Supporting Information

A New Turn-On Fluorescent Probe for Selective Detection of Glutathione and Cysteine in Living Cells

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Reagents and apparatus

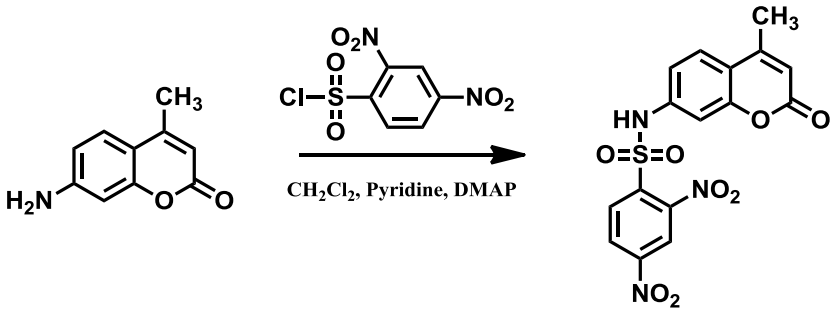
7-Amino-4-methylcoumarin, N-methylmaleimide, 2,4-dinitrobenzenesulfonyl and 4-Dimethylaminopyridine were obtained from Sigma-Aldrich company. Glutathione (GSH), cysteine (Cys), homocysteine (Hcy), histidine (His), glycine (Gly), alanine (Ala), glutamic (Glu), arginine (Arg), dopamine (DA), proline (Pro), methionine (Met), bovine serum albumin (BSA), uric acid, and vitamin C were purchased from Wako Pure Chemical Industries (Osaka, Japan) and used without further purification. Pyridine and dichloromethane were obtained from Sinopharm chemical reagent company. All other chemicals used in this work were of analytical grade. The detection buffer was PBS buffer (0.01 M, pH 9.0). Milli-Q ultrapure water (Millipore, ≥ 18 MΩ□ cm) was used throughout. Except the specific statement, solvents were purified by distillation.

The synthesis of N-(4-methyl-2-oxo-2H-chromen-7-yl)-2,4-dinitrobenzenesulfonamide) (NDS) was carried out under a nitrogen atmosphere. Silica gel 300-400 mesh (37-54 μm) was used for column chromatography. The thin-layer chromatography (TLC) was carried out on silica gel plates (60F-254) using UV-light to monitor the reaction. Bruker AVB-500 spectrometer, API 4000 QTRAP LC/MS/MS System with ESI lon Source (AB SCIEX Co., U. S. A.), Nexus 670 Fourier Transform Infrared Spectrometer (Nicolet Co., U. S. A.) and F-4500 FL spectrophotometer (Hitachi Co., Japan) were used to characterize the probe NDS. Fluorescence images of HeLa cells were performed by fluorescence microscopy (Nikon, Eclipse Ti-S)

Experimental section

Synthesis and characterization of the N-(4-methyl-2-oxo-2H-chromen-7-yl)-2,4-dinitrobenzenesulfonamide (NDS): 7-Amino-4-methylcoumarin (32 mg, 0.188 mmol) and 4-Dimethylaminopyridine (27.6 mg, 0.226 mmol) were added to a solution of dichloromethane (6 mL)

and pyridine (3 mL) at 0 ^{o}C. After stirring for 0.5 h, 2,4-dinitrobenzensufonyl chloride (76 mg, 0.282 mmol) in 3.0 mL of anhydrous dichloromethane was added dropwise to the reaction mixture. And the reaction mixture was stirred at 0 °C for 1 h. Then, the mixture was stirred at room temperature for 8 h, and it was extracted with dichloromethane. The organic layer was washed twice with brine, dried with magnesium sulfate, filtered and evaporated in *vacuo*. The crude product was purified by column chromatography and NDS (24 mg, 31% yield) was obtained**.** ^{1}H NMR (500 MHz, DMSO) δ 11.74 (1H, s), 8.93 (1H, d, *J* = 1.6 Hz), 8.6 (1H, dd, *J* = 1.8, 8.6 Hz), 8.31 (1H, d, *J* = 8.7 Hz), 7.71 (1H, d, *J* = 8.6 Hz), 7.13 (1H, d, *J* = 8.6 Hz), 7.08 (1H, s), 6.30 (1H, s), 2.35 (3H, s); ^{13}C NMR (126 MHz, DMSO) δ 159.5, 153.6 152.9, 150.3, 147.8, 139.6, 135.8, 131.6, 127.5, 126.8, 120.6, 116.2, 115.5, 113.1, 106.36, 17.9; IR (neat): 3431 cm^{-1}, 3259 cm^{-1}, 3104 cm^{-1}, 2932 cm^{-1}, 1680 cm^{-1}, 1600 cm^{-1}, 1542 cm^{-1}, 1341 cm^{-1}, 1152 cm^{-1}, 865 cm^{-1}, 733 cm^{-1}; MS (ESI^{+}) [M-H]$^{-}$: 405.4.



