Electronic Supporting Information (ESI)：

Homocysteine-specific fluorescence detection and quantification for evaluating S-Adenosylhomocysteine hydrolase activity

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Determination of quantum yields and fluorescence lifetime

Quantum yields and fluorescent lifetime were determined at 25 °C, Excitation was chosen at 480 nm; the emission spectra were corrected and integrated from 460 nm to 750 nm.

Kinetics constant[1]

The reaction of F_{542}-Cu^{2+} (10 μM) with 10 equivalents Hcy in Tris-HCl buffer solution (20 mM, pH 7.4) was monitored using the fluorescence spectrophotometer. Fluorescence intensity at 523 nm were recorded in the course of the reaction. The apparent rate constant of the reaction was determined by fitting the fluorescence intensities to the pseudo first-order equation:

$$\mathrm{Ln}\left((F_{max}-F_t) / F_{max}\right)= -k_t$$

Where F_t is the fluorescence intensity at 523 nm at times t, F_{max} is the fluorescence intensity at 523 nm obtained after the reaction was complete at the maximum value. k is the apparent rate constant.

Binding constant[2]

The binding constant was calculated from the emission intensity-titration curves according to the Benesi-Hildebrand plots. F_{max} is the emission intensity of probe L at 523 nm, F is the emission intensity of probe L at 523 nm upon the addition of different amount of Cu^{2+}, [Cu^{2+}] is the concentration of Cu^{2+}. The association constant values K_S is given by the ratio intercept/slope.

Determination of the detection limit

The detection limit was calculated according to the fluorescence titration curve. To determine the S/N ratio, the emission intensity of F_{542}-Cu^{2+} at 523 nm without any analyte was measured 10 times and the standard deviation of blank measurement was obtained.Three independent duplication measurements of emission intensity were made for different concentrations of Hcy and Plot the average intensity as a function of [Hcy] for determine the slope. The detection limit was calculated with the

following equation: LOD=3σ/k. Where σ is the standard deviation of blank measurement, k is the slop between the fluorescence intensity versus Hcy concentrations. The detection limit was estimated to be 116.0 nM.

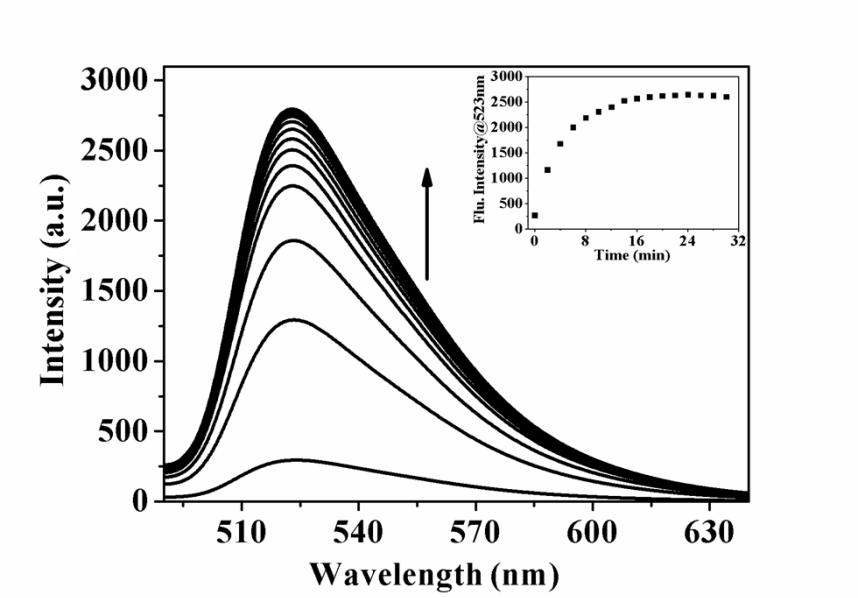


Figure S1. Time-dependent fluorescence changes of F_{542}-Cu^{2+} (10 μM) after the addition of 20 equivalents Hcy in Tris-HCl buffer solution (20 mM, pH 7.4); Inset: fluorescence intensity at 523 nm. Excitation wavelength was 480 nm.

