# Electronic Supplementary Information

## Dual modulation sites for reversible fluorescent probe for GSH over Cys/Hcy

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**Contents:**

**Figure S5**: Cell viability estimated by MTT-8 assay with HL-7702 cells, which were cultured in the presence of 5-50.0 $\mu$M **HBT-COU-N(Et)$_2$** for 5 h and 10 h.

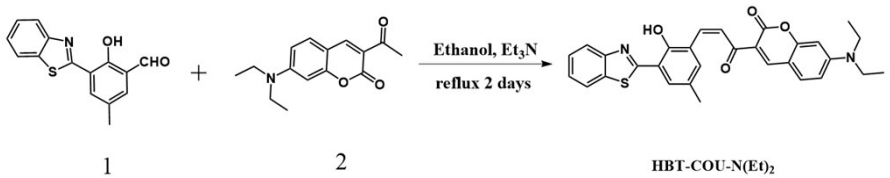
## I: Material and Methods

*Materials and Physical measurements*

All the regents and solvents were commercially available. Naphthalene-1,6-diol and ethyl acetoacetate were got from Aladdin Industrial Corporation (Shanghai, China). Amino acids were got from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). Fluorescence spectra were recorded by HITACHI F-7000 fluorescence spectrophotometer. Ultraviolet-visible spectra were detected by Hitachi U-3900 UV spectrophotometer. $^{1}$H NMR and $^{13}$C NMR data were obtained by Bruker AVANCE-600 MHz NMR spectrometers (Bruker, Billerica, MA). HR-MS determinations were implemented on an AB SCIEX Tripple TOF5600 Instruments. The cell imaging experiments were measured by Zeiss LSM880 Airyscan confocal laser scanning microscope.

## II: Synthesis

Compounds 1was synthesized with reference to literature[1]. Compounds 1 and 2 were refluxed in ethanol under the catalysis of triethylamine to obtain **HBT-COU-N(Et)$_2$** (**Scheme S1**), which was confirmed by NMR and HR-MS. (**Figure S1**)




1 2 HBT-COU-N(Et)$_2$

**Scheme S1** the synthesis of **HBT-COU-N(Et)$_2$**

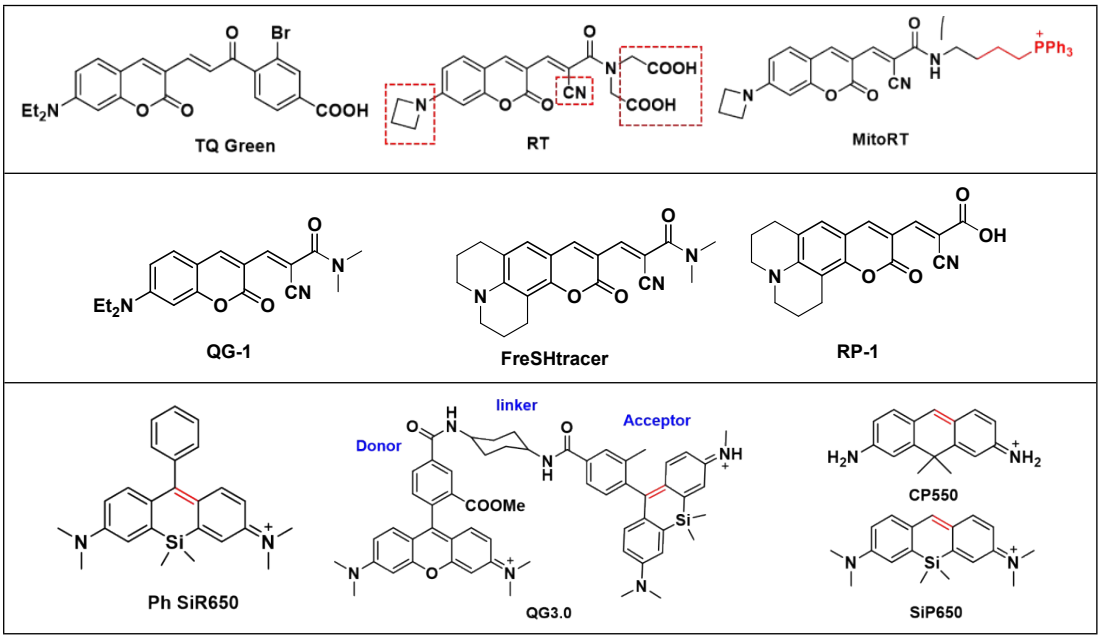



**Table S1**

* The relative refs were shown below.

