

## Electronic Supplementary Information for

### A two-photon ratiometric fluorescent probe for highly selective sensing mitochondrial cysteine in live cells

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Chuan Dong<sup>a</sup> and Shaomin Shuang<sup>\*a</sup>

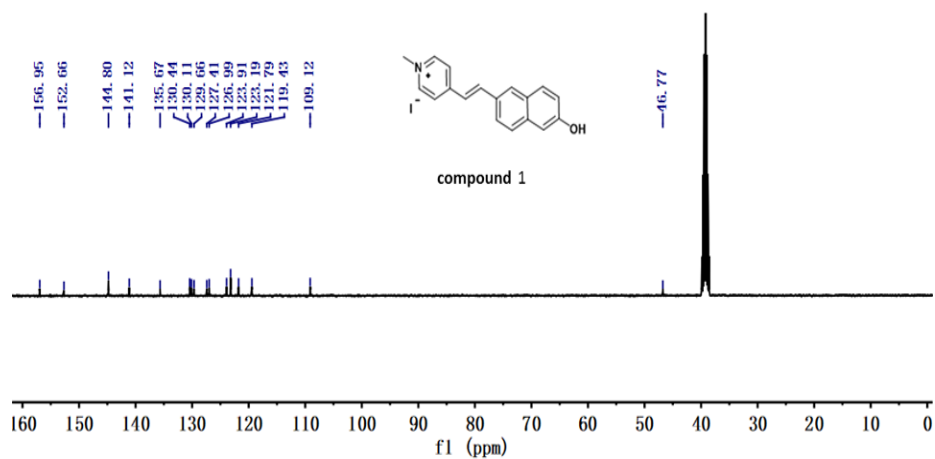
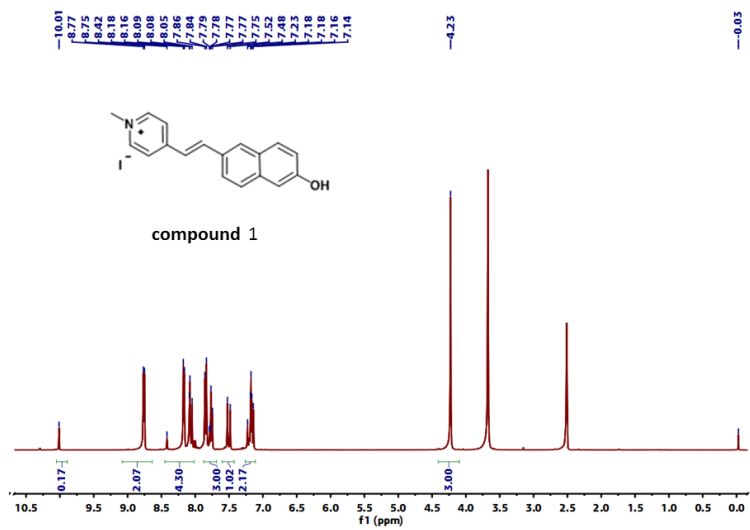
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- Fig. S14** Two-photon action cross-section excited spectra of DNEPI and its released fluorophore compound 1 in DMSO:H<sub>2</sub>O (1:1), respectively.



