Electronic Supplementary Information For

Fluorescent chemodosimeter for Cys/Hcy with a Large Absorption Shift and Imaging in Living Cells

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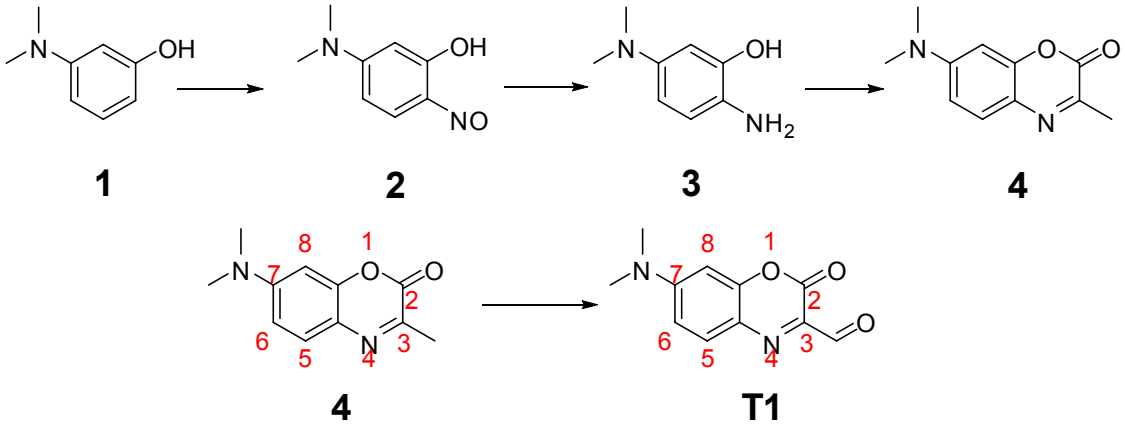
Materials and general methods:

All solvents used were of analytical grade. Analyte solutions were prepared from L-Cysteine (Cys), L-Aspartic acid (Asp), L-Glutamic acid (Glu), L-Asparagine (Asn), L-Glutamine (Gln), L-Tryptophan (Trp), L-isoleucine (Ile), L-Leucine (Leu), L-Valine (Val), L-Methionine (Met), L-Tyrosine (Tyr), L-Histidine (His), L-Lysine (Lys), L-Threonine (Thr), L-Proline(Pro), L-alanine (Ala), L-Arginine (Arg), L-Serine (Ser), L-Phenylalanine (Phe), L-Glycin (Gly), DL-Homocysteine (Hcy), reduced Glutathione (GSH), L-cystine by separately dissolved in distilled water. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a VARIAN INOVA-400 spectrometer chemical shifts reported as ppm (in $CDCl_3$ or CD_3OD, TMS as internal standard). Mass spectrometry data were obtained with a HP1100LC/MSD mass spectrometer and a LC/Q-TOF MS spectrometer. Fluorescence measurements were performed on a VAEIAN CARY Eclipse Fluorescence Spectrophotometer (Serial No. FL0812-M018) and the slit width was 5 nm for both excitation and emission. All pH measurements were made with a Model PHS-3C meter.

Synthetic procedures:



Scheme S1. Synthesis of **T1**.

Synthesis of 2

8.6 g **1** (62.72 mmol) was dissolved in 30 mL concentrated hydrochloric acid and 10 mL water, dropped with 30 mL aqueous solution of sodium nitrite 4.36 g (63.20 mmol) below 5°C, the mixture were stirred for 1 h. The yellow solid was washed with 100 mL saturated sodium acetate solution, then recrystallization with acetone to give red acicular crystal (9.2 mg, yield: 89.2%), mp. 170°C.

