

*Supporting Information for*

# A Fast-response Two-photon Fluorescent Probe for the Detection of Cys over GSH / Hcy with a Large Turn-on Signal and Its Application in Living Tissues

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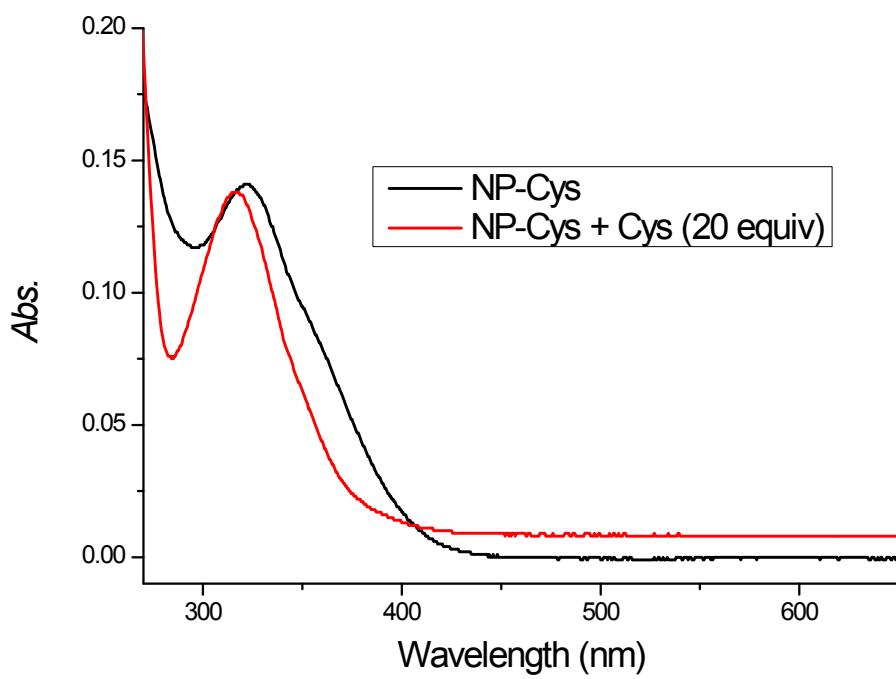
Email: [weiyinglin2013@163.com](mailto:weiyinglin2013@163.com)

