Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A Novel Strategy of Rhodamine B-based Fluorescent Probe for Selective Response of Glutathione to Serve as Bioimaging in Living Cells

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Equipments:

NMR spectra were recorded on a Bruker Avance II spectrometer. LCMS spectra were recorded on a Waters QTof spectrometer. UV-Vis spectra were recorded on an INESA spectrophotometer. Fluorescence spectra were recorded on a FluoroMax-4 fluorescence spectrophotometer. Confocal images were taken by a Nikon A1 laser confocal scanning microscope (red channel, 20× objective lens).

synthetic methods:

Synthesis of *compound **RBN***

A mixture of 1,2-diaminoethane (167 μL, 2.5 mmol) and rhodamine B (240 mg, 0.5 mmol) in 5.5 mL of alcohol was heated at 79 °C for 6 h. The solution was combined with 2 g SiO_2 gel and concentrated to dry powder, then which was purified by preparation chromatography (n-hexane /ethyl acetate = 2:1 →1:1) to get target product **RBN** as an orange powder solid (170 mg, yield 70%).

Synthesis and characterization of *probe **RBA**:*

A mixture of 2-chloro-1-methylpyridinium iodide (382 mg, 1.5 mmol) and 3-butynoic acid (84 mg, 1.0 mmol) in 3.5 mL of ultra-dry dichloromethane was stirred for 65 min. Next, a ultra-dry dichloromethane (1.2 mL) solution of **RBN** (484 mg, 1.1 mmol) and of triaethylamin (TEA, 200 μL, 1.5 mmol) was added to above reaction intermediator, and continue stirring for 35 min. The solution was combined with 5 g SiO_2 gel and concentrated to dry powder, which was purified by preparation chromatography (n-hexane /ethyl acetate = 6:1 →3:1) to get target probe **RBA** as a white powdery solid (210 mg, 38 % yield).

^{1}H NMR (300 MHz , $CDCl_3$, 298 K) δ (ppm): 7.83 (br. s., 1 H), , 7.37 (br. s., 2.75 H), 7.01 (br. s., 1 H), 6.36 (m, *J* = 8.80 Hz, 2 H) , 6.30 (s, 2 H), 6.20 (m, *J* = 8.62 Hz, 2 H), 5.46 (t, *J* = 6.42 Hz, 0.25 H), 5.17 (d, *J* = 6.42 Hz, 0.5 H), 3.24 - 3.33 (m, 8 H), 3.23 (br. s., 3.5 H), 3.02 (br. 0.75 H), 2.30 (br. s., 0.75 H), 1.09 (t, *J* = 6.88 Hz, 12 H), ^{13}C NMR (75 MHz , $CDCl_3$, 298 K) δ (ppm): 169.58, 166.55, 153.81, 153.29, 148.93, 132.74, 130.56, 128.46, 128.14, 123.91, 122.86, 108.25, 104.85, 97.80, 77.26, 73.68, 39.59, 65.48, 44.37, 40.41, 27.34, 12.62. HRMS (ESI) calculated for $C_{34}H_{39}N_4O_3^+$, [**RBA**+H]$^+$, 551.3017, found, 551.3014.

