Electronic Supplementary Information (ESI)

A Merocyanine-based Colorimetric and Fluorescent Probe for Specifically Distinguishing Cysteine from Biothiols in Water and Imaging Application in Living Cells

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Experimental

1. Synthesis

1.1 (E)-2-(4-hydroxystyryl)-1,3,3-trimethyl-3H-indolium iodide (compound 2) was synthesized according to the literature procedure\[^1\].

\[^{1}\]H NMR (400 MHz, d\(_6\)-DMSO) \(\delta = 1.784\) (s, 6H), 4.102 (s, 3H), 6.967 (d, \(J = 8.8\) Hz, 2H), 7.475 (d, \(J = 16.4\) Hz, 1H), 7.561–7.638 (m, 2H), 7.843–7.865 (m, 2H), 8.150 (d, \(J = 8.4\) Hz, 2H), 8.375 (d, \(J = 16\) Hz, 1H), 10.815 (s, 1H). \(^{13}\)C NMR (d\(_6\)-DMSO, 100 MHz) \(\delta = 25.1, 33.6, 51.2, 108.8, 114.5, 115.9, 122.3, 125.5, 128.2, 128.3, 133.1, 141.3, 142.7, 153.2, 162.7, 180.9\). ESI-MS \(m/z\) [(M-I\(^+\)]: 278.2.

1.2 Synthesis of (E)-2-(4-acryloyloxystyryl) -1,3,3-trimethyl-3H-indolium iodide (probe 3).

To a solution of 2 (132 mg, 0.325 mmol) in 2 mL of DMF, and then added 25 ml of CH\(_2\)Cl\(_2\). After added anhydrous K\(_2\)CO\(_3\) (45 mg, 325 mmol) and acryloyl chloride (0.2 ml, mixed with 5 mL of CH\(_2\)Cl\(_2\)) the reaction mixture was stirred at 0 °C and warmed to rt overnight. After removal of the insoluble materials by filtration, the filtrate was evaporated. The crude product was purified by chromatography (SiO\(_2\), CH\(_2\)Cl\(_2\)/MeOH 20:1, v/v) to afford 3 as a orangered solid (106 mg, 75%).

\(^{1}\)H NMR (400 MHz, d\(_6\)-DMSO) \(\delta = 1.809\) (s, 6H), 4.181 (s, 3H), 6.224 (d, \(J = 10.4\) Hz, 1H), 6.457 (dd, \(J = 17.2\) Hz, \(J = 10.0\) Hz, 1H), 6.598 (d, \(J = 17.2\) Hz, 1H), 7.459 (d, \(J = 8.4\) Hz, 2H), 7.561–7.638 (m, 2H), 7.711 (d, \(J = 16.4\) Hz, 1H), 7.889–7.940 (m, 2H), 8.321 (d, \(J = 8.4\) Hz, 1H), 8.842 (d, \(J = 16.4\) Hz, 1H). \(^{13}\)C NMR (d\(_6\)-DMSO, 100 MHz) \(\delta = 24.7, 34.2, 51.8, 112.9, 114.8, 122.1, 122.2, 122.4, 126.8, 128.4, 129.0, 133.8, 141.3, 143.1, 151.2, 153.2, 163.2, 181.3\). ESI-MS \(m/z\) [(M-I\(^+\)]: 332.3.
2. Limit of detection in analysis

The limit of detection, expressed as the concentration \( c_L \), or the quantity \( q_L \), is derived from the smallest measure \( x_L \), that can be detected with reasonable certainty for a given analytical procedure. The value of \( x_L \) is given by the equation:

\[
x_L = \bar{x}_{bi} + ks_{bi}
\]

Where, \( \bar{x}_{bi} \) is the mean of the blank measures, \( s_{bi} \) is the standard deviation of the blank measures, and \( k \) is a numerical factor chosen according to the confidence level desired.

\[
c_L = \left( x_L - \bar{x}_{bi} \right) / m
\]

Where, \( m \) is the slope of the linear regression equation. Combine two of the equations, Long and Winefordner defined \( c_L \):

\[
c_L = (k \cdot s_{bi}) / m
\]

Generally, \( k=3, \ P < 0.01 \), we obtained \( c_L = 0.5 \mu M \).


Single-crystal X-ray diffraction measurements were carried out on a Bruker APEX-II CCD diffractometer operating at 50 KV and 30 mA using Mo K\(\alpha \) radiation (\( \lambda = 0.71073\text{Å} \)). Data collection and reduction were performed using SMART and SAINT software.\(^2\) An empirical absorption correction was applied using the SADABS program.\(^3\) The structure was solved by direct methods and refined by full-matrix least-squares on \( F^2 \) using the SHELXL-97 program package.\(^4\)

Crystal data and details of the structure determination for compound 2 are summarized in Table S1. CCDC 970856 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi.

