Supplementary Information

A simple selenamorpholine-based fluorescent probe for targeting lysosome and visualizing hydrogen peroxide in living cells and zebrafishes

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Scheme S1. Synthetic route of fluorescent probe BODIPY-Se.

Table S1. Properties of the reported lysosome-targeted fluorescent probes for H₂O₂.

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<th>Probes</th>
<th>Signaling mode</th>
<th>λ_{em-max}</th>
<th>LOD</th>
<th>Response time</th>
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<th>References</th>
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<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td>Turn-on</td>
<td>676</td>
<td>0.21 μM</td>
<td>25 min</td>
<td>Monitoring H₂O₂ in living cells</td>
<td>Anal. Methods, 2018, 10, 3754–3758.</td>
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<td><img src="image2.png" alt="Image" /></td>
<td>Turn-on</td>
<td>606</td>
<td>0.06 μM</td>
<td>9 min</td>
<td>Monitoring H₂O₂ in living cells</td>
<td>Analyst, 2017, 142, 4522–4528</td>
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<td><img src="image3.png" alt="Image" /></td>
<td>Turn-on</td>
<td>537</td>
<td>0.22 μM</td>
<td>60 min</td>
<td>Detecting endogenous/exogenous H₂O₂; Tissue-imaging</td>
<td>Chem. Commun., 2017, 53, 3701–3704</td>
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<td><img src="image4.png" alt="Image" /></td>
<td>Turn-on</td>
<td>584</td>
<td>0.23 μM</td>
<td>10 min</td>
<td>Detecting endogenous/exogenous H₂O₂</td>
<td>Anal. Chem. 2016, 88, 5865–5870</td>
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<td><img src="image5.png" alt="Image" /></td>
<td>Turn-on</td>
<td>550</td>
<td>1.21 μM</td>
<td>160 seconds</td>
<td>Monitoring H₂O₂ in living cells; Detecting endogenous/exogenous H₂O₂</td>
<td>Biosensors and Bioelectronics, 2016, 79, 79, 237–243.</td>
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<td><img src="image6.png" alt="Image" /></td>
<td>Turn-on</td>
<td>528</td>
<td>--</td>
<td>30 min</td>
<td>Detecting endogenous/exogenous H₂O₂</td>
<td>Scientific reports, 2015, 5, 8488.</td>
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<tr>
<td><strong>This work</strong></td>
<td>Turn-on</td>
<td>504</td>
<td>0.13 μM</td>
<td>5 min</td>
<td>Monitoring H₂O₂ in living cells; Detecting endogenous/exogenous H₂O₂; Monitoring H₂O₂ in living zebrafishs.</td>
<td></td>
</tr>
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</table>
Quantum yield
The quantum yield ($\Phi_F$) was calculated according to the equation: $\Phi_F = \Phi_{ref} \left( \frac{A_{ref} S_{sample}}{A_{sample} S_{ref}} \right) \left( \frac{n_{sample}}{n_{ref}} \right)$. Where $A$ is the absorbance at the excitation wavelength, $S$ is the area under the corrected emission curve, and $n$ is the refractive index of the solvents used. Subscripts “Sample” and “ref” refer to the standard and to the unknown, respectively.

Cell culture
Cells were cultured in DMEM (Dulbecco’s modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO$_2$ and 95% air at 37 °C.

Imaging of H$_2$O$_2$ in living cells
a) Imaging of exogenous H$_2$O$_2$ in MCF-7 cells
The MCF-7 cells were plated on 6-well plates and allowed to adhere for 24 h. The cells were washed with PBS (pH=7.4) buffer three times. Subsequently, incubating with the probe BODIPY-Se (2 μM) (containing 0.1 % DMSO as a co-solvent) for another 30 min at 37 °C, the MCF-7 cells were rinsed with PBS three times, and the cells were incubated with (0, 10, 30 and 50 μM) H$_2$O$_2$ for 30 min at 37 °C, and then washed with PBS three times, and the fluorescence images were acquired through confocal microscopy fluorescence imaging.

b) Imaging of endogenous H$_2$O$_2$ in MCF-7 cells
The MCF-7 cells were plated on 6-well plates and allowed to adhere for 24 h and then PMA treated for 30 min. The cells were washed with PBS (pH=7.4) buffer. Subsequently, incubating with probe BODIPY-Se (2 μM) (containing 0.1 % DMSO as a co-solvent) for another 30 min at 37 °C, and then washed with PBS three times, and the fluorescence images were acquired through confocal microscopy fluorescence imaging.

c) Imaging of MCF-7 cells under stimulus of ascorbic acid
The MCF-7 cells were plated on 6-well plates and allowed to adhere for 24 h and then different concentrations of VC (0 mM, 0.5 mM, 0.7 mM and 1.0 mM) for 24 h. The cells were washed with PBS (pH=7.4) buffer. Subsequently, incubating with probe BODIPY-Se (2 μM) (containing 0.1 % DMSO as a co-solvent) for another 30 min at 37 °C, and then washed with PBS three times, and the fluorescence images were acquired through confocal microscopy fluorescence imaging.

