Electronic Supplementary Information for:

Intracellular endogenous glutathione detection and imaging by a simple and sensitive spectroscopic off-on probe

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1. Synthesis of probe 1

Fig. S1 ^{1}H NMR spectrum of **1** (600 MHz, DMSO-d_6, 298K).

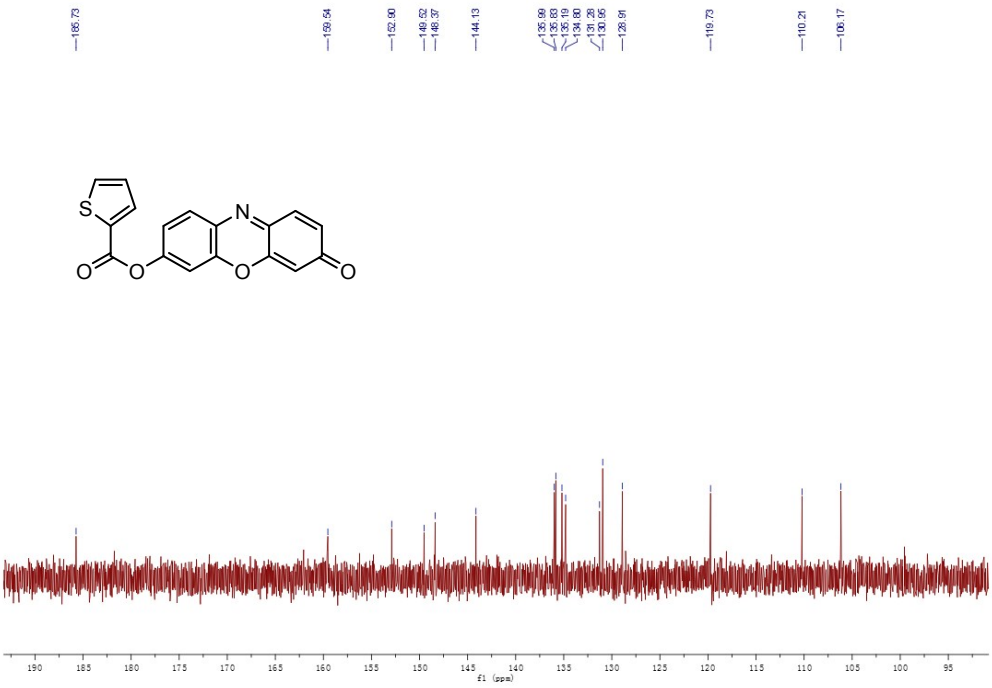

Fig. S2 ^{13}C NMR spectrum of **1** (150 MHz, DMSO-d_6, 298K).

2. Effects of pH and temperature

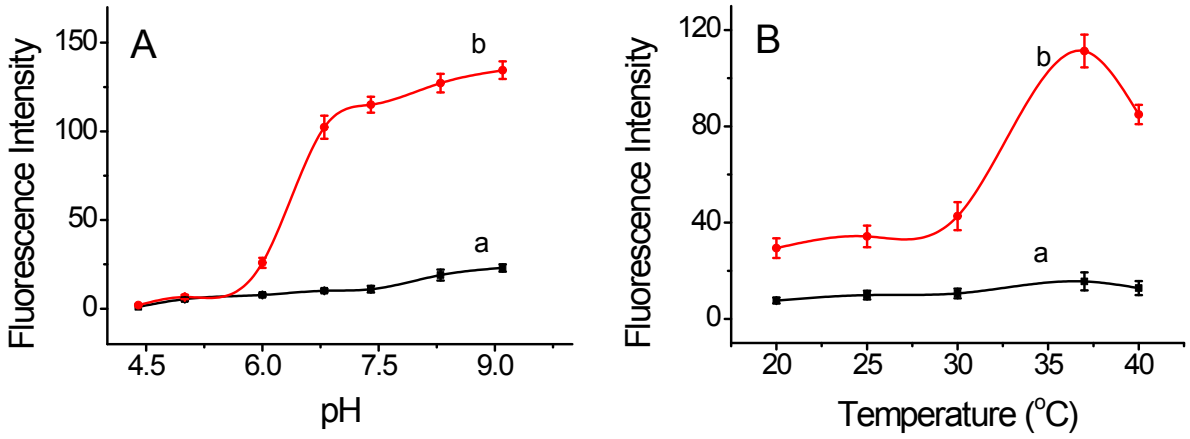

Fig. S3 Effects of (A) pH and (B) temperature on the fluorescence of 10 μM **1** (a) before and (b) after reaction GSH (100 μM). The results are the mean ± standard deviation of three separate measurements. $\lambda_{ex/em}$ = 550/585 nm.