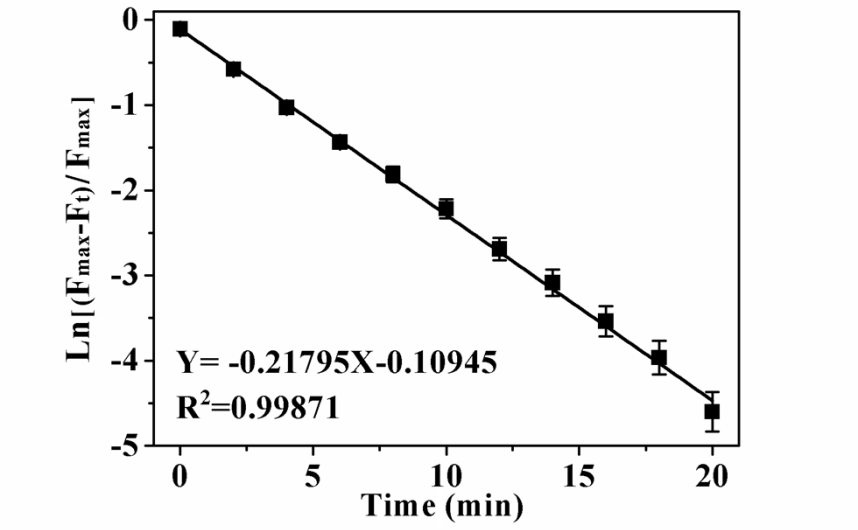


Figure. S2. Pseudo first-order kinetic plots of reaction of 10 μM F_{542}-Cu^{2+} with 20 equivalents Hcy in Tris-HCl buffer solution (20 mM, pH 7.4). Excitation wavelength was 480 nm, slit width 5.0 nm. Slope=-0.21795± 0.00298 min^{-1}.

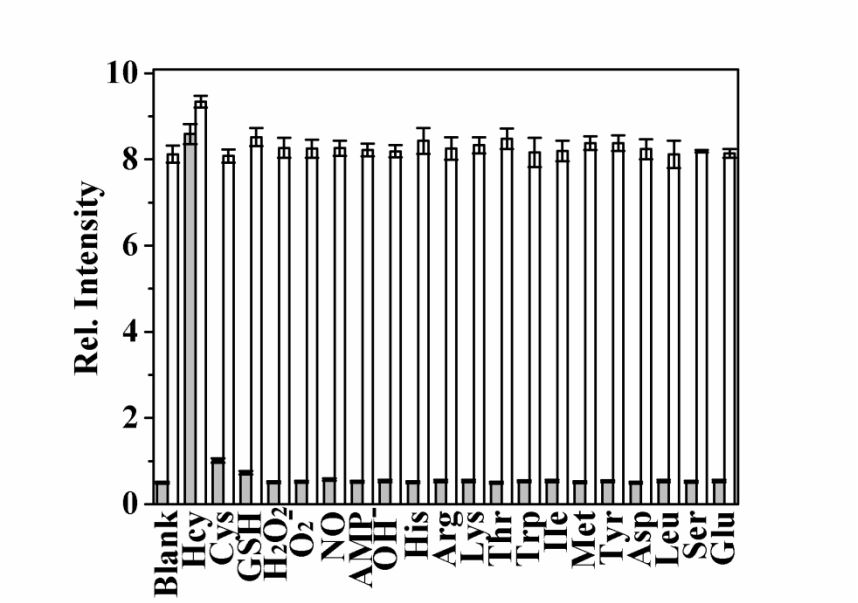


Figure S3. Competitive fluorescence responses of 10 μM the F_{542}-Cu^{2+} ensemble in the presence (white bar) of Hcy (20 equivalents) and presence (black bar) of biothiols (20 equivalents) and various substrates (100 equivalents) in Tris-HCl buffer solution (20 mM, pH 7.4). Fluorescence intensities at 523 nm were recorded 20 minutes after Hcy (20 equivalents) was added. Excitation wavelength was 480 nm. Slit width 5.0 nm. The data represents the average of three independent experiments. The error bars represent ± S.D.

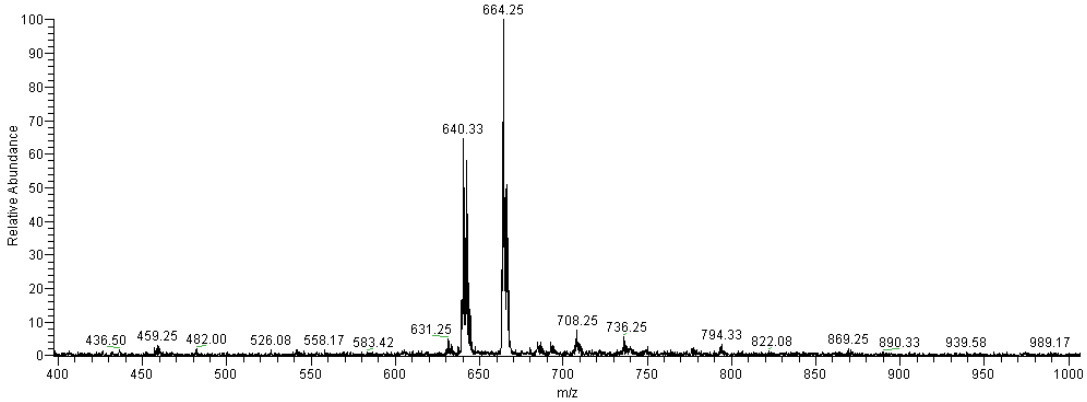


Figure S4. ESI-MS spectrum of [F_{542}+$CuCl^+$], m/z= 640.33 and [F_{542}+$CuCl^+$+Na], m/z= 664.25.