(1) Jiang, X.; Chen, J.; Bajić, A.; Zhang, C.; Song X.; Carroll, S. L.; Cai, Z. L.; Tang, M.; Xue, M.; Cheng, N.; Schaaf, C. P.; Li, F.; MacKenzie, K. R.; Ferreon, A.; Xia, F.; Wang, M. C.; Maletić-Savatić, M.; Wang, J. *Nat. Commun.* 2017, *8*, 16087-16098.

(2) Jiang, X.; Yu, Y.; Chen, J.; Zhao, M.; Chen, H.; Song, X.; Matzuk, A.; Carroll, J.; Tan, X.; Sizovs, A.; Cheng, N.; Wang, M. C.; Wang, J. *ACS. Chem. Bio.* 2015, *10 (3)*, 864-874.

(3) Liu, Z.; Zhou, X.; Miao, Y.; Hu, Y.; Kwon, N.; Wu, X.; Yoon, J. *Angew. Chem., Int. Ed.* 2017, *56*, 5812-5816.

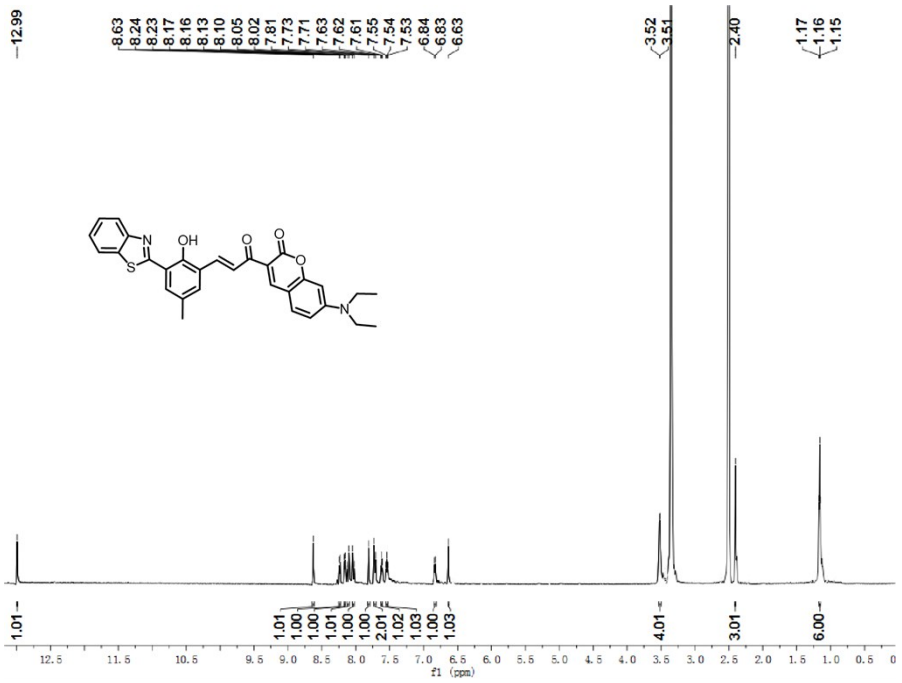
(4) Jeong, E. M.; Yoon, J. H.; Lim, J.; Shin, J. W.; Cho, A. Y.; Heo, J.; Lee, K. B.; Lee, J. H.; Lee, W. J.; Kim, H. J.; Son, Y. H.; Lee, S. J.; Cho, S. Y.; Shin, D. M.; Choi, K.; Kim, I. G. *Stem Cell Rep.* 2018, *10*, 600-614.

(5) Tian, M.; Yang, M.; Liu, Y.; Jiang, F. L. *ACS Appl. Bio Mater.* 2019, *2*, 4503-4514.

