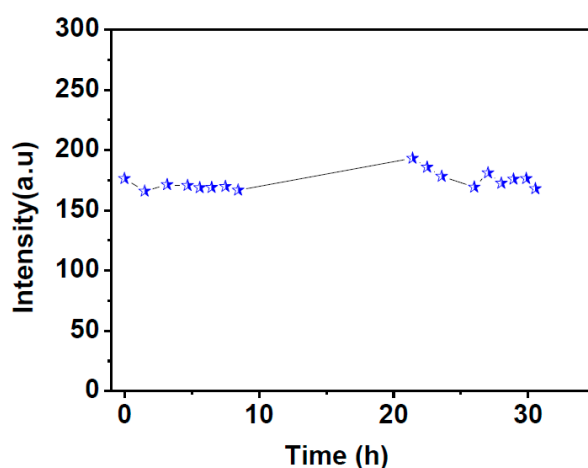
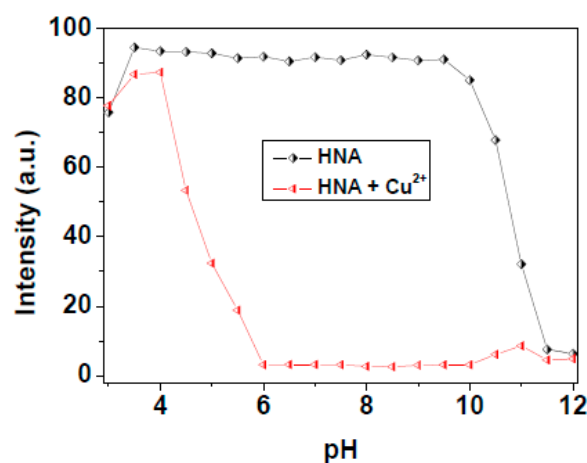


# Supplementary Materials: Synthesis and Application of an Aldazine-Based Fluorescence Chemosensor for the Sequential Detection of $\text{Cu}^{2+}$ and Biological Thiols in Aqueous Solution and Living Cells

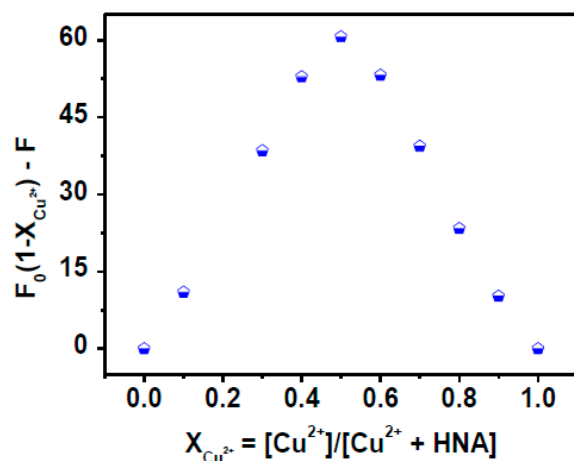
Hongmin Jia <sup>1</sup>, Ming Yang <sup>1</sup>, Qingtao Meng <sup>1,\*</sup>, Guangjie He <sup>2</sup>, Yue Wang <sup>1</sup>, Zhizhi Hu <sup>1</sup>, Run Zhang <sup>3</sup> and Zhiqiang Zhang <sup>1,\*</sup>



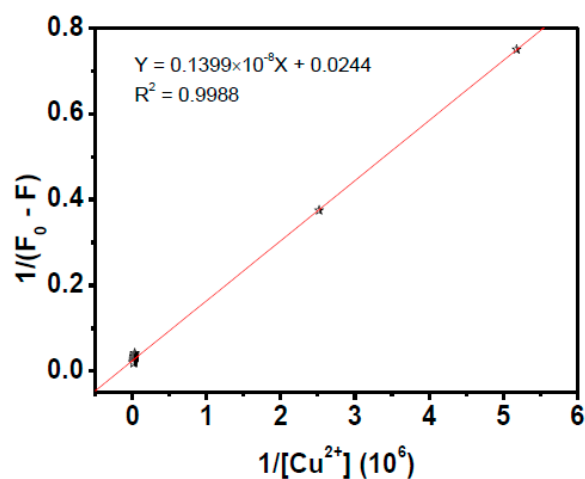
**Figure S1.** Fluorescence intensity of HNA (10  $\mu\text{M}$ ) at different time in DMF-HEPES buffer (20 mM, pH = 7.4, 3:7 v/v). The intensities were recorded at 513 nm, excitation at 411 nm.