Synthesis of 4

Compound **3** was easily oxidized, and its fast degradation leads to highly-blue-colored product. To avoid of oxidative degradation, use **3** readily for subsequent cyclization in to **4**. Put 5 g **2** (30.11 mmol) into 30 mL absolute ethyl alcohol, dropped 20 mL 85% hydrazine hydrate, added 0.5 g palladium-carbon catalyst under nitrogen atmosphere at 30~40 °C. Refluxed until the red color of solution vanished. Then dropped 20 mL ethyl pyruvate, refluxed another 3 h. After removal of ethanol under vacuum, the residue was purified by flash chromatography with petroleum ether/ethyl acetate = 3/1 as eluent to give the yellow powder **4** (4.06 g, yield: 66.2%), mp. 124°C. ^{1}H-NMR (400 MHz, $CDCl_3$), δH (ppm): d=2.48 (s, 6H), d=3.06 (s, 6H), d=6.41 (s, 1H), d=6.67 (d, 1H, *J*=8.8Hz), d=7.49 (d, 1H, *J*=8.8Hz). ^{13}C-NMR (100 MHz, $CDCl_3$), δC(ppm): 154.57, 151.86, 148.78, 147.57, 129.14, 122.81, 109.73, 97.45, 77.20, 40.45, 20.90. Q-TOF MS：$[M+Na]^+$ 227.0796; found 227.0805.

Synthesis of T1

1.5 g **4** (7.34 mmol) and 1.0 g SeO_2 were dissolved in 25 mL dioxane, stirred at 75°C for 7 h. The solution turned from light yellow to orange color. After removal of ethanol under vacuum, the residue was purified by flash chromatography with petroleum ether/ethyl acetate = 1/2 as eluent to give the brownish-orange powder **T1** (0.88 g, yield:

54.6%).^{1}H-NMR (400 MHz, $CDCl_3$), δH (ppm): δ=3.22 (s, 6H, $N(CH_3)$), δ=6.43 (s, 1H), δ=6.79 (d, 1H,*J*=8.8Hz), δ=7.71 (d, 1H, *J*=8.8Hz), δ=10.10 (s, 1H) ^{13}C-NMR (100 MHz, $CDCl_3$), δC(ppm): 187.97, 155.61, 152.61, 151.29, 135.44, 124.62, 111.72, 96.99, 77.30, 40.82, Q-TOF MS：[M+H]+ 219.0770; found 219.0766.

Experimental procedure

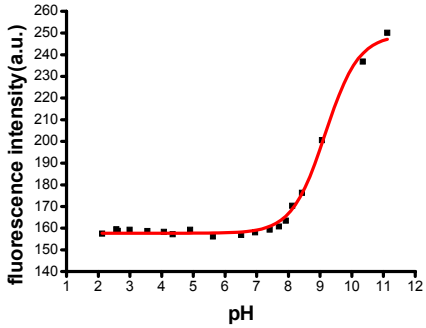
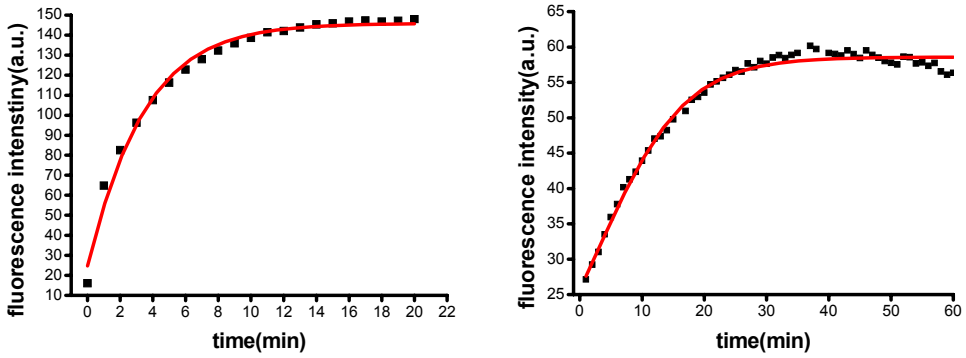


Fig. S1. Influence of pH on fluorescence at 560 nm for T1 (10 μM) in acetonitrile-HEPES buffer (20 mM, pH=7.4) solution (3:7, v/v, rt), λex = 430 nm. The pH of solution was adjusted by aqueous solution of NaOH (1 M) or HCl (1 M).