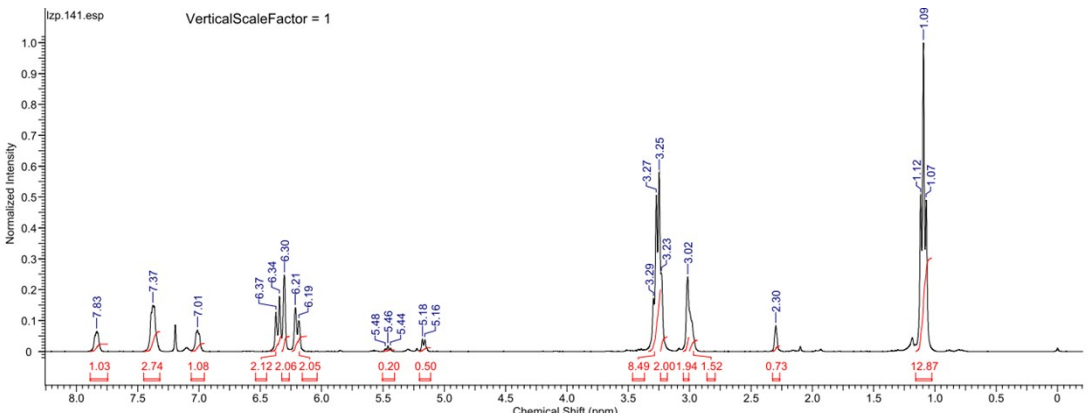

Fig. S1. ^{1}H NMR spectrum of compound **RBA.**

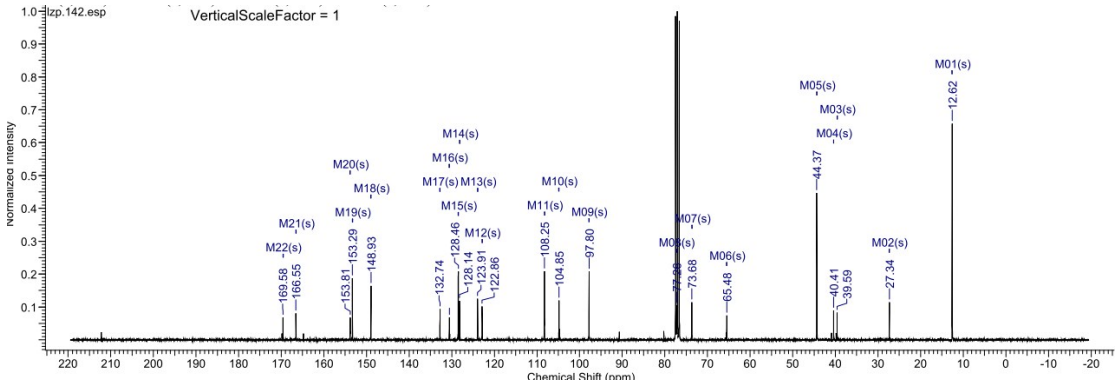

Fig. S2. ^{1}C NMR spectrum of compound **RBA.**

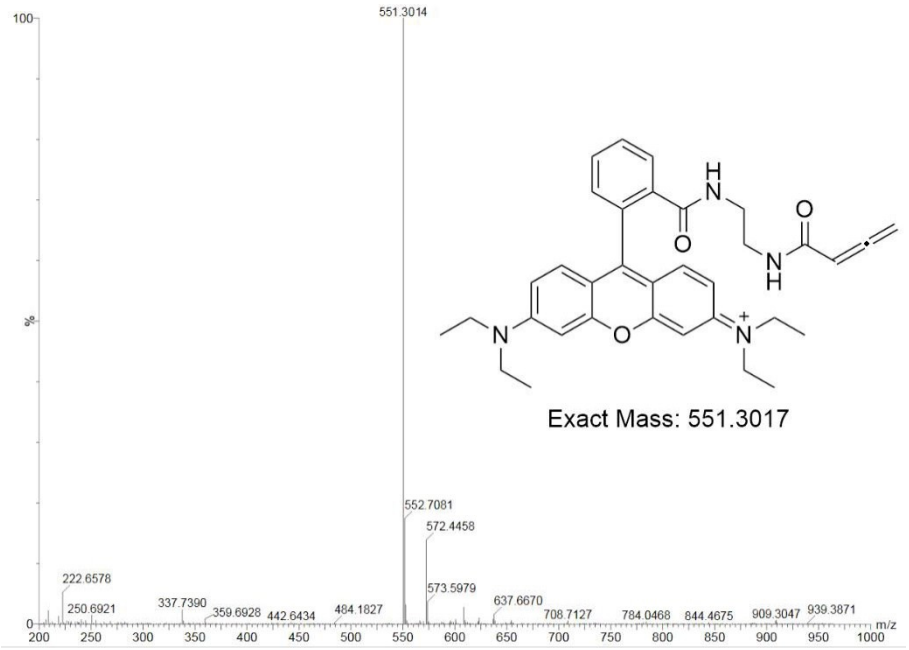

Fig. S3. ESI-HRMS spectrum of probe **RBA.**

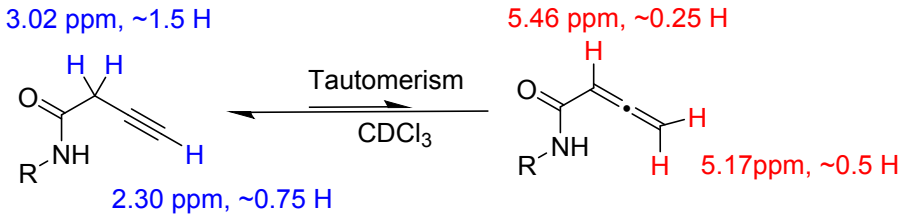

Fig.S4. Tautomerism between butynamide and allenamide

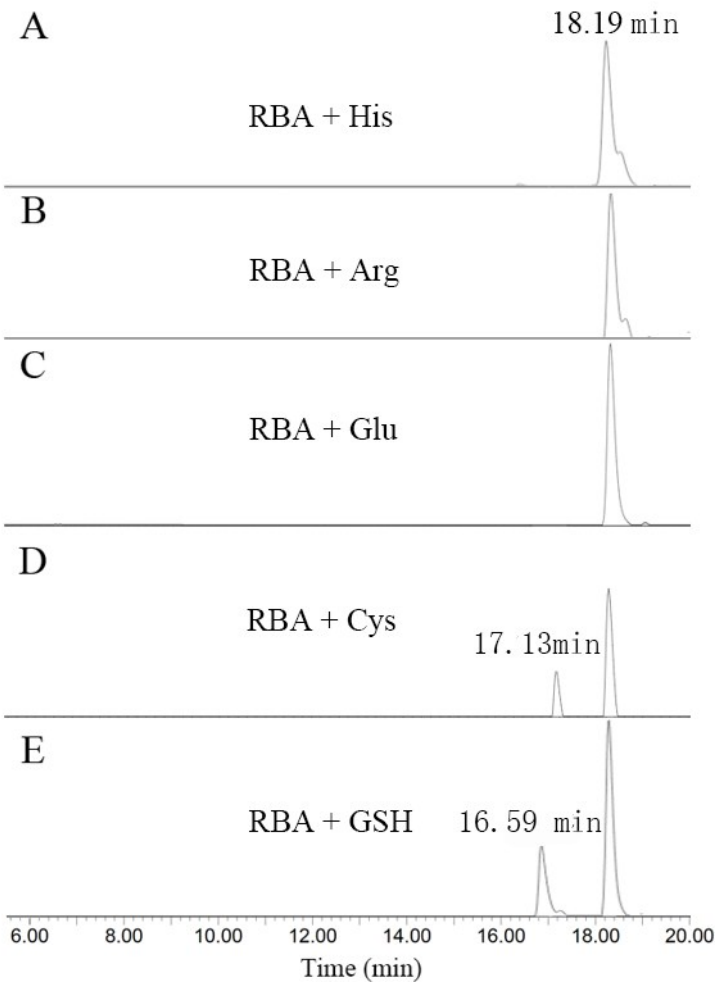

Fig. S5. LCMS analysis of **t**he products of **RBA** incubated with some representative molecules. (A) **RBA+His**; (B) **RBA+Arg**; (C) **RBA+Glu**; (D) **RBA+Cys**; (E) **RBA+GSH**. The reaction mixture was analyzed by LCMS with the detection wavelength at 254 nm. The HPLC conditions were as follows: mobile phase composition was MeOH/H_2O:20/80~90/10 (formic acid 0.1%); a temperature of 40 ^oC; Agilent RP-C18 column of 4.6*150 mm; and flow rate of 1 mL/min.

