Supplementary data

Novel pyrazoline-based selective fluorescent sensor for detecting reduced glutathione and its application in cells

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Table of Contents

- 3. Fig. S-3 Fluorescence spectra of probe 3 (10 μ M) in a buffer solution (10 mM PBS

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buffer, pH 7.4) after the addition of Cys, GSH, mercaptoacetic acid, (each 100 μ M) and Cys, GSH, mercaptoacetic acid with *N*-ethylmaleimide, (1 mM) for 12 h.4

- **4. Fig. S-4** Photographs of the tested samples after addition of amino acid, K^+ , Ca^{2+} , Na^+ , Mg^{2+} , Fe^{3+} and Zn^{2+} taken by irradiating the samples with a UV lamp at 365 nm.4
- **5. Fig. S-5** Fluorescence responses of **3** (10 μ M) to various analytes. Red bars display fluorescence intensity of the products of **3** (10 μ M) reacted with Cys (100 μ M) in the presence of other amino acids and metal ions (100 μ M). The black bars display the fluorescence intensities of the products of **3** (10 μ M) reacted with GSH (100 μ M) in the presence of other amino acids and metal ions(100 μ M); blue bars display the fluorescence intensities of **3** (10 μ M) in the presence of other amino acids and metal ions(100 μ M); blue bars display the fluorescence intensities of **3** (10 μ M) in the presence of different amino acids and metal ions (100 μ M), respectively.
- **6. Fig. S-6** Fluorescence spectra (10 μ M) of probe **3** recorded upon addition of Cys (0-600 equiv.) in a buffer solution (10 mM PBS buffer, pH 7.4). The individual spectra were recorded after incubation of probe **3** with Cys for 24 h. Excitation wavelength was 370 nm (slit = 15.0/2.5). Inset: Linear regression equation of probe **3** (10 μ M) upon addition of Cys (2-18 equiv.), in CH₃CN/H₂O (3:7, v/v), R = 0.99805 (I₄₆₄).



Fig. S-1 Fluorescence spectra of probe **3** (10 μ M) and probe **3** + Cys (100 M) in different ratios of CH₃CN/H₂O solution at pH 7.4 (10 mM PBS buffer)



Fig. S-2 The ¹H NMR spectra of probe **3** (in DMSO- d_6) in the presence of different concentrations of Cys and the ratio of Cys to **3** is 0, 0.5, 1.0 and 2.0 respectively.



Fig. S-3 Fluorescence spectra of probe **3** (10 μ M) in a buffer solution (10 mM PBS buffer, pH 7.4) after the addition of Cys, GSH, mercaptoacetic acid, (each 100 μ M) and Cys, GSH, mercaptoacetic acid with *N*-ethylmaleimide, (1 mM) for 12 h.



Fig. S-4 Photographs of the tested samples after addition of amino acid, K^+ , Ca^{2+} , Na^+ , Mg^{2+} , Fe^{3+} and Zn^{2+} taken by irradiating the samples with a UV lamp at 365 nm.



Fig. S-5 Fluorescence responses of **3** (10 μ M) to various analytes. Red bars display fluorescence intensity of the products of **3** (10 μ M) reacted with Cys (100 μ M) in the presence of other amino acids and metal ions (100 μ M). The black bars display the fluorescence intensities of the products of **3** (10 μ M) reacted with GSH (100 μ M) in the presence of other amino acids and metal ions(100 μ M); blue bars display the fluorescence intensities of **3** (10 μ M) in the presence of other amino acids and metal ions(100 μ M); blue bars display the fluorescence intensities of **3** (10 μ M) in the presence of different amino acids and metal ions (100 μ M); respectively.



Fig. S-6 Fluorescence spectra (10 μ M) of probe **3** recorded upon addition of Cys (0-600 equiv.) in a buffer solution (10 mM PBS buffer, pH 7.4). The individual spectra were recorded after incubation of probe **3** with Cys for 24 h. Excitation wavelength was 370 nm (slit = 15.0/2.5). Inset: Linear regression equation of probe **3** (10 μ M) upon addition of Cys (2-18 equiv.), in CH₃CN/H₂O (3:7, v/v), R = 0.99805 (I₄₆₄).



Fig. S-7 Time-dependent changes in the fluorescence intensity (I₄₆₄) of probe 3 (10 μ M) observed upon addition of Cys (100 μ M) in CH₃CN/H₂O (3:7, v/v). All experiments were performed at room temperature.



Fig. S-8 Fluorescence intensity (464 nm) of free probe **3** (10 μ M) and probe **3** + 10 equiv. of GSH in a mixture of CH₃CN/H₂O (3:7, v/v) with different pH conditions.



Fig. S-9 Fluorescence intensity (464 nm) of free probe **3** (10 μ M) and probe **3** + 10 equiv of Cys in a mixture of CH₃CN/H₂O (3:7, v/v) with different pH conditions.