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# A novel near-infrared fluorescent probe based on isophorone for bioassay of endogenous cysteine

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**1. General Methods.**

Unless otherwise specified, all chemical reagents are purchased from Beijing Innochem Technology Co., Ltd. All the reagents were directly used without further purification. In the titration experiment, Pro, Ala, Thr, Phe, His, Ser, Asn, Asp, Lys, Arg, Hcy, GSH and Cys were dissolved in deionized water (10 mL), and the concentration of all amino acids was $1.0 \times 10^{-3}$ μM. $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded on Bruker am 400 spectrometers. Chemical shifts were reported in ppm (TMS as internal standard). The UV-Vis absorption spectra were recorded on Shimadzu UV-2450 spectrophotometer. The fluorescence spectra were recorded by Shimadzu RF-5310pc and F-320 (Guangdong Science and Technology) spectrophotometer. Laser confocal imaging was performed by Olympus fv1200 laser confocal microscope.

**DDP** was accurately weighed and dissolved in DMSO to prepare 1.0 mM stock solution. Amino acids were dissolved in distilled water to prepare 0.01 mol / L solution for standby. **DDP** stock solution was diluted to 10.0 μM with DMSO-PBS (4:1, v/v pH 7.4), in which was added 200.0 μM of different biothiols respectively. The resulting solution was shaken at 37 ℃ for 30 minutes. For all fluorescence spectra, the excitation is set at 550 nm, the emission wavelength is in the range of 560-850 nm, and the excitation and emission gaps are 5/5 nm.

HeLa cells were purchased from Shanghai iCell Bioscience Inc., HeLa cells in logarithmic growth phase were counted, and the cell concentration was adjusted. HeLa cells were inoculated into a confocal special culture dish according to 4 × 104 / plate, and cultured overnight in 5% $CO_2$, 37 $^{o}$C incubator containing 10% fetal bovine seru. **DDP** (10.0 μM) was added to the cells and incubated at 37 $^{o}$C for 30 minutes. After washing with PBS buffer (pH 7.2-7.4) to remove the free **DDP**, the cells were excited at 559 nm and imaged with confocal fluorescence microscopy at 680-750 nm. In the first control group, HeLa cells were pretreated with N-ethyl maleimide (NEM, 500.0 μM) at 37 $^{o}$C for 30 minutes, then washed with PBS buffer, incubated with **DDP** (10.0 μM) for 40 minutes, and imaged with confocal fluorescence microscope.

As another control group, HeLa cells were co-incubated with **DDP** (10.0 μM) for 40 minutes, washed with PBS buffer, and then incubated with Cys (200.0 μM) for 30 minutes.

