Electronic Supplementary Material (ESI) for Analytical Methods.

A fluorescent probe for the discrimination between Cys and GSH

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Supporting Information

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Experiment

Reagents and methods

Unless otherwise specified, all the commercial reagents (Aladdin Corporation) were of analytical grade or above and used without further purification. Ultra-pure water was prepared through Sartorius Arium 611DI system.
Accurately weighted amount of **RTP** was dissolved in MeOH to obtain 1×10^{-3} M stock solutions. Thiols and other analytes were dissolved in phosphate buffer solution (PBS 20 mM, pH 7.4 containing 1 mM CTAB) to obtain stock solutions with appropriate concentrations. The stock solution of RTP was diluted with PBS containing 1 mM CTAB to acquire 20 μM dye solutions. In the kinetic measurements, 60 μL of GSH or Cys or Hcy stock solution was added to 3 mL of 20 μM dye aqueous solution to keep the thiol concentration to be 400 μM. In the titration experiments, appropriate volume of GSH/Cys stock solution was added into 3 mL of 20 μM probe aqueous solution.

Instruments

Absorption spectra were measured with an Evolution 220 UV-vis spectrophotometer (Thermo Scientific). Fluorescence spectra were carried out on a Lumina Fluorescence Spectrometer (Thermo Scientific). All the fluorescence spectra were uncorrected. NMR spectra were performed with a Bruke AV-400 spectrometer (400M Hz). Mass spectra were recorded on a MA 1212 Instrument on standard condition (ESI, 70ev). The experiments were performed at 37°C using non-degassed samples.

Living cell culture and fluorescence imaging

Hela cells were cultured in Dulbcco's modified Eagle's (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C and under 5% CO_2 in a CO_2 incubator. The cells were washed with phosphate buffered saline (PBS) and then incubated with 20 μM **RTP** in DMEM medium for 80 min at 37 °C and washed 3 times with PBS. For control experiment, the cells were pretreated with 0.5 mM Maleimide (or Cys) for 30 min at 37°C followed by further incubated with 20 μM **RTP** for 80 min. Cells imaging was then carried out after washing cells with PBS. Fluorescent imaging was performed with red channel.