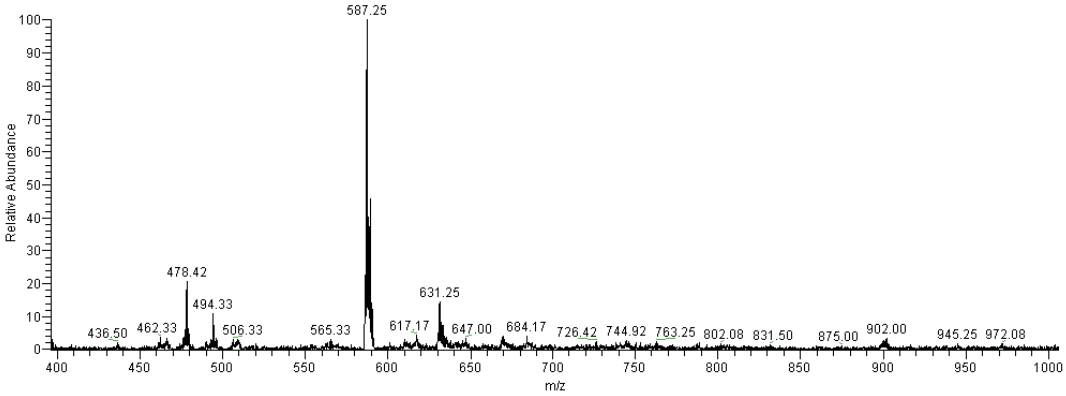


Figure. S5. ESI-MS spectrum of [F_{465}+$CuCl^{+}$], m/z= 565.33 and [F_{465}+$CuCl^{+}$+Na], m/z= 587.25.

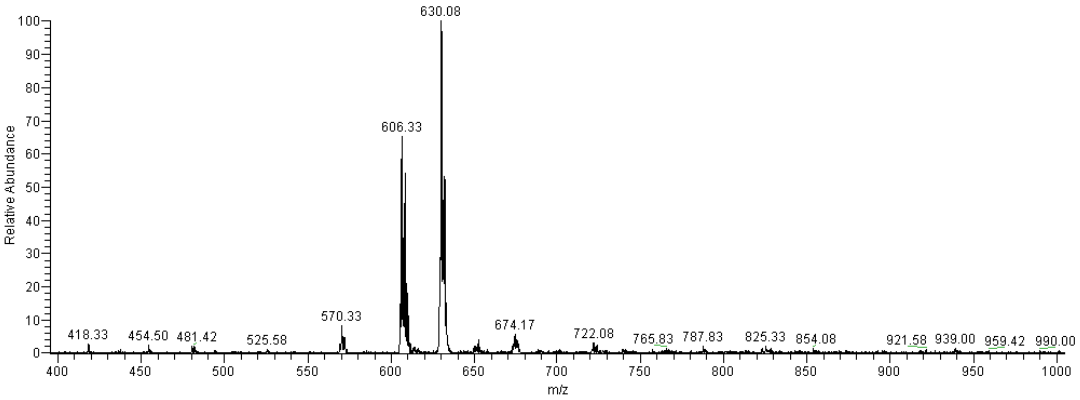


Figure S6. ESI-MS spectrum of [F_{508}+$CuCl^{+}$], m/z= 606.33 and [F_{508}+$CuCl^{+}$+Na], m/z= 630.08.

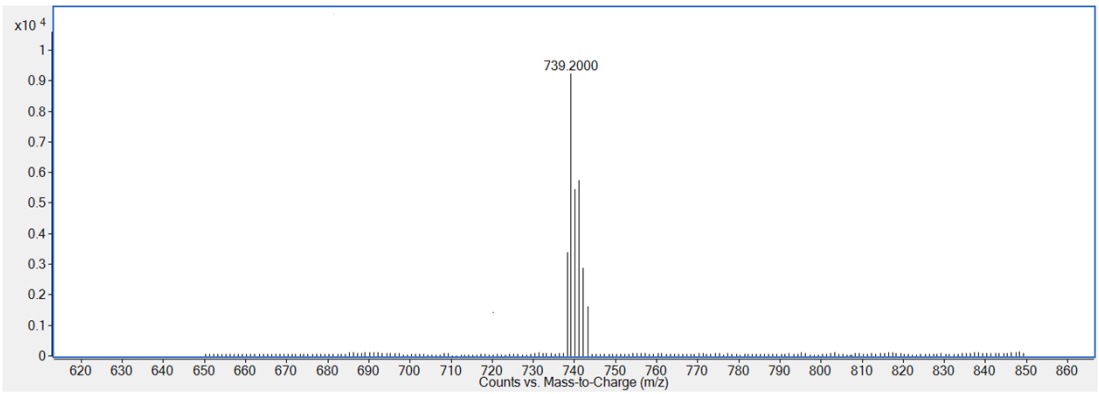


Figure S7. ESI-MS spectrum of [F_{542}+Cu^{+}+Hcy], m/z= 739.20.