3. Fluorescence kinetic curves of 1 reacting with GSH

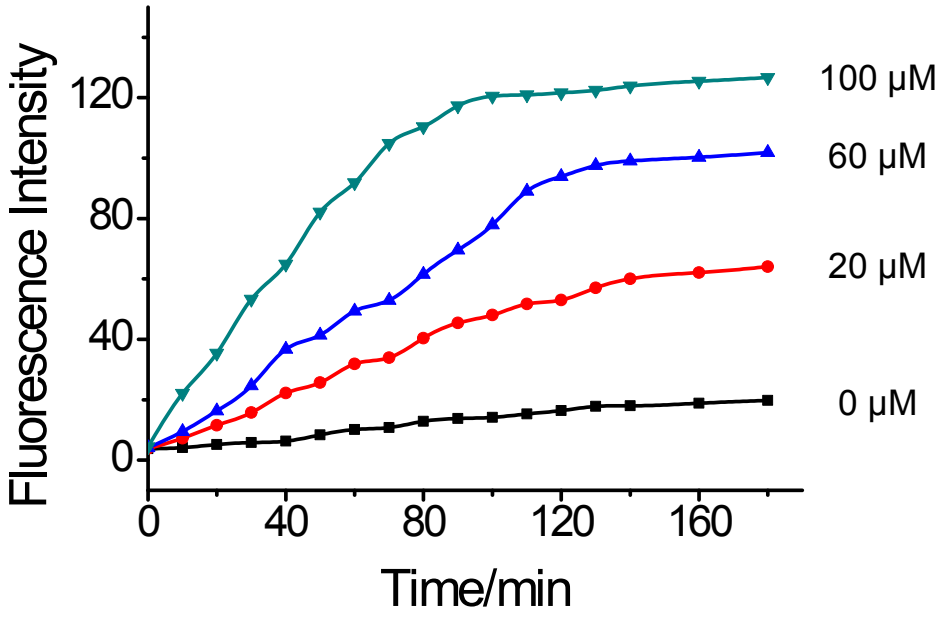

Fig. S4 Plots of fluorescence intensity of **1** (10 μM) *vs.* the reaction time in the presence of varied concentrations of GSH (0-100 μM). The measurements were performed at 37 °C in 10 mM PBS (pH 7.4). $\lambda_{ex/em}$ = 550/585 nm.