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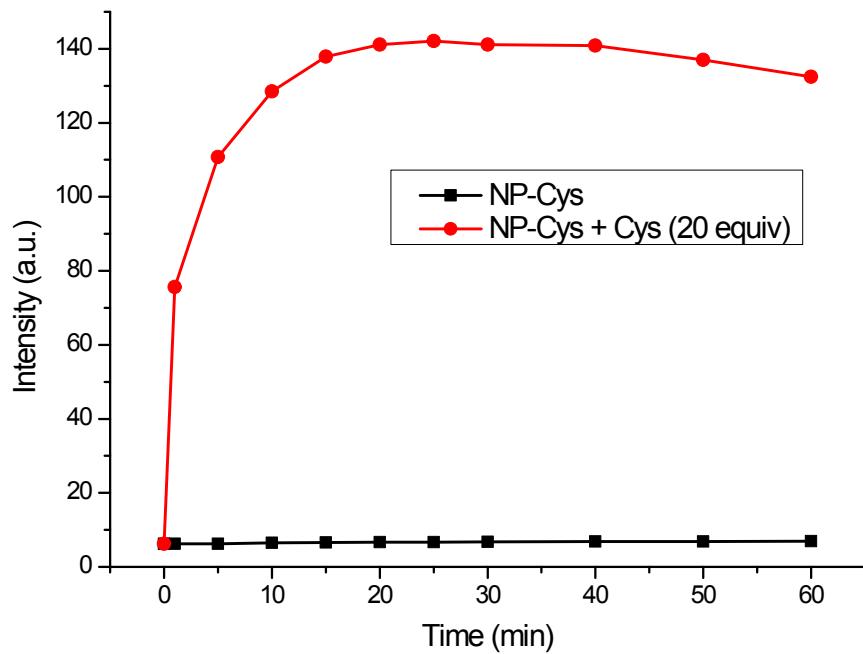
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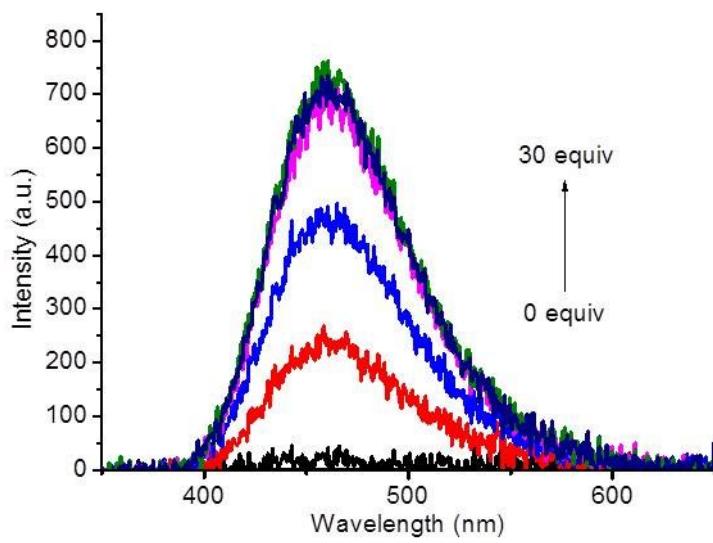
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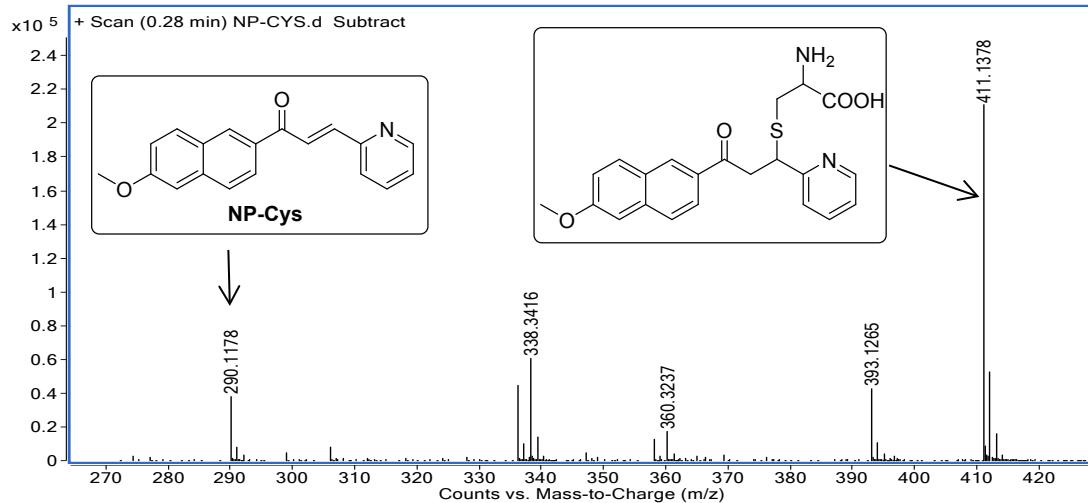
**Fig. S1.** The absorption spectra of NP-Cys (10 μM) in pH 7.4 PBS buffer (containing 5% MeOH) in the absence or presence of Cys (20 equiv).