Scheme S1. Synthesis of N-(4-methyl-2-oxo-2H-chromen-7-yl)-2,4-dinitrobenzenesulfonamide (NDS)

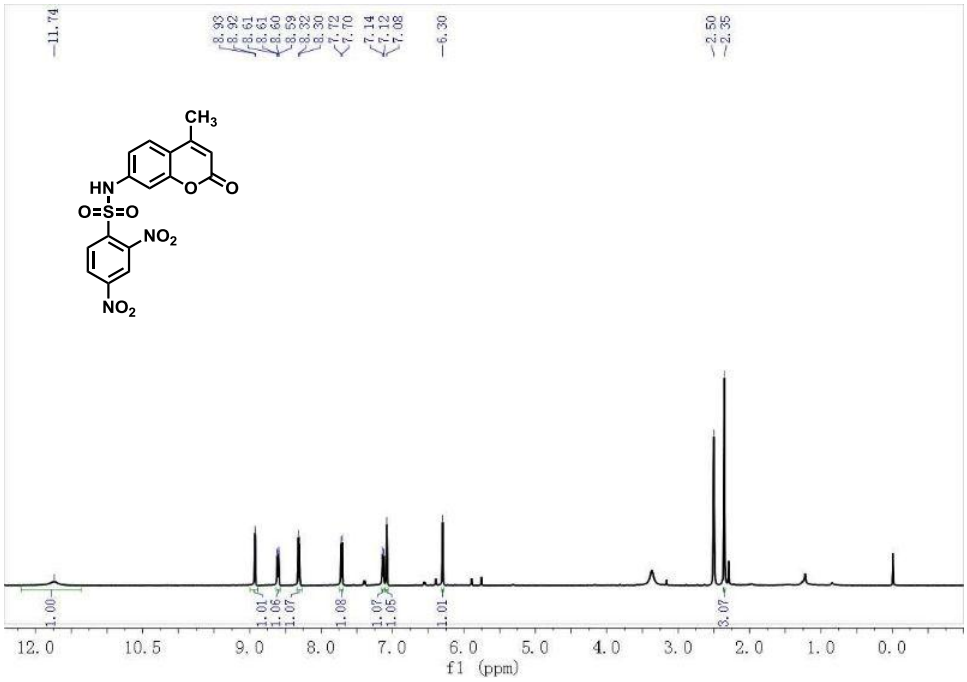


Fig. S1. ^{1}H NMR of NDS (DMSO-d_6, 500 MHz).