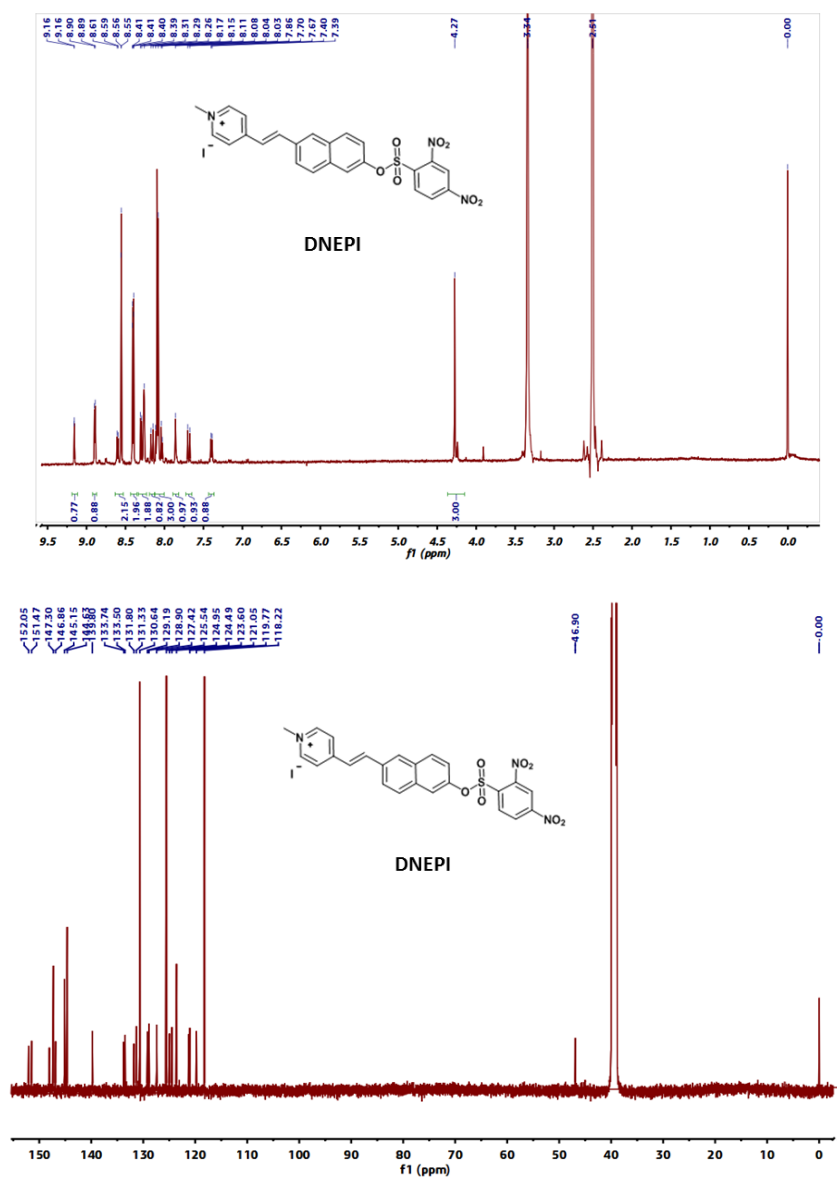
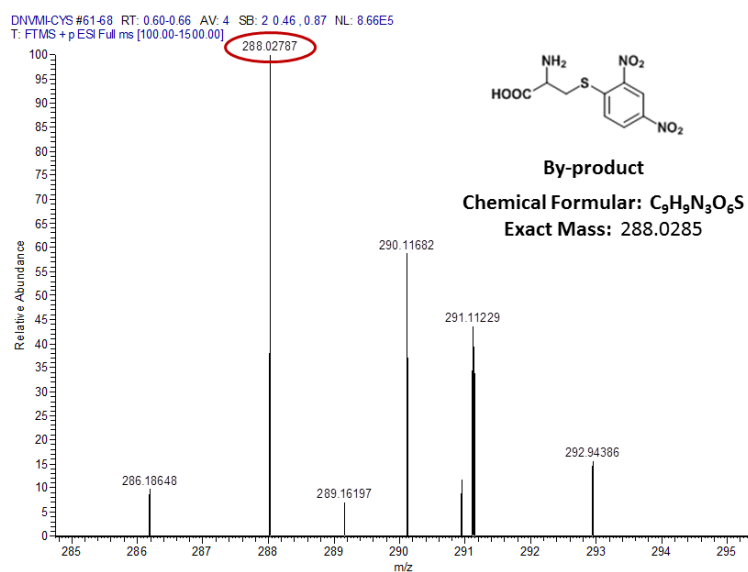
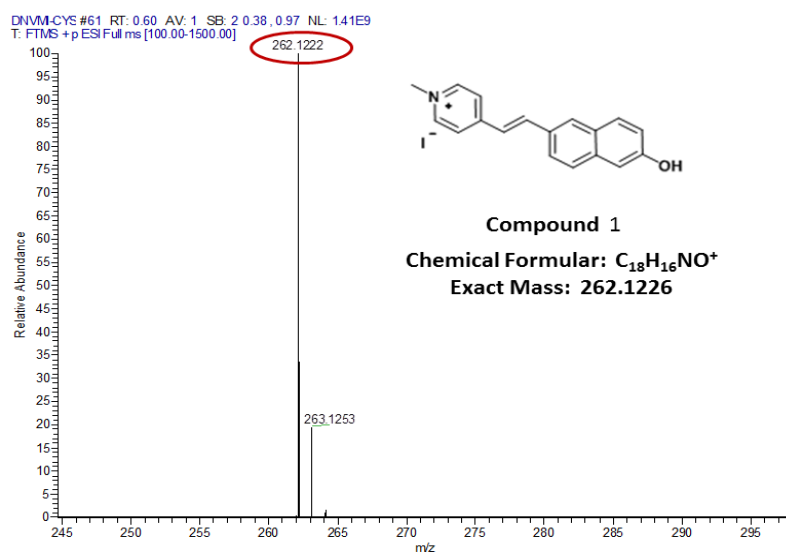
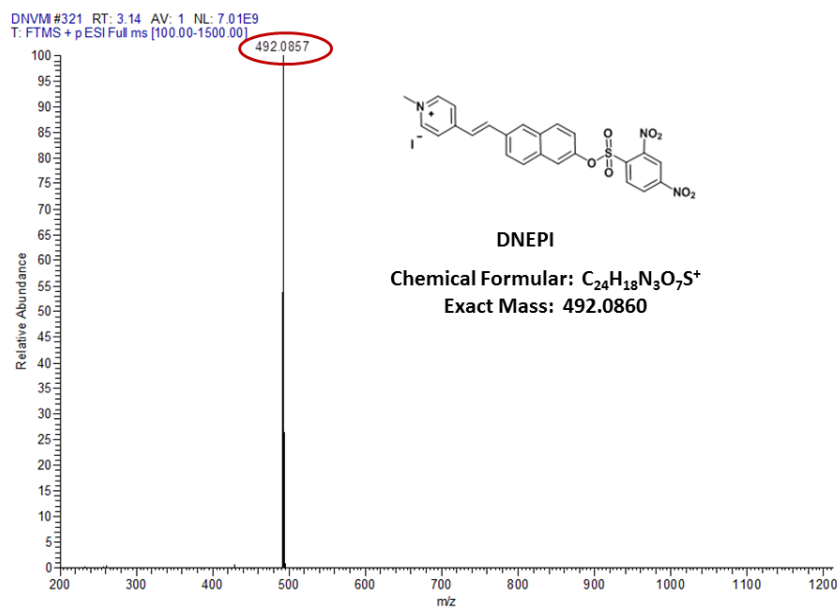


Fig. S1 <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of compound 1 and DNEPI.