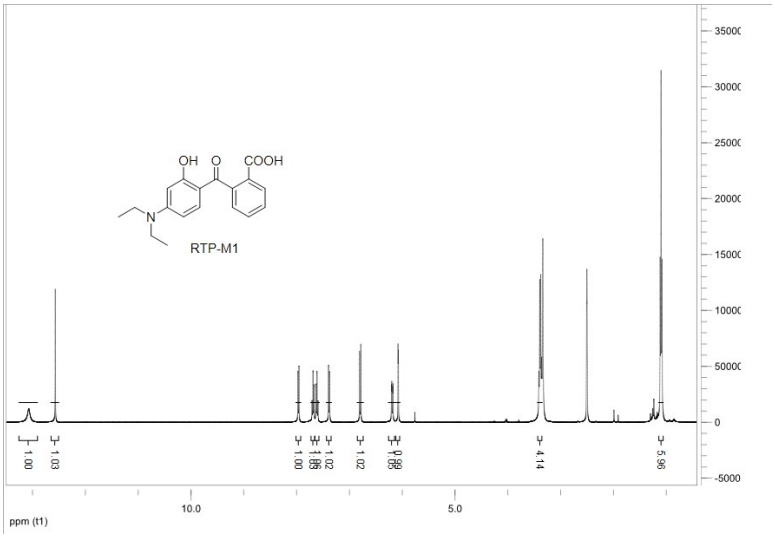


Fig. S1 ^{1}H-NMR of **RTP-M1**

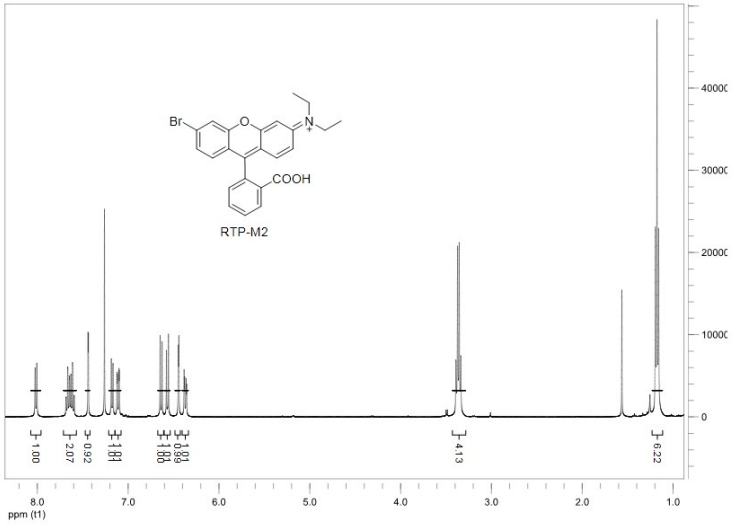
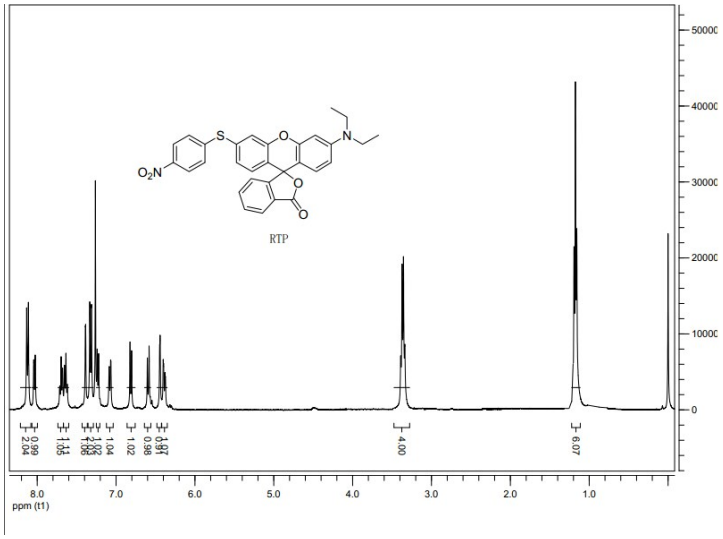
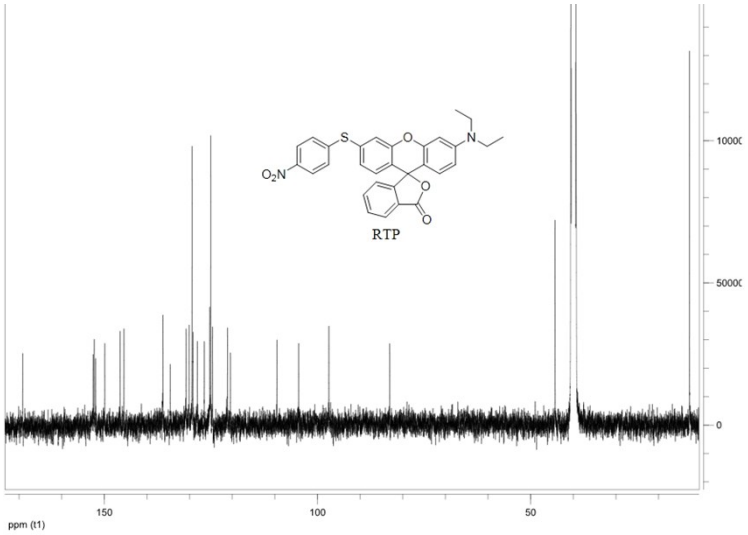


Fig. S2 ^{1}H-NMR of **RTP-M2**





Monoisotopic Mass, Even Electron Ions
47 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-30 H: 0-90 N: 0-2 O: 0-5 S: 0-1

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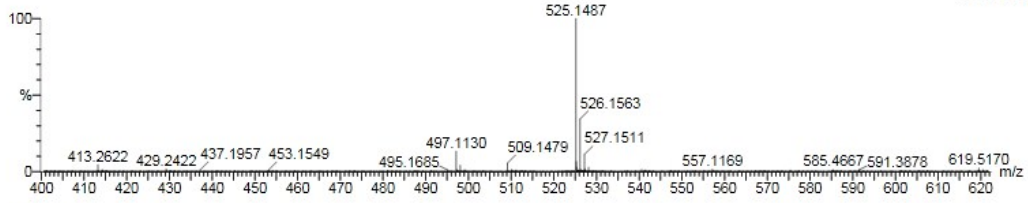
ZWB-SL-99 17 (0.620) Cm (13:19) 1: TOF MS ES+ 1.52e+004



Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
525.1487	525.1484	0.3	0.6	19.5	227.0	0.0	C30 H25 N2 O5 S

Fig. S3 ^{1}H NMR, ^{13}C NMR and ESI spectra of **RTP**

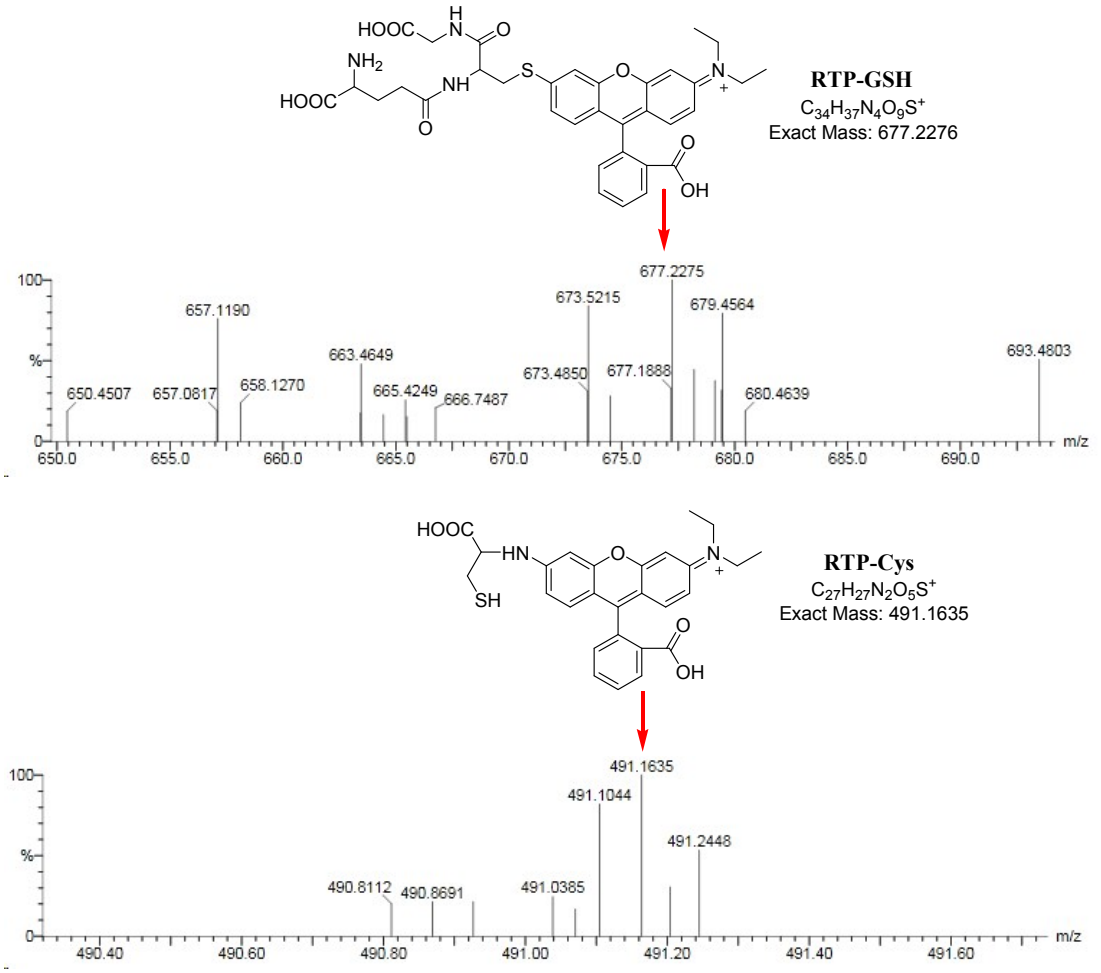


Fig. S4 MS of **RTP** mixed with GSH and Cys for 80 min. [**RTP**] = 20 μM, [GSH] = [Cys] = 400 μM, equilibrated in 20 mM PBS (pH 7.4) containing 1 mM CTAB, 37 °C.

