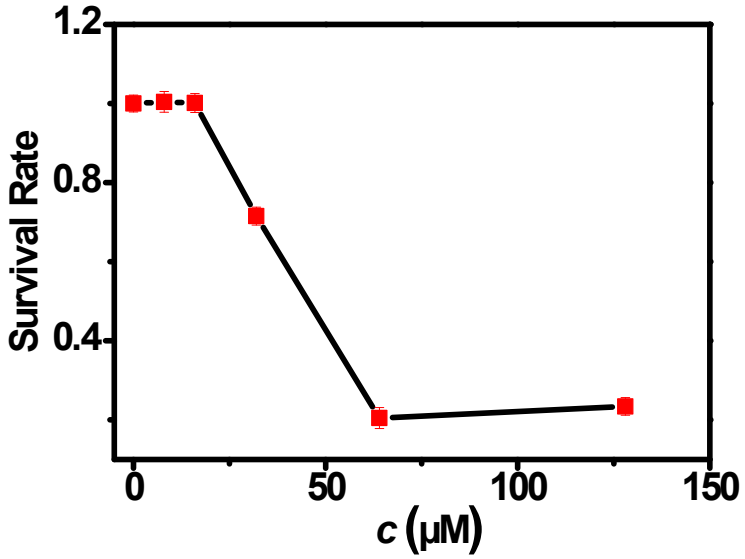


Fig. S5 Cell viability of Hela cells treated by **RhB-1** at different concentrations (0, 8, 16, 32, 64, 128 μM) after 24 h.

5. Cell imaging

Hela cells were cultured in Dulbeccos Modified Eagle Medium containing 10% fetal bovine serum, as well as 1% amphotencin and 1% penicillin. The cells were seeded in culture dishes and incubated at 37 °C with 5% CO_2 for 12 h prior to imaging experiments. The sensor solution in DMSO (20 mM) was diluted with ultrapure water to the specific concentrations (50 μM). After 30 min incubation of GSH at 37 °C, Lysosensor Green DND-189 was firstly added into the dishes to stain the cells. 30 min later, the prepared sensor solution (50 μM) was added. After an incubation of 30 min, the cells were washed with phosphate buffered saline (PBS) three times to remove the remaining medium and used for confocal fluorescence imaging (Leica SP8).

6. Photostability of RhB-1

Prior to incubation at 37 °C with 5% CO_2 for 12 h, Hela cells were treated with Lyso-tracker Red and **RhB-1** (50 μM) for 30 min. After washed with PBS, the Hela cells were irradiated by confocal laser microscope (Zeiss LSM710) with excitation at 561 nm through the red channel (570 - 630 nm). The excitation power of the microscope at 561 nm is 25 mW. Nearly 0.6% of the power was used to irradiate cells stained with Lyso-tracker Red, while almost 100% was used to irradiate cells stained with **RhB-1**. As shown in **Fig. S6**, during the operation, a cell (1) as the target area would be irradiated; another cell (2) as control would not be irradiated; and area 3 was made as the reference context. Each irradiation had an interval of 15 s and cost about 7.2 s.