**Fig. S2 LC-MS analysis of DNEPI, compound 1 and by-product in Scheme 1.**

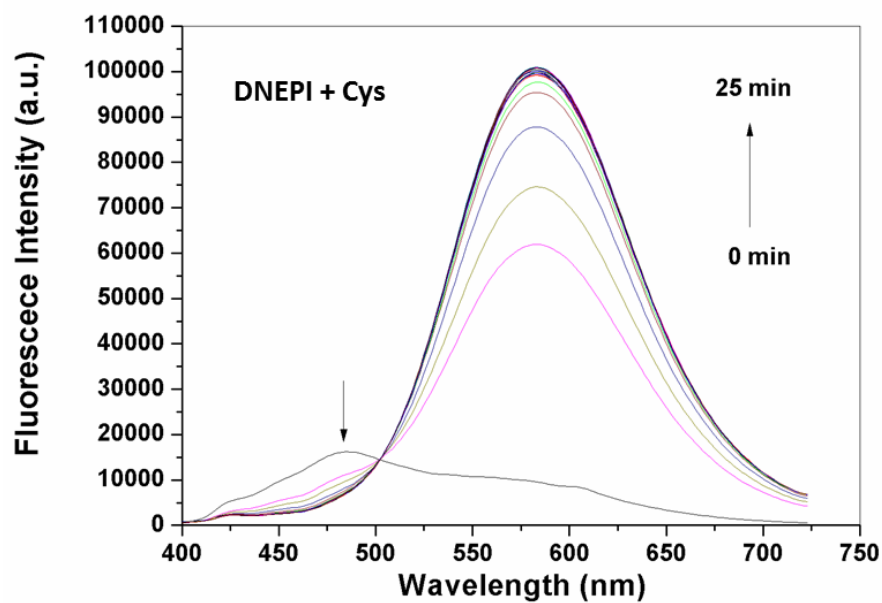


Fig. S3 Fluorescence spectral changes of DNEPI (10  $\mu$ M) against time of 25 min in the presence of Cys (100  $\mu$ M) in DMSO/PBS (1/1, v/v, pH 7.4).