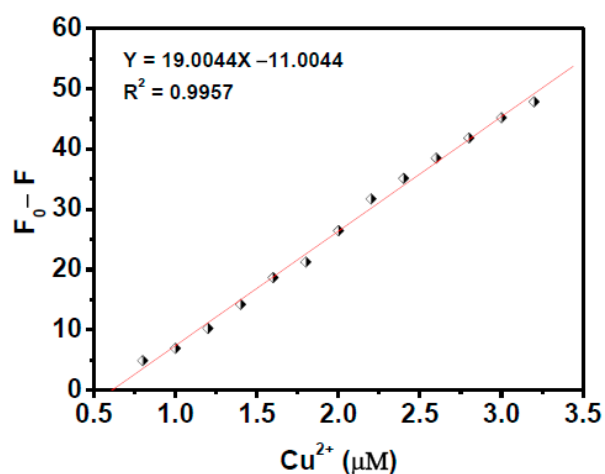
**Figure S2.** Variations of fluorescence intensity of HNA (10  $\mu\text{M}$ ) at 513 nm in aqueous solution with (bottom) and without (up)  $\text{Cu}^{2+}$  (0–20  $\mu\text{M}$ ) as a function of pH. Excitation at 411 nm.



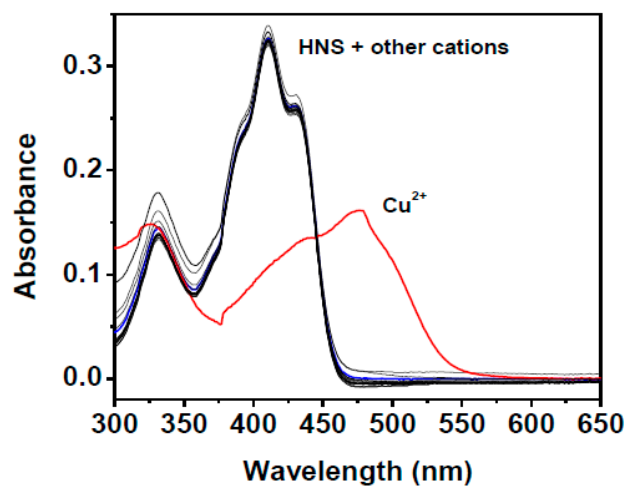
**Figure S3.** Job's plots according to the method for continuous variations. The total concentration of HNA and  $Cu^{2+}$  is 10  $\mu M$ . The intensities were recorded at 513 nm, excitation at 411 nm.



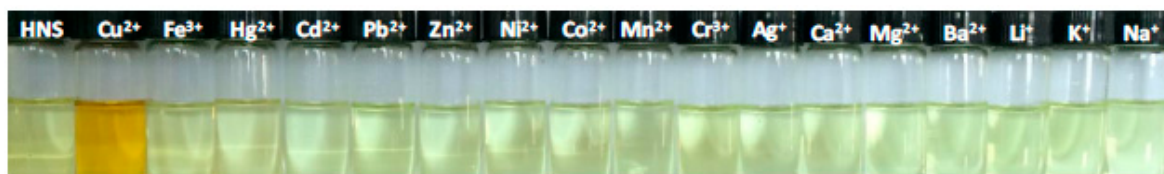
**Figure S4.** Benesi-Hildebrand plot of HNA (10  $\mu M$ ) based on 1:1 binding stoichiometry with  $Cu^{2+}$  ions. The intensities were recorded at 513 nm, excitation at 411 nm.



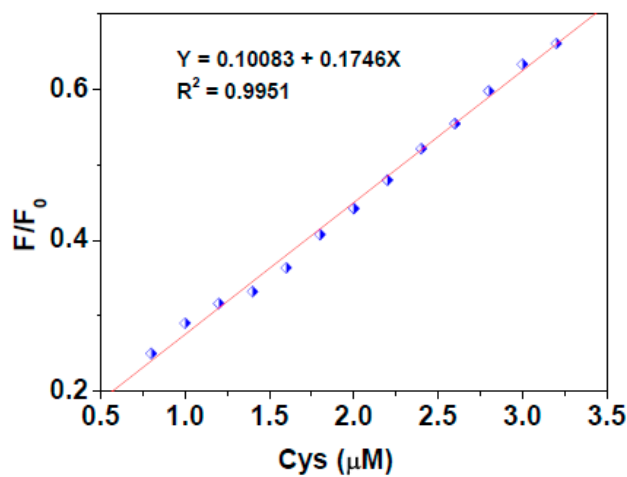
**Figure S5.** Linear relationship between fluorescence intensity of HNA (1  $\mu M$ ) at 513 nm *versus* the concentration of  $Cu^{2+}$  (0-3.5  $\mu M$ ) in DMF-HEPES buffer (20 mM, pH = 7.4, 3:7 v/v). Excitation was performed at 411 nm.