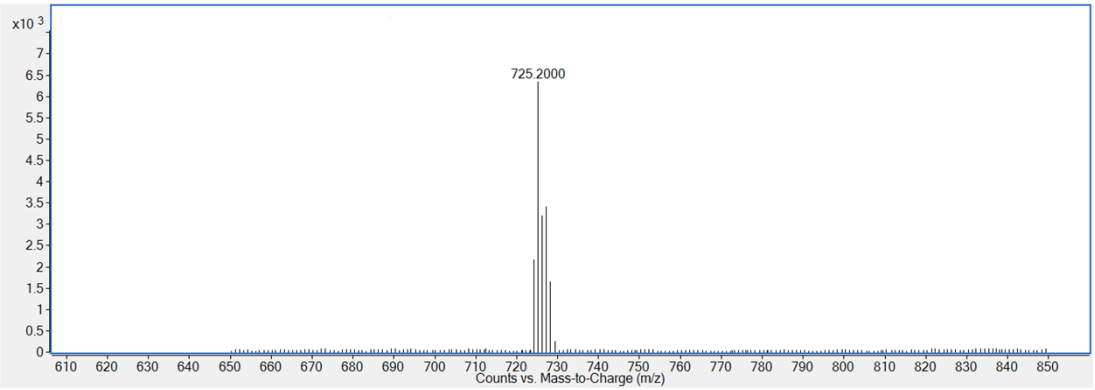


Figure S8. ESI-MS spectrum of [F_{542}+Cu^{+}+Cys], m/z= 725.20.

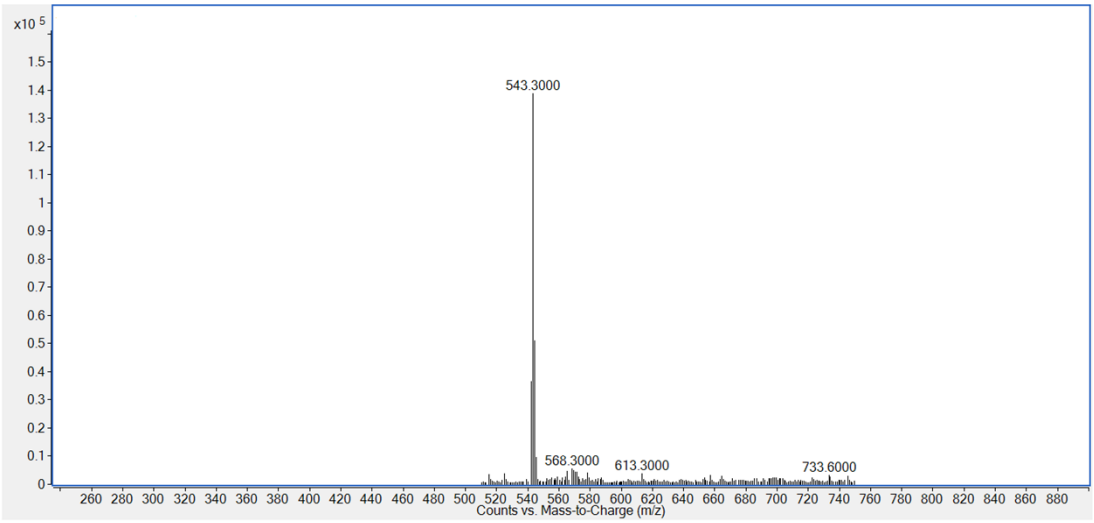


Figure S9. ESI-MS spectrum of [F_{542}+H^{+}], m/z= 543.30.

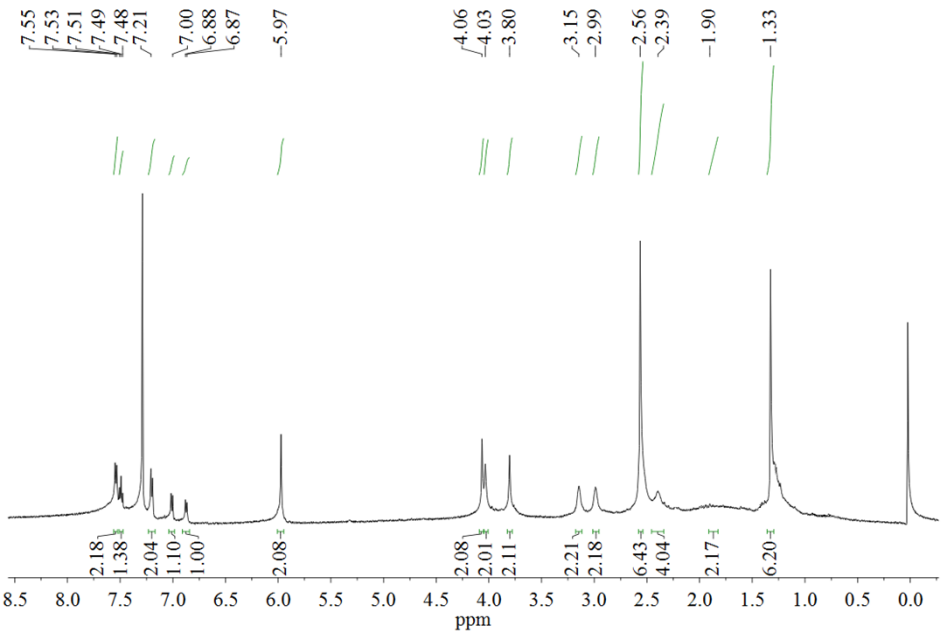


Figure S10. ^{1}H NMR spectrum of 8-[3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

(F_{542}) in chloroform.

