Supplementary Material (ESI) for Chemical Communications

Supporting Information

Fluorescence Turn-On Probe for Homocysteine and Cysteine in Water

Kyung-Sik Lee,[a] Tae-Ki Kim,[b] Jong-Ho Lee,[c] Hae-Jo Kim,[*,b] and Jong-In Hong[*,a]

[*]To whom correspondence should be addressed.

E-mail: *jihong@snu.ac.kr, haejkim@kgu.ac.kr*

Experimental

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded using an Advance 300MHz Bruker spectrometer in chloroform-d_3, D_2O. ^{1}H NMR chemical shifts are reported as parts per million (δ) and referenced to residual solvent peak (δ 4.80 for D_2O and 7.28 for $CDCl_3$). and ^{13}C NMR chemical shifts in $CDCl_3$ were reported relative to $CHCl_3$ (77.23 ppm). UV visible spectra were recorded on a Beckman DU 800 spectrophotometer. Fluorescence spectra were recorded on a Jasco FP-6500 spectrophotometer. Analytical thin-layer chromatography was performed using Kieselgel 60_F-254 plates from Merck. Column chromatography was carried out on Merck silica gel 60 (70 - 230 mesh). All solvents and reagents were commercially available and used without further purification unless otherwise noted.

Fluorescence titration experiment

Probe **1** was dissolved in DMSO to afford a concentration of 10 mM stock solution, which was diluted with distilled water up to 10 μM. Guests were dissolved in 10 μM host (**1**) solution and used for the fluorescence titration experiment. Fluorescence titration was performed with Jasco FP-6500 spectrofluorometer at rt.

Human blood serum treatment

Venous blood specimens were collected in EDTA-treated tubes after a 12-hour fast. The tubes were immediately covered with aluminum foil and placed on ice until they arrived at the laboratory room (within 1-3 hours) for separating plasma. The tubes centrifuged at for 15minutes at 2-8℃ at 1,000×g. The separated plasmas were immediately stored at -70°C until analysis.

Fig S1. ^{1}H NMR spectra of **1** after addition of Cys. (a) **1** only (b) **1** and 2 equiv. L-cysteine in D_2O with HEPES buffer (pH=7.4). Peaks below 4.79 ppm are from those of HEPES.

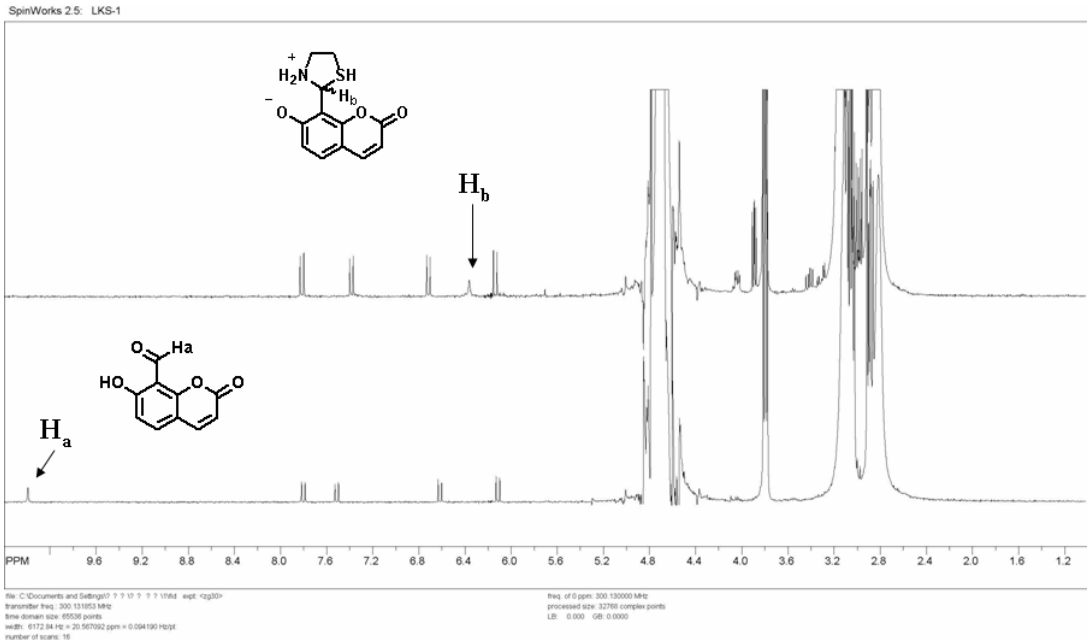


Fig S2. ^{1}H NMR spectra of **1** after addition of Cys. (a) **1** only (b) **1** and 2 equiv. D/L-homocysteine in D_2O with HEPES buffer (pH=7.4). Peaks below 4.79 ppm are from those of HEPES.