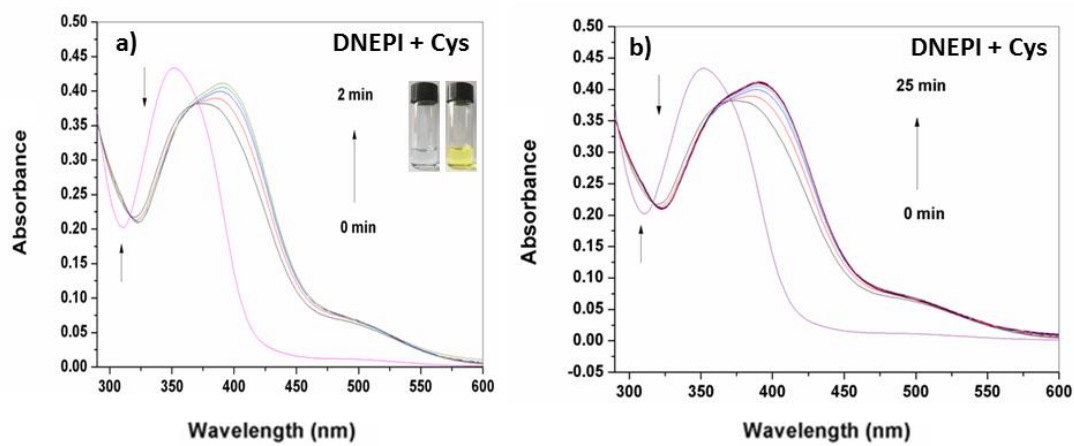
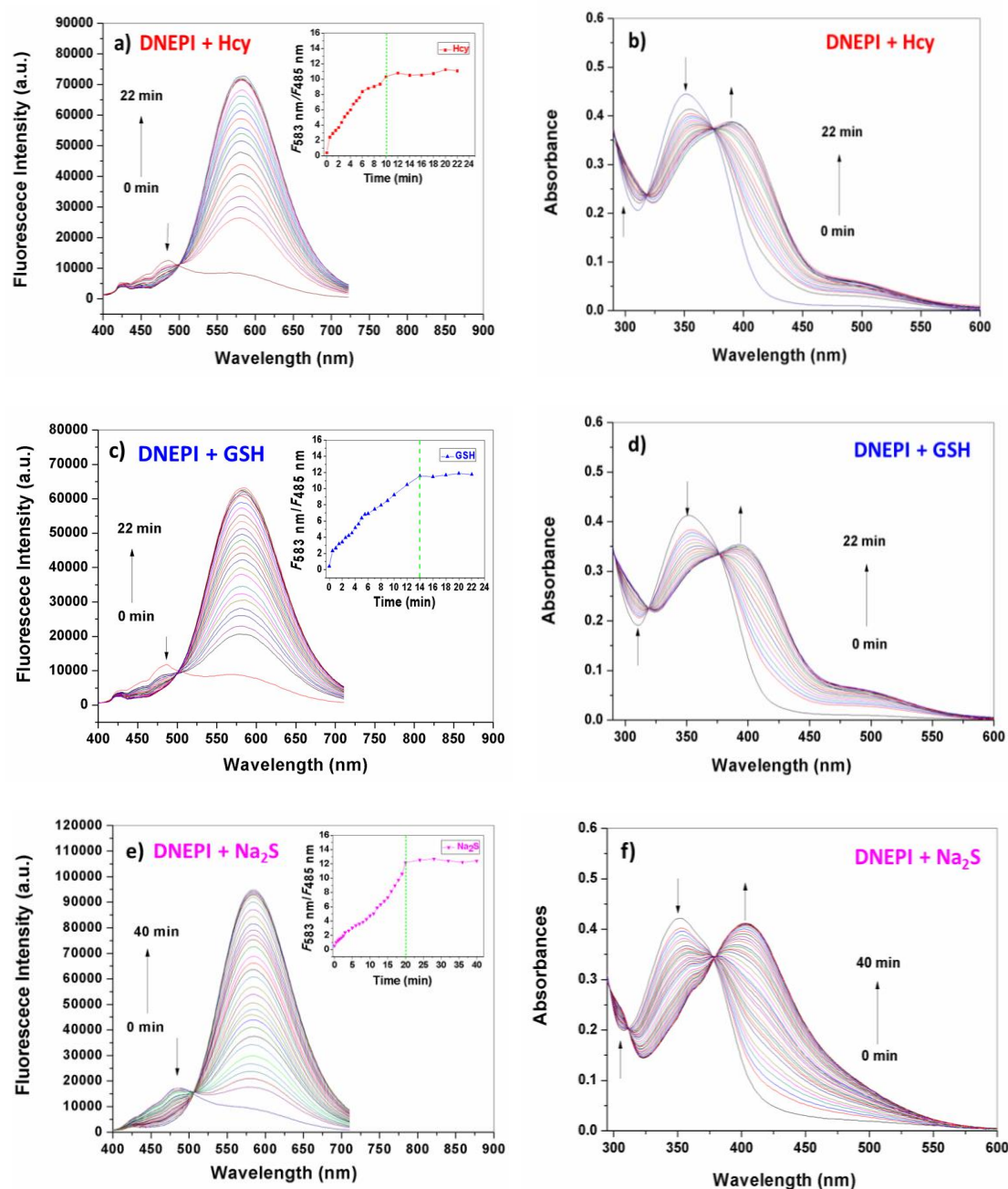


Fig. S4 UV-vis spectral changes of DNEPI (10  $\mu$ M) against time of 2 min (a) and 25 min (b) in the presence of Cys (100  $\mu$ M) in DMSO/PBS (1/1, v/v, pH 7.4), respectively.



**Fig. S5** Fluorescence and UV-vis spectral changes of DNEPI (10  $\mu\text{M}$ ) against time in the presence of 100  $\mu\text{M}$  Hcy (a, b), 100  $\mu\text{M}$  GSH (c, d) and 100  $\mu\text{M}$   $\text{Na}_2\text{S}$  (e, f) in DMSO/PBS (1/1, v/v, pH 7.4), respectively. Inset: fluorescence intensity ratio ( $F_{583\text{nm}}/F_{485\text{nm}}$ ) vs time.

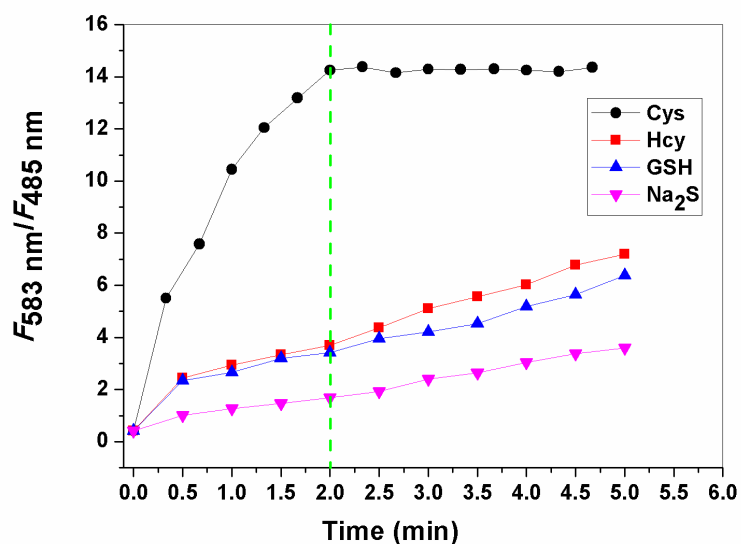


Fig. S6 Fluorescent kinetic of DNEPI (10  $\mu$ M) with 100  $\mu$ M of Cys, Hcy, GSH and  $\text{Na}_2\text{S}$  in DMSO/PBS (1/1, v/v, pH 7.4) within 5 min.