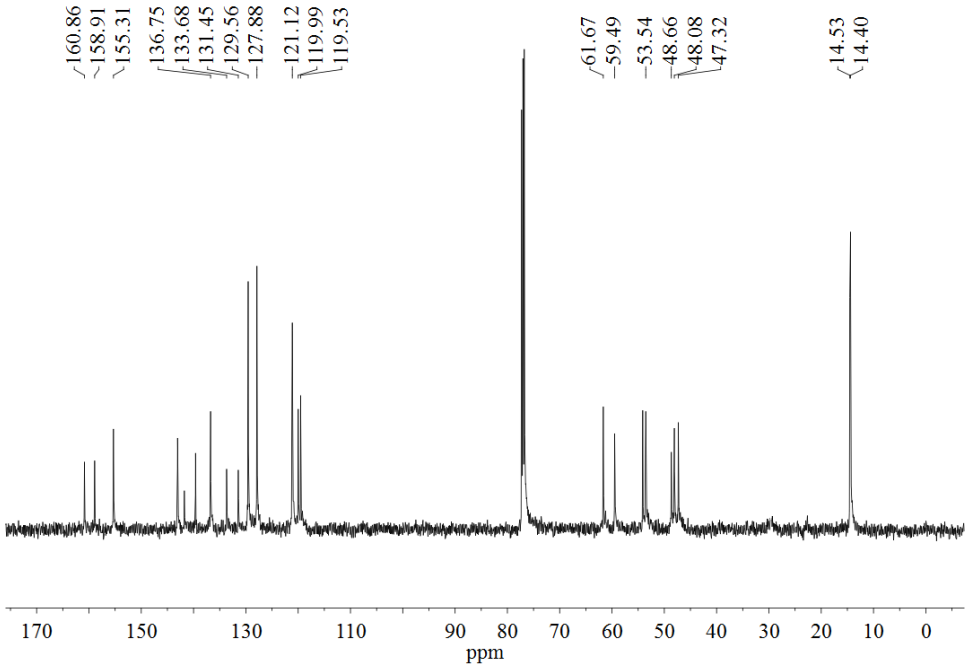


Figure S11. ^{13}C NMR spectrum of 8-[3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15), 11,13-triene]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (F_{542}) in chloroform.

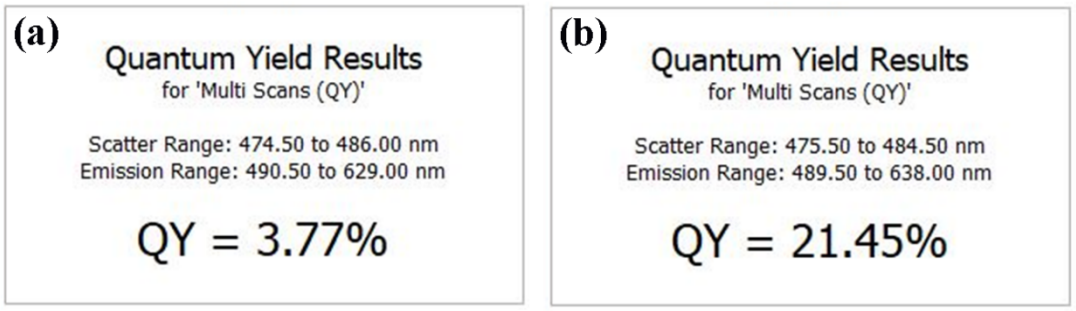


Figure S12. Fluorescence quantum yields of F_{542}-Cu^{2+} (10 μM) (a), F_{542}-Cu^{2+} (10 μM) with 20 equivalents Hcy (b) in Tris-HCl buffer solution. All measurements were taken at 25 °C.