4. Selectivity study

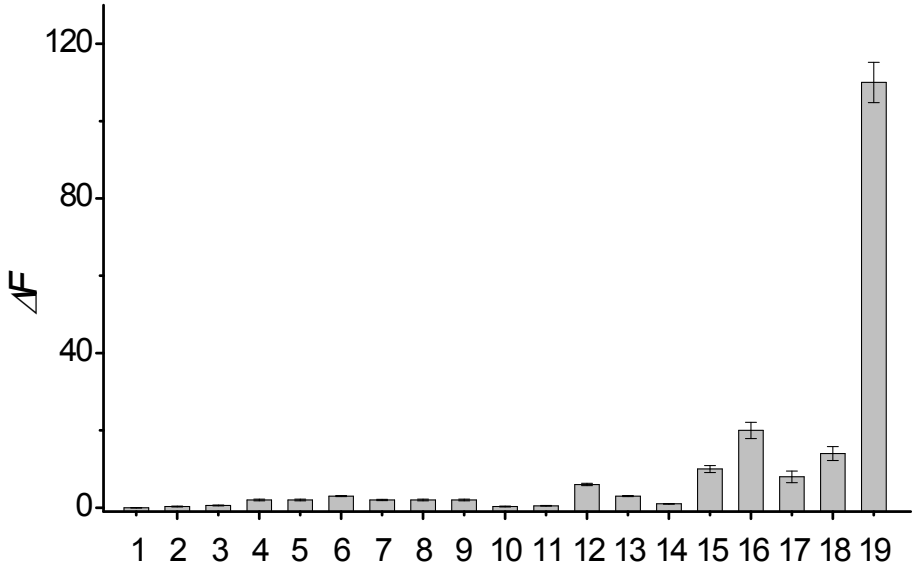

Fig. S5 Fluorescence responses of **1** (10 μM) to various species: (1) blank (probe **1** only), (2) 100 μM HOCl, (3) 100 μM $ONOO^-$, (4) 100 μM *tert*-butyl hydroperoxide, (5) 100 μM $^1O_2$, (6) 100 μM H_2O_2, (7) 150 mM KCl, (8) 0.1 mM $FeCl_3$, (9) 100 μM $CuCl_2$, (10) 100 μM $CaCl_2$, (11) 100 μM $ZnCl_2$, (12) 100 μM $MgCl_2$, (13) 10 nM Cytochrome C, (14) 10 mM glucose, (15) 100 nM human serum albumin, (16) 5 nM creatine phosphokinase, (17) 0.5 μg/mL nitroreductase, (18) 5 nM thrombin, (19) 100 μM GSH. The results are the mean ± standard deviation of three separate measurements. $\lambda_{ex/em}$ = 550/585 nm.

5. Studies on reaction mechanism of probe 1 with GSH

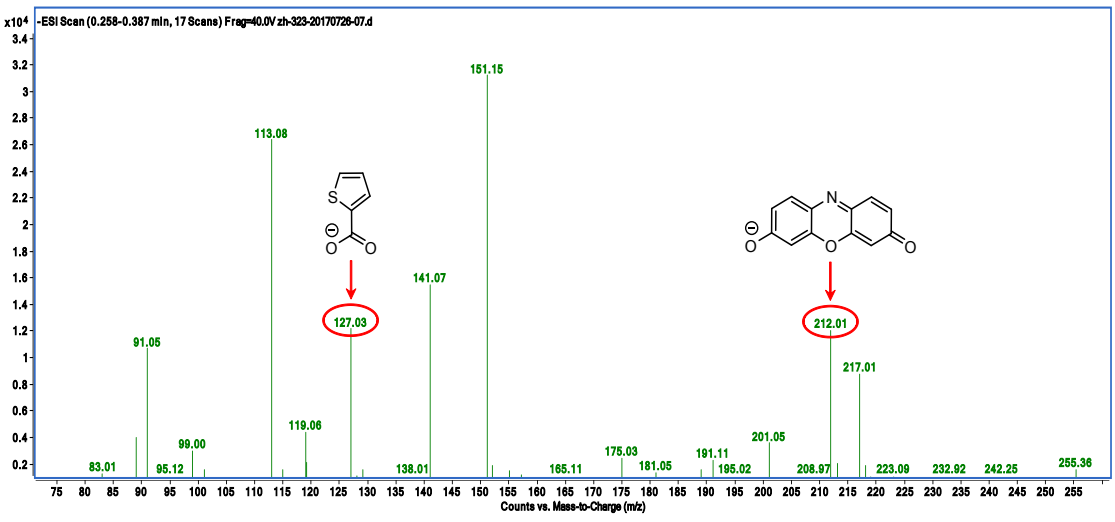

Fig. S6 ESI-MS of the reaction solution of **1** (10 μM) with GSH (50 μM/mL).

6. Cytotoxicity assay

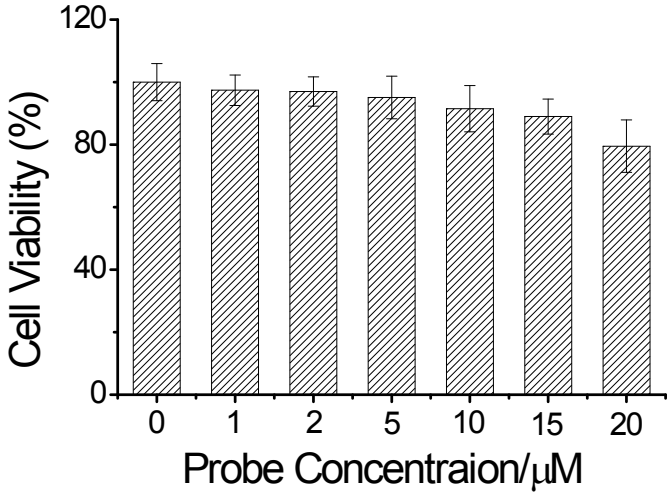

Fig. S7 Effects of **1** with varied concentrations (1-20 μM) on the viability of HeLa cells. The viability of the cells without **1** is defined as 100%. The results are the mean ± standard deviation of five separate measurements.

