A fluorescent probe for the discrimination between Cys and GSH

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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 $1 \times 10^{-3}$ 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 $\mu$M dye solutions. In the kinetic measurements, 60 $\mu$L of GSH or Cys or Hcy stock solution was added to 3 mL of 20 $\mu$M dye aqueous solution to keep the thiol concentration to be 400 $\mu$M. In the titration experiments, appropriate volume of GSH/Cys stock solution was added into 3 mL of 20 $\mu$M 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 Dulbeco’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 $\mu$M 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 $\mu$M 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
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. \([\text{RTP}] = 20 \, \mu\text{M}, \, [\text{GSH}] = [\text{Cys}] = 400 \, \mu\text{M}\), 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, $\lambda_{\text{ex}}$ = 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<sub>ex</sub> = 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<sub>ex</sub> = 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.