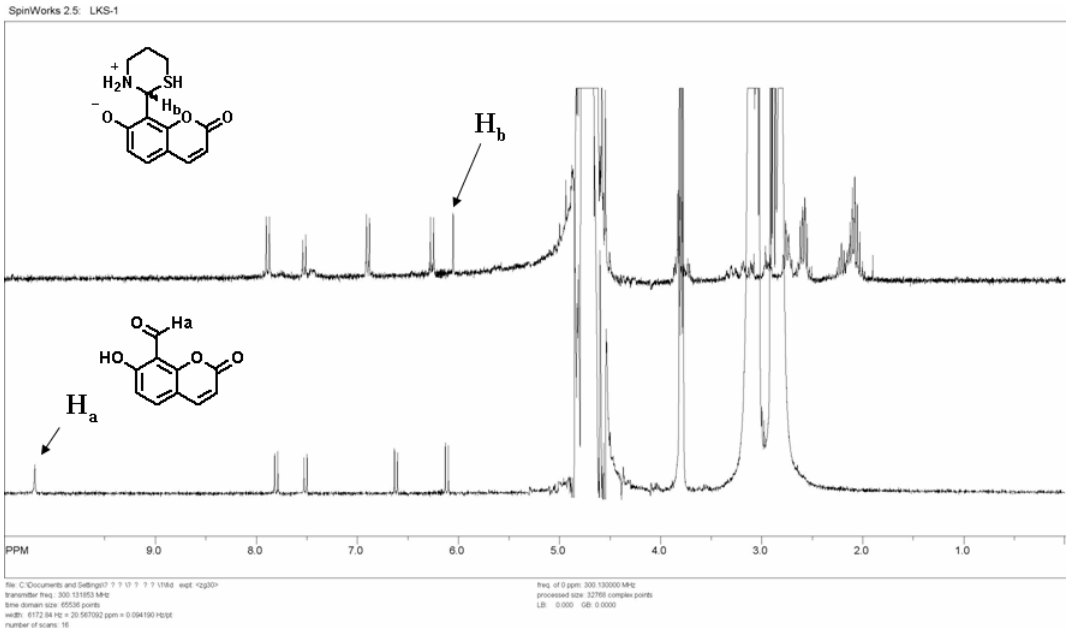


Fig S3. UV-vis absorbance and fluorescence spectra of probe **1**.

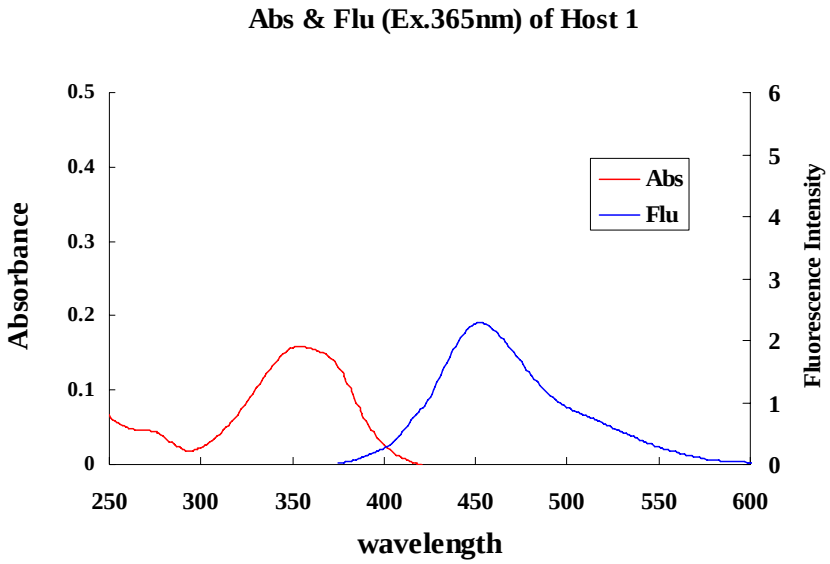


Fig S4. Fluorescence emission change of probe **1** vs D/L-homocysteine equiv at 500 nm.

