An interface-targeting and H$_2$O$_2$-activatable probe liberating AIEgen: enabling on-site imaging and dynamic movement tracking of lipid droplet

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1. Experimental section

1.1. Instrumentation and materials

All starting materials were purchased from commercial suppliers and used without further purification. PBS buffer (pH = 7.4, 10 mM) was prepared with Double distilled (deionized) water. $^1$H NMR and $^{13}$C NMR were recorded on Bruker Avance III 400 MHz, and TMS was used as an internal standard. UV–vis absorption spectra and fluorescence spectra were recorded using a Helios Alpha UV-Vis scanning spectrophotometer and a Hitachi F-4500 FL spectrophotometer, respectively.

1.2. Preparation of assay solutions

Stock solutions of TPY and TPYS (1.0 mM) were prepared in DMSO. The stock solution of 30% H$_2$O$_2$ was 1 mM in water. The concentration of H$_2$O$_2$ was determined by standard solution of potassium permanganate calibrated with oxalic acid. Then, a stock solution of the H$_2$O$_2$ was prepared at 0.1 M in water.

In a typical assay of H$_2$O$_2$, stock solutions of ions (500 μM Fe$^{2+}$, Fe$^{3+}$, Zn$^{2+}$, Ca$^{2+}$, NH$_4^+$, Na$^+$, F$^-$, Cl$^-$, I$^-$, C$_6$H$_5$O$_7$$^{3-}$, ClO$_3^-$, NO$_3^-$, HCO$_3^-$, CO$_3^{2-}$, S$_2$O$_3^{2-}$, and NO$_2^-$), aminoacid (500 μM GSH, Cys, and Glu), and ROS/RNS (500 μMH$_2$O$_2$, TBHP, 'BuOOH, HO$, ONOO^-$, and $^1$O$_2$) were prepared in double-distilled water. Stock solution of TPYS (1 mM) was diluted with a mixed solution of DMSO/H$_2$O (1:1, v/v, HEPES 10 mM, pH = 7.4) to make a final concentration at 20 μM. The fluorescence selectivity experiments were conducted by adding the same doses of ions, aminoacid, and ROS/RNS into the TPYS assay solution. For titration experiments, different concentrations of H$_2$O$_2$ were added into the TPYS solution and measure the
fluorescence changes. Moreover, in a typical assay of saccharides (1.0 mM D-glucose, D-mannose, D-fructose, and D-galactose) and GOx (4 U/mL) were prepared in double-distilled water. Stock solution of TPYS (1 mM) was diluted with a mixed solution of DMSO/H₂O (1:1, v/v, HEPES 10 mM, pH = 7.4) to make a final concentration at 20 μM. The fluorescence selectivity experiments were conducted by adding GOx (4 U/mL) and the same doses of saccharides into the TPYS assay solution. For titration experiments, different concentrations of D-glucose were added into the TPYS solution and measure the fluorescence changes.

1.3 Cytotoxicity assay by MTT

The cytotoxicity of the probe was examined by Cell Counting MTT method. HeLa Cells were grown in 96-well plates with a confluence of about 1.0 × 10³ cells/well. Then, 100 μL fresh culture medium containing different concentrations of TPYS (0, 0.1, 0.5, 1 and 10 μM), TPY (0, 2, 5, 10, 30 and 50 μM) and TPYS (10 μM) with H₂O₂ (0, 10, 20, 30, 50, 100, 150, 200, 300) were added into different cell plates. After incubation for 24 h at 37 °C, the old cell culture medium was discarded, and the cells were washed twice or more with PBS and further incubated with 100 μL fresh medium containing 5 mg mL⁻¹ of MTT at 37 °C for another 4 h. Then the medium was removed and 100 μL DMSO was added. Finally, the absorbance at 570 nm was measured by a microplate Reader. Each experiment was run in triplicate. The equation: Cell viability (%) = (A_probe−A_blank)/(A_control−A_blank)×100% was used to calculate the Cell viability.

1.4. Cell culture and fluorescence imaging

HeLa cells were purchased from ATCC. Cells were cultured in Dulbecco’s...
Modified Eagle Medium (DMEM) and 10% fetal bovine serum (FBS) with 5% CO₂ at 37 °C. For imaging studies, HeLa cells (1 × 10³ cells/well) were passed onto the culture dishes and incubated for 24 h, and then the culture medium was discarded. To explore the effect of H₂O₂ in vitro, HeLa cells were incubated with TPYS (10 μM) for 1 h, then the probe treated cells were incubated with different concentrations of H₂O₂ (0, 50, 100, 200 μM) for another 30 min. For the endogenous H₂O₂ detection, cells were successively incubated with N-acetyl-cysteine (NAC, 1 mM, H₂O₂ scavenger) at 37 °C for 30 min, TPYS (10 μM) for 1 h and PMA (phorbol myristate acetate, trigger production of ROS) for 30 min. After washing the culture dishes three times with PBS, fluorescence imaging experiments were carried out on a LSM710 confocal microscope (Carl Zeiss, Germany).