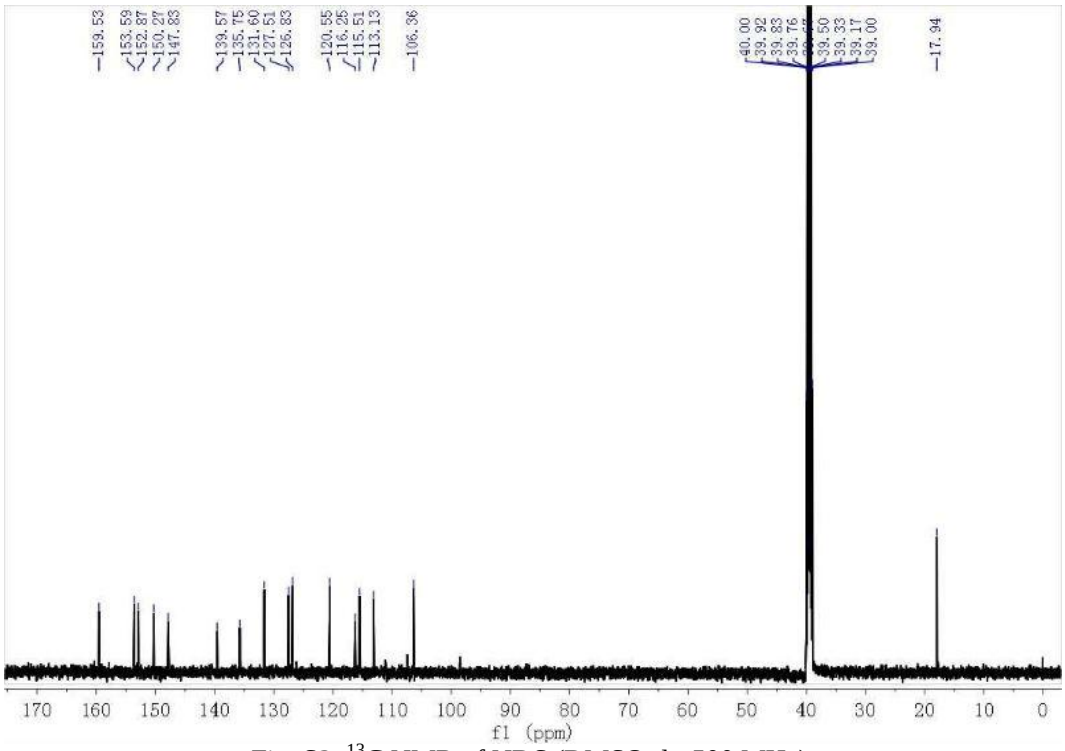


Fig. S2. ^{13}C NMR of NDS (DMSO-d_6, 500 MHz).

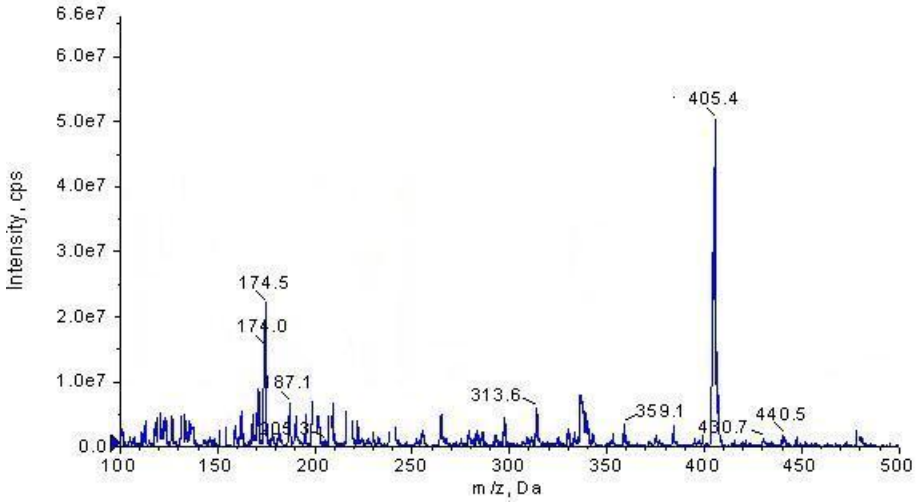


Fig. S3. Mass spectrometry spectrum of NDS.

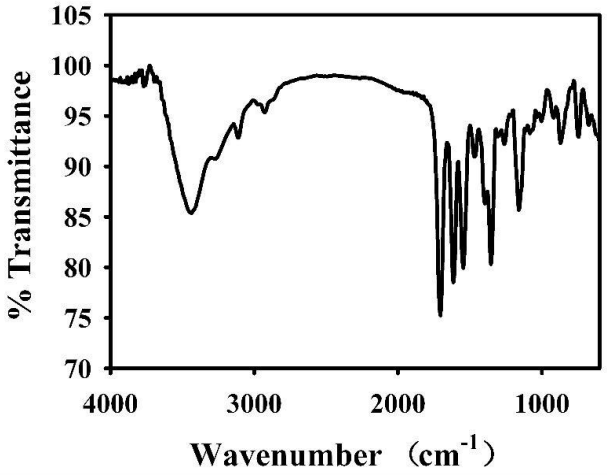
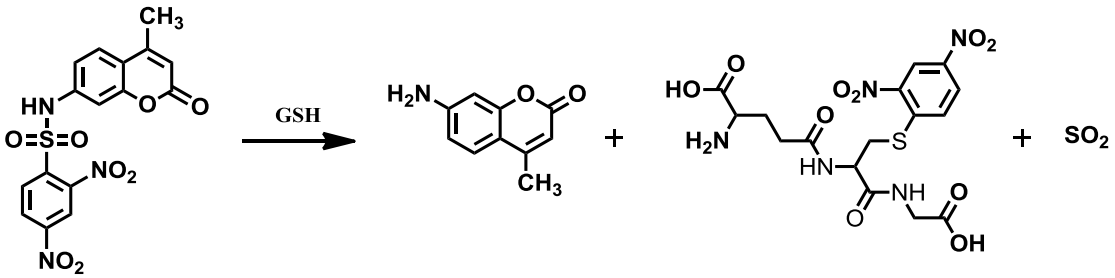