## 2. $^{1}H$, $^{13}C$ NMR and ESI-MS copies of DDP.

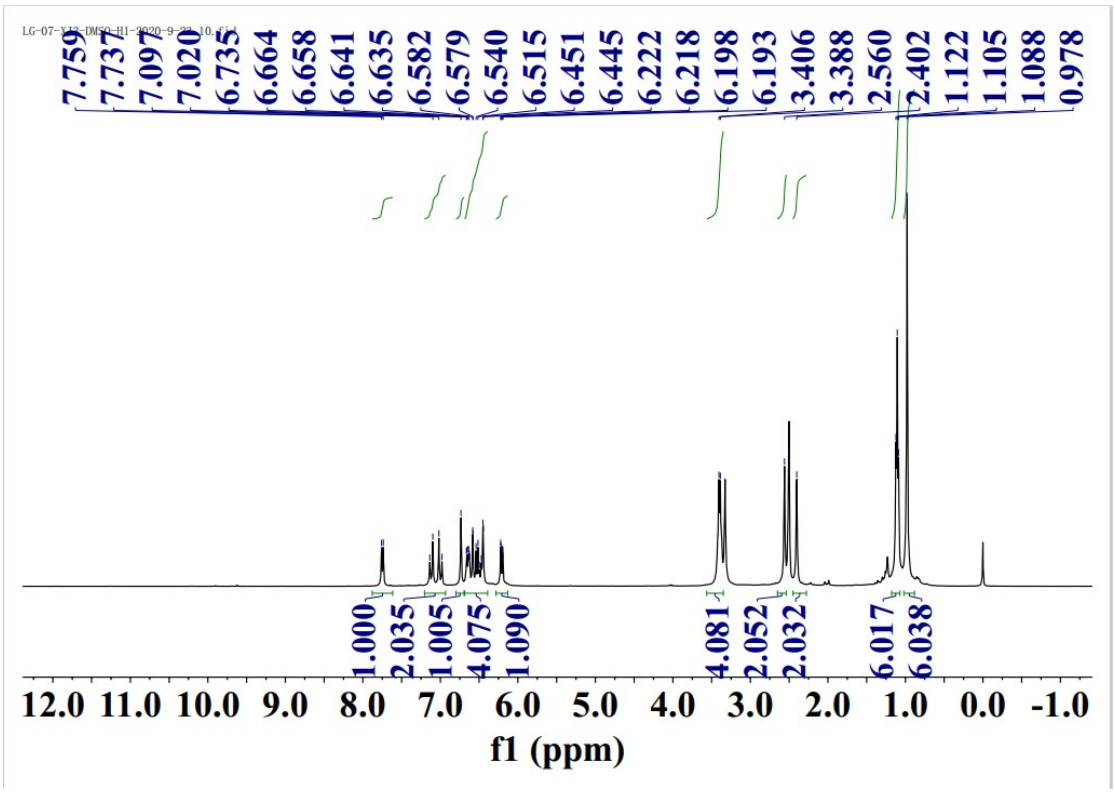


**Figure. S1** $^{1}H$ NMR spectrum of **DDP** in DMSO-$d_6$.

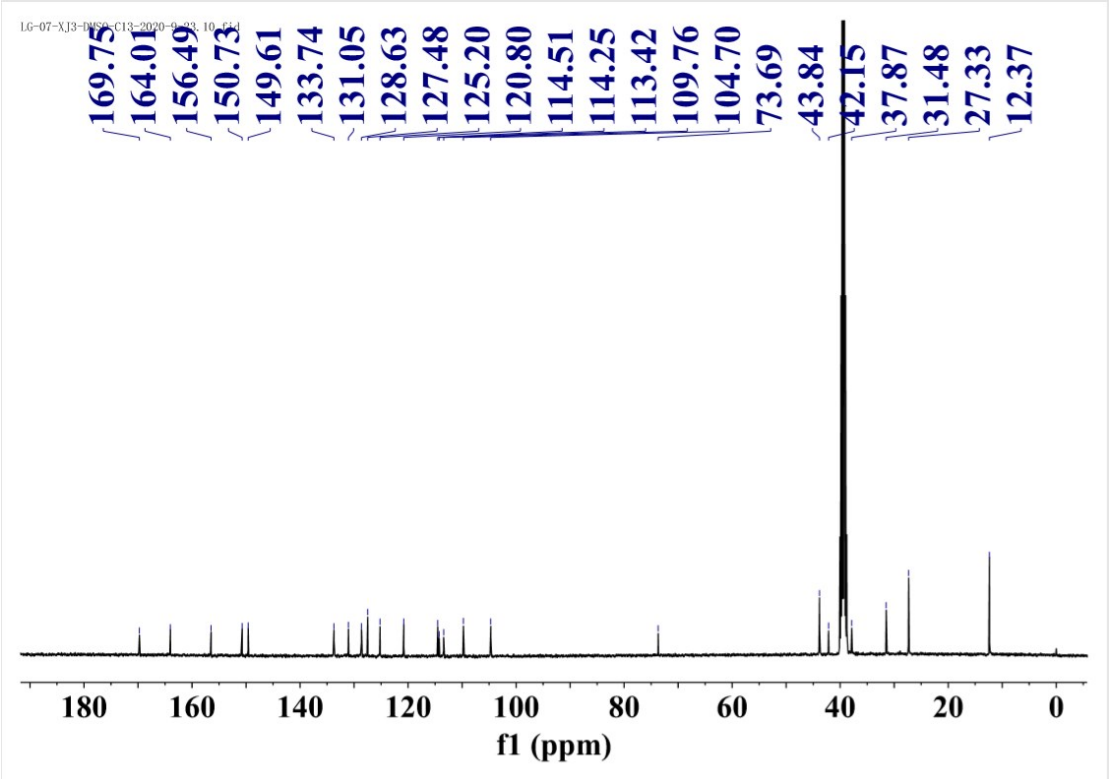


**Figure S2.** $^{13}$C NMR spectrum of **DDP** in DMSO-$d_6$.

