Supporting Information

Constructing a FRET-based molecular chemodosimeter for cysteine over homocysteine and glutathione by naphthalimide and phenazine

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Table of Contents

Instrumentation and materials ............................................................................. S3

Synthesis of PHSN ............................................................................................... S4

Characterization of Intermediates and PHSN .................................................. S4

Excitation spectra of PHSN when treated with cysteine .......................... S11

Absorption spectra of PHSN when treated with cysteine ....................... S12

Emission spectra of PHSN when treated with Hcy and GSH ............ S12

Comparison of $^1$H NMR spectra of PHSN and compound 4 and 6 .... S13

$^1$H NMR titration spectra of PHSN with MPA .................................................. S14

Emission and excitation titration spectra of PHSN when treated with

cysteine .................................................................................................................. S14

Excitation spectra of PHSN when treated with various amino acids

............................................................................................................................... S15

Supplementary data of Cell imaging ................................................................. S16
Instrumentation and materials

$^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AM 400MHz spectrometer with tetramethysilane (TMS) as internal reference. Absorption spectra were measured on a Varian Cary 500 UV-Vis spectrophotometer. Fluorescence spectra were measured on a Cary Eclipse Fluorescence spectrometer. Electrospray ionization and time-of-flight analyzer (ESI-TOF) mass spectra were recorded with a Waters Micromass LCT mass spectrometer. Matrix assisted laser desorption ionization and time-of-flight analyzer (MALDI-TOF) were recorded by an Applied Biosystems 4700 Proteomics Analyzer.

$N$, $N$-dimethylformamide (DMF) was refluxed with calcium hydride and distilled before use. All other reagents and reactant including phenazine were purchased as commercial products from Aldrich and used as received without further purification. All synthetic reactions were conducted under protection of Argon shield.

Absorbance and fluorescence spectra were measured in HEPES buffer which is 20 mM with 50% EtOH (v/v) at 37.4 °C.

Selectivity Measurements

The changes in the absorption and fluorescence spectra caused by Cys, Hcy, GSH and various other amino acids including Ala, Arg, Asp, Glu, Gly, Leu, Met, Pro, Ser, Thr and Try in water solutions were recorded.

Cell imaging

Hela cells were cultured at 37 °C, in 5 % CO$_2$ air condition and maintained one day before imaging by the confocal laser scanning microscopy. The cells were plated on 14 mm glass coverslips and incubated with PHSN for 40 min then thiols for 60 min respectively, at 37 °C in PBS (pH = 7.4).

Synthesis of PHSN
Scheme S1 The synthesis procedures of PHSN: (i) EtOH, 70 °C, overnight; (ii) trifluoroacetic acid, room temperature, 10 min; (iii) acetonitrile, piperidine, cyanoacetic acid, 12 h; (iv) DMF, EDC, DMAP, NHS, ice bath to rt, 6 h; (v) DMF, Et3N, 0 °C to rt, 4h.

Characterization of PHSN

Compound 3: 1H in CDCl3 and 13C NMR in DMSO-d6 spectrum, high-res ESI-TOF mass spectrum
## Elemental Composition Report

### Single Mass Analysis

- **Tolerance:** 30.0 mDa / DBE: min = -1.5, max = 100.0
- **Element prediction:** Off
- **Number of isotope peaks used for i-FIT:** 2

**Monoisotopic Mass, Even Electron Ions**

| 177 formula(s) evaluated with 11 results within limits (up to 1 best isotopic matches for each mass) |
| Elements Used: |

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**JL-HUAN**

| ECUST institute of Fine Chem |
| 10-Sep-2014 |
| 10-19-54 |
| 1: TOF MS ES+ |
| 6.05e+003 |

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**Minimum:** -1.5

**Maximum:** 0.0 50.0 100.0

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S5
Compound 4: $^1$H and $^{13}$C NMR spectrum in DMSO-$d_6$, high-res ESI-TOF mass spectrum
Compound 6: $^1$H and $^{13}$C NMR spectrum in DMSO-$d_6$, ESI-TOF mass spectrum
Single Mass Analysis
Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
156 formula(e) evaluated with 11 results within limits (up to 1 closest result for each mass)
Elements Used:
C: 0-30  H: 0-60  N: 0-5  O: 0-5