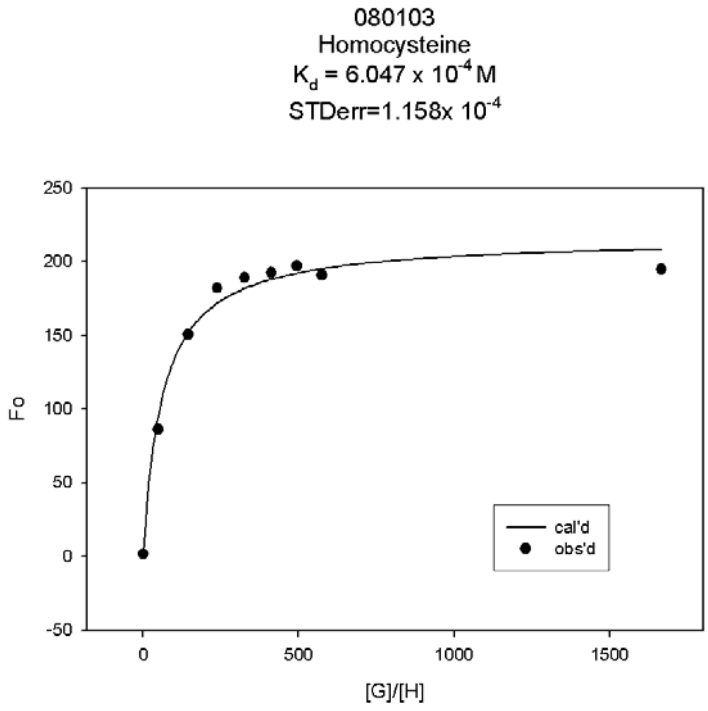


Fig S5. Fluorescence titration spectra of the probe **1** upon addition of L-cysteine. ([**1**] = 10 μM, λ_{ex} = 365 nm, λ_{em} = 450 nm)

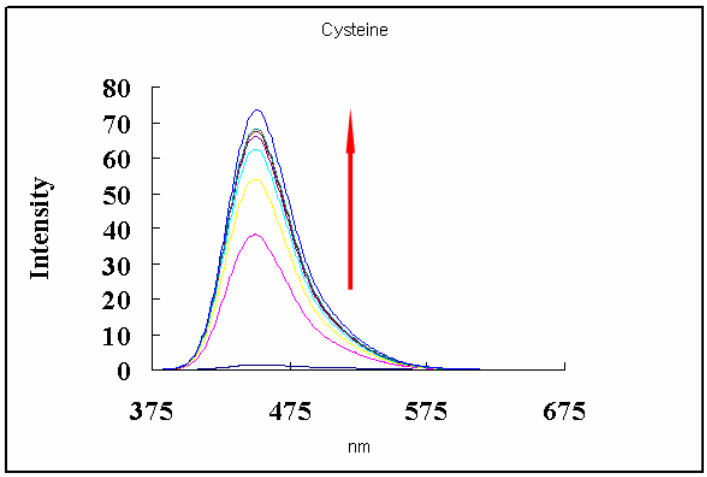


Fig S6. Fluorescence emission change of probe **1** vs L-cysteine equiv at 450 nm.