<table>
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<th>Formula</th>
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<td>( \text{Pnma} )</td>
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<td>( a ) (Å)</td>
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Table 1. Crystal Data and Details of the Structure Determination for compound 2.

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<td>(b) (Å)</td>
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<td>(c) (Å)</td>
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<td>(\alpha) (°)</td>
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<td>(\gamma) (°)</td>
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<td>(Z)</td>
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<td>(R) indices (all data)</td>
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<td>Goodness-of-fit on (F^2)</td>
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4. Distribution Coefficients of Probe 3 in Octanol/Water

The retention measurements by HPLC were made with a Waters 1525 (Breeze™ HPLC system) equipped with a C\(_{18}\) reversed phase HPLC column (75 mm \(\times\) 4.6 mm Symmetry\(^R\) C\(_{18}\) 3.5 \(\mu\)m) and UV detector (Waters 2998 PDA) set at 384 nm. An injector with 5 \(\mu\)L sample loop was used for the upper and the lower phases sample injections. The mobile phase was CH\(_3\)CN 50\% (v/v) in distilled water and the flow rate was 0.5 mL/min.

Partition coefficients of the probe 3 in n-octanol/water system (log \(P_{o/w}\)) were determined by
a conventional shake-flask method. Briefly, probe 3 (10 mg) was dissolved in octanol saturated distilled water (5 ml) and mixed with water saturated n-octanol (10 ml). The mixture was shaken on 3D shaking-table overnight. The absorbances of the upper and the lower phases showed on any other compound. In order to determine the concentration, the two phases were carefully separated. The Fig. S23-24 shows that the retention time of probe 3 in the water and octanol are appropriate and consistent with each other, peak shape is normal, shows that this method is applicable to the fat water distribution coefficient of determination of the sample. All values correspond to the mean of 3 measurements.

References


Fig. S1. Time-dependent fluorescence spectral and UV-vis spectral changes of probe 3 (10 μM) with Hcy (10 equiv) or GSH (10 equiv) in phosphate buffer (10 mM, pH 7.40). (a) Fluorescence spectral of Hcy (λex = 418 nm). Inset: Fluorescence intensity ratios $R (I_{552}/I_{502})$ with addition of 100 μM Hcy depending on time. (b) Fluorescence spectral of Hcy within 4 min, λex = 418 nm. (c) Fluorescence spectral of GSH (λex = 418 nm). Inset: Fluorescence intensity ratios $R (I_{552}/I_{502})$ with addition of 100 μM GSH depending on time. (d) Fluorescence spectral of GSH within 10 min, λex = 418 nm. (e) UV-vis spectral of Hcy. Inset: Absorbance ratio $(A_{512}/A_{384})$ with addition of 100 μM Hcy depending on time. (f) UV-vis spectral of GSH. Inset: Absorbance ratio $(A_{512}/A_{384})$ with addition of 100 μM GSH depending on time.
**Fig. S2.** HPLC chromatograms (from highest to lowest) of probe 3 and Cys (a) Before addition of Cys. (b) Reaction mixture after incubation for 15 min at room temperature. (c) Compound 2 prepared as the authentic sample. The reaction was carried out in water. The HPLC gradient was as follows: solvent A (water), solvent B (CH$_3$CN); 0-12 min, 80% B. The peaks were monitored by UV absorbance at 490 nm.

**Fig. S3.** Fluorescence images of living Hela cells incubated at different time. The cells were pretreated with 100 μM N-ethylmaleimide for 1.0 h, and then incubated with 50 μM probe 3. (a, b) 40 min; (c, d) 40 min; (e, f) 50 min; (g, h) 60 min; (i, j) 70 min; (k, l) 80 min; (k, l) 90 min. Scale bar is 20 mm. Brightfield images (a, c, e, g, i, k), fluorescence images (b, d, f, h, j, l).
**Fig. S4.** Fluorescence intensity ratio $R (I_{552}/I_{502})$ of probe 3 (■) and probe 3 (10 μM) with Cys (0.1 mM) (●) at various pH values. Effect of pH on the Fluorescence intensity ratio $R (I_{552}/I_{502})$ of probe 3 (■) and probe 3 (10 μM) with Cys (0.1 mM) after 10 min (●), respectively. The excitation wavelength was 418 nm.
Fig. S5. (a) Pseudo first-order kinetic plot of reaction of probe 3 (10^{-5} M) with Cys (10^{-4} M), slope = -0.192 so $k' = -0.192 \text{ min}^{-1}$ and $k = 3.2 \times 10^{3} \text{ M}^{-1}\text{s}^{-1}$. (b) Pseudo first-order kinetic plot of reaction of probe 3 (10^{-5} M) with Hcy (10^{-4} M), slope = -0.031 so $k' = -0.031 \text{ min}^{-1}$ and $k = 5.2 \times 10^{2} \text{ M}^{-1}\text{s}^{-1}$. (c) Pseudo first-order kinetic plot of reaction of probe 3 (10^{-5} M) with GSH (10^{-4} M), slope = -0.006 so $k' = -0.006 \text{ min}^{-1}$ and $k = 1.3 \times 10^{2} \text{ M}^{-1}\text{s}^{-1}$. 
Fig. S6. (a) Time-dependent UV-vis spectral changes of probe 3 (10 μM) with Cys (10 equiv) in phosphate buffer (10 mM, pH 7.4). (b) Absorption spectra of 490 nm with addition of 100 μM Cys depending on time.