(a) (b)

Fig. S2.Time dependent fluorescence intensity changes of **T1** (10 μM) in the presence of (a) 200 equiv Cys and (b) 50 equiv Cys, Condition: acetonitrile-HEPES buffer (20 mM, pH=7.4) solution (3:7, v/v, rt), λ_{ex} = 430 nm.

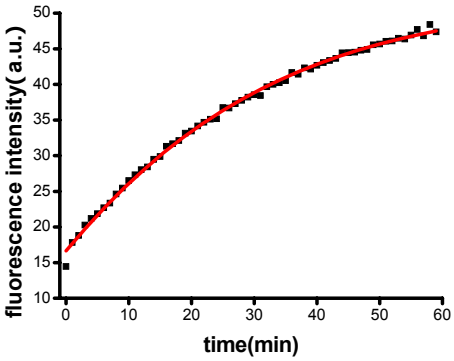


Fig. S3. Time dependent fluorescence intensity changes of **T1** (10 μM) in the presence of 50 equiv Hcy. Condition: acetonitrile-HEPES buffer (20 mM, pH=7.4) solution (3:7, v/v, rt), λ_{ex} = 430 nm.

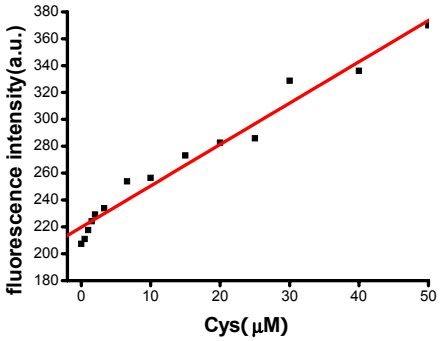


Fig. S4. Fluorescence spectral changes of **T1** (10 μM) upon addition of Cys (0-50 μM). Each spectrum was recorded after 60 min. Condition: acetonitrile-HEPES buffer (20 mM, pH=7.4) solution (3:7, v/v, rt), λex = 430 nm.

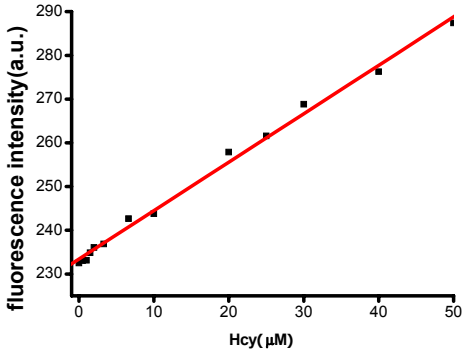
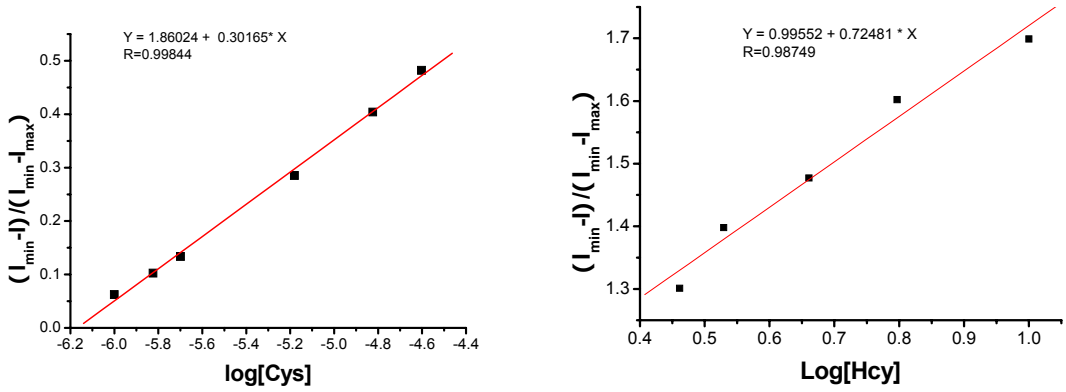


Fig. S5. Fluorescence spectral changes of **T1** (10 μM) upon addition of Hcy (0-50 μM). Each spectrum was recorded after 60 min. Condition: acetonitrile-HEPES buffer (20 mM, pH=7.4) solution (3:7, v/v, rt), λex = 430 nm.