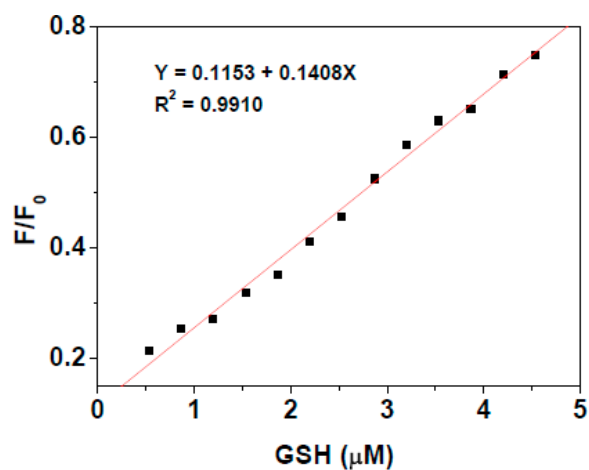
**Figure S6.** Absorption spectra of **HNA** (10  $\mu\text{M}$ ) in DMF-HEPES buffer (20 mM, pH = 7.4, 3:7 v/v) upon addition of various metal ions (30  $\mu\text{M}$ ).



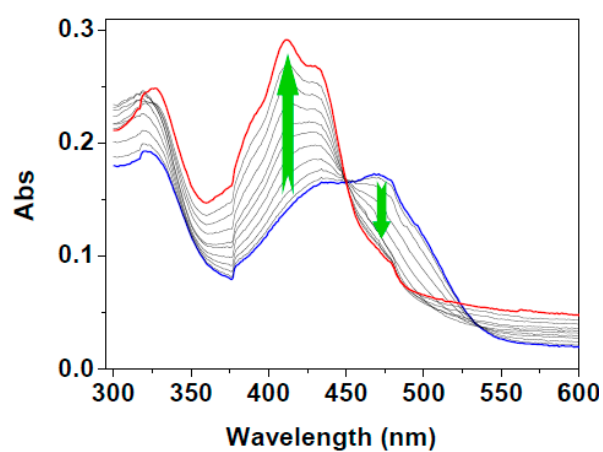
**Figure S7.** The colour changes of **HNA** (10  $\mu\text{M}$ ) in DMF-HEPES buffer (20 mM, pH = 7.4, 3:7 v/v) upon addition of various metal ions (30  $\mu\text{M}$ ).



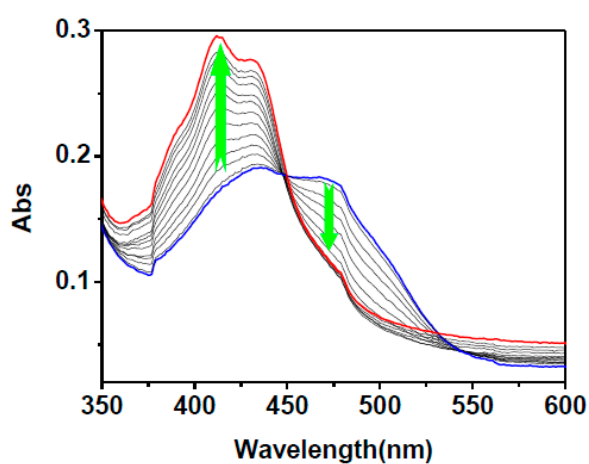
**Figure S8.** The linear fluorescence responses of **HNA-Cu<sup>2+</sup>** (3  $\mu\text{M}$ ) *versus* low concentration Cys (0–3.3  $\mu\text{M}$ ) at 513 nm. Excitation was performed at 411 nm.



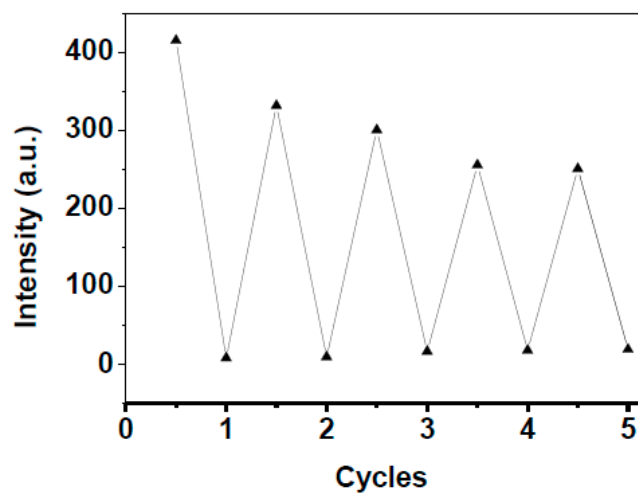
**Figure S9.** The linear fluorescence responses of HNA-Cu<sup>2+</sup> (3 μM) *versus* low concentration GSH (0–4.6 μM) at 513 nm. Excitation was performed at 411 nm.