1.5. Colocalization

For the co-staining experiment, HeLa cells were incubated with TPYS (10 μM) for 1 h and the commercially lipid dye nile red (6 μM) for 20 min, after the cells were washed with PBS twice, then the H₂O₂ (200 μM) was incubated the cells for another 30 min. The cells were washed twice with PBS, and then imaged by a LSM710 confocal microscope (Carl Zeiss, Germany).

1.6. In Situ Spectra Measurement.

The in situ emission spectra inside cells were recorded by means of the spectral imaging function of an LSM710 confocal microscope. With the excitation wavelength of 405 and 543 nm, the in situ emission spectra can be obtained.

1.7. Synthesis
(1-(4-bromophenyl)-2-(4-methoxyphenyl)ethene-1,2-diyl)dibenzene (1), 4-bromo-7-(pyridin-4-yl)benzothiadiazole (3) were prepared as previously described.

Synthesis of compound 2.

A Schlenk tube was charged with 1 (1.081 g, 2.435 mmol), potassium acetate (0.95 g, 9.74 mmol), bis(pinacolato)diboron (0.745 g, 2.945 mmol) and Pd(dppf)Cl₂ (88.5 mg, 0.12 mmol) in anhydrous dioxane (10 mL). The reaction was performed at 85 °C for 24 h under nitrogen. After cooling down to room temperature, the mixture was subsequently diluted with water, extracted with dichloromethane, which washed with brine and dried over MgSO₄. After solvent removal under reduced pressure, the residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 4/1) to afford 2 (0.87 g, yield: 73%) as white solid.

¹H NMR (400 MHz, CDCl₃) δ: 7.54-7.53 (d, J = 4.0 Hz, 2H), 7.10 (d, 6H), 7.04-7.02 (d, J = 8.0 Hz, 6H), 6.94-6.92 (d, J = 8.0 Hz, 2H), 3.74 (s, 3H), 1.32 (s, 12H).

¹³C-NMR (100 MHz, CDCl₃): 158.21, 147.10, 143.97, 143.84, 141.04, 136.13, 134.12, 132.58, 131.41, 130.78, 127.74, 127.64, 126.53, 126.33, 113.18, 113.09, 83.72, 83.70, 55.11, 55.09, 24.95. HRMS (ESI): m/z [M+Na]⁺ calcd for C₃₃H₃₃BO₃: 511.2421, found: 511.2425.

Synthesis of compound TPY.

A mixture of 2 (0.98, 2 mmol), 3 (0.58 g, 2 mmol), Pd(PPh₃)₄ (200 mg, 0.2 mmol), and potassium carbonate (1.4 g, 10 mmol) in 24 mL of degassed toluene/ethanol/water (10:1:1 v/v/v) was stirred and reflux for 12 h under nitrogen. After reaction finished, the mixture was cooled to room temperature, and then poured into water, and extracted
with dichloromethane by above three times. The organic layers were washed with brine and dried by magnesium sulfate anhydrous overnight. After filtration and solvent evaporation, the residue was purified by silica-gel column chromatography using dichloromethane as eluent. **TPY** was obtained as yellow solid in 40% yield.

\[ \text{1H-NMR (400 MHz, CDCl}_3\text{): } \delta 8.78–8.76 (d, J = 8.0 Hz, 2H), 7.93–7.92 (d, J = 4.0 Hz, 2H), 7.87–7.85 (d, J = 8.0 Hz, 2H), 7.79–7.76 (t, J = 12.0 Hz, 2H), 7.20–7.18 (d, J = 8.0 Hz, 2H), 7.16–7.13 (m, 10H); 6.97–6.95 (d, J = 8.0 Hz, 2H), 6.66–6.64 (d, J = 8.0 Hz, 2H) 3.75 (s, 3H). \]

\[ \text{13C-NMR (100 MHz, CDCl}_3\text{): } \delta 158.36, 158.24, 153.96, 153.59, 153.57, 150.20, 144.64, 144.61, 143.92, 143.83, 141.36, 139.55, 139.53, 132.65, 132.60, 131.74, 131.52, 131.48, 131.43, 128.78, 128.61, 128.50, 127.89, 127.83, 127.77, 127.53, 123.51, 113.29, 113.12, 55.12. \]

HRMS (ESI): m/z [M]+ calcld for C\textsubscript{38}H\textsubscript{27}N\textsubscript{3}O\textsubscript{5}: 574.1948, found: 574.1951.