(a) (b)

Fig. S6. Normalized response of the fluorescence signal to changing Cys concentrations (a) and Hcy concentrations (b). A linear regression curve was then fitted to these normalized fluorescence intensity data, and the point at which this line crossed the ordinate axis was considered as the detection limit 6.8×10^{-7} M for Cys and 4.2×10^{-2} M for Hcy.[1]

Fig.S7. Left: color change after addition of different analytes: blank, GSH, Cys, Hcy 500 μM separately; Right: fluorescent emission intensity after adding 500 μM Cys. Condition: acetonitrile-HEPES buffer (20 mM, pH= 7.4) solution (3:7, v/v, rt).

Fig. S8. Selective Cys/Hcy detection in human plasma without prior deproteinization. Only the Cys/Hcy-spiked sample turns (added thiol left to right: none, GSH, Hcy, Cys, 800 μM separately). Left: samples before adding trifluoroacetic acid

Detection of Cys/Hcy in Human Blood Plasma :

Three 0.5-mL aliquots of reconstituted human blood plasma are spiked with GSH, Cys and Hcy, 800 μM respectively. Each spiked sample is mixed with 0.5 mL of a

solution of **T1** in acetonitrile-HEPES buffer (20 mM, pH = 7.4) solution (3:7, v/v). The samples are added 500 μM trifluoroacetic acid separately to make protein subsided.

Cell incubation and fluorescence imaging:

Osteoblasts were seeded onto the cover slips at a concentration of 2×10^4 cells·mL^{-1} and cultured in DMEM, supplemented with 10% neonatal bovine serum in an incubator (37^oC, 5% CO_2 and 20% O_2). After 24 h, the cover slips were rinsed slightly three times with PBS to remove the media and then cultured in PBS for later use. In respect to the verification procedure, 10 μM of probes were added to above cellular samples and incubated for 20 min, then the samples were slightly rinsed 3 times with PBS and observed under an inverted and fluorescent microscope (IX70-131 Olympus, Japan) to get pictures with white light (a, c) and fluorescence (b, d), respectively, by digital color camera system (Sony 3 CCD).

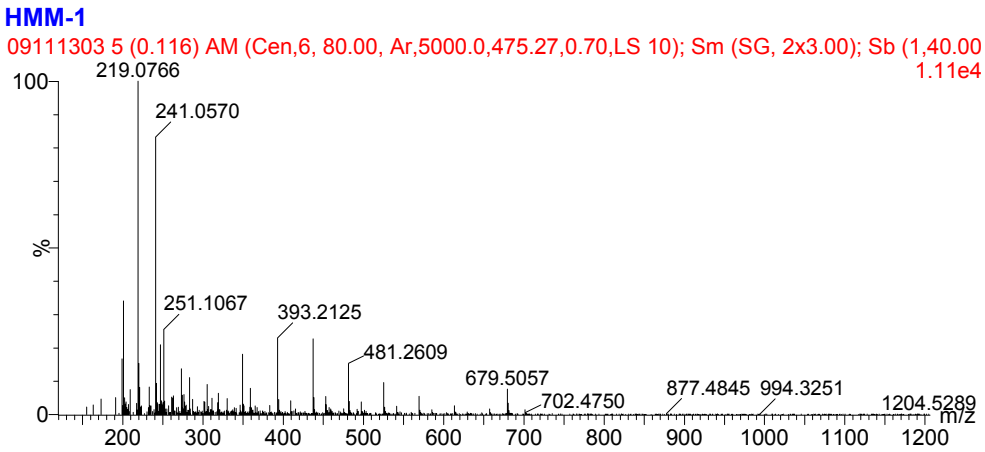


Fig. S9. TOF-MS of **T1**$[M+H]^+$219.0770; found 219.0766.