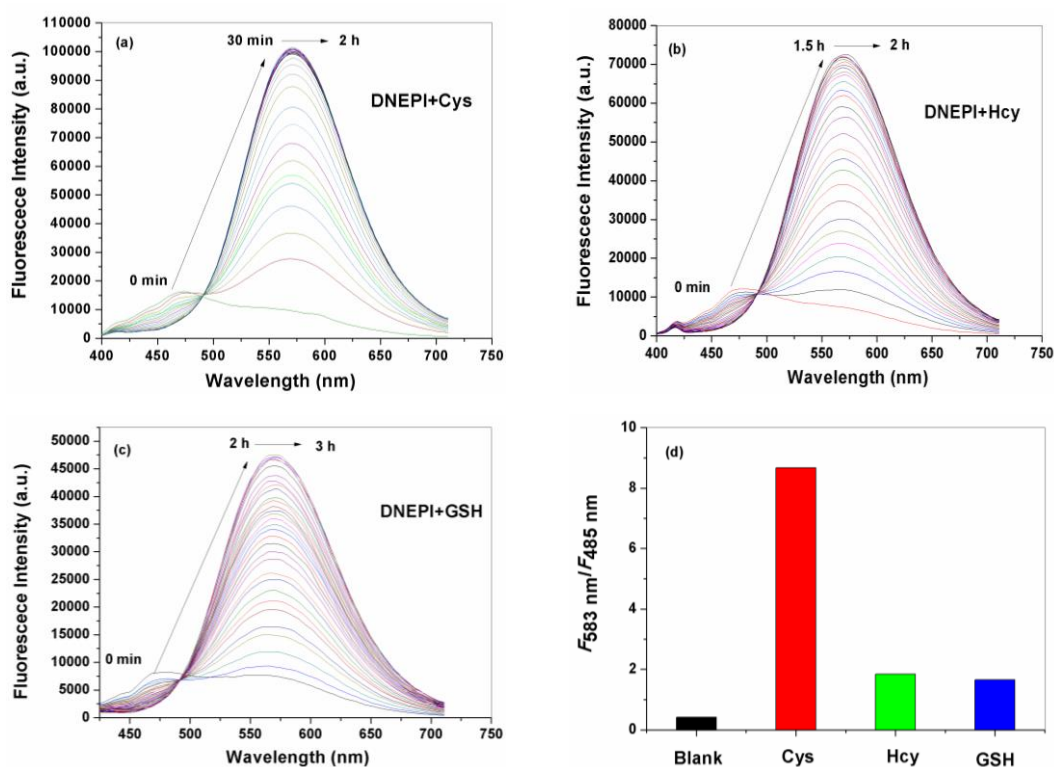


Fig. S7 Fluorescence spectral changes of DNEPI (10  $\mu$ M) against time in the presence of 100  $\mu$ M of (a) Cys, (b) Hcy, (c) GSH in DMSO/PBS (1/99, v/v, pH 7.4). (d) Fluorescence responses of DNEPI toward thiols after 30 min in DMSO/PBS (1/99, v/v, pH 7.4).



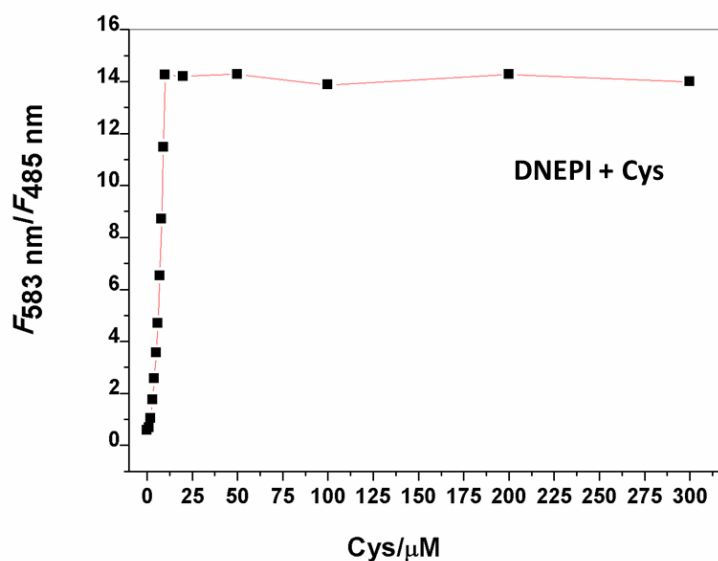


Fig. S8 Fluorescence intensity ratio ( $F_{583\text{nm}}/F_{485\text{nm}}$ ) of DNEPI with Cys concentration in the range of 0-300  $\mu\text{M}$ .