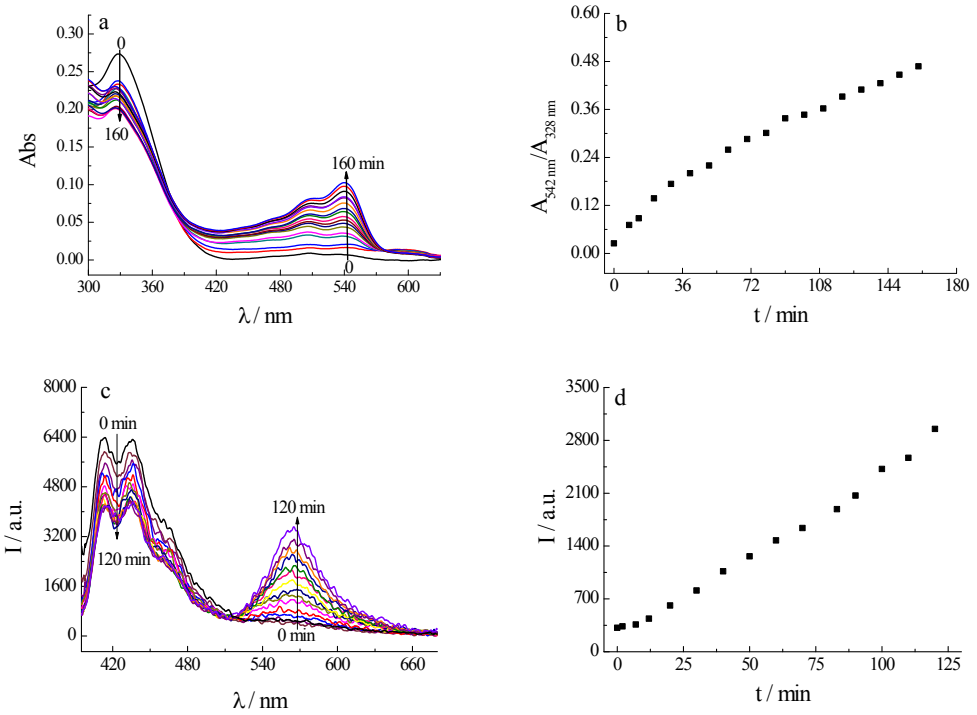


Fig. S5 Time-dependent absorption (a) and emission (c) spectra of RTP mixed with Hcy; (b) and (d) are the ratio of 542 nm and 328 nm and the fluorescence intensity at 570 nm as a function of time, respectively. [RTP] = 20 μM, [Hcy] = 400 μM, 20 mM PBS (pH 7.4) containing 1 mM CTAB, λ_{ex} = 385 nm, 37 °C.