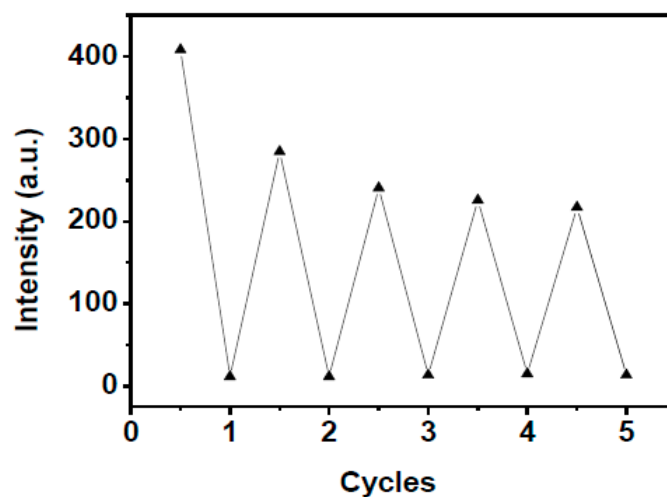
**Figure S10.** UV-Vis absorption spectra of HNA-Cu<sup>2+</sup> (10 μM) in the presence of increasing amount of GSH (40 μM) in DMF-HEPES buffer (20 mM, pH = 7.4, 3:7 v/v).



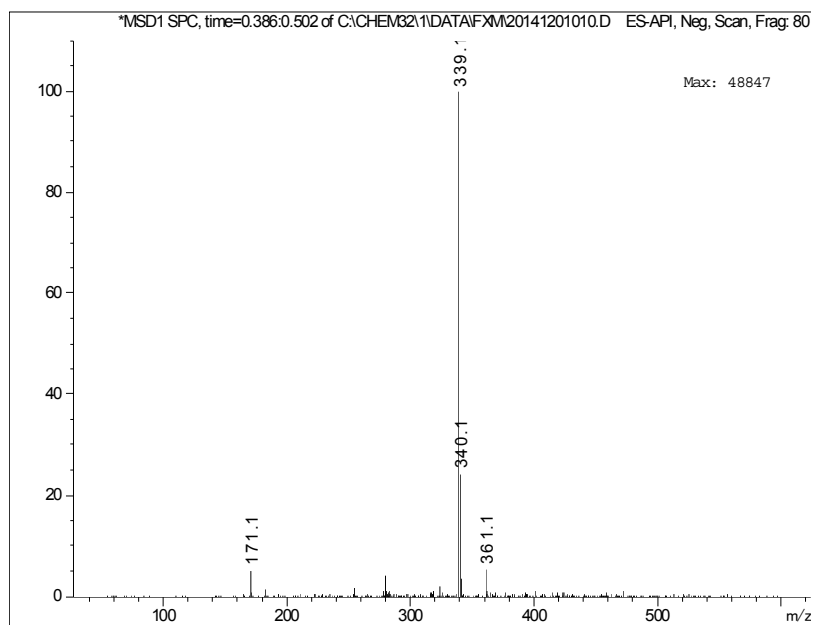
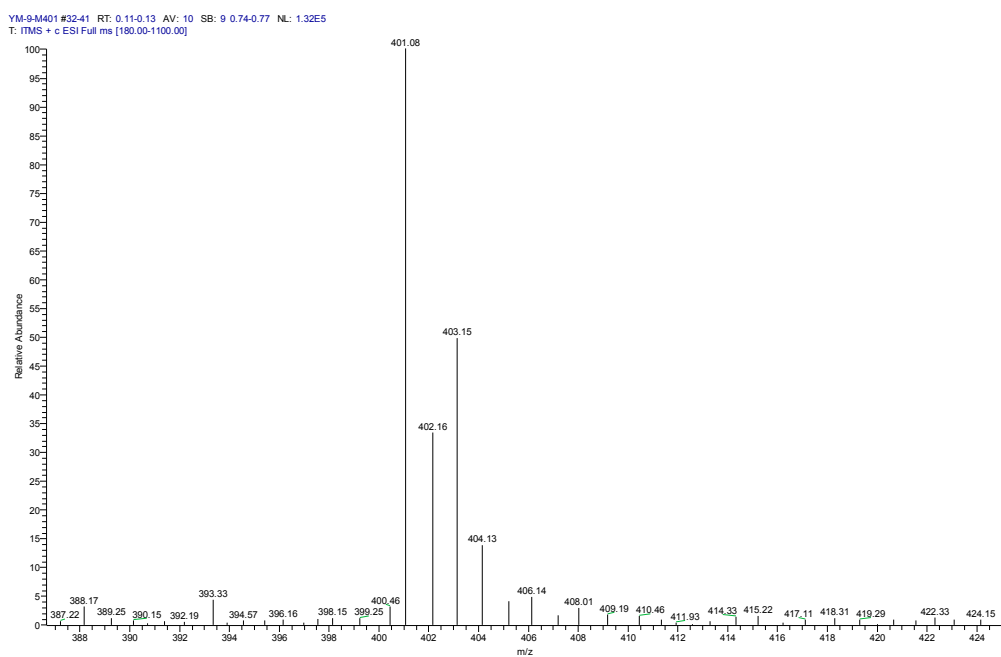
**Figure S11.** UV-Vis absorption spectra of HNA-Cu<sup>2+</sup> (10 μM) in the presence of increasing amount of Cys (40 μM) in DMF-HEPES buffer (20 mM, pH = 7.4, 3:7 v/v).