Fig. S4. IR Spectrum of NDS

The detailed investigation for the reaction of GSH with NDS: 20.25 mg (0.05 mmol) NDS was dissolved in 3 mL DMSO-PBS (1:5, V/V, pH 9), and **GSH** (18.45 mg, 0.06 mmol) was added into the solution. After stirring at 45 ^{o}C for 2 h, the reaction mixture was cooled to room temperature and extracted with dichloromethane twice. The combined organic layers were concentrated under reduced pressure, and the crude product was purified by column chromatography to afford compound 1. The water layers were acidified to pH 2 with dilute hydrochloric acid. The resulting crystalline product was collected by filtration, sequentially washed with water, ,ether and acetone, and then dried *in vacuo* to afford compound 2.[1] ^{1}H NMR (500 MHz, DMSO) of compound 1 δ 7.40 (1H, d, *J* = 8.6 Hz), 6.56 (1H, dd, *J* = 2.2, 8.7 Hz), 6.40 (1H, d, *J* = 2.1 Hz), 6.11 (2H, s), 5.90 (1H, d, *J* = 1.1 Hz), 2.29 (3H, d, *J* = 1.1 Hz): ^{13}C NMR (126 MHz, DMSO) of compound 1 δ 160.77, 155.52, 153.78, 153.13, 126.25, 111.23, 108.91, 107.54, 98.60, 18.05.: ^{1}H NMR (500 MHz, DMSO) of compound 2 δ 8.92 (1H, t, *J* = 5.4 Hz), 8.86 (1H, d, *J* = 2.5 Hz), 8.76 (1H, d, *J* = 8.5 Hz), 8.46 (1H, dd, *J* = 2.6, 9.2 Hz), 7.97 (1H, d, *J* = 9.2 Hz), 4.61(1H, m), 3.73 (2H, d, *J* = 6.4 Hz), 3.62 (1H, dd, *J* = 4.3, 13.2 Hz), 3.37-3.32 (2H, m), 2.33-2.30 (2H, m), 1.96-1.92 (1H, m), 1.84-1.80 (1H, m). ^{13}C NMR (126 MHz, DMSO) of compound 2 δ 172.2, 171.1, 170.9, 170.0, 145.3, 144.8, 143.8, 128.3, 127.7, 121.5, 53.1, 51.1, 41.5, 34.2, 31.4, 26.7.



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Scheme S2. Reaction equation of GSH with NDS.