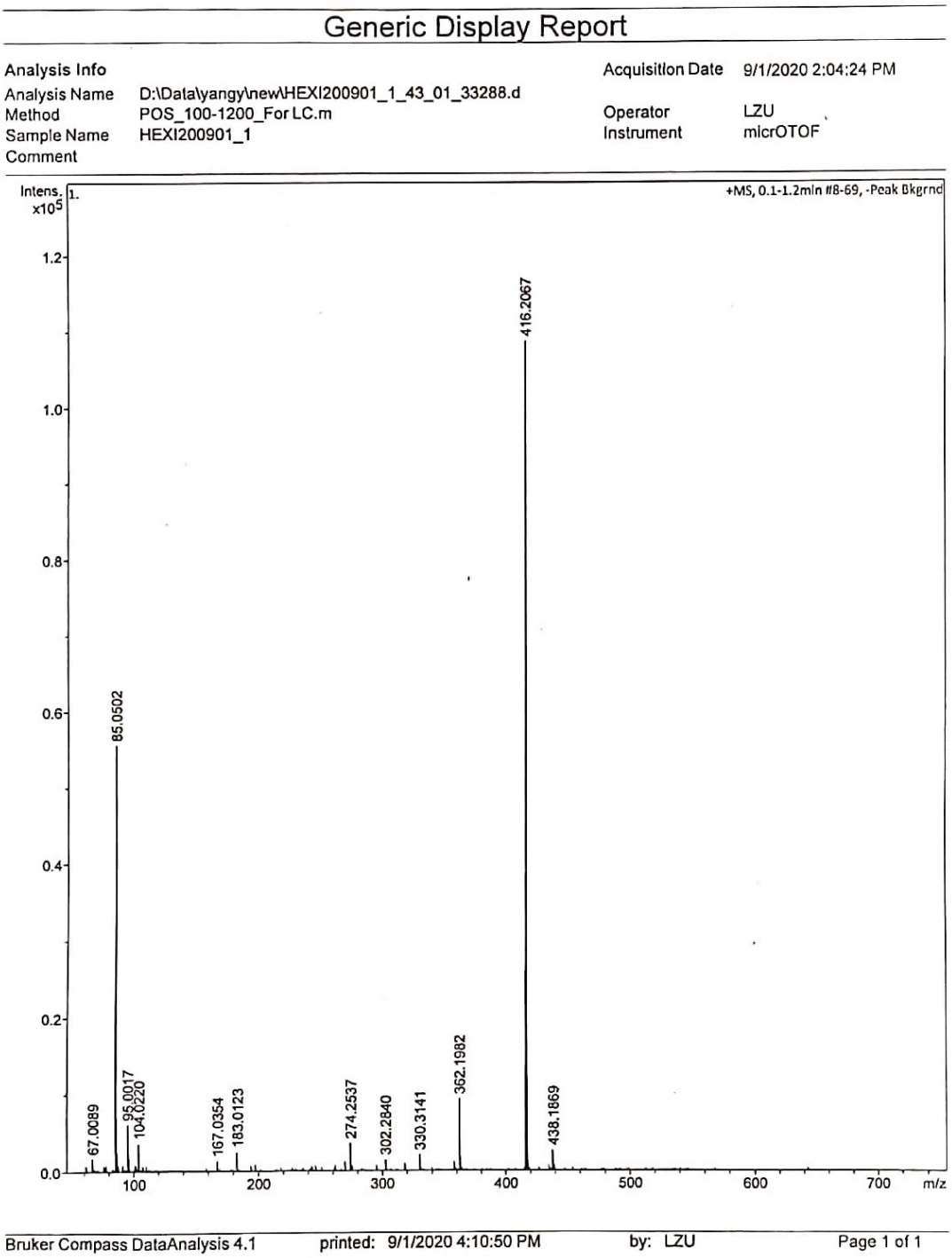


**Figure S3.** ESI-MS spectrum of **DDP**.

## 3. Effect of different solvents on fluorescence intensity

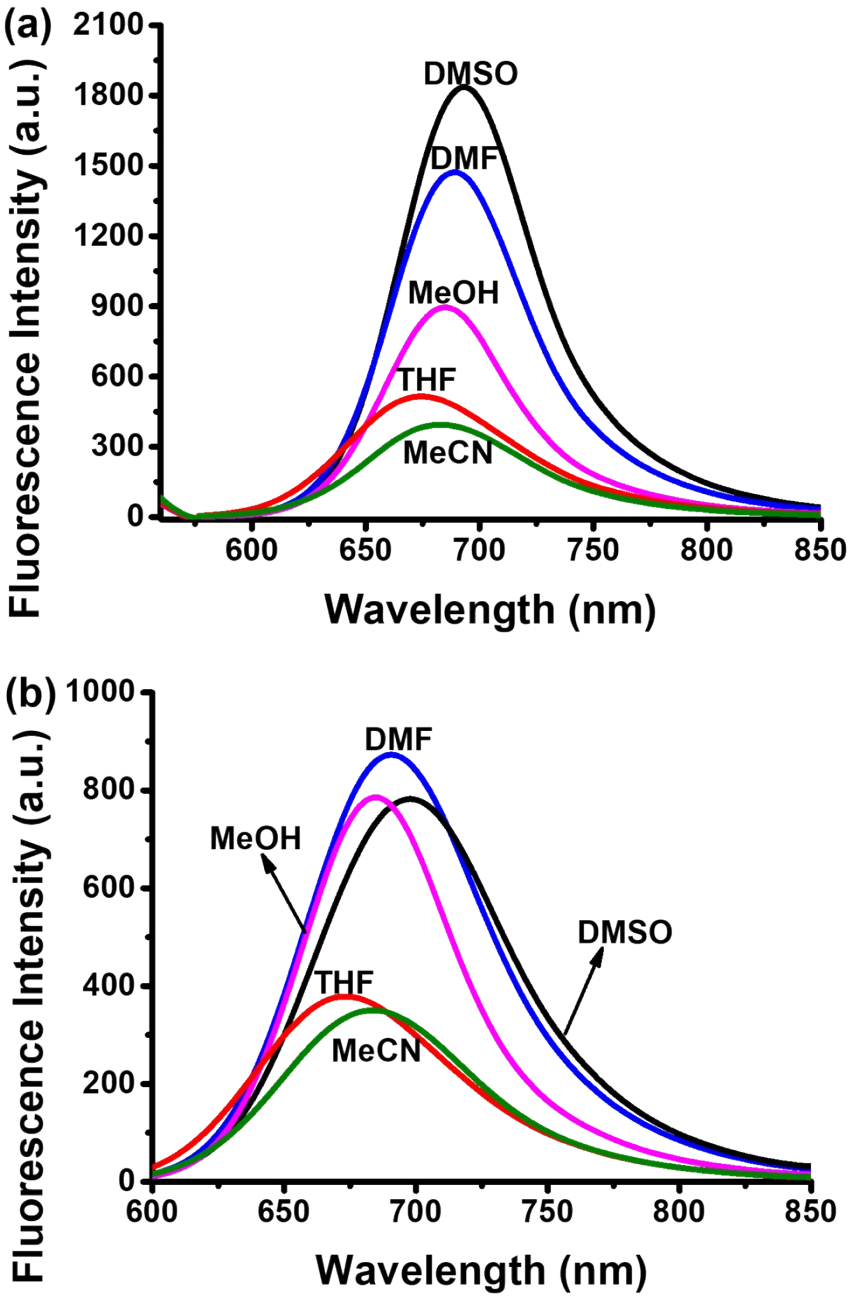


**Figure S4.** (a) Fluorescence spectra of **DDP** (10.0 μM) in PBS buffer with 80% various organic solvent incubation with Cys (200.0 μM) for 0.5 h at pH 7.4. (b) Fluorescence spectra of **DDP** (10.0 μM) in PBS buffer with 80% various organic solvent incubation (200.0 μM) for 0.5 h at pH 7.4 ($\lambda_{ex}$ = 550 nm).