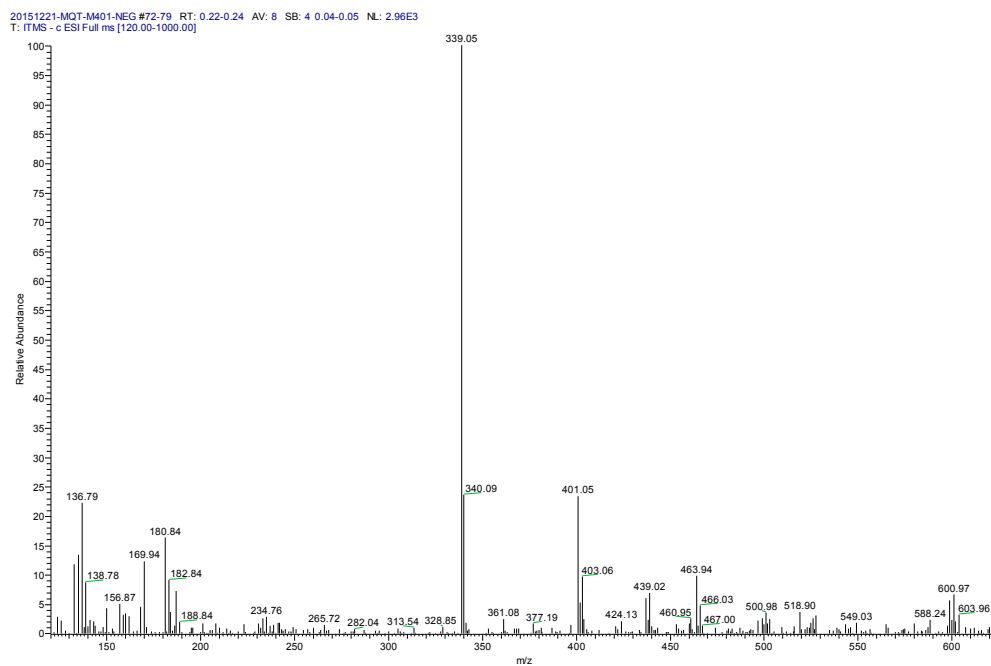


**Figure S12.** Fluorescence intensity of HNA-Cu<sup>2+</sup> (10 µM) at 513 nm in DMF-HEPES buffer (20 mM, pH = 7.4, 3:7 v/v) upon the alternate addition of Cys/Cu<sup>2+</sup> with several concentrations ratio (0:0, 20:0, 20:40, 80:40, 80:160, 160:160, 160:320, 320:320, 320:640, 640:640 µM, respectively). Excitation at 411 nm.



**Figure S13.** Fluorescence intensity of HNA-Cu<sup>2+</sup> (10 µM) at 513 nm in DMF-HEPES buffer (20 mM, pH = 7.4, 3:7 v/v) upon the alternate addition of GSH/Cu<sup>2+</sup> with several concentrations ratio (0:0, 20:0, 20:40, 80:40, 80:160, 160:160, 160:320, 320:320, 320:640, 640:640 µM, respectively). Excitation at 411 nm.

**Figure S14.** ESI-mass spectra of HNA.**Figure S15.** ESI-mass spectra of HNA-Cu<sup>2+</sup> ensemble.



**Figure S16.** ESI-mass spectra of HNA-Cu<sup>2+</sup> ensemble in the presence of Hcy.