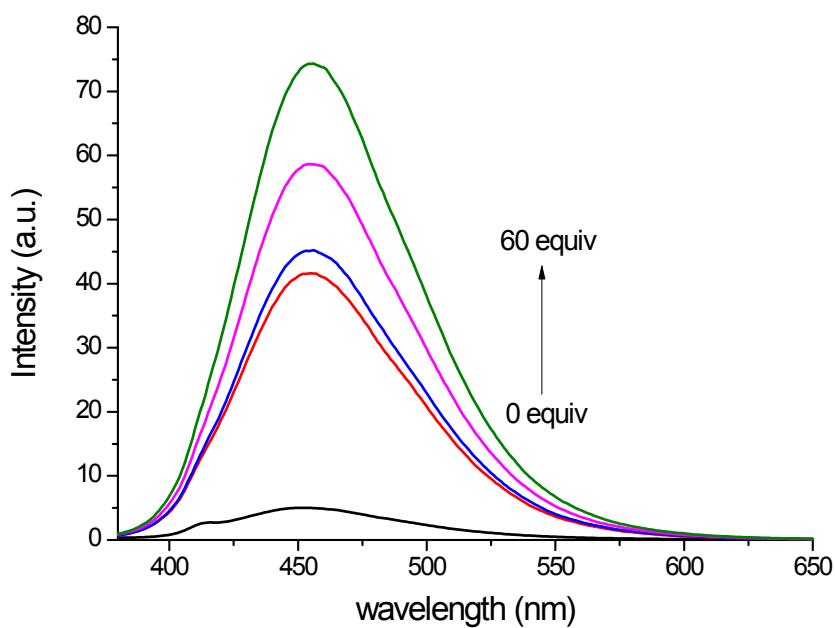
**Fig. S2.** The stability spectra of NP-Cys (10 μM) in pH 7.4 PBS buffer (containing 5% MeOH as a cosolvent) in the absence or presence of Cys (20 equiv).



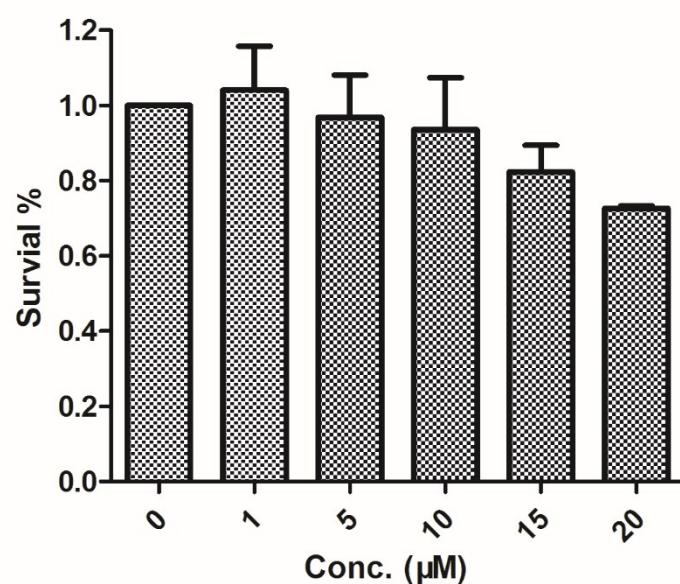
**Fig. S3.** Two-photon fluorescence spectra of the **NP-Cys** (10  $\mu\text{M}$ ) in the absence or presence of Cys for 20 min in PBS buffer (containing 5% MeOH as cosolvent).