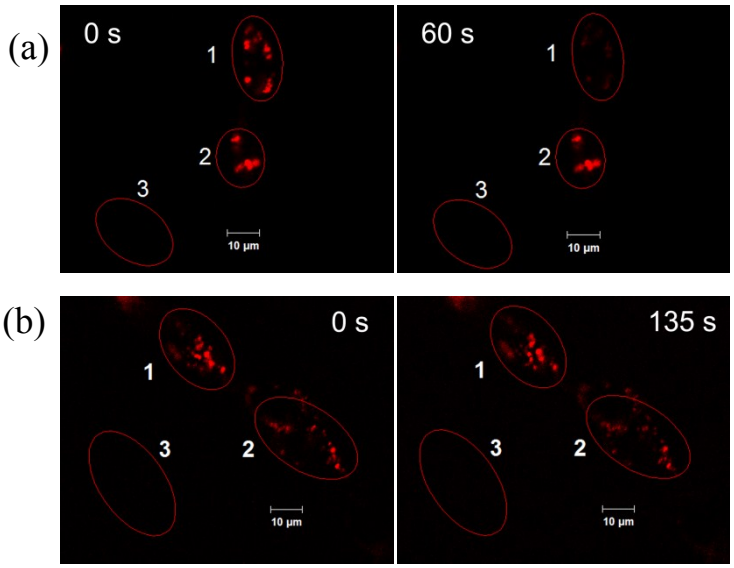
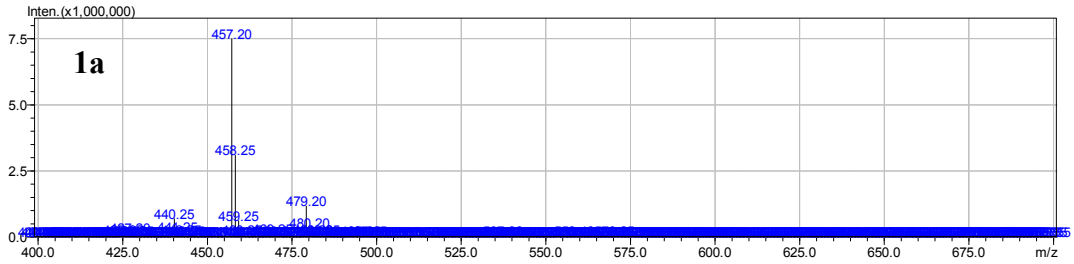
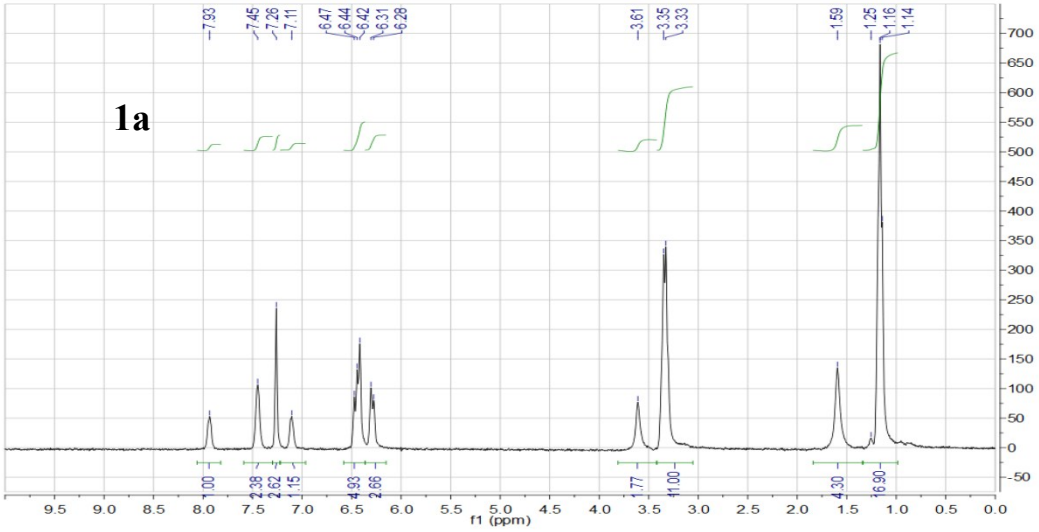


Fig. S6 (a) Fluorescence images of Hela cells treated with 100 μM of Lyso-tracker Red irradiated after 0 s and 60 s. (b) Fluorescent images of Hela cells treated with 50 μM of **RhB-1** irradiated after 0 s and 135 s. Both of (a) and (b) were excited at the same wavelength (561 nm) for the red channel (560 - 610 nm). Scale bars: 10 μm.

(a)


Inten.(x1,000,000)
1a
457.20
458.25
440.25
459.25
479.20
480.20
m/z

(b)