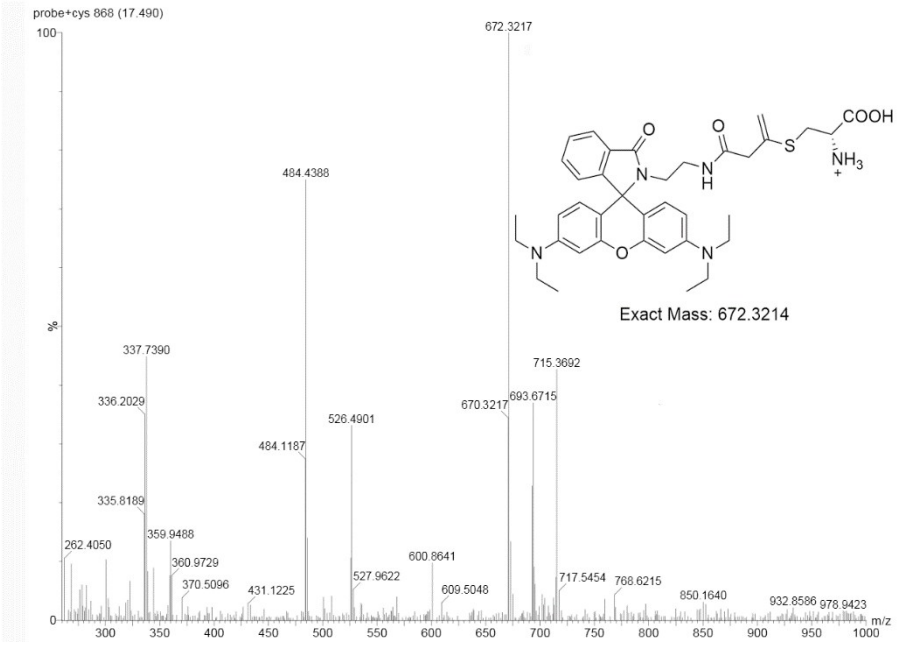

Fig. S6. ESI-HRMS spectrum of **RBA-Cys.**

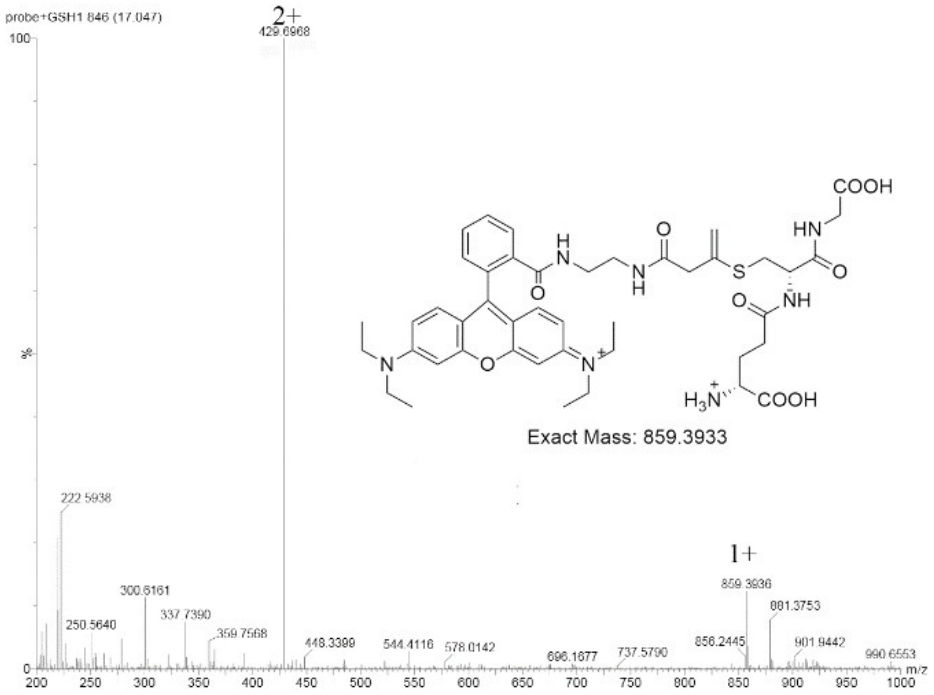

Fig. S7. ESI-HRMS spectrum of **RBA-GSH.**

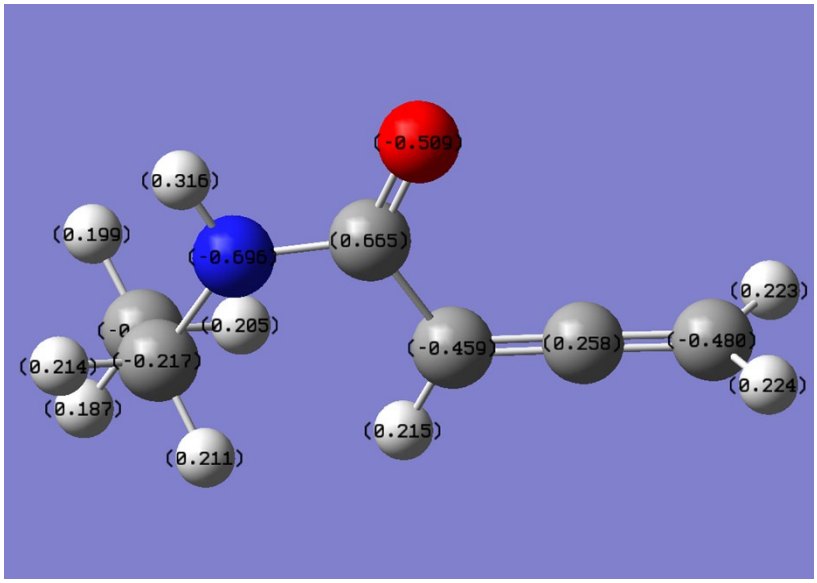

Fig. S8. Hückel charge distribution of the allenamide.