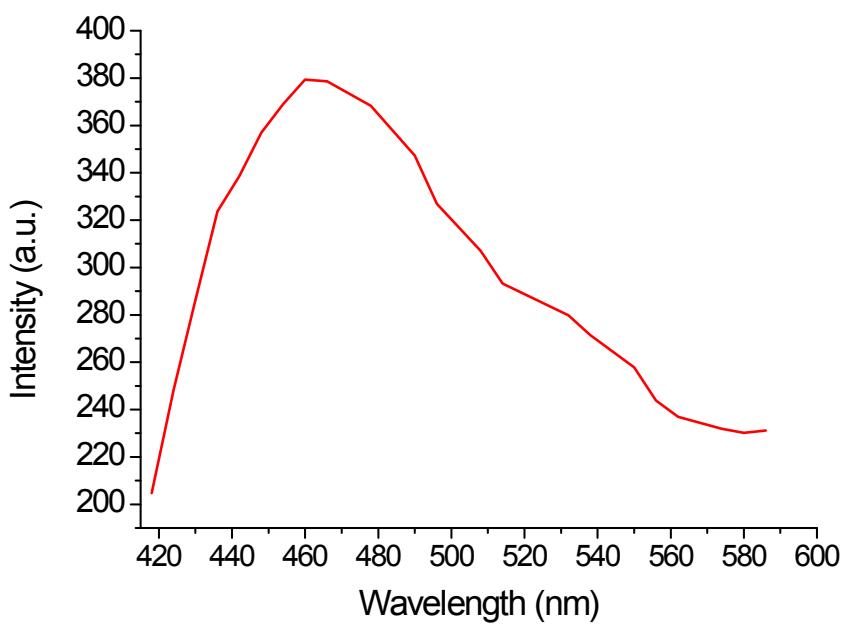
**Fig. S4.** HRMS (positive ion mode) spectrum of **NP-Cys** (20  $\mu\text{M}$ ) after treatment with Cys (400  $\mu\text{M}$ ) in pH 7.4 PBS/MeOH (19: 1) for 60 min. The peak at m/z 411.1378 corresponds to **NP-Cys-adduct**; The peak at m/z 290.1178 corresponds to the probe **NP-Cys**.



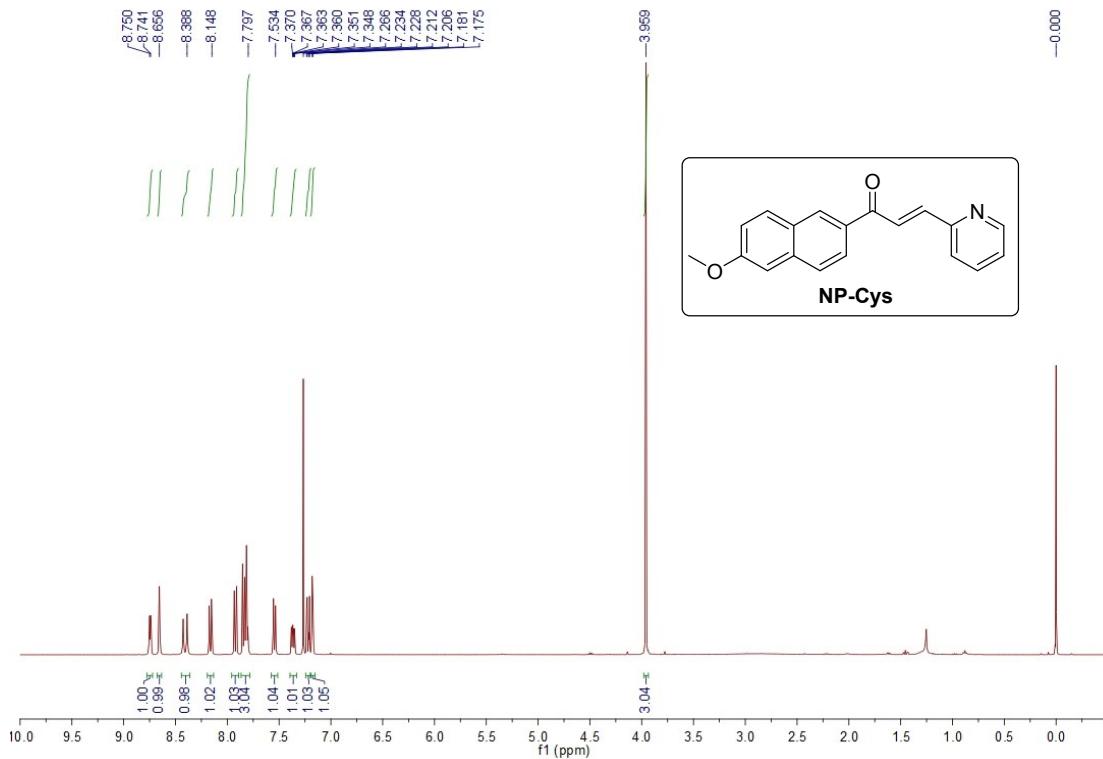
**Fig. S5.** The fluorescence spectra of **NP-Cys** (10  $\mu\text{M}$ ) in pH 7.4 PBS buffer (containing 5% MeOH) in the absence or presence of GSH (0-60 equiv).