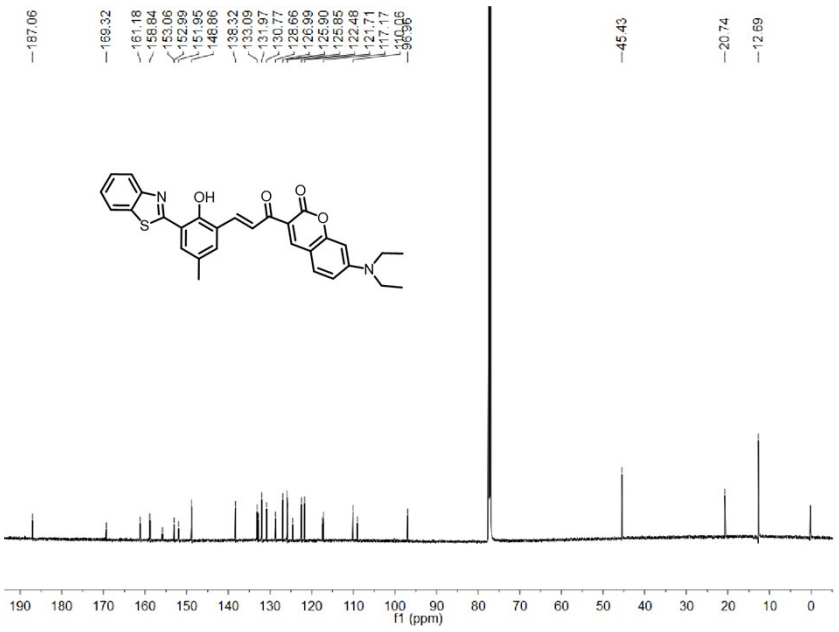
(6) Umezawa, K.; Yoshida, M.; Kamiya, M.; Yamasoba, T.; Urano, Y. *Nat. Chem.* 2017, *9*, 279-286.

(7) Morozumi, A.; Kamiya, M.; Uno, S.; Umezawa, K.; Kojima, R.; Yoshihara, T.; Tobita, S.; Urano, Y. *J. Am. Chem. Soc.* 2020, *142*, 9625-9633.