**4. The pH dependence of the fluorescence intensity change.**

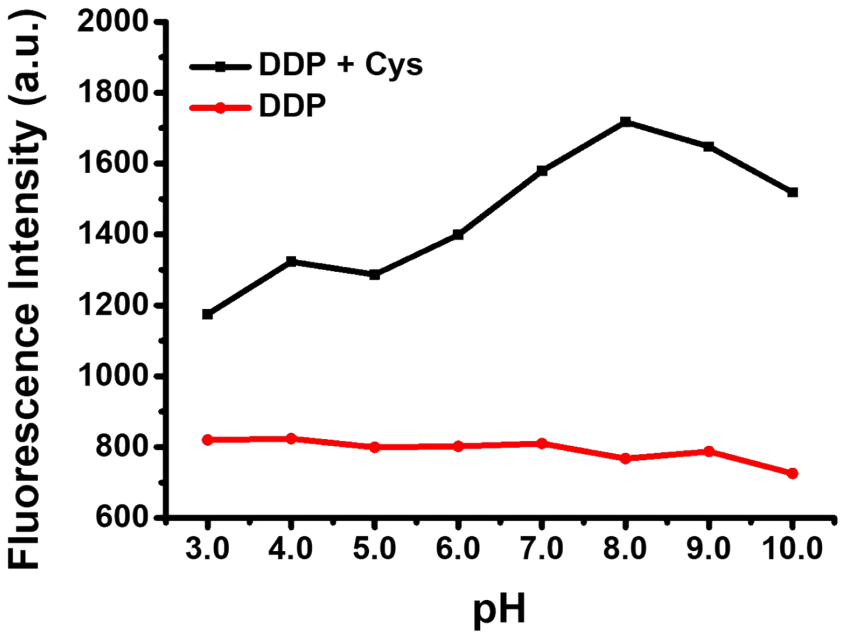


**Figure S5.** Fluorescence intensity of **DDP** (10.0 μM) at 550 nm in the absence (red) or presence (black) of Cys (200.0 μM) at various pH values at room temperature ( $\lambda_{ex}$ = 550 nm).