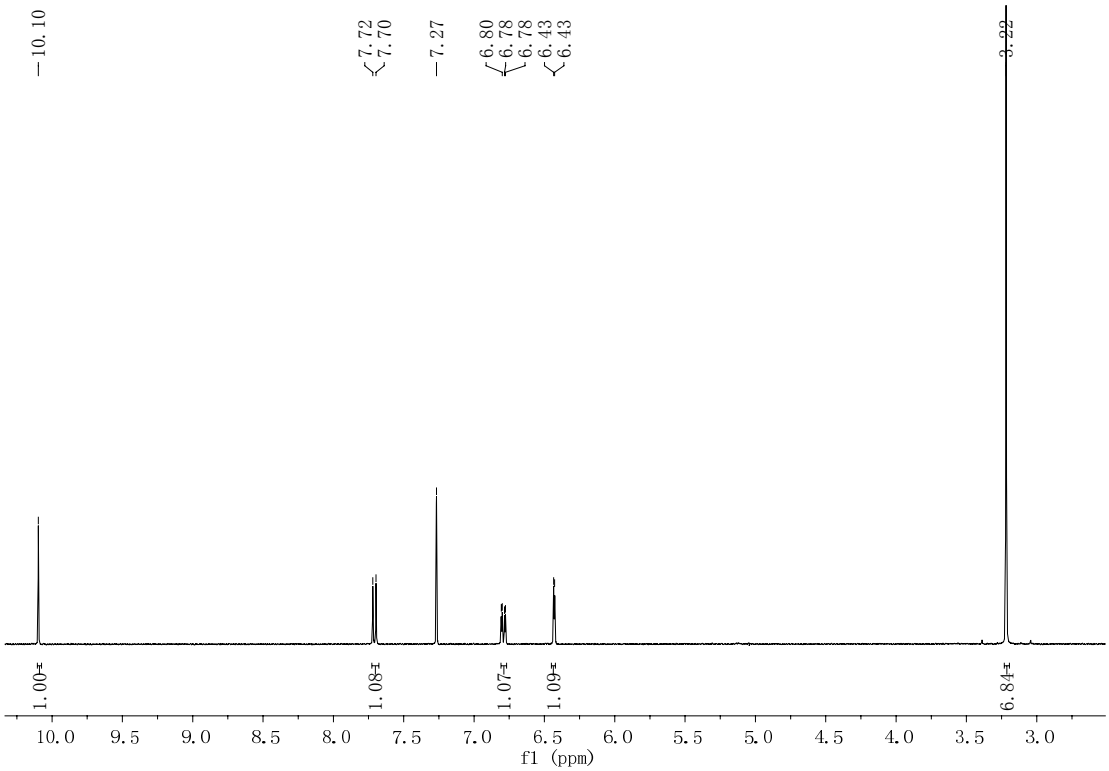


Fig. S10 ^{1}H-NMR of **T1** in $CDCl_3$

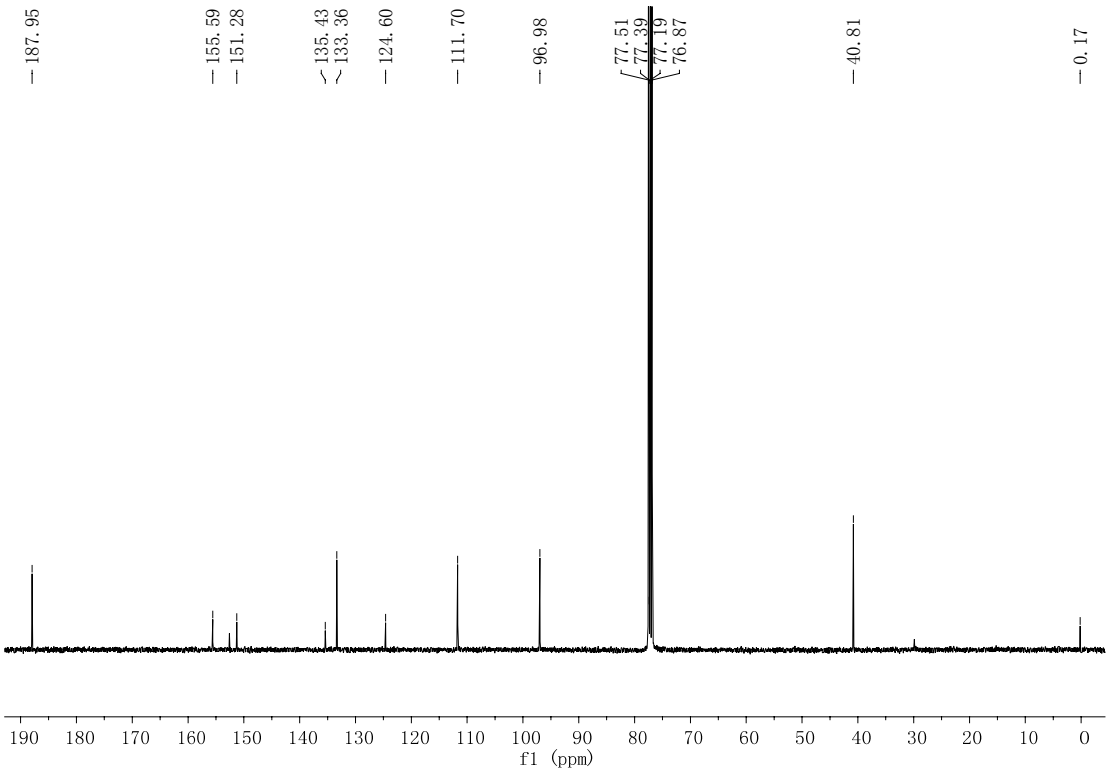


Fig. S11. ^{13}C-NMR of **T1** in $CDCl_3$