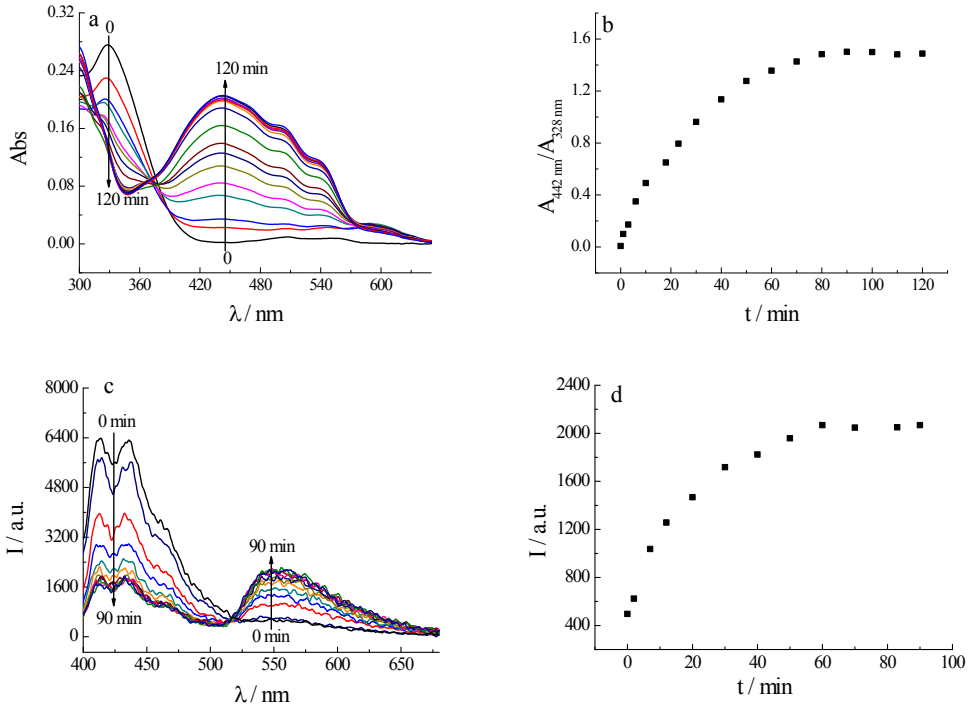


Fig. S6 Time-dependent absorption (a) and emission (c) spectra of RTP mixed with GSH; (b) and (d) are the ratio of 442 nm and 328 nm and the fluorescence intensity at 542 nm as a function of time, respectively. [RTP] = 20 μM, [GSH] = 400 μM, 20 mM PBS (pH 7.4) containing 1 mM CTAB, λ_{ex} = 370 nm, 37 °C.

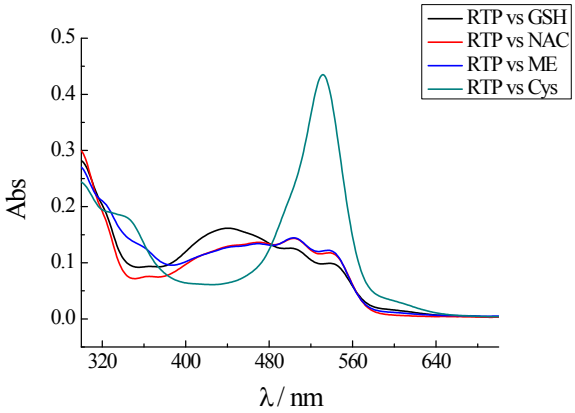


Fig. S7 The absorption spectra of RTP mixed with different analytes for 80 min. [RTP] = 20 μM, [GSH] = [NAC] = [ME] = [Cys] = 400 μM, equilibrated in 20 mM PBS (pH 7.4) containing 1 mM CTAB, 37 °C.