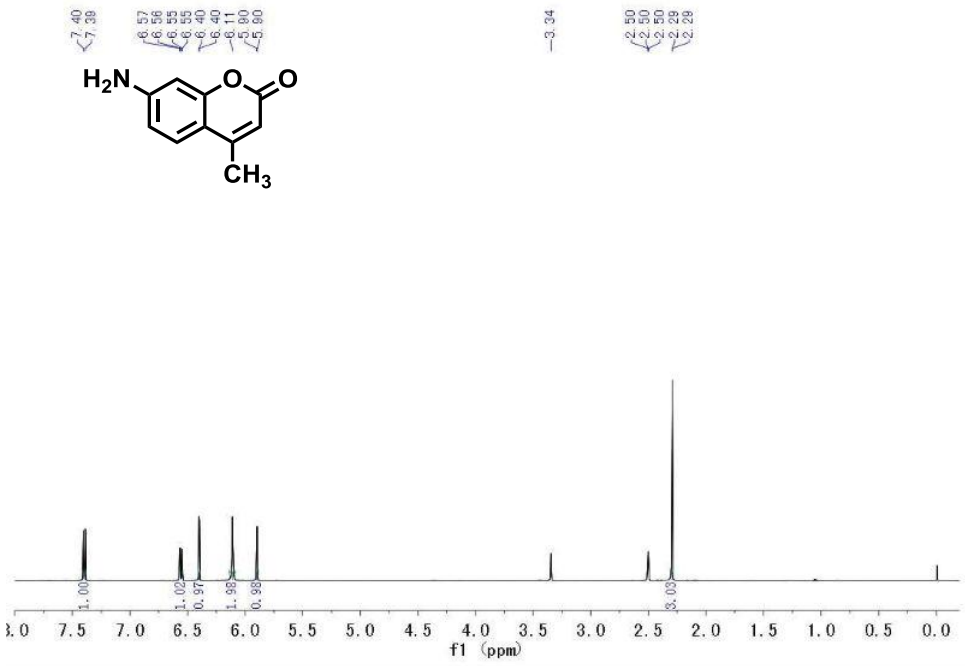

Fig. S5. ^{1}H NMR of compound 1 (DMSO-d_6, 500 MHz).

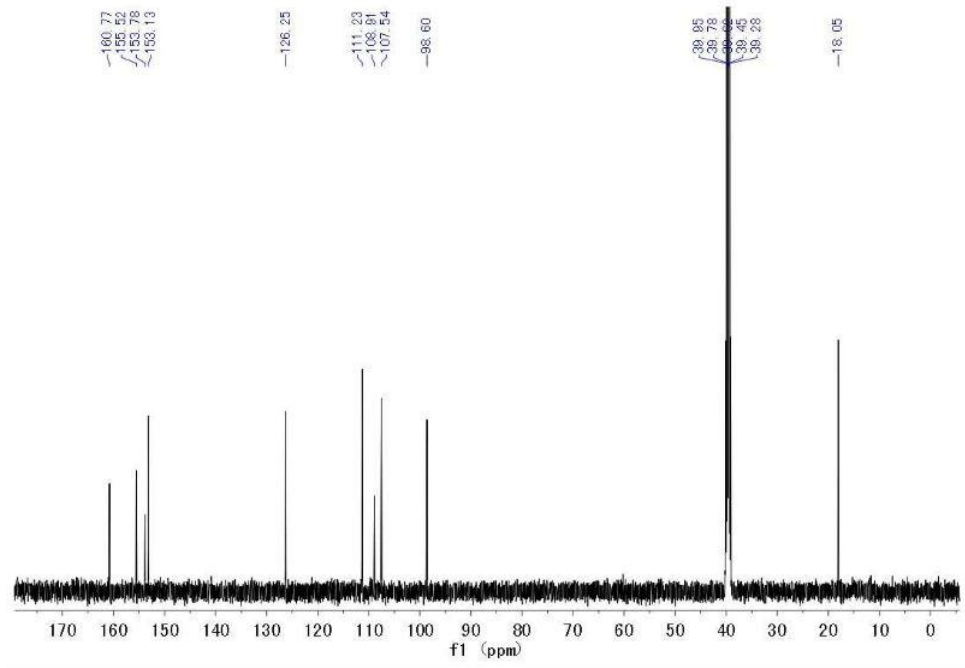

Fig. S6. ^{13}C NMR of compound 1 (DMSO-d_6, 500 MHz).