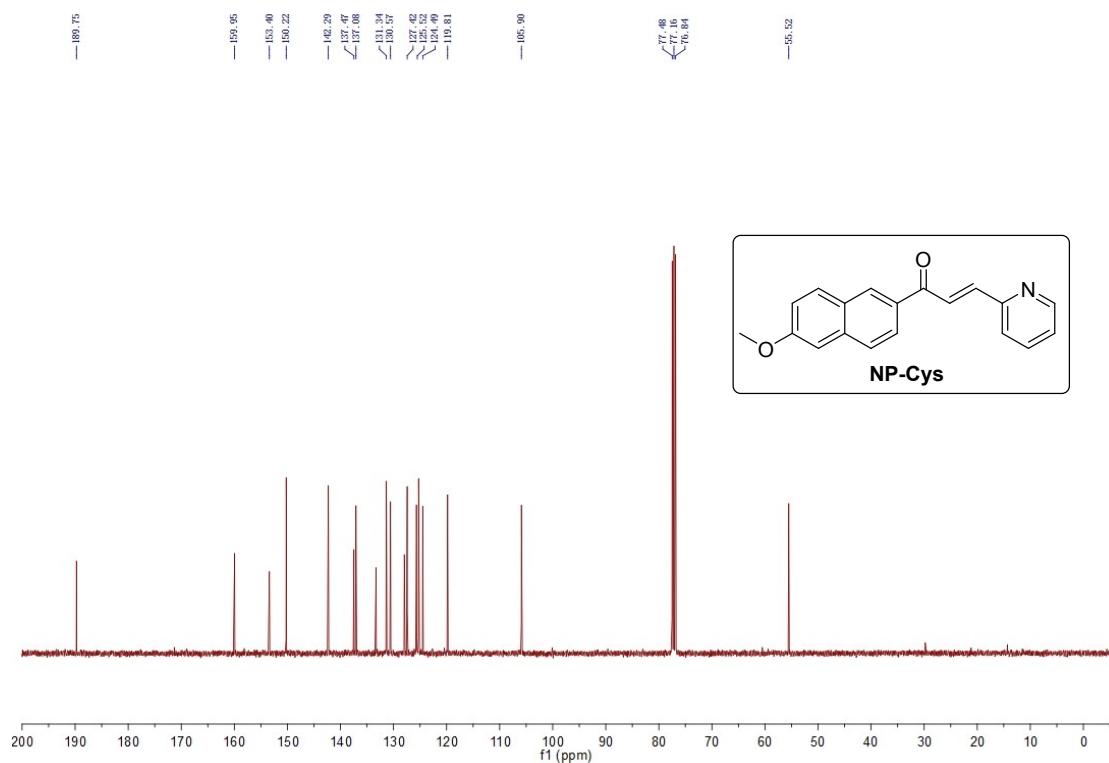
**Fig. S6.** Cytotoxicity assays of **NP-Cys** at different concentrations (0  $\mu\text{M}$ ; 1  $\mu\text{M}$ ; 5  $\mu\text{M}$ ; 10  $\mu\text{M}$ ; 15  $\mu\text{M}$ ; 20  $\mu\text{M}$ ) for HeLa cells.



**Fig. S7.** Two-photon excited fluorescence spectrum from the HeLa cells incubated with both the probe **Np-Cys** and Cys upon excitation at 800 nm.



**Fig. S8.** <sup>1</sup>H-NMR ( $\text{CDCl}_3$ ) spectrum of **NP-Cys**.



**Fig. S9.** <sup>13</sup>C-NMR ( $\text{CDCl}_3$ ) spectrum of **NP-Cys**.