**5. Fluorescence responses of DDP to other amino acids in PBS buffer solution**

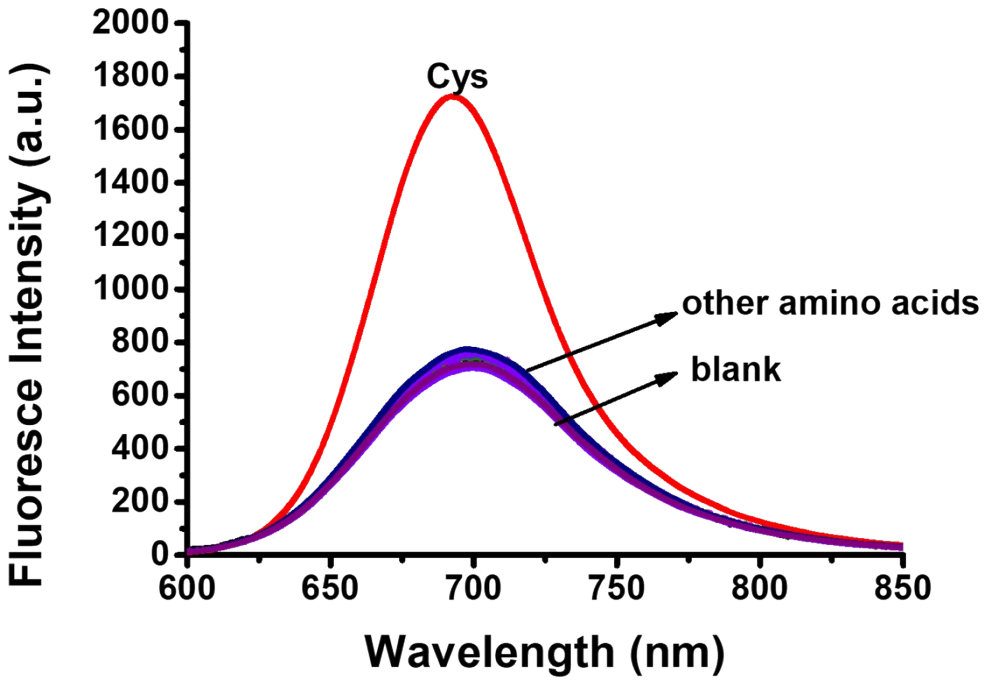


**Figure S6.** Fluorescence spectral changes of the **DDP** (10.0 μM) after addition of Cys and other amino acids (200.0 μM) in DMSO : PBS = 4:1 (v/v) solution at pH 7.4.

**6. Linear concentration range of DDP with Cys in PBS buffer solution.**

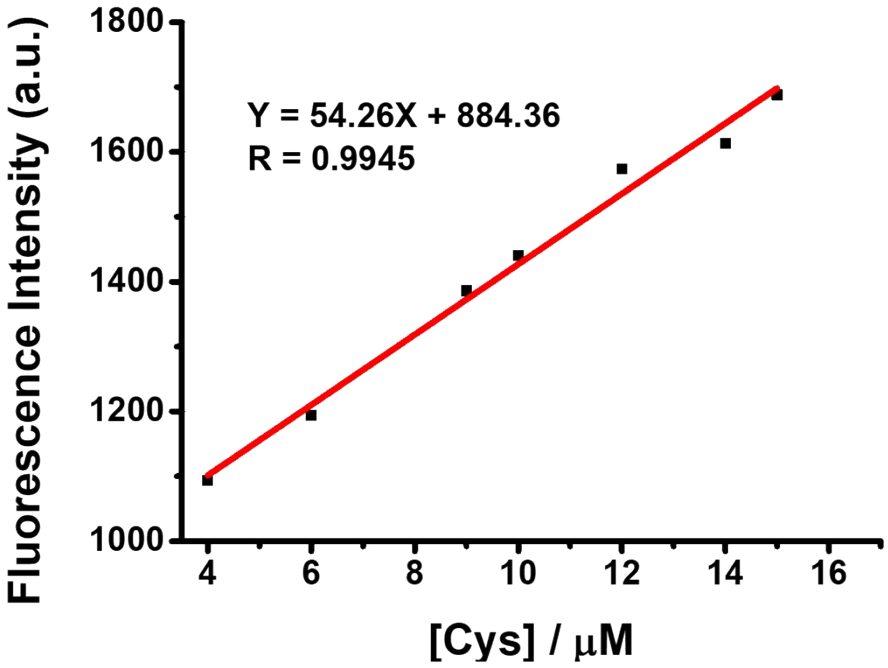


**Figure S7.** The linear relationship of fluorescence intensity the concentration of Cys (4.0-15.0 μM) ($\lambda_{ex}$ = 550 nm).

**7. The UV-vis titrations of DDP with Cys.**

(a)

(b)