Table S1. Photophysical data for DNEPI and compound **1**

| Solvent  | $\lambda_{\text{ex}}$ (nm) | $\lambda_{\text{em}}$ (nm) <sup>a</sup> | $F_{\text{max}}$ <sup>b</sup> | $\Phi_{\text{F}}$ <sup>c</sup> (DNEPI) | $\Phi_{\text{F}}$ (compound <b>1</b> ) |
|----------|----------------------------|-----------------------------------------|-------------------------------|----------------------------------------|----------------------------------------|
| PBS(7.4) | 350                        | 472                                     | $3.91 \times 10^3$            | 0.0021                                 | 0.0126                                 |
| MeOH     | 350                        | 460                                     | $2.44 \times 10^4$            | 0.0114                                 | 0.0825                                 |
| DMSO     | 354                        | 462                                     | $2.73 \times 10^4$            | 0.0201                                 | 0.0620                                 |
| DCM      | 356                        | 460                                     | $1.49 \times 10^4$            | 0.0091                                 | --                                     |

a)  $\lambda_{\text{em}}$  is the maximum emission wavelength. b)  $F_{\text{max}}$  is the maximum fluorescence intensity of **DNEPI**. c)  $\Phi_{\text{F}}$  is the fluorescence quantum yield. "--" represents that compound **1** is insoluble in DCM.

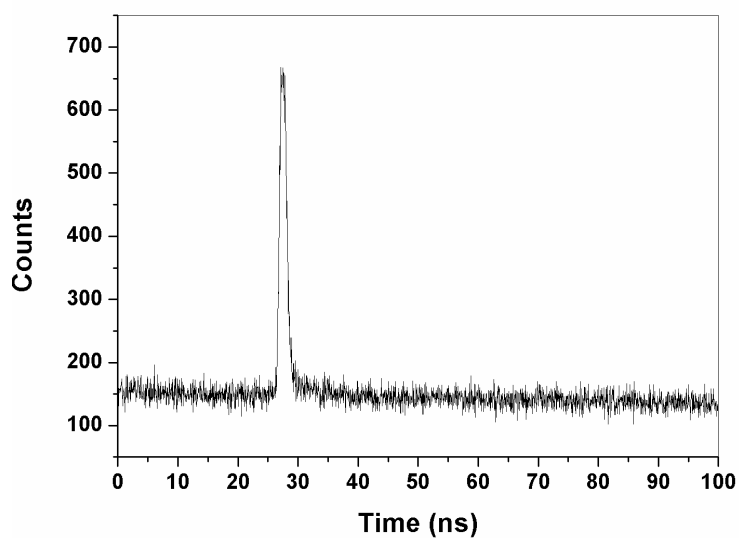
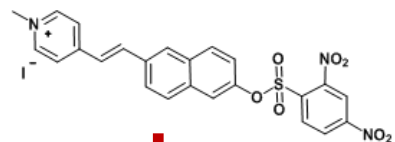
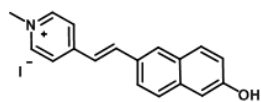
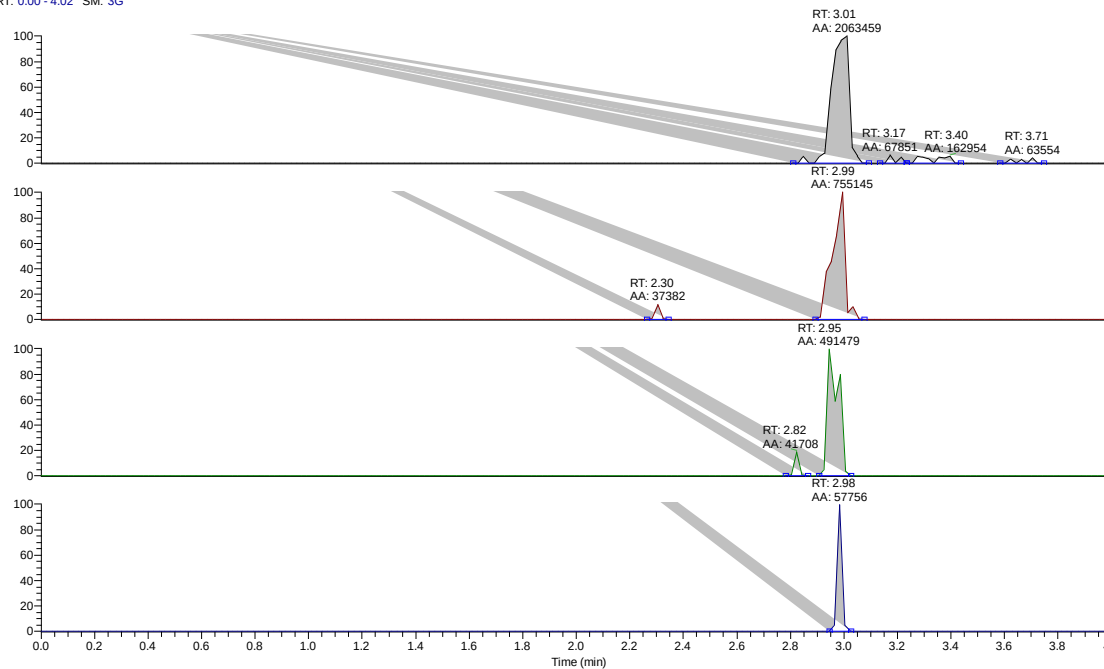


Fig. S9 Fluorescence decay as a function of lifetime of DNEPI in DMSO/PBS (1/1, v/v, pH 7.4).