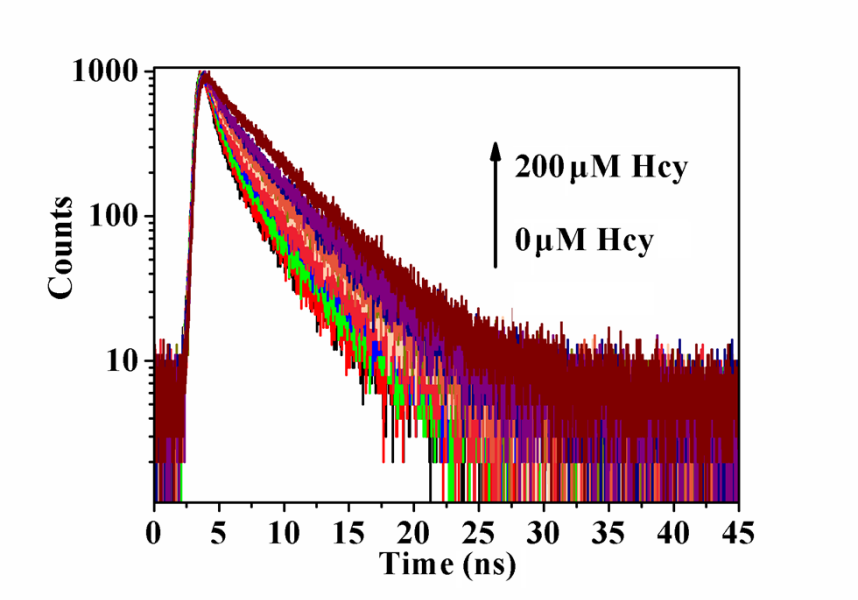


Figure S13. Fluorescence lifetime spectra of F_{542}-Cu^{2+} (10 μM), measured after adding concentrations of Hcy (0–200 μM) for 20 minutes in Tris-HCl buffer solution (20 mM, pH 7.4).

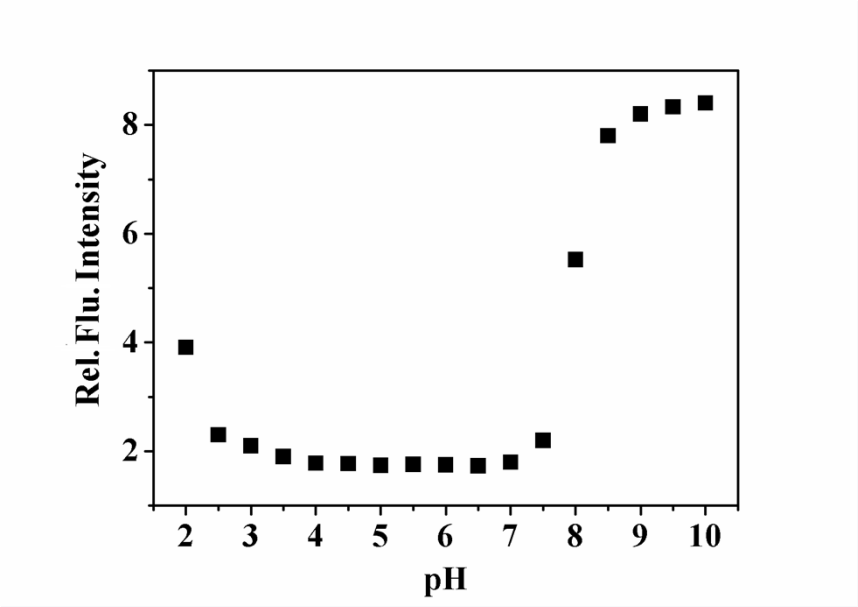


Figure S14. Relative fluorescence intensity at 523 nm of F_{542}-Cu^{2+} as a function of pH. Excitation wavelength was 480 nm. Slit width 5.0 nm. All measurements were taken at 25 °C.

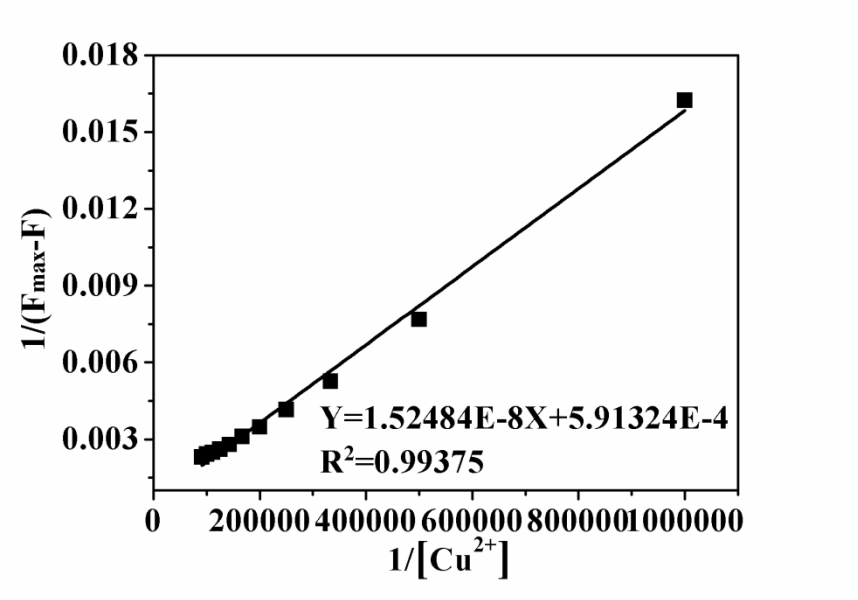


Figure S15. Fitting of fluorescence titration curve of F_{542} in Tris-HCl buffer (20 mM, pH 7.4). The association constant (Ks) is 3.68×10^4 M^{-1}.

