Supplementary Information

Proton donor modulating ESIPT-based fluorescent probes for highly sensitive and selective detection of Cu$^{2+}$

Liyan Huang, Biao Gu, Wei Su, Peng Yin, Haitao Li*

Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research (Ministry of Education), College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410081, PR China
Fig. S1. $^1$H NMR spectrum of Pi-A (500 MHz, CDCl$_3$).

Fig. S2. $^{13}$C NMR spectrum of Pi-A (126 MHz, CDCl$_3$).
**Fig. S3.** ESI-MS spectrum of Pi-A.

**Fig. S4.** $^1$H NMR spectrum of Pi-E (500 MHz, CDCl$_3$).
**Fig. S5.** $^{13}$C NMR spectrum of Pi-E (126 MHz, CDCl$_3$).

**Fig. S6.** ESI-MS spectrum of Pi-E.
Fig. S7. Absorption spectra of 10 µM Pi-A (A) and Pi-E (B) in the absence and presence of 10 µM Cu²⁺. Buffer: Tris-HCl (10 mM, pH 7.4), 2% (v/v) DMSO/water.
Fig. S8. (A) Job’s plot of Pi-A and Cu$^{2+}$ ([Pi-A] + [Cu$^{2+}$] = 10 μM) and (B) Job’s plot of Pi-E and Cu$^{2+}$ ([Pi-E] + [Cu$^{2+}$] = 10 μM). Buffer: Tris-HCl (10 mM, pH 7.4), 2% (v/v) DMSO/water.
Fig. S9. ESI-MS spectrum of the reaction products of \( \text{Pi-E} \) with \( \text{Cu}^{2+} \).

Fig. S10. ESI-MS spectrum of compound 2.
Fig. S11. Fluorescence spectra of 10 µM Pi-E (a), 10 µM Pi-E with 10 µM Cu$^{2+}$ (b) and 10 µM compound 2 (c). Conditions: (A) in DMSO/Tris-HCl (v/v = 9:1) solution, $\lambda_{ex}/\lambda_{em} = 296/389$ nm; (B) in DMSO/Tris-HCl (v/v = 1:49) solution at pH 7.4. $\lambda_{ex}/\lambda_{em} = 296/481$ nm.
Figure (A) shows the normalized intensity as a function of wavelength (nm) for different solvents: DCM (red), DMSO (black), and CH$_3$OH (blue). Figure (B) illustrates the normalized intensity for DMSO (black), CH$_3$OH (red), and DCM (blue) with similar wavelength dependence.
Fig. S12. Fluorescence spectra of 10 µM Pi-A (A) or compound 2 (B) in different solvents. Fluorescence spectra of 10 µM Pi-A (C) or compound 2 (D) in DMSO/water mixture of varying water proportions from 0 to 99%. $\lambda_{ex} = 296$ nm.
Fig. S13. Effect of pH on the fluorescence intensity of 10 µM Pi-A (A) and Pi-E (B) in the absence and presence of 10 µM Cu^{2+}. Buffer: 10 mM NaAc-HAc for pH 4.0-6.0 and 10 mM Tris–HCl buffer for pH 7.0-10.0. Conditions: for Pi-A, $\lambda_{\text{ex}}/\lambda_{\text{em}} = 296/455$ nm; for Pi-E, $\lambda_{\text{ex}}/\lambda_{\text{em}} = 296/481$ nm.
Fig. S14. Time–dependent fluorescence intensity of 10 µM Pi-A (A) and Pi-E (B) in the presence of 10 µM Cu²⁺.

Conditions: for Pi-A, λ<sub>ex</sub>/λ<sub>em</sub> = 296/455 nm; for Pi-E, λ<sub>ex</sub>/λ<sub>em</sub> = 296/481 nm. Buffer: Tris-HCl (10 mM, pH 7.4), 2% (v/v) DMSO/water. T = 25 °C.
**Fig. S15.** Fluorescence microscope images of living HeLa cells. Cells incubated with PBS (A); 10 µM Pi-A (B); 10 µM Pi-E (C) for 30 min at 37 °C. Top: bright field image, Bottom: fluorescence image.