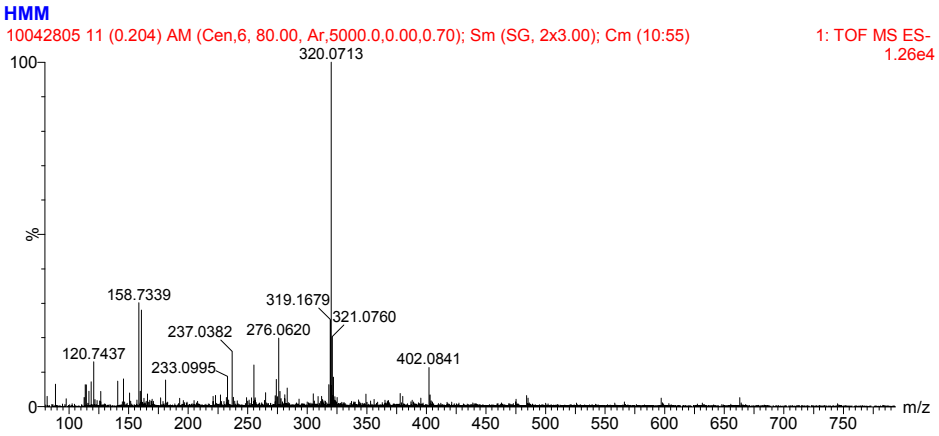


Fig. S12. TOF-MS of **T1-p,** $[M-H]^-$ 320.0705; found 320.0713.

1. M. Shortreed, R. Kopelman, M. Kuhn and B. Hoyland. *Anal. Chem*. 1996, **68**, 1414.