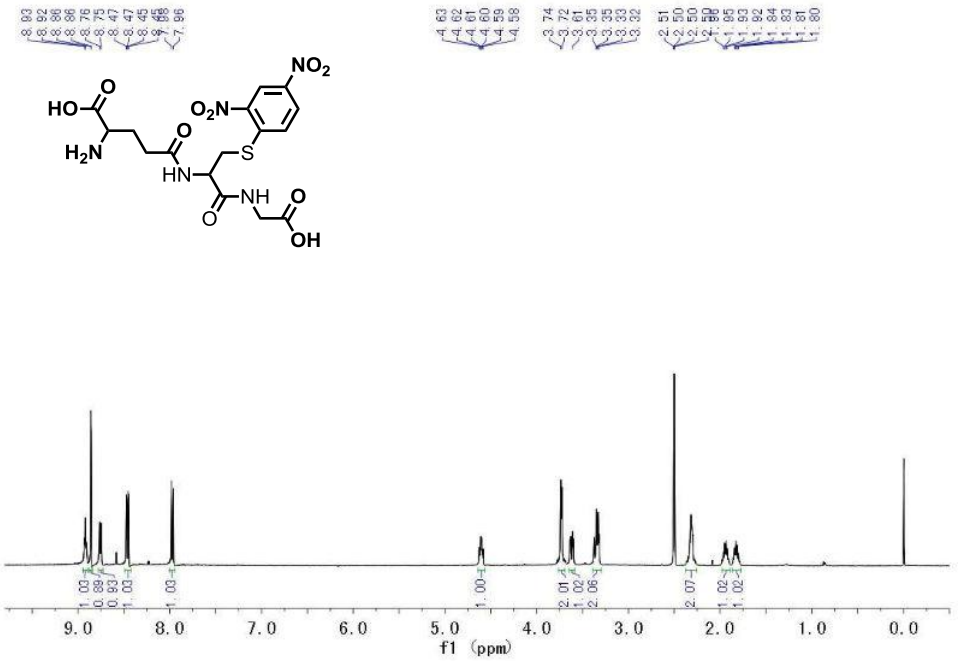

Fig. S7. ^{1}H NMR of compound 2 (DMSO-d_6, 500 MHz).

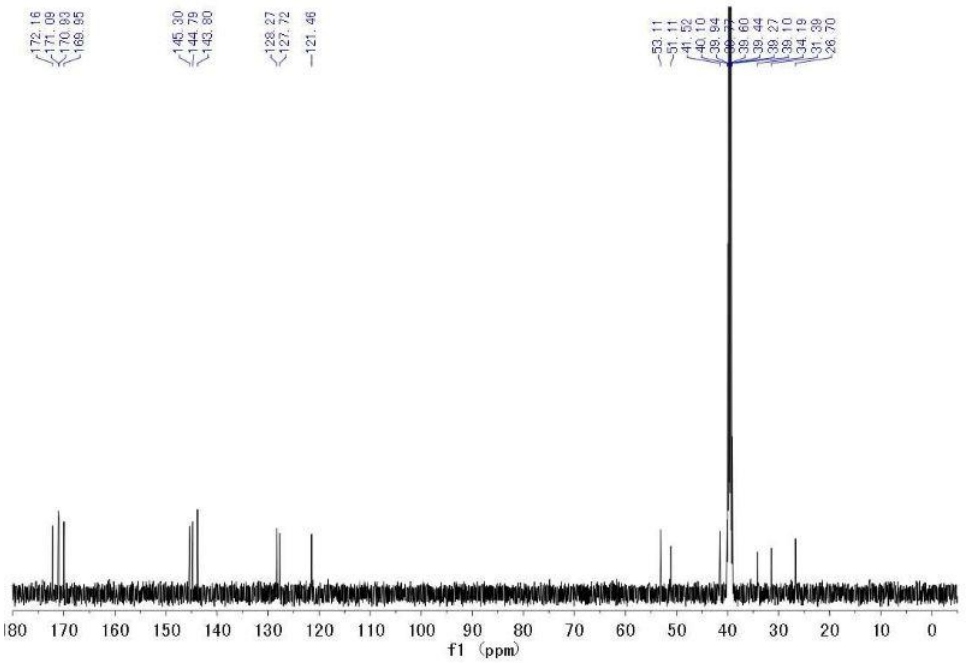

Fig. S8. ^{13}C NMR of compound 2 (DMSO-d_6, 500 MHz).

Fluorescent detection of GSH/Cys: For all tests and reactions, the experiments were repeated at least three times to ensure the accuracy of the measurements. All of the fluorescent spectra were recorded on a fluorescence spectrophotometer. The fluorescent measurements for GSH/Cys were carried out as follows: NDS was dissolved in methanol to prepare a stock solution (200 μM). The fluorescence of

20 μM NDS was detected after incubating 2h at 45 ^{o}C in phosphate buffer (0.01 M, pH 9.0) in the presence and absence of GSH/Cys. Then fluorescence spectra were recorded with excitation wavelength at 353 nm. The fluorescence emission intensity at 450 nm was used for quantitative analysis of GSH/Cys.