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RT: 0.00 - 4.02 SM: 5G

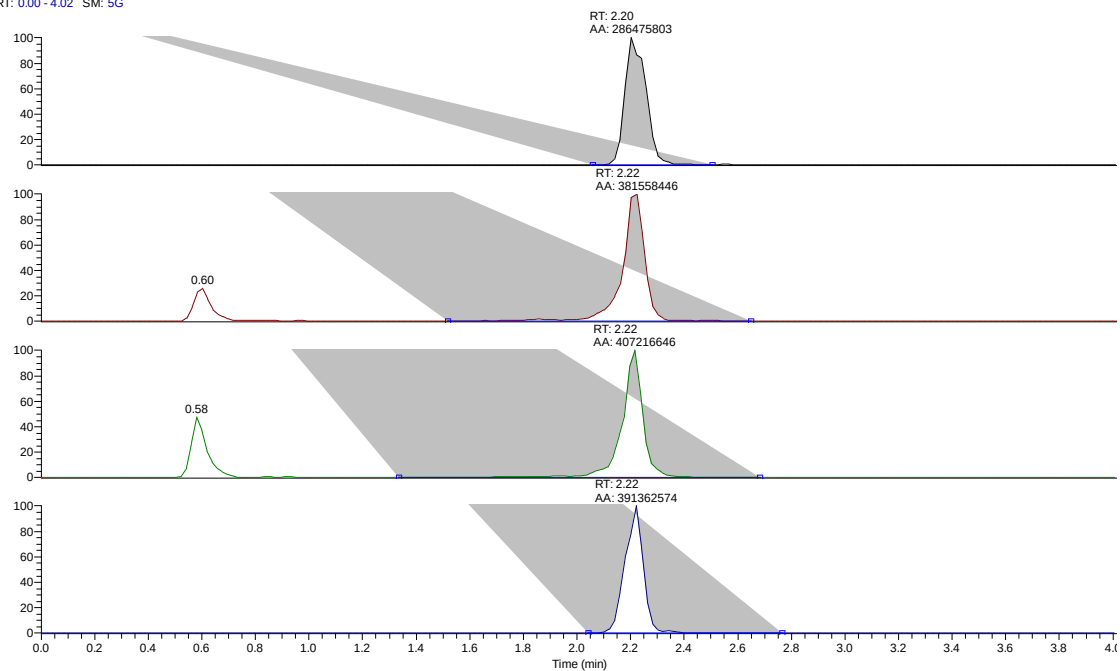


Fig. S10 UPLC-MS spectral changes for DNEPI upon addition of Cys. The interactions were monitored at 0 min, 5 min, 10 min and 15 min, respectively. The integral area of DNEPI around 3.0 min decreased markedly from  $2.06 \times 10^6$  to  $5.78 \times 10^4$ , concomitant with a new peak of compound 1 at 2.2 min appeared and over time, and its integral area increased gradually from  $2.86 \times 10^8$  to  $3.91 \times 10^8$ .

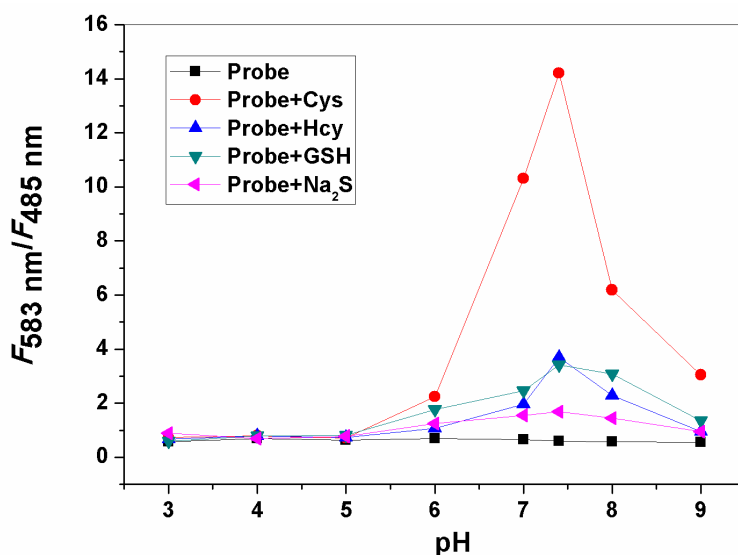


Fig. S11 The pH effect on the fluorescence ratio ( $F_{583\text{nm}}/F_{485\text{nm}}$ ) changes of DNEPI (10  $\mu\text{M}$ ) in the absence and presence of Cys, Hcy, GSH and Na<sub>2</sub>S (100  $\mu\text{M}$ ) in DMSO/PBS (1/1, v/v, pH 7.4) at different pH, respectively.