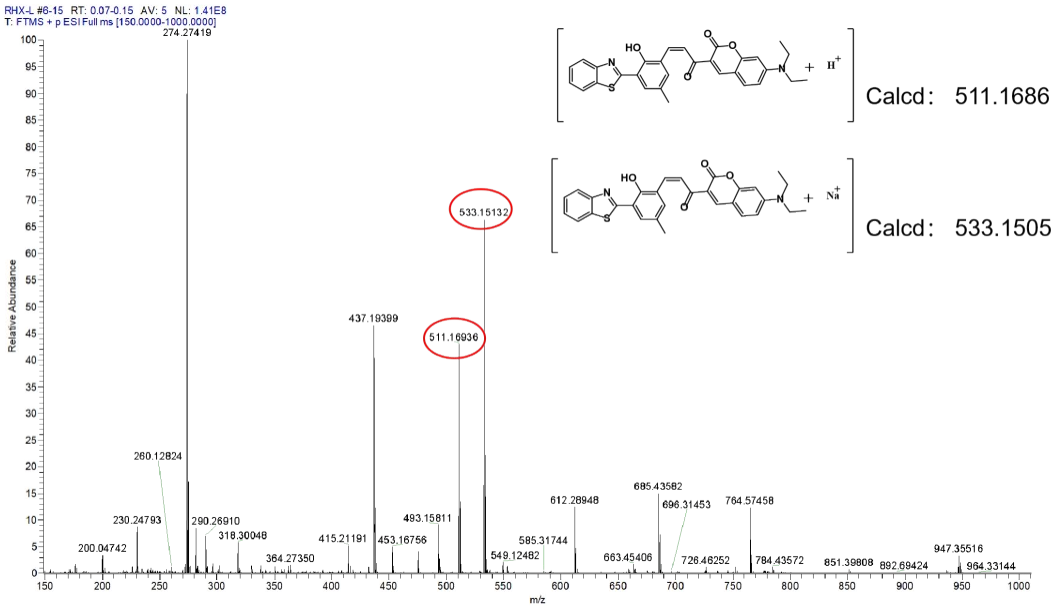
Figure S1




$^{1}$H-NMR of the probe **HBT-COU-N(Et)$_2$**




$^{13}$C-NMR of the probe **HBT-COU-N(Et)$_2$**




HR-MS of the probe **HBT-COU-N(Et)$_2$**

**Figure S2:**

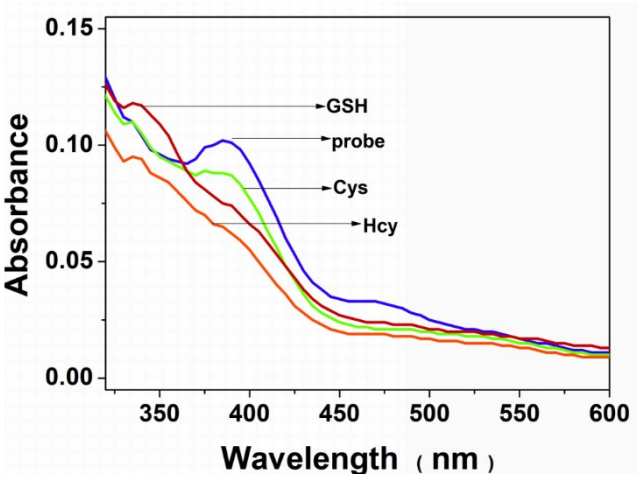



Figure S3

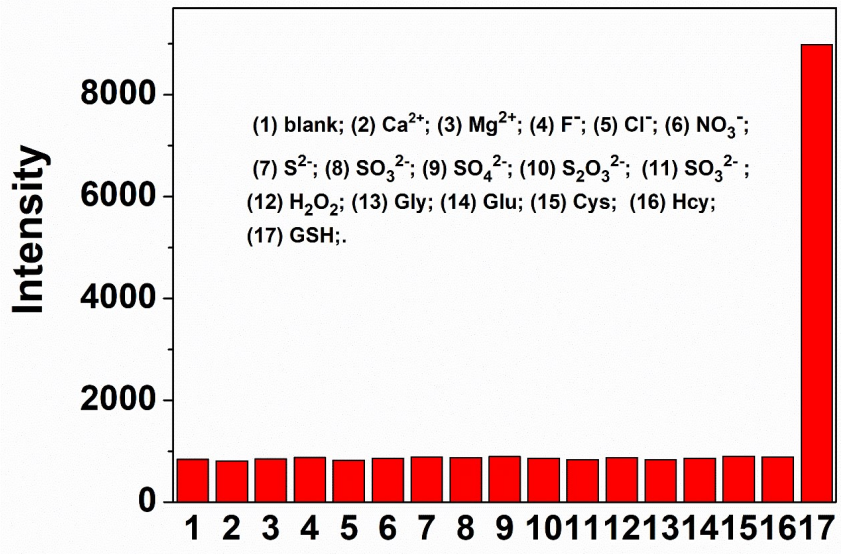


Figure S4

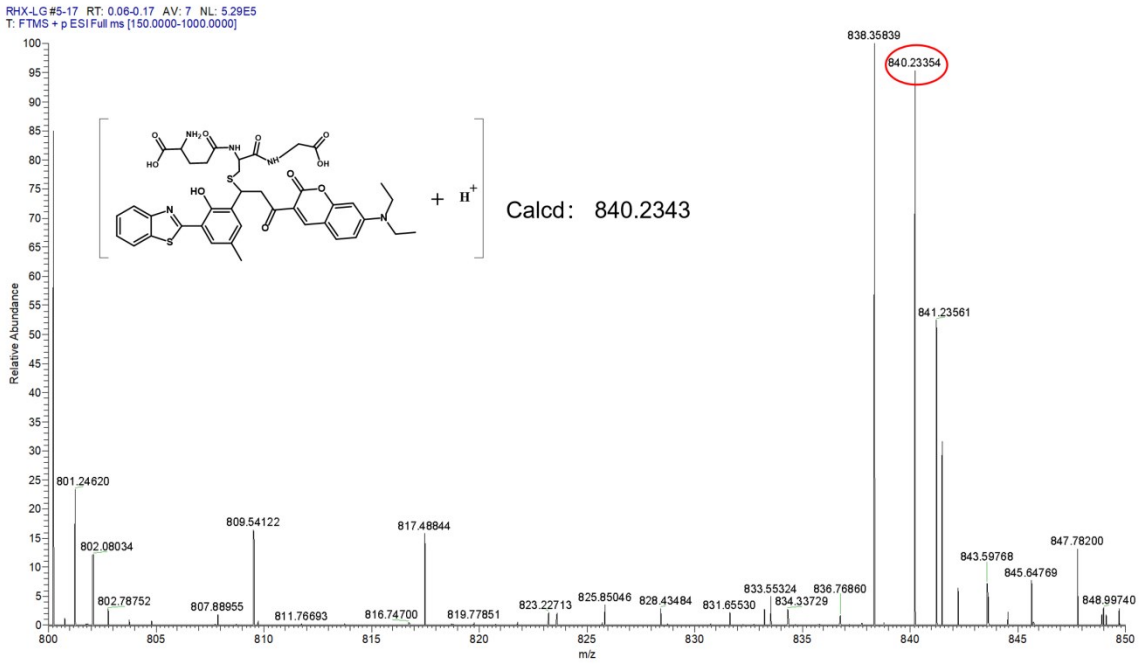



Figure S5