Analysis of GSH/Cys in living cells: The cells were incubated on 96-well plate and allowed to adhere for 24 h at 37 ^{o}C, and then were washed with PBS and incubated at 37 ^{o}C in the presence of NDS (30 μM, 1:99 DMSO/PBS, V/V, pH 7.4) for overnight. In a control experiment, the cells were pretreated with 600 μM N-methylmaleimide in DMSO-PBS solution (1:99, V/V, pH 7.4) at 37 ^{o}C for 2 h. Then the cells were washed three times with PBS buffer and incubated at 37 °C in the presence of NDS (30 μM, 1:99 DMSO/PBS, V/V, pH 7.4) for overnight. The fluorescence images were recorded on an inverted fluorescence microscopy (NIKON Eclipse Ti-S, Japan) with a 40x objective lens.

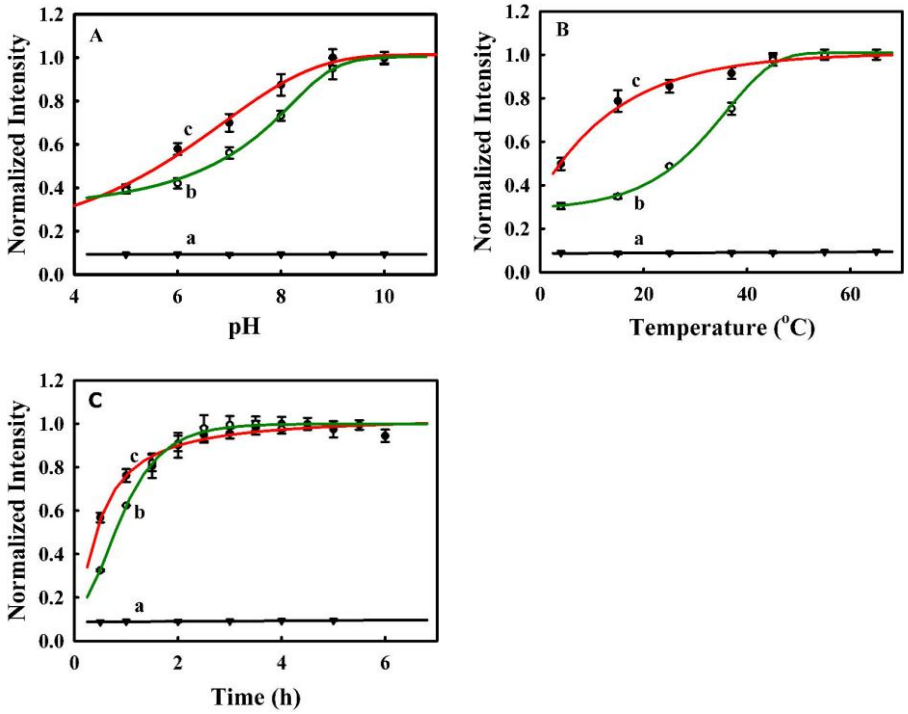

Fig. S9. Effects of pH value of the incubation buffer (A), the incubation temperature (B), the incubation time (C). 20 μM NDS in the presence of 20 μM GSH or Cys. (a) NDS blank. (b) with Cys. (c) with GSH.

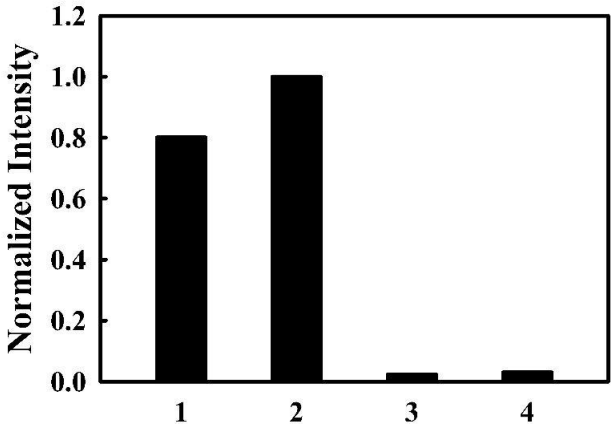

Fig. S10. Fluorescence responses of NDS to different analytes. The concentration of NDS was 20 μM, and other analytes were 20 μM. 1, 2, 3, 4, represent fluorescence responses of NDS to GSH, Cys, mixture of GSH and N-Methylmaleimide, mixture of Cys and N-Methylmaleimide, respectively.

5

Reference

1 I. Mancini, G. Guella, G. Chiasera and F. Pietra, *Tetrahedron Letters.*, 1998, **39**, 1611.