1a
7.93
7.45
7.26
7.11
6.47
6.44
6.42
6.31
6.28
3.61
3.35
3.33
1.59
1.25
1.16
1.14
1.00
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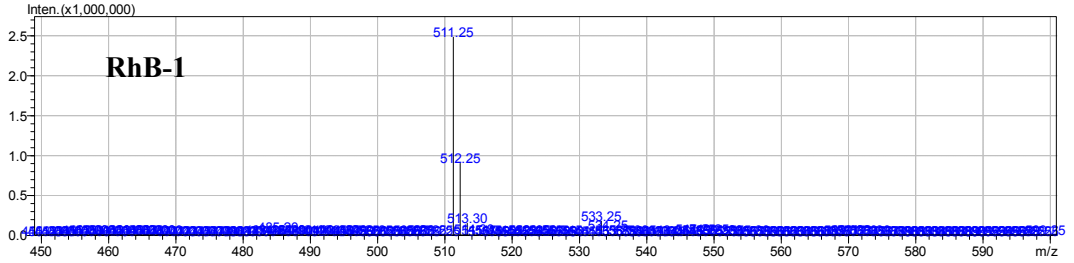
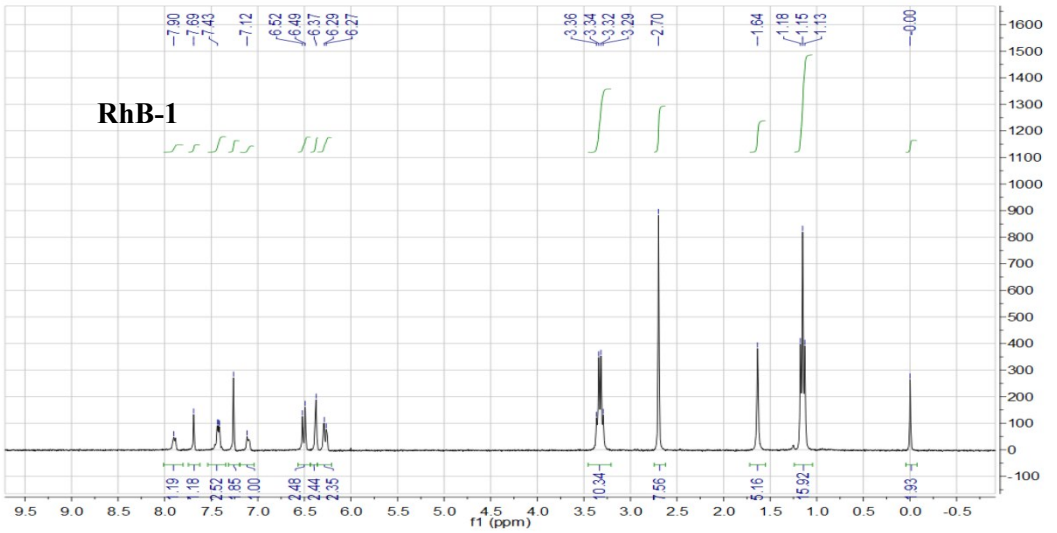
1.77
11.00
4.30
16.90
f1 (ppm)

(c)


Inten.(x1,000,000)
RhB-1
511.25
512.25
513.30
533.25
m/z

(d)



(e)

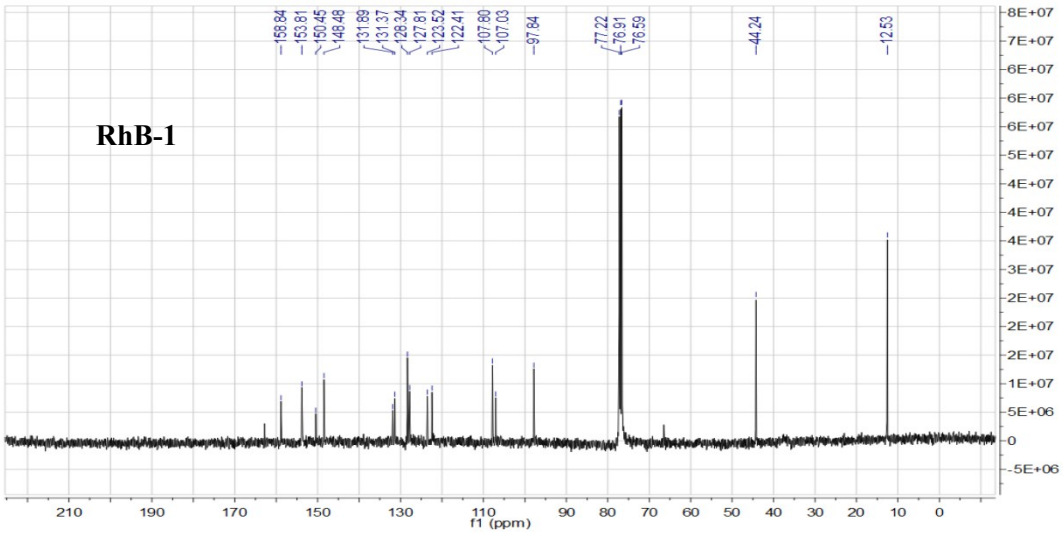


Fig. S7 Mass spectra of **1a** (a) and **RhB-1** (c). ^{1}H NMR spectra of **1a** (b) and **RhB-1** (d) and ^{13}C NMR spectrum of **RhB-1** (e).