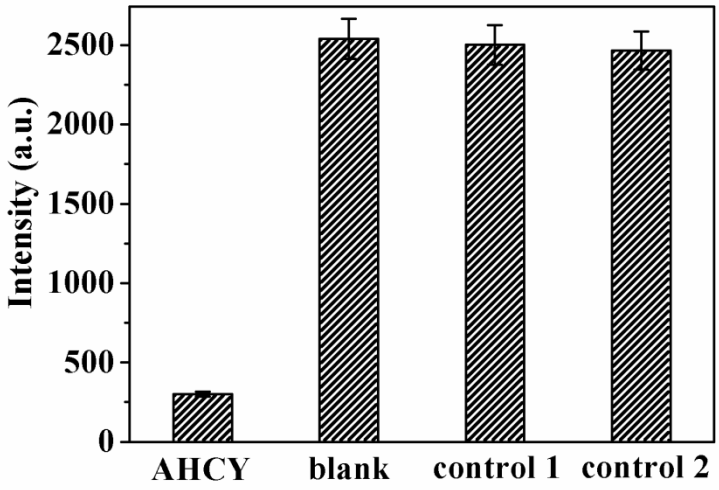


Figure S16. Fluorescence intensity of F_{542}-Cu^{2+} (10 μM) at 523 nm upon adding the resultant solutions. The solution consisted of L-homocysteine (2.5 mM) and adenosine (6 mM). Note: AHCY: adding 2 μL AHCY (2.5 mg/mL); blank: adding no AHCY; control 1: 2 μL AHCY (2.5 mg/mL) was heated in boiling water for 15 minutes and then added into the solution; control 2: 10 μl AHCY (2.5 mg/mL) was heated in boiling water for 15 minutes and then added into the solution. All the solution was incubated at 37 °C.

