Supporting Information

A self-referenced nanodosimeter for reaction based ratiometric imaging of hypochlorous acid in living cells

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Scheme S1. Synthesis of ThioRB-APTS

Fig. S1 Kinetic analysis of the reaction rates between ThioRB-FITC-MSN (1 mg ml⁻¹) and NaOCl (0-100 μM as indicated). The fluorescence emission intensity at 586 nm was recorded as a function of time using an excitation wavelength of 560 nm.

Fig. S2 Genesis of RB-CM from ThioRB-ester in aqueous acetonitrile (50%) supplemented with NaOCl.
**Fig. S3** Fluorescence emission spectra of FITC and activated ThioRB displayed on MSN (1 mg ml⁻¹) in Na₂HPO₄-citrate buffer (100 mM, pH 5) containing various amounts of NaOCl. Analyte concentration: 0, 5, 10, 20, 30, 40, 50, 60, and 80 μM. (A) The fluorescence emission intensity of FITC was obtained using an excitation wavelength at 490 nm; (B) the fluorescence emission intensity of activated ThioRB was obtained using an excitation wavelength at 560 nm; (C) the titration curve was plotted by fluorescence emission intensity at 586 nm over that at 526 nm as a function of HOCl concentration.

**Fig. S4** Fluorescence emission spectra of FITC and activated ThioRB doped in MSN (1 mg ml⁻¹) in PBS (pH 7.4) containing various amounts of NaOCl (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, and 80 μM). (A) The fluorescence emission intensity of FITC was obtained using an excitation wavelength of 490 nm; (B) the fluorescence emission intensity of activated ThioRB was obtained using an excitation wavelength at 560 nm; (C) the titration curve was plotted by fluorescence emission intensity at 586 nm over that at 526 nm as a function of HOCl concentration.

**Fig. S5** Fluorescence emission of ThioRB-ester (10 μM) in PBS (100 mM, pH 7.4) buffered CH₃CN (0, 25%, 50%, 75% and 100%, v/v of PBS/CH₃CN) supplemented with or without NaOCl (100 μM). The blue columns showed the fluorescence of ThioRB-ester in PBS buffered CH₃CN spiked with NaOCl. The dark columns showed the fluorescence emission of ThioRB-ester in PBS buffered CH₃CN with no addition. The fluorescence emission intensity at 586 nm was recorded using an excitation wavelength of 560 nm.
Fig. S6 Visual images of ThioRB-FITC-MSN (1 mg ml⁻¹) in DMEM medium spiked with or without NaOCl (0.5 mM). The images of ThioRB-FITC-MSN in PBS supplemented with or without NaOCl (0.5 mM) in PBS (100 mM, pH 7.4) were used as the controls.

Fig. S7 Fluorescence emission of ThioRB-FITC-MSN (0.5 mg ml⁻¹) in PBS (pH 7) supplemented with one of the following species: H₂O₂ (1 mM), OH⁻ (1 mM, in blue), ROO• (1 mM, in black, bottom), O₂•⁻ (0.1 mM) or OCl⁻ (0.1 mM, in red); λex@490 nm; (B) H₂O₂ (1 mM), OH• (1 mM), ROO• (1 mM), NO• (1 mM), O₂•⁻ (0.1 mM) or OCl⁻ (0.1 mM, in red); λex@560 nm. (A) The fluorescence spectra of ThioRB-FITC-MSN were recorded using an excitation wavelength of 490 nm, showing the effects of the species on the emission of fluorescein; (B) the fluorescence spectra of ThioRB-FITC-MSN were recorded using an excitation wavelength of 560 nm, showing the degree of ThioRB activation.

Fig. S8 Fluorescence of ThioRB-FITC-MSN (0.5 mg ml⁻¹) in PBS buffer (pH 7.4) supplemented with each of the following species: (A) K⁺ (1 mM), Na⁺ (1 mM), Cu²⁺ (1 mM), Mn²⁺ (1 mM), Mg²⁺ (1 mM), Ca²⁺ (1 mM), Zn²⁺ (0.1 mM), Fe³⁺ (0.1 mM), Fe²⁺ (0.1 mM), Co²⁺ (0.1 mM), Ni²⁺ (0.1 mM), Pb²⁺ (0.1 mM) or OCl⁻ (0.1 mM, in red); λex@490 nm; (B) K⁺ (1 mM), Na⁺ (1 mM), Cu²⁺ (1 mM), Mn²⁺ (1 mM), Mg²⁺ (1 mM), Ca²⁺ (1 mM), Zn²⁺ (1 mM), Fe³⁺ (1 mM), Fe²⁺ (1 mM), Co²⁺ (1 mM), Ni²⁺ (1 mM), Pb²⁺ (1 mM) or OCl⁻ (0.1 mM, in red); (A) The fluorescence spectra of ThioRB-FITC-MSN were recorded using λex@490 nm, showing the effects of the species on the emission of fluorescein; (B) the fluorescence spectra of ThioRB-FITC-MSN were recorded using λex@560 nm, showing the degree of ThioRB activation.
Fig. S9 Flow cytometry analysis of lysosomal HOCl in HeLa (A) and L929 cells (B) with ThioRB-FITC-MSN under single wavelength excitation ($\lambda_{ex}$@488 nm). The cell populations marked in green were incubated in PBS supplemented with 0.5 mM NaOCl and the cell populations marked in red were treated with 1 mM NaOCl. The control cell populations shown in dark was incubated in PBS with no addition. The fluorescence of FITC (FL1) was collected @510-535 nm while that of activated ThioRB signal was collected @565-625 nm (FL2).

Fig. S10 Cytotoxicity of NaOCl on L929 cells. L929 cells were incubated with various amounts of NaOCl in PBS (0-1 mM) for 0-30 min as indicated and then stained with trypan blue. Cell viability was determined by the trypan blue exclusion assay.