7. Determination of GSH in cell lysate with assay kit

The whole procedure is carried out according to the instruction of the assay kit as follows:

(1) *Standards*. First dilute GSH standard to 300 μM by mixing 3 μL 100 mM Standard with 997 μL dH2O. Next, prepare the 3 μM Premix by mixing 5 μL of the 300 μM GSH with 495 μL 1 × Assay Buffer. Dilute standards in 1.5-mL centrifuge tubes as described in the Table 1. Transfer 200 μL of each Standard to separate wells in a 96 well plate.

Table S1 Determination of GGT level in human serum samples.

No	Premix + 1 × Assay Buffer	GSH (μM)
1	250 μL + 0 μL	3.0
2	150 μL + 100 μL	1.8
3	75 μL + 175 μL	0.9
4	0 μL + 250 μL	0

(2) *Glutathione Detection*. Prepare enough working reagent (WR) for 4 standards and all samples. For each reaction combine the following: 105 μL 1 × Assay Buffer, 1 μL GR Enzyme, 0.25 μL NADPH and 0.5 μL DTNB. Mix WR immediately after adding the DTNB. Add 100 μL of WR to each Standard and Sample well. Mix well.

(3) Read OD_{412nm} at 0 min and again at 10 min.

(3) *Calculation*. Subtract OD_{0min} from OD_{10min} for each Standard and sample. Next subtract the ΔOD_{BLANK} (Std 4) from the ΔOD values of all Standards and plot the $\Delta\Delta OD$'s against standard concentrations. Determine the slope using linear regression fitting. The GSSG and GSH concentrations of the Samples are calculated as follows:

$$[GSH_{TOTAL}] = \frac{\Delta OD_{SAMPLE} - \Delta OD_{BLANK}}{Slope} \times n\ (\mu M)$$

$$[GSSG] = 0.5 \times \frac{\Delta OD_{S(GSSG)} - \Delta OD_{BLANK}}{Slope} \times n\ (\mu M)$$

$$[GSH] = [GSH_{TOTAL}] - 2 \times [GSSG]\ (\mu M)$$

ΔOD_{SAMPLE}, ΔOD_{BLANK} and $\Delta OD_{S(GSSG)}$ are the change in optical density values of the sample, water (Std 4) and sample treated with Scavenger, respectively. n is the dilution factor. For all samples treated with Scavenger, n = 165. Conversions: 1 mg/dL glutathione equals 32.5 μM, 0.001% or 10 ppm.

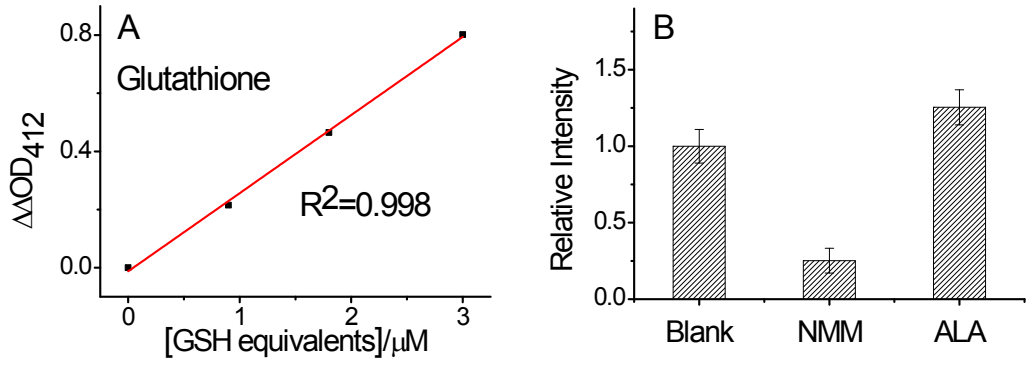

Fig. S8 Determination of GSH in cell lysate with assay kit. (A) The linear regression fitting of the $\Delta\Delta OD$'s against standard concentrations; (B) The relative intensity of absorbance of cell lysate under different treatment conditions then incubated with assay kit.

8. Determination of GSH in cell lysate with probe 1

Subsequently, the cell lysate of NMM and ALA pre-treated A549 cells was diluted to appropriately to meet the linear detection requirement, incubated with the probe and measured with the Hitachi F-7000 spectrofluorimeter.