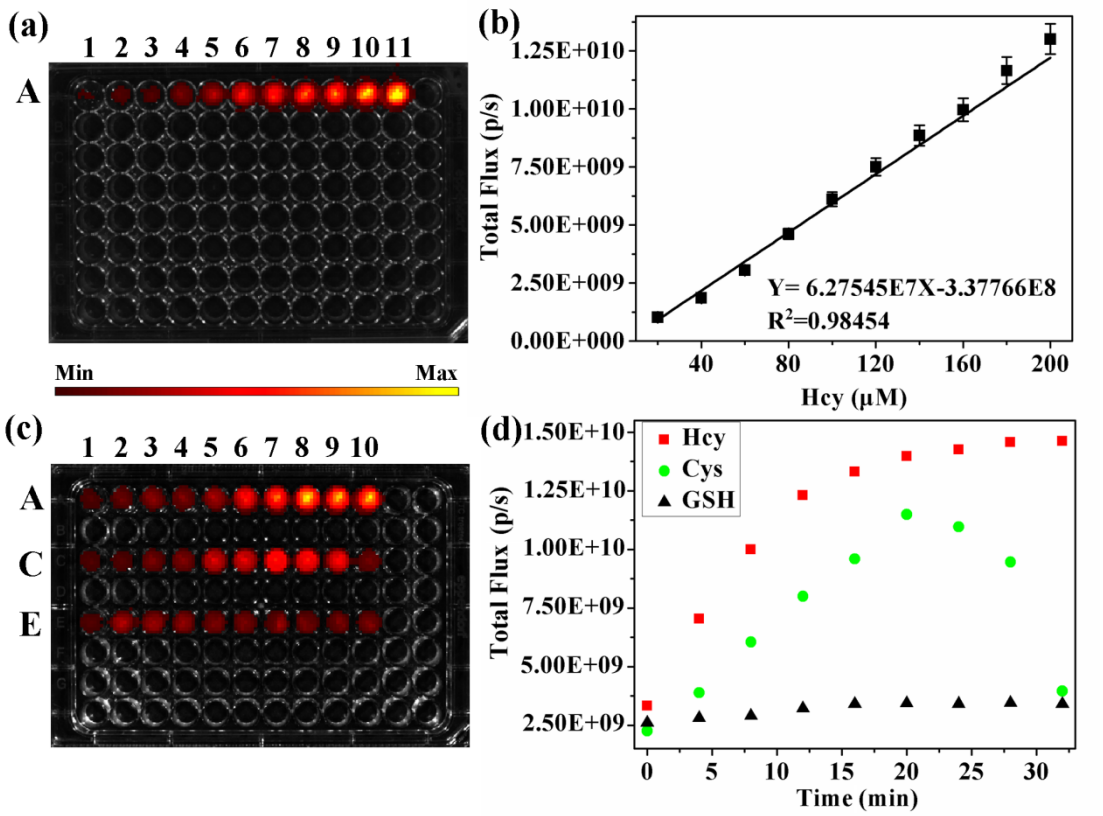
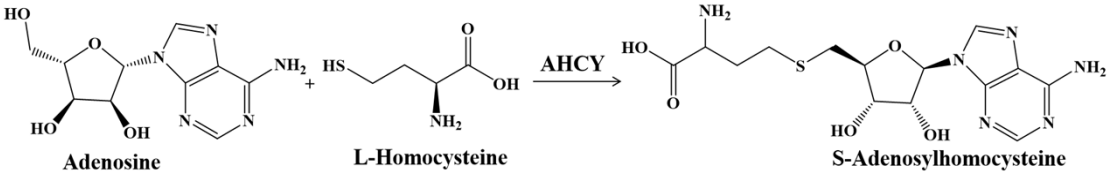
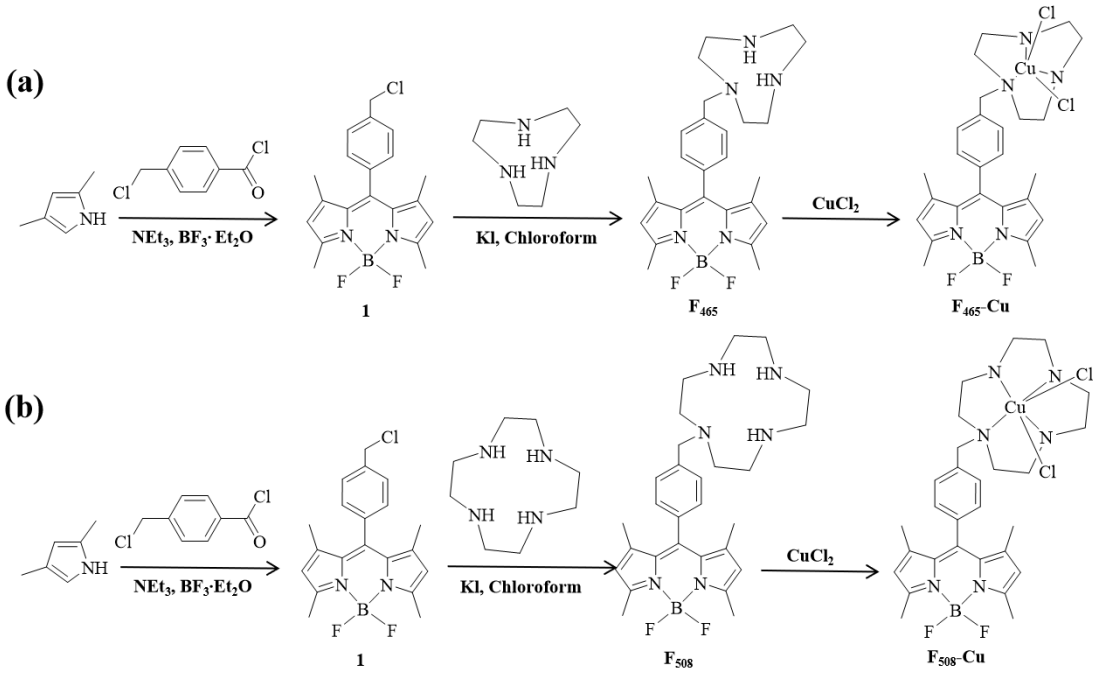


Figure S17. (a) Concentration-dependent in vitro imaging of F_{542}-Cu^{2+} (10 μM) toward Hcy (0–200 μM, from A1-A11); (b) Linear calibration curve of fluorescence intensity in F_{542}-Cu^{2+} (10 μM) solution versus concentrations of Hcy (0–200 μM); (c) Time-dependent fluorescence imaging of F_{542}-Cu^{2+} (10 μM) solution after addition of Hcy, Cys and GSH (200 μM) respectively. Note: reaction times (0–32 minutes from left to right). Hcy was A2–A10, and A1 represent blank; Cys was C2–C10, and C1 represent blank; GSH was E2–E10, and E1 represent blank; (d) Time-dependent fluorescence intensity in F_{542}-Cu^{2+} (10 μM) solution after adding 20 equivalents of Hcy, Cys and GSH respectively. Ex was 480 nm, Em was 523 nm. The data were acquired in Tris-HCl buffer solution (20 mM, pH 7.4).



Scheme S1. The route of S-Adenosylhomocysteine (SAH) forming catalyzed by S-Adenosylhomocysteine Hydrolase (AHCY).



Scheme S2. Synthetic procedure for F_{465}, F_{508}, F_{465}-Cu^{2+} and F_{508}-Cu^{2+}.

Reference

[1] X. F. Yang, Q. Huang, Y. G. Zhong, Z. Li, H. Li, M. Lowry, J. O. Escobedo and R. M. Strongin, Chem. Sci., 2014, 5, 2177–2183.

[2] Y. Fu, Q. C. Feng, X. J. Jiang, H. Xu, M. Li and S. Q. Zang, Dalton Trans., 2014, 43, 5815–5822.