**Synthesis of compound TPYS**

A mixture of compound **TPY** (0.36 g, 0.64 mmol) and compound 4-(bromomethyl) benzene boronic pinacol ester (0.22 g, 0.70 mmol) in DMF (15.0 mL) was refluxed for 8.0 h. After cooling to room temperature, the precipitate was filtered, washed with acetone to get a red solid in 70 % yields.

\[ \text{1H-NMR (400 MHz, CDCl}_3\text{): } \delta 9.49–9.47 (d, J = 8.0 Hz, 2H), 8.82–8.81 (d, J = 4.0 Hz, 2H), 8.27–8.26 (d, J = 4.0 Hz, 1H), 7.78–7.75 (m, 5H), 7.63–7.61 (d, J = 8.0 Hz, 2H), 7.20–7.12 (m, 12H), 6.97–6.95 (d, J = 8.0 Hz, 2H), 6.66–6.64 (d, J = 8.0 Hz, 2H), 6.30 (s, 2H), 3.75 (s, 3H), 1.31 (s, 12H). \]

\[ \text{13C-NMR (100 MHz, CDCl}_3\text{): } \delta 162.56, 158.27, 153.74, 152.73, 144.44, 143.74, 139.28, 135.92, 132.57, 131.83, 131.43, 128.81, 128.61, 128.50, 127.89, 127.83, 127.77, 127.53, 123.51, 113.29, 113.12, 55.12. \]
HRMS (ESI): m/z [M-Br]^+ calcd for C_{51}H_{45}BN_{3}O_{3}S: 790.3278; found: 790.3288.
Scheme S1. Synthetic of compound TPYS.
Figure S1 (a) Normalized absorbance spectra of TPYS in solvents with different polarity. (b) Normalized emission spectra of TPYS in solvents with different polarity: hexane (black line), toluene (red line), dioxane (blue line), tetrahydrofuran (green line), chloroform (magenta line), dichloromethane (olive line), and acetone (navy line).

Figure S2 (a) Emission spectra and (b) changes in the fluorescent intensities of TPYS in DMSO/PBS buffer mixtures with different PBS buffer fractions.

Figure S3 Solid-state emission spectrum TPYS. Inset: photo images of the powder solids for TPYS ($\lambda_{ex} = 420$ nm).
**Figure S4** (a) Normalized absorbance and (b) emission spectra of TPY in solvents with different polarity: hexane (black line), toluene (red line), dioxane (blue line), tetrahydrofuran (green line), chloroform (magenta line), dichloromethane (olive line), and acetone (navy line).

**Figure S5** (a) Emission spectra and (b) intensity at 590 nm changes of TPY in DMSO/Glycerol mixtures with different glycerin fractions ($f_g$).
Figure S6 (a) Emission spectra (b) Changes in the fluorescent intensities at 590 nm of TPY in DMSO/PBS buffer mixtures with different PBS buffer fractions ($f_w$). (c) Solid-state emission spectrum TPYS. Inset: photoimages of the powder solids for TPY ($\lambda_{ex}$ = 365 nm).
Figure S7 Plot of the intensity at 590 nm of compound TPYS with different amount of H$_2$O$_2$.

Figure S8 Plot of the intensity at 590 nm for a mixture of the sensor TPYS (20 μM) and H$_2$O$_2$ in DMSO/PBS buffer (1:1, v/v, pH 7.0) solution at 37 °C in the range of 0-9 equivalents. Fluorescence intensity at 590 nm was measured with excitation at 420 nm.

Linear Equation: $y = -1.6888 + 41.4869 \times x$ 
$R = 0.9969$

$S = 4.15 \times 10^8$
$\delta = \sqrt{\frac{\sum (F_0 - F_1)^2}{N - 1}} = 11.11862$ (N = 10)  
$K = 3$

$LOD = K \times \delta / S = 3 \times 11.11862 / 4.15 \times 10^8 = 0.083$ μM

$F_0$ is the fluorescence intensity of TPYS; $F_1$ is the average of the $F_0$. 
Figure S9 Time-dependent fluorescence intensity at 590 nm of TPYS (20 μM) after incubation with different concentration of hydrogen peroxide. The reaction was performed at 37 °C in DMSO/PBS buffer (1:1, v/v, pH 7.0). Fluorescence intensity at 590 nm was measured with excitation at 420 nm.

Figure S10 Effect of different pH from 2 to 14 on the fluorescence intensity at 590 nm of TPYS, TPYS+H₂O₂.

