Supporting Information

Dynamic Mapping of Spontaneous H$_2$S in Entire Cell Space and Live Animal by a Rationally Designed Molecular Switch

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Fig. S1 $^1$H NMR spectrum of compound C-OH.

Fig. S2 $^{13}$C NMR spectrum of compound C-OH.

Fig. S3 HR-MS spectrum of compound C-OH (negative mode, calculated for C-OH [M-H]$^-$ = 233.0450).
Fig. S4 $^1$H NMR spectrum of compound Cda-OH.

Fig. S5 $^{13}$C NMR spectrum of compound Cda-OH.

Fig. S6 HR-MS spectrum of compound Cda-OH (negative mode, calculated for Cda-OH [M-H]$^- = 275.1032$).
**Fig. S7** $^1$H NMR spectrum of probe Cda-DNP.

**Fig. S8** $^{13}$C NMR spectrum of probe Cda-DNP.

**Fig. S9** HR-MS spectrum of probe Cda-DNP (positive mode, calculated for Cda-DNP [M+H]$^+$ = 443.1203).
**Fig. S10** HR-MS spectrum of the products DNP-SH and Cda-OH resulting from the reaction of probe with H$_2$S (negative mode, calculated for DNP-SH [M-H]$^-$ = 198.9814 and Cda-OH [M-H]$^-$ = 275.1032).

**Fig. S11** (A) Absorption spectra of 20 μM Cda-DNP with the addition of HS$^-$ (0-500 μM) at pH 7.4 in 10 mM HEPES/THF (7:3). (B) The plot of absorbances vs HS$^-$ concentrations. Inset: the liner relationship between absorbances (at 405 nm) and HS$^-$ concentrations.
Fig. S12  Time-dependent fluorescence responses of Cda-DNP (1 µM) to 50 µM HS\(^{-}\). Inset is the plots of fluorescence enhancement vs the time of 50 µM HS\(^{-}\) response to Cda-DNP.

Fig. S13 Fluorescence spectra of Cda-DNP (1 µM) at pH 7.4 in 10 mM HEPES/THF (7:3) in the presence of HS\(^{-}\) (50 µM) and other species (1 mM anions and NO, 200 µM Cys and Hcy, and 2 mM GSH).
Fig. S14 Interference tests of Cda-DNP (1 μM) in the presence of various species (Blank, 1 mM F⁻, Cl⁻, Br⁻, I⁻, HCO₃⁻, NO₂⁻, NO₃⁻, SO₄²⁻, HSO₄⁻, S₂O₃²⁻, SCN⁻; 200 μM Cys, Hcy; 2 mM GSH; 50 μM HS⁻) in 10 mM HEPES/THF (7:3).

Fig. S15 Viability of A549 cells treated with different concentrations of Cda-DNP for 24 h by MTT assay.
**Fig. S16** Screening of the appropriate dosage of H$_2$S scavenger (NMM) to clean up endogenous H$_2$S in A549 cells. (A) Fluorescent intensity changes of cells with the addition of different concentrations of NMM. The cells were separately incubated with 0, 20, 40, 50, 60 and 80 μM NMM for 30 min, and then cultured with 5 μM Cda-DNP for another 30 min after cells were washed with medium two times. (B) Dose-dependent evolutions of mean fluorescence intensities. Thirty cells were manually selected to collect the fluorescent intensities, and the mean fluorescent intensities at each concentration of NMM were calculated from the 30 intensities. The error bars represent standard deviation (±SD). All these above quantification experiments were repeated with three batches of cells.

**Fig. S17** Quantitative determination of exogenous H$_2$S in living cells. (A) The fluorescent intensity changes of A549 cells incubated with different concentrations of exogenous H$_2$S after removed endogenous H$_2$S. A549 cells were first pretreated with 60 μM NMM, and then incubated separately with 0, 5, 10, 15 and 20 μM NaHS, followed by the addition of 5 μM Cda-DNP. For above tests, all incubation periods were 30 min. (B) Dose-dependent evolutions of mean fluorescence intensities. Thirty cells were manually selected to collect the fluorescent intensities, and the mean fluorescent intensities at each concentration of H$_2$S were calculated from the 30 intensities. The error bars represent standard deviation (±SD). All these above quantification experiments were repeated with three batches of cells.
**Fig. S18** Time-dependent fluorescence changes of A549 cells. The cells were incubated with 4 μL Reddot (a commercial nucleus tracker) for 30 min, and then the images were obtained from blue and red channel of A549 cells incubated with 5 μM probe Cda-DNP for different times. Blue channel, $\lambda_{\text{ex}} = 405$ nm, $\lambda_{\text{em}} = 410-580$ nm; Red channel, $\lambda_{\text{ex}} = 633$ nm, $\lambda_{\text{em}} = 640-750$ nm.

**Fig. S19** Time-dependent fluorescence changes of A549 cells. The cells were incubated with 1 μL LysoTracker Deep Red (a commercial lysosome tracker) for 30 min, and then the images were obtained from blue and red channel of A549 cells incubated with 5 μM probe Cda-DNP for different times. Blue channel, $\lambda_{\text{ex}} = 405$ nm, $\lambda_{\text{em}} = 410-580$ nm; Red channel, $\lambda_{\text{ex}} = 633$ nm, $\lambda_{\text{em}} = 640-750$ nm.
Fig. S20 Time-dependent fluorescence intensity changes of A549 cells. The cells were incubated with 1 μL MitoTracker (a commercial mitochondria tracker) for 30 min, and then the images were obtained from blue and red channel of A549 cells incubated with 5 μM probe Cda-DNP for different times. Blue channel, $\lambda_{ex} = 405$ nm, $\lambda_{em} = 410$-580 nm; Red channel, $\lambda_{ex} = 633$ nm, $\lambda_{em} = 640$-750 nm.

Fig. S21 Dose-dependent fluorescent images of probe for the detection of spontaneous H$_2$S in normal zebrafish. The seven-day old zebrafish were cultured with 0, 5, 10, 20, 30 and 40 μM Cda-DNP for 60 min, respectively.