Supplementary Material

A two-photon off-on fluorescence probe for imaging thiols in live cells and tissues

Xinyue Zhu, a Yan Li, a Wenyan Zan, a Jianjian Zhang, a Zhenjie Chen, b Xiaoyan Liu,
Fengchao Qi, a Xiaojun Yao, a Xiaoyu Zhang b and Haixia Zhang a

a Key Laboratory of Nonferrous Metal Chemistry and Resources Utilization of Gansu Province,
College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, China
b Institute of Physiology, School of Basic Medical Sciences, Lanzhou University, Lanzhou 730000, China

* Corresponding author. E-mail address: zanghx@lzu.edu.cn
Tel: +86-931-8912058; Fax: +86-931-8912582

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1. Sensing Mechanism of Z1 with Thiols

Scheme S1. Proposed mechanism of Z1 responding to Cys

Figure S1. HRMS of probe Z1.
Figure S2. HRMS of probe Z1 adding with excess of Cys.
2. Spectroscopic Measurement

UV-Vis absorption spectra

The UV-Vis absorption spectra of probe $Z_1$ toward Cys/Hcy/GSH were examined. As displayed in Figure S2, the UV-Vis absorption of $Z_1$ increased with the increasing concentration of three kinds of analytes at around 409 nm.

Figure S3. UV-Vis absorption spectra change of probe $Z_1$ (10 μM) toward Cys (A)/Hcy (B)/GSH (C) with different concentrations (2.0, 4.0, 6.0, 8.0, 10, 20, 40, 60, 80, 100, 150, 200 μM, bottom to up). (20 mM PBS, pH 7.4, 5% DMSO, $\lambda_{ex} = 409$ nm)
Fluorescence spectra

The fluorescence change of probe Z1 toward different reaction times.

**Figure S4.** Fluorescence change of Z1 toward different reaction times (probe Z1: 10μM, Cys: 100 μM).

The Fluorescence spectra of probe Z1 toward Hcy/GSH were obtained using the method described in text. The fluorescence intensities increased with the increasing concentration of Hcy/GSH at around 530 nm.

**Figure S5.** OP fluorescence spectra of probe Z1 (10 μM) toward Hcy (A)/GSH (B) with different concentrations (2.0, 4.0, 6.0, 8.0, 10, 20, 40, 60, 80, 100, 150, 200μM, bottom to up). (20 mM PBS, pH 7.4, 5% DMSO, λex = 409 nm).
4. Measurement of Two-Photon TPA Value

The two-photon absorption cross section (δ) was determined by using femto second (fs) fluorescence measurement technique as described.\textsuperscript{1} To measure the two-photon absorption cross section (δ) of the reaction product of probe Z1 (5.0 × 10\textsuperscript{-6} M) and Cys (1.0 × 10\textsuperscript{-4} M), the reaction mixture dissolved in 20 mM PBS (5% DMSO, pH 7.4) was kept at 37°C for 2 h before the measurement was conducted (NAP-P, QY\%: 11.11). The two-photon induced fluorescence intensity was measured at 700-880 nm by using Rhodamine 6G (1.3 × 10\textsuperscript{-6} M, QY\%: 95, ethyl alcohol) as the reference, whose two-photon property has been well characterized in the literature.\textsuperscript{2} The intensities of the two-photon induced fluorescence spectra of the reference and sample emitted at the same excitation wavelength were determined. The TPA cross section was calculated according to Equation S1.

\[
\delta_s = \delta_r \frac{\phi_s C_r n_s S_s}{\phi_r C_s n_s S_r}
\]  
(Equation S1)

Where the subscript s and r standed for Z1 and reference (Rhodamine 6G), respectively. δ was the TPA value, φ was the fluorescence quantum yield, n was the refractive index of the solvents, C was the concentration and S represented the intensity of TPE fluorescence emission.

References

1. Characteristic of Z1

Figure S6. ^1^H NMR spectrum of compound 1 in CDCl\textsubscript{3}.

Figure S7. ^13^C NMR spectrum of compound 1 in CDCl\textsubscript{3}.
Figure S8. \(^1\)H NMR spectrum of NAP-P in CDCl\(_3\).

Figure S9. \(^{13}\)C NMR spectrum of NAP-P in CDCl\(_3\).
Figure S10. $^1$H NMR spectrum of probe Z1 in CDCl$_3$.

Figure S11. $^{13}$C NMR spectrum of probe Z1 in CDCl$_3$. 