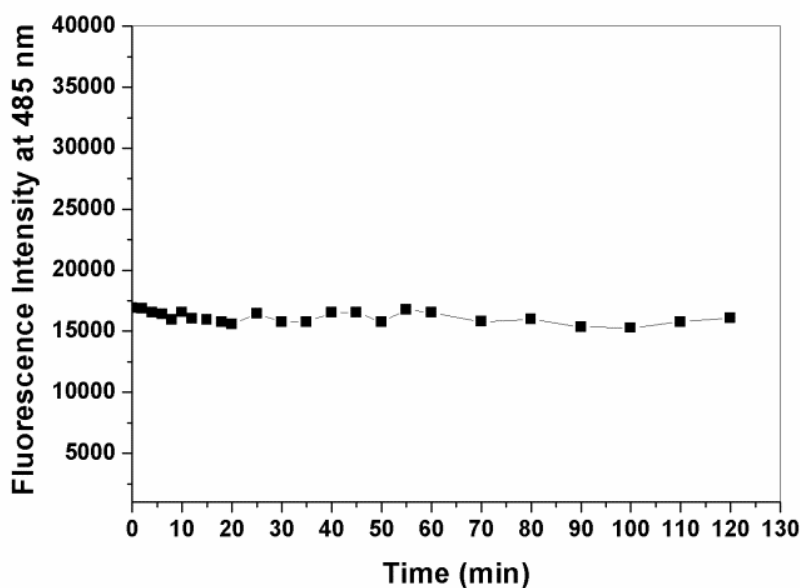
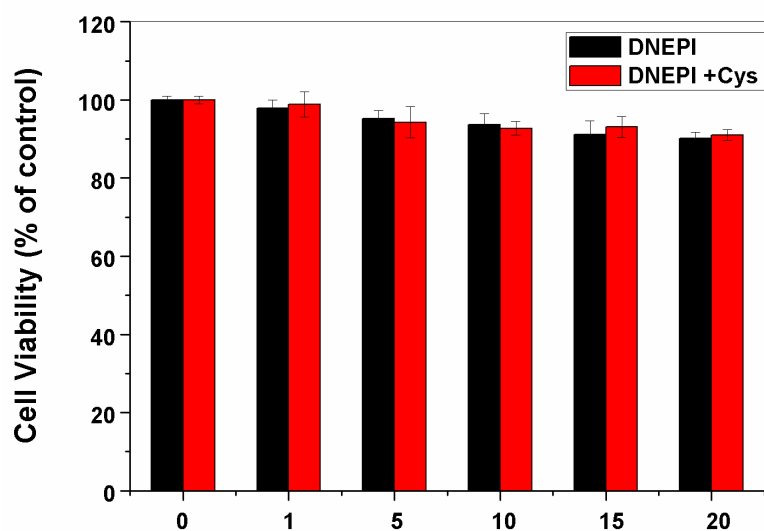
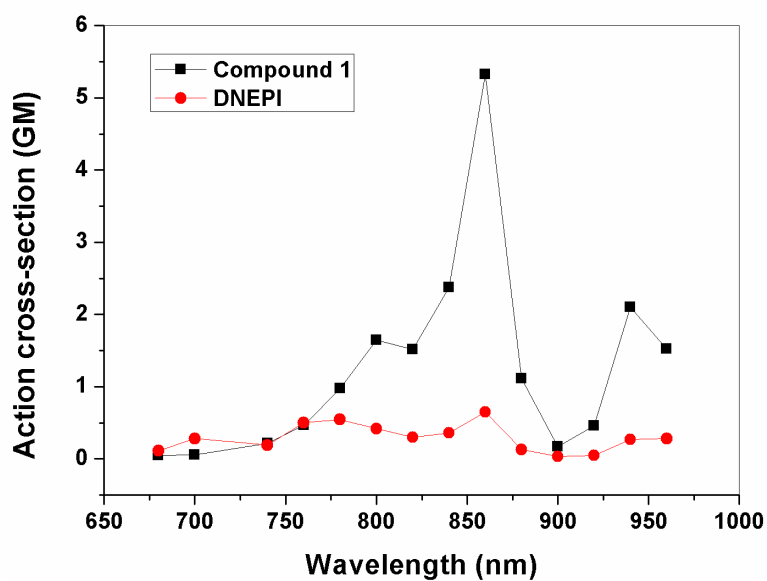


Figure S12 Changes in fluorescence intensity of DNEPI (10  $\mu\text{M}$ ) against time in DMSO/PBS (1/1, v/v, pH 7.4) ( $\lambda_{\text{ex}} = 370 \text{ nm}$ ,  $\lambda_{\text{em}} = 485 \text{ nm}$ ).



**Fig. S13** Cell viability of SMMC7721 cells treated with different concentration of DNEPI (0, 1, 5, 10, 15 and 20  $\mu\text{M}$ ) in the absence (black bars) and presence (red bars) of 100  $\mu\text{M}$  Cys for 24 h in fresh medium.



**Fig. S14** Two-photon action cross-section excited spectra of DNEPI and its released fluorophore compound 1 in DMSO:H<sub>2</sub>O (1:1), respectively.