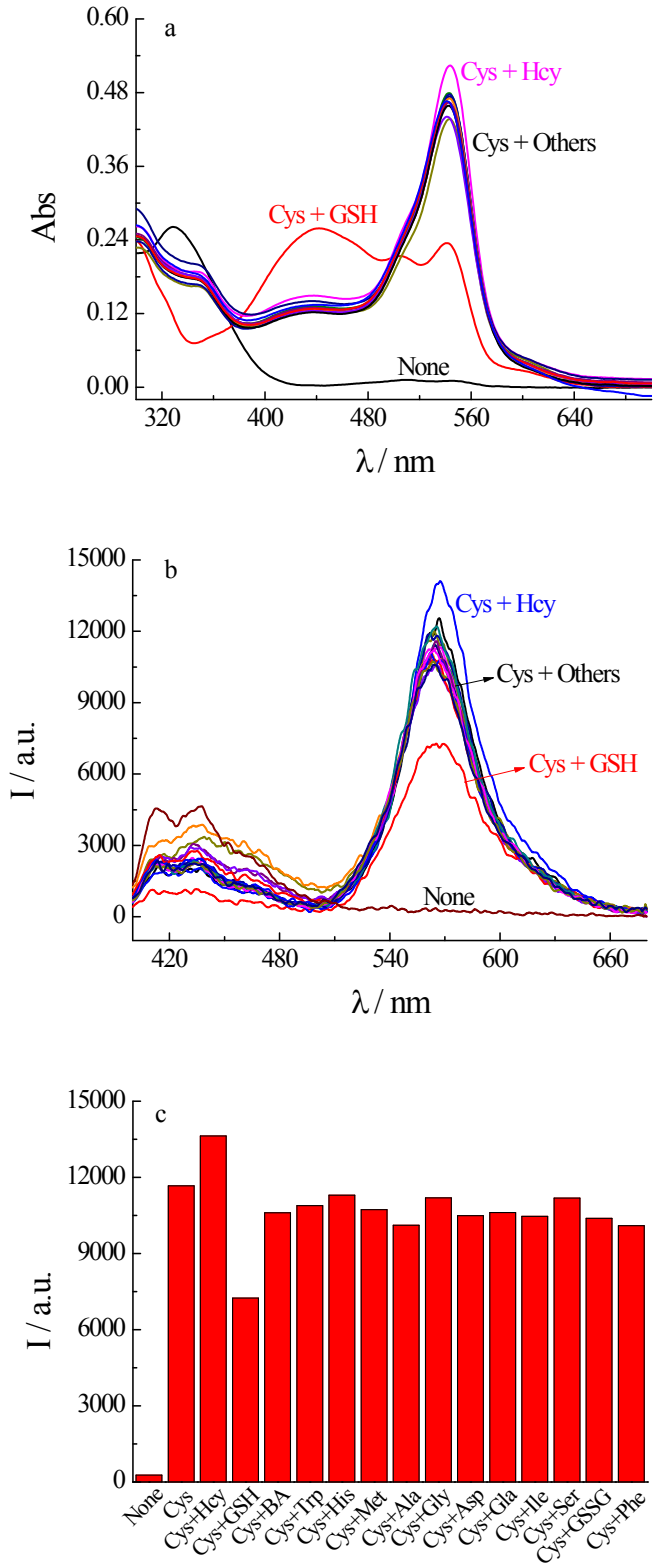


Fig. S8 The absorption (a), emission (b) spectra and the fluorescence intensity at 570 nm (c) of RTP-Cys in the presence of 400 μM different additives in 20 mM PBS (pH 7.4) contain 1 mM CTAB, [RTP] = 20 μM, GSH = 400 μM, λ_{ex} = 385 nm, recorded 80 min after each addition, 37 °C.

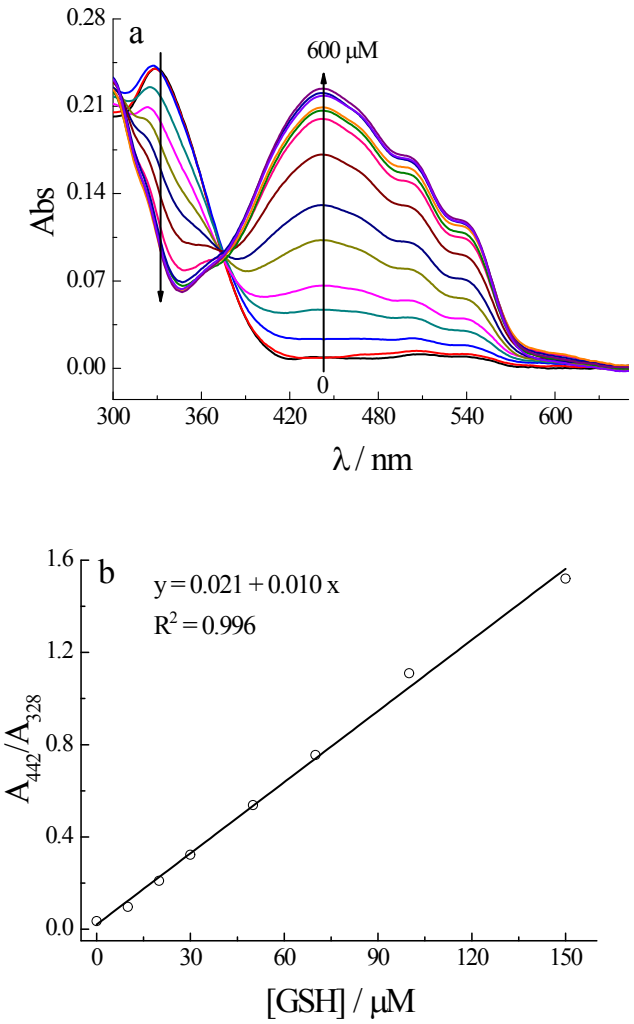


Fig. S9 (a) The absorption spectra of RTP with different concentrations of GSH and (b) the ratio of absorbance at 442 nm and 328 nm as a function of GSH concentration. [RTP] = 20 μM, 20 mM PBS (pH 7.4) contain 1 mM CTAB, recorded 60 min after each addition, 37°C.