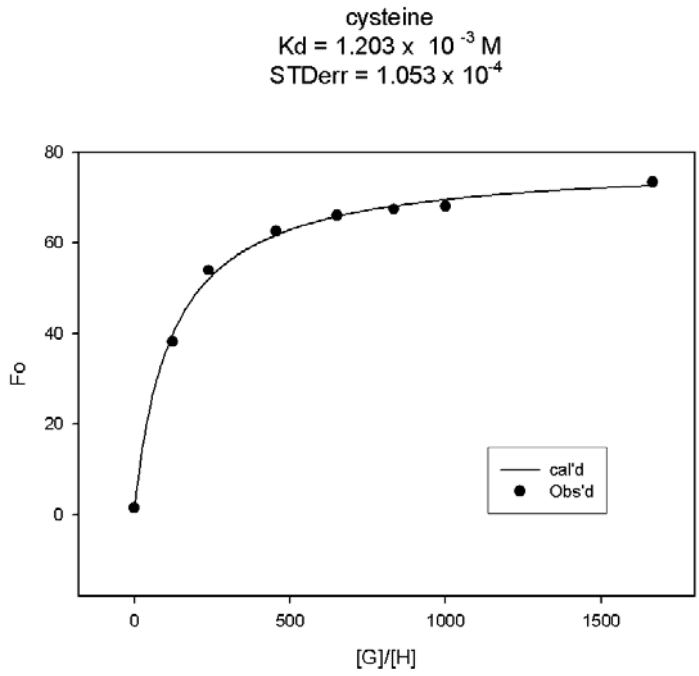


Fig S7. Homocysteine and cysteine selectivity of **1** in the presence of other amino acids and glutathione. **[1]** = 10 μM, Hcy, Cys = 500 equiv in the presence of each amino acids and GSH (500 equiv). Light blue bar upon the addition of each analyte to **1** and brown bar upon the addition of Hcy to a solution of **1** and each analyte.

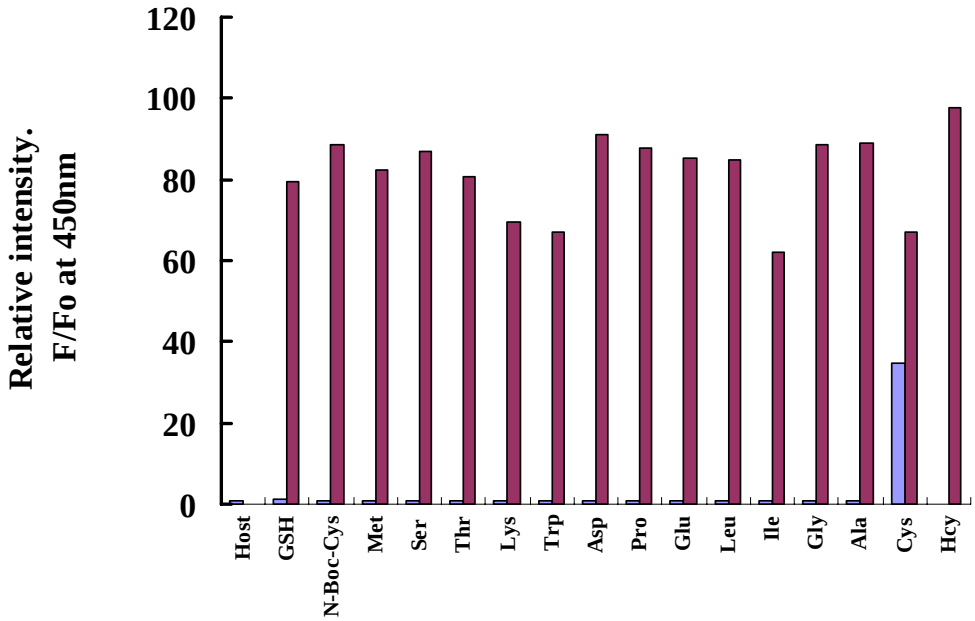
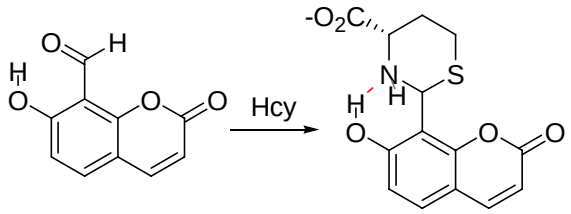
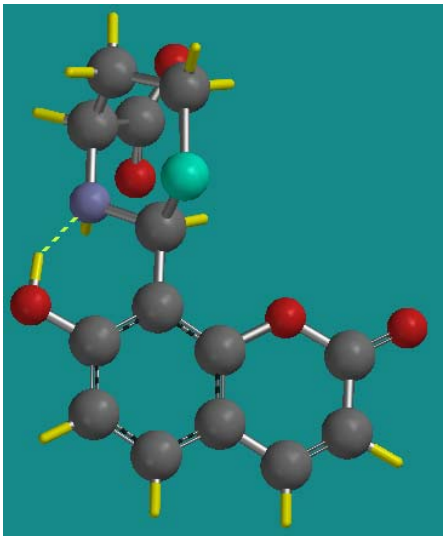


Fig S8. (a) Proposed **1**-Hcy binding mode, (b) its computational model structure.



(a)



(b)

Fig S9. The HOMO and LUMO orbitals of probe **1** and its homocysteine adduct. DFT calculation results based on B3LYP 6-31G* in Spartan 2004, Wavefunction Inc.