Cells cytotoxicity assay
The (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) MTT assay was used to test the cytotoxicity of probe to HepG2 cells. Cells were planted in 36-well flat-bottomed plates and maintained at 37 °C under 5% CO$_2$ atmosphere. After 24 h, different concentration probes were incubated with HepG2 cells for 3 h in fresh medium, respectively. Then, 10 μL of MTT solution (10 mg/ml, PBS) was added into each well after cells were rinsed with cold phosphate buffered saline (PBS, pH 7.40) 5 times and further incubated for 24 h. Afterwards, the remaining MTT solution was removed from wells, and 150 μL of DMSO was added into each well to dissolve the intracellular blue-violet formazan crystals. The absorbance of the solution is observed at 490 nm
via a microplate reader.

**Fig. S1** Absorption spectra of BODIPY-Se at different concentrations of H$_2$O$_2$ in EtOH-PBS solution (v/v, 1/1, pH=5.0). [H$_2$O$_2$]= 0-250 μM.

**Fig. S2** Fluorescence responses of probe (10 μM) toward H$_2$O$_2$ and other substances in EtOH-PBS solution (v/v, 1/1, pH=5.0) for 30 min at room temperature. (0) Probe only. (1-5) Fe$^{3+}$, Hg$^{2+}$, Cr$^{3+}$, Mg$^{2+}$, Cu$^{2+}$ (500 μM for each). (6-10) GSH, Hcy, SO$_4^{2-}$, S$^{2-}$ (500 μM for each). (11-20) NO$_2^-$, NO$_3^-$, NO, TBO, TBOH, O$_2^-$, ONOO$^-$, ClO$^-$, OH$^-$, H$_2$O$_2$ (200 μM for each). $\lambda_{ex}$= 460 nm.
The molecular orbitals for the ground states of BODIPY-Se, BODIPY-SeH, BODIPY-SeOH based on DFT (B3LYP/6-31G*) calculations.

Fig. S3 The molecular orbitals for the ground states of BODIPY-Se, BODIPY-SeH, BODIPY-SeOH based on DFT (B3LYP/6-31G*) calculations.

Fig. S4 Cytotoxicity of different concentrations of BODIPY-Se to HepG 2 cells by a standard MTS assay, the experiment was repeated five times and the data are shown as mean (±S.D.).
Fig. S5 (a) Fluorescence images of HepG 2 cells treated with 2 μM BODIPY-Se and then incubated with PBS solution, 10 μM, 30 μM, and 50 μM H₂O₂. Fluorescence intensities were collected at 490–530 nm in green channel. (b) Average fluorescence intensities of images under different concentrations of H₂O₂ in green channels.
**Fig. S6** (a) Fluorescence images of MCF-7 cells treated with 2 μM BODIPY-Se and then incubated with different concentrations of vitamin C (0 mM, 0.5 mM, 0.7 mM and 1.0 mM). Fluorescence intensities were collected at 490–530 nm in green channel. (b) Average fluorescence intensities of images under different concentrations of vitamin C in green channels.

**Fig. S7** Confocal fluorescence images in zebrafishs incubated with probe BODIPY-Se and subsequently treated with 10 μM H₂O₂ for different time: 30 min, 60 min, 90 min. Top: Bright field. Middle: Fluorescence imagines of zebrafishs in Green channel. Bottom: Merged imagines between fluorescence imagines and bright field. Scar bar: 200 μm. Confocal image from green channel (λex = 488 nm, λem = 500-600 nm).
Fig. S8 $^1$H NMR spectrum (CDCl$_3$, 300 MHz) of BODIPY-Cl

Fig. S9 $^1$H NMR spectrum (CDCl$_3$, 300 MHz) of BODIPY-Se
**Fig. S10** $^{13}$C NMR spectrum (CDCl$_3$, 75 MHz) of BODIPY-Se

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