HJL-YL-220-2 17 (0.640) Cm (17.35)  ECUST institute of Fine Chem

Minimum: 30.0  Max: 100.0

Mass  Calc. Mass  mDa  PPM  DBE  i-FIT  i-FIT (Norm) Formula
416.1960  416.1974  0.6  1.4  14.5  7.5  0.0  C25 H26 N3 O3
Compound 7: $^1$H NMR in CDCl$_3$, $^{13}$C NMR spectrum in DMSO-$d^6$, ESI-TOF mass spectrum
PHSN: $^1$H NMR in DMSO-$d^6$, $^{13}$C NMR in CDCl$_3$ and MALDI-TOF mass spectrum
Excitation spectra of PHSN when treated with cysteine

Figure S1. Left: Excitation spectra of PHSN with 40.0 equivalents of cysteine at 37 °C. Right: the corresponding dot plot of intensity at 400 nm against reaction minute. PHSN (5 μM) was in HEPES buffer (20 mM, 50% EtOH, pH 7.4). Cysteine was dissolved in distilled water and added by microsyringe.

Absorption spectra of PHSN when treated with cysteine

Figure S2. Unchanged absorption spectra of PHSN when treated with 40.0 equivalents of cysteine at 37 °C.
Emission spectra of PHSN when treated with Hcy and GSH

**Figure S3.** Time-dependent fluorescence emission spectra of PHSN (5 μM) toward Hcy (a) and GSH (b), respectively, from 0 – 60 min in HEPES buffer (50% EtOH v/v, pH 7.4) at 37.4 °C.

Comparison of $^1$H NMR spectra of PHSN, compounds 4 and 6

**Figure S4.** $^1$H NMR spectra of compound 4 (above), compound 6 (below) and PHSN (middle) from 9.0 to 6.0 ppm.
$^1$H NMR titration spectra of PHSN with MPA

Figure S5. $^1$H NMR spectra of PHSN with MPA for 30 min and 1.5 h, the spectra was shown from 13.0 to 6.0 ppm. The small peaks marked by stars showed the appearance of carboxyl proton.

Emission and excitation titration spectra of PHSN when treated with 0-0.6 eq. cysteine
Figure S6. Emission spectra (a) and excitation spectra (b) of PHSN (5 μM) reacted with 0 – 0.6 eq Cys in HEPES buffer (50% EtOH v/v, pH 7.4) at 37.4 °C. Each spectrum was recorded after 1 h of titration; c) excitation corresponding scattered plot of intensity at 400 nm against Cys equivalents.

Excitation spectra of PHSN when treated with various amino acids

Figure S7. Left: Fluorescence excitation spectra of PHSN (5 μM) against 40 equivalents of Cys, Hcy, GSH and various other amino acids in HEPES buffer (50% EtOH v/v, pH 7.4) at 37.4 °C. Right: Corresponding histogram demonstrated the selectivity of PHSN for Cys over other amino acids.
Cell imaging

Figure S8. Confocal fluorescence Z-scan images of Hela cells in two fluorescence channels (Green Channel: $\lambda_{ex} = 488.0$ nm, $\lambda_{em} = 525-575$ nm; Red channel: $\lambda_{ex} = 561$ nm, $\lambda_{em} = 600-700$ nm). Hela cells were incubated with PHSN (10$^{-5}$ M) for 40 min then added and incubated with Cys (about 10$^{-5}$ M) for another 60 min.

Figure S9. Confocal laser scan fluorescence ratio images of Hela cells incubated with PHSN (10 $\mu$M) in PBS buffer pH 7.4 at 37.4 °C for 40 min then added and incubated with cysteine (10 $\mu$M), homocysteine (10 $\mu$M) or glutathione (10 $\mu$M) respectively for another 60 min. The colorful bar indicated the ratio scale of Green / Red from 0 to 2.0 for PHSN + Cys and 0 to 0.2 for PHSN + Hcy and PHSN + GSH. Green channel ($\lambda_{ex} = 488$ nm, $\lambda_{em} = 525-575$ nm), red channel ($\lambda_{ex} = 561$ nm, $\lambda_{em} = 600-700$ nm).