Fig. S7. The color change of probe 3 in pH 7.40 of phosphate buffer solution in the presence of 10 equiv. of certain amino acids, important metal ions or redox species at 10 min.

Fig. S8 (a) Fluorescence spectra of probe 3 (10 μM) with the titration of Cys (1, 2, 4, 7, 10, 15, 20, 30, 40, 55, 75, 100, 150, 200, 300 μM) (λex=418 nm) in phosphate buffer (10 mM, pH 7.4). (b) Fluorescence intensity ratio R \( (I_{552}/I_{502}) \) changes of probe 3 as a function of concentration Cys (0-40μM). Each spectrum was recorded at 10 min after the addition of Cys to probe 3.
Fig. S9. Fluorescence intensity ratio R ($I_{552}/I_{502}$) changes of probe 3 (10 μM) with addition of 100 μM Cys, Hcy and GSH depending on time in phosphate buffer (10 mM, pH 7.4). $\lambda_{ex} = 418$ nm. The spectrum was recorded at 10 min.

Fig. S10. Fluorescence spectra of probe 3 (10 μM) and Cys (0–40 μM) in diluted deproteinized human plasma. The plasma was diluted with phosphate buffer (10 mM, pH 7.4) and the reaction monitored at 10 min. (a) $\lambda_{ex} = 418$ nm. (b) $\lambda_{ex} = 490$ nm.
Fig. S11. Fluorescence spectra study upon excitation at 490 nm. (a) Time-dependent fluorescence spectral changes of probe 3 (10 μM) with Cys (10 equiv) in phosphate buffer (10 mM, pH 7.40). Inset: Fluorescence intensity at 552 nm vs time; (b) Fluorescence spectra of probe 3 (10 μM) with the titration of Cys (1, 2, 4, 7, 10, 15, 20, 35, 50, 75, 100, 125, 150 μM) (λ<sub>ex</sub>=490 nm) in phosphate buffer (10 mM, pH 7.4). Each spectrum was recorded at 10 min after the addition of Cys to probe 3.

Fig. S12. Plot of fluorescence intensity/ intensity ratio as a function of Cys concentration in diluted deproteinized human plasma. Phosphate buffer (10 mM, pH 7.4) was used to dilute the plasma sample and the reaction was monitored at 10 min, λ<sub>ex</sub> = 490 nm.
Fig. S13. Fluorescence images of living Hela cells incubated with various concentrations of probe 3. Hela cells loaded with probe 3 for 30 min. (a, d) 10 μM, (b, e) 30 μM, (c, f) 50 μM. Scale bar is 20 mm. Brightfield images (a, b, c), fluorescence images (d, e, f).
Fig. S14. $^1$H MNR (400 MHz) spectrum of compound 2 in d$_6$-DMSO.
Fig. S15. $^{13}$C MNR (100 MHz) spectrum of compound 2 in d$_6$-DMSO.
Fig. S16. $^1$H MNR (400 MHz) spectrum of probe 3 in $d_6$-DMSO.
Fig. S17. $^{13}$C MNR (100 MHz) spectrum of probe 3 in $d_6$-DMSO.
Fig. S18. $^1$H – $^1$H COSY NMR spectrum of probe 3 in D$_2$O/CD$_3$OD (1:1, V/V).
Fig. S19. Mass spectrum (ESI) of compound 2.
Fig. S20. Mass spectrum (ESI) of probe 3.
Fig. S21. Mass spectrum (ESI) obtained from the reaction of 4 with Cys.
Fig. S22. The retention measurements of probe 3 in water phase.
Fig. S23. The retention measurements of probe 3 in octanol phase.