A NIR fluorescent chemodosimeter for imaging endogenous hydrogen polysulfides via the CSE enzymatic pathway

Jianhua Ma, a Jiangli Fan, a Haidong Li, a Qichao Yao, a Feng Xu, a Jingyun Wang b and Xiaojun Peng* a

a State Key Laboratory of Fine Chemicals, Dalian University of Technology, 2 Linggong Road, Dalian 116024, P.R. China.

b School of Life Science and Biotechnology, Dalian University of Technology, 2 Linggong Road, Dalian 116024, P.R. China.

Email: Pengxj@dlut.edu.cn.

Contents

Scheme S1 The synthetic route of probe Cy-Sn.................................................................S2

Fig. S1 Photostability test of Cy-Sn and traditional cyanine dye Cy7-Cl................................S2

Fig. S2 HRMS analysis of Cy-Sn before and after addition of Na2S4........................................S3

Fig. S3 Fluorescence intensity of Cy-Sn and Cy-Sn treated with Na2S4 at various pH values........S3

Fig. S4 Fluorescence intensity of Cy-Sn and Cy-Sn treated with various RSS..........................S4

Fig. S5 Fluorescence intensity of Cy-Sn and Cy-Sn treated with Na2S4 and various RSS............S4

Fig. S6 Fluorescence intensity of Cy-Sn and Cy-Sn treated with various ROS............................S5

Fig. S7 Fluorescence intensity of Cy-Sn and Cy-Sn treated with H2S and various ROS...............S5

Fig. S8 Confocal images of exogenous H2Sn in RAW264.7 cells........................................S6

Fig. S9 Confocal images of H2Sn from H2S/ClO− system in RAW264.7 cells............................S6

Fig. S10 Confocal images of endogenous H2Sn in RAW264.7 cells.......................................S7

Fig. S11 1H NMR and 13C NMR spectrum of compound Cy-Sn in CDCl3..............................S8
Scheme S1. The synthetic route of probe Cy-S<sub>n</sub>. Reagent and conditions: (a) Et<sub>3</sub>N, DMF, 50°C, 5 h, 60.2%; (b) DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 30 min, r.t., 77.1%.

Fig. S1. (a) Time-dependent normalized absorbance intensity of probe Cy-S<sub>n</sub> (5 μM, monitored at 590 nm), Cy-S<sub>n</sub> (5 μM) treated with Na<sub>2</sub>S<sub>4</sub> (300 μM, monitored at 700 nm), and Cy<sub>7</sub>-Cl (10 μM, monitored at 780 nm) in solution (DMSO/PBS = 5/5, pH = 7.4) under sustained illumination; (b) the structure of compound Cy<sub>7</sub>-Cl as reference for photo-stability experiment.
Fig. S2. HRMS spectra of Cy-S$_n$ (a) and Cy-S$_n$ + Na$_2$S$_4$ (b) in solution (DMSO/PBS = 5/5, pH = 7.4).

Fig. S3. Fluorescence intensity of Cy-S$_n$ (5 μM) and Cy-S$_n$ treated with Na$_2$S$_4$ (300 μM) in 5 min in solution (DMSO/PBS = 5/5, pH = 7.4) at various pH values, respectively. The excitation wavelength was 680 nm.
**Fig. S4.** Fluorescence intensity of Cy-Sₙ (5 μM) and Cy-Sₙ treated with 300 μM of Na₂S₂, Na₂S₄, ascorbic acid, tocopherol, GSSG, GSH, HCY, Cys, H₂S, SO₄²⁻, S₈ in 5 min in solution (DMSO/PBS = 5/5, pH = 7.4). The excitation wavelength was 680 nm.

**Fig. S5.** Fluorescence intensity of Cy-Sₙ (5 μM) and Cy-Sₙ with the mixture of Na₂S₄ and various RSS (ascorbic acid, tocopherol, GSSG, GSH, HCY, Cys, H₂S, SO₄²⁻, S₈ 300 μM) in 5 min in solution (DMSO/PBS = 5/5, pH = 7.4). The excitation wavelength was 680 nm.
Fig. S6. Fluorescence intensity of Cy-S (5 μM) and Cy-S treated with 300 μM of Na2S4, H2O2, ·OH, O2-, 1O2 and ClO- in 5 min in solution (DMSO/PBS = 5/5, pH = 7.4). The excitation wavelength was 680 nm.

Fig. S7. Fluorescence intensity of Cy-S (5 μM) and Cy-S with the mixture of H2S (300 μM) and various ROS (H2O2, ·OH, O2-, 1O2 and ClO-, 300 μM) in solution (DMSO/PBS = 5/5, pH = 7.4). The reactions were carried out for 20 min at 37 °C. The excitation wavelength was 680 nm.
Fig. S8. Confocal microscope images of RAW264.7 cells. Cells were treated with Cy-Sn for 15 min, then PBS (a), (d), (g), Na₂S₂ (b), (e), (h) and Na₂S₄ (c), (f), (i) for 20 min. Confocal microscope image of cells upon excitation at 635 nm, emission window 650 nm—750 nm.

Fig. S9. Confocal microscope images of RAW264.7 cells. Cells were treated with Cy-Sn for 15 min, then NaClO (a), (d), (g), H₂S (b), (e), (h) and mixture of NaClO and H₂S (c), (f), (i) for 20 min. Confocal microscope image of cells upon excitation at 635 nm, emission window 650 nm—750 nm.
Fig. 510. Confocal microscope images of RAW264.7 cells. Cells were treated with Cy-5 for 15 min, then PBS (a), (e), (i); 1 μg/mL LPS for 24 h (b), (f), (j); 1 μg/mL LPS and 1 nM dexamethasone for 24 h (c), (g), (k); pretreated with 1mM PPG 8 h then 1 μg/mL LPS for 24 h (d), (h), (l). Confocal microscope image of cells upon excitation at 635 nm, emission window 650 nm—750 nm.
Fig. S11. $^1$H NMR (top) and $^{13}$C NMR (down) spectrum of compound Cy-S$_n$ in CDCl$_3$. 