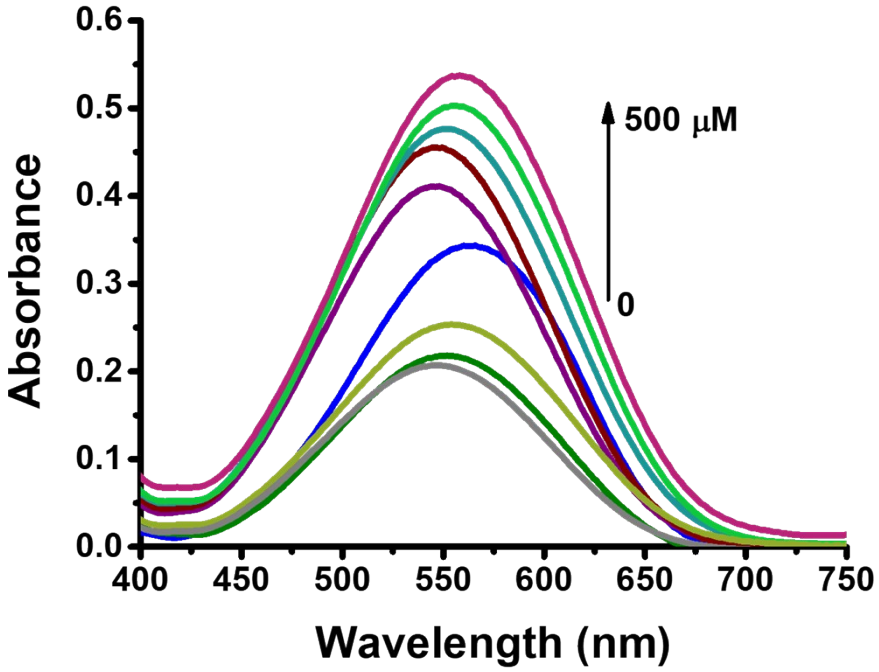


**Figure S8.** (a) Photographs observed of **DDP** (10.0 μM) after the addition of Cys (from left to right : 0.0, 0.50, 1.0, 3.0, 5.0, 10.0, 15.0, 30.0, 50.0, 100.0, 200.0, 500.0 μM) at pH 7.4 ( $\lambda_{ex}$ = 550 nm). (b) Absorbance spectra of **DDP** (10.0 μM) after the addition of Cys (0 – 500.0 μM) at pH 7.4.

**8. Time dependence of the fluorescence intensity change.**

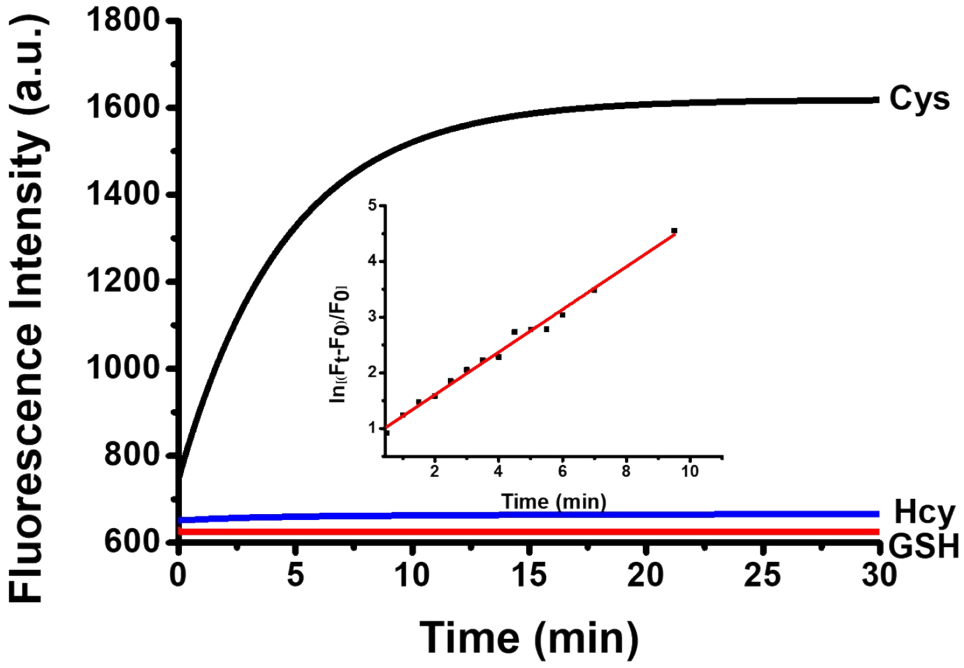


**Figure S9.** Time dependence of fluorescence spectrum of **DDP** (10.0 μM) in DMSO : PBS = 4:1 (v/v) solution at pH 7.4 ( $\lambda_{ex}$ = 550 nm). Inset: The pseudo-first-order kinetics equation of **DDP**.