Figure S12 HRMS (ESI$^+$) of TPYS+H$_2$O$_2$
Figure S13 TEM images of TPYS (20.0 μM) (a) before and after incubation with H$_2$O$_2$ (400.0 μM) (b); DLS data of TPYS (20.0 μM) (c) before and after incubation with H$_2$O$_2$ (400.0 μM) (d). The reaction between TPYS and H$_2$O$_2$ was performed at 37 °C for 30 min in DMSO/PBS buffer (1:1, v/v, pH 7.0) before the collection of DLS data.
Figure S14 Calculated the energy gap between HOMO and LUMO of compound TPYS and compound TPY.
**Figure S15** Relative cell viability of HeLa cells in vitro after incubation with (b) **TPY** at various concentrations for 24 h.

**Figure S16** Relative cell viability of HeLa cells in vitro after incubation with different concentrations of **TPYS** and H\(_2\)O\(_2\): 1. **TPYS** (0 uM) and H\(_2\)O\(_2\) (0 uM), 2. **TPYS** (10 uM) and H\(_2\)O\(_2\) (10 uM), 3. **TPYS** (10 uM) and H\(_2\)O\(_2\) (20 uM), 4. **TPYS** (10 uM) and H\(_2\)O\(_2\) (30 uM), 5. **TPYS** (10 uM) and H\(_2\)O\(_2\) (50 uM), 6. **TPYS** (10 uM) and H\(_2\)O\(_2\) (100 uM), 7. **TPYS** (10 uM) and H\(_2\)O\(_2\) (150 uM), 8. **TPYS** (10 uM) and H\(_2\)O\(_2\) (200 uM), 9. **TPYS** (10 uM) and H\(_2\)O\(_2\) (300 uM) for 24 h.
Figure S17 Confocal fluorescence images of Hela cells incubated with probe TPYS (10 μM) and different concentrations of (a) 0 μM, (b) 50 μM, (c) 100 μM, (d) 200 μM H$_2$O$_2$. Images were collected from yellow (λ$_{em}$ = 550–600 nm) channels. λ$_{ex}$ = 405 nm, scale bar: 20 μm. (e) The fluorescence intensity ratio in the cells treated with H$_2$O$_2$ for different concentration to that in untreated cells.
**Figure S18** Confocal fluorescence images of Hela cells under different conditions with probe TPYS. (a) Cells treated with TPYS (10 μM); (b) NAC (10 μM)-pretreated cells further incubated with TPYS (10 μM); (c) cells sequentially treated with TPYS (10 μM) and PMA (1 mM); (d) cells sequentially treated with NAC (10 μM), TPYS and PMA (1 mM). Fluorescence images were collected from yellow (λ_{em} = 550–600 nm) channels. λ_{ex} = 405 nm, scale bar: 20 μm. (i) The ratio of fluorescence intensity in the cells treated with NAC, PMA or NAC/PMA to that in untreated cells.
Figure S19  Confocal fluorescence images of HeLa cells untreated and treated with 0.1 mM oleic acid for different time intervals of (a) 0, (b) 2, (c) 4, and (d) 6 h and then stained with probe TPYS (10 μM) and H₂O₂ (200 μM). λ_{ex} = 405 nm, scale bar: 20 μm. (i) The ratio of fluorescence intensity of confocal fluorescence images of HeLa cells untreated and treated with 0.1 mM oleic acid for different time intervals of 0, 2, 4, and 6 h and then stained with probe TPYS (10 μM) and H₂O₂ (200 μM).
Figure S20 CLSM images of HeLa cells stained with TPYS (20 μM) + H₂O₂ system. (a–d) Different pseudo-colors are used to illustrate the fluorescence images at different times of 0, 2, 4, and 6 min. Merging images at two different times: (e) 0 and 2 min, (f) 2 and 4 min, (g) 4 and 6 min, and (h) bright-field image. Scale bar = 10 μm. i – k: Amplified fluorescence images of white rectangular areas in e – g.
Figure S21 $^1$H NMR of compound TPYS
Figure S22 $^{13}$C NMR of compound TPYS
**Figure S23** HRMS(ESI⁺) of compound TPYS.
Table S1. Comparison of detection performance for H$_2$O$_2$ by our work and those reported in literature

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<th>Structure</th>
<th>Strategy</th>
<th>Limit of detection</th>
<th>Reference</th>
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<td><img src="image1" alt="Chemical Structure 1" /></td>
<td>Fluorescence “Turn on” at 580 nm, specific lipid droplet (LD)-targeting,</td>
<td>0.083 μM for H$_2$O$_2$</td>
<td>This work</td>
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<td><img src="image8" alt="Chemical Structure 8" /></td>
<td>Fluorescence “Turn on” at 510 nm</td>
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Fluorescence “Turn on” at 540 nm 100 nM for H$_2$O$_2$

Emission red-shifted from 475 nm to 520 nm.

Emission red-shifted from 565 nm to 579 nm. 6 μM

References