**9. ESI-MS spectrum of DDP + Cys.**

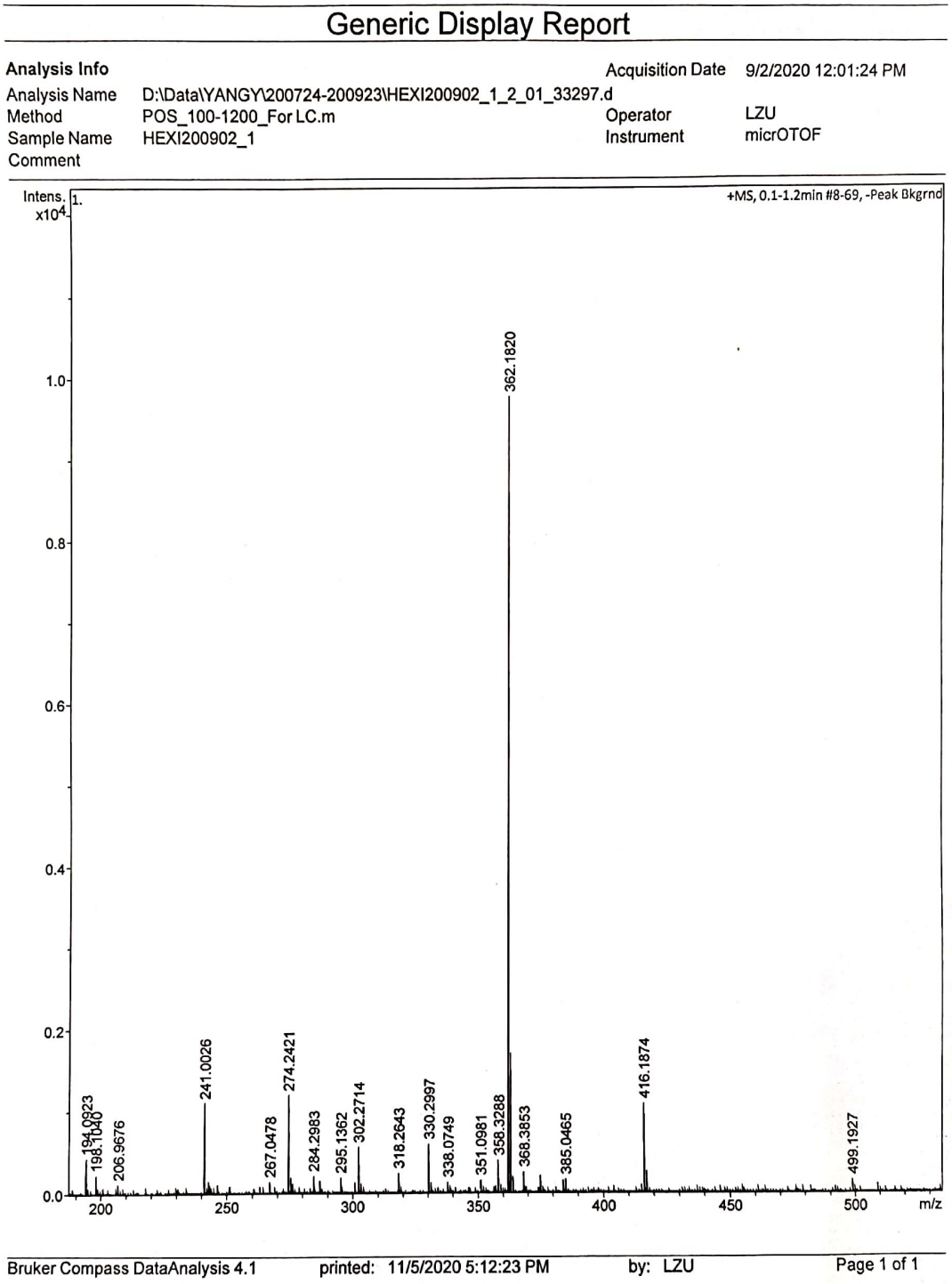


**Figure S10.** ESI-MS spectrum of **DDP** + Cys.