Electronic Supplementary Material (ESI) for ChemComm.

An interface-targeting and H_2O_2-activatable probe liberating AIEgen: enabling on-site imaging and dynamic movement tracking of lipid droplet

Wenting Li, Lingyun Wang *, Hao Tang and Derong Cao

Key Laboratory of Functional Molecular Engineering of Guangdong Province, School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou, China, 510641

*Corresponding author: Tel. +86 20 87110245; fax: +86 20 87110245. E-mail: lingyun@scut.edu.cn

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_2O_2 was 1 mM in water. The concentration of H_2O_2 was determined by standard solution of potassium permanganate calibrated with oxalic acid. Then, a stock solution of the H_2O_2 was prepared at 0.1 M in water.

In a typical assay of H_2O_2, stock solutions of ions (500 μM Fe^{2+}, Fe^{3+}, Zn^{2+}, Ca^{2+}, NH_4^+, Na^+, F^-, Cl^-, I^-, $C_6H_5O_7^{3-}$, ClO_3^-, NO_3^-, HCO_3^-, CO_3^{2-}, $S_2O_3^{2-}$, and NO_2^-), aminoacid (500 μM GSH, Cys, and Glu), and ROS/RNS (500 μMH_2O_2, TBHP, $^{t}BuOOH^{\bullet}$, $HO^{\bullet}$, $ONOO^-$, and $^1O_2$) were prepared in double-distilled water. Stock solution of **TPYS** (1 mM) was diluted with a mixed solution of DMSO/H_2O (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_2O_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/H_2O (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 1.0×10^3 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 H_2O_2 (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 (%) = $(A_{probe}–A_{blank})/(A_{control}–A_{blank})\times100\%$ 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% CO_2 at 37 °C. For imaging studies, HeLa cells (1×10^3 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 H_2O_2 in vitro, HeLa cells were incubated with **TPYS** (10 μM) for 1 h, then the probe treated cells were incubated with different concentrations of H_2O_2 (0, 50, 100, 200 μM) for another 30 min. For the endogenous H_2O_2 detection, cells were successively incubated with N-acetyl-cysteine (NAC, 1 mM, H_2O_2 scavenger) at 37 °C for 30 min, **TPYS** (10 μM) for 1h 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 H_2O_2 (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 $Pd(dppf)_2Cl_2$ (88.5 mg, 0.12 mmol) in anhydrous dioxane (10 mL). The reaction was performed at 85 ^oC 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 $MgSO_4$. 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, $CDCl_3$) 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). ^{13}C-NMR (100 MHz, $CDCl_3$): 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 $C_{33}H_{33}BO_3$: 511.2421, found: 511.2425.

*Synthesis of compound **TPY.***

A mixture of **2** (0.98, 2 mmol), **3** (0.58 g, 2 mmol), $Pd(PPh_3)_4$ (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.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 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 2H) 3.75 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 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 $[M]^+$ calcd for $C_{38}H_{27}N_3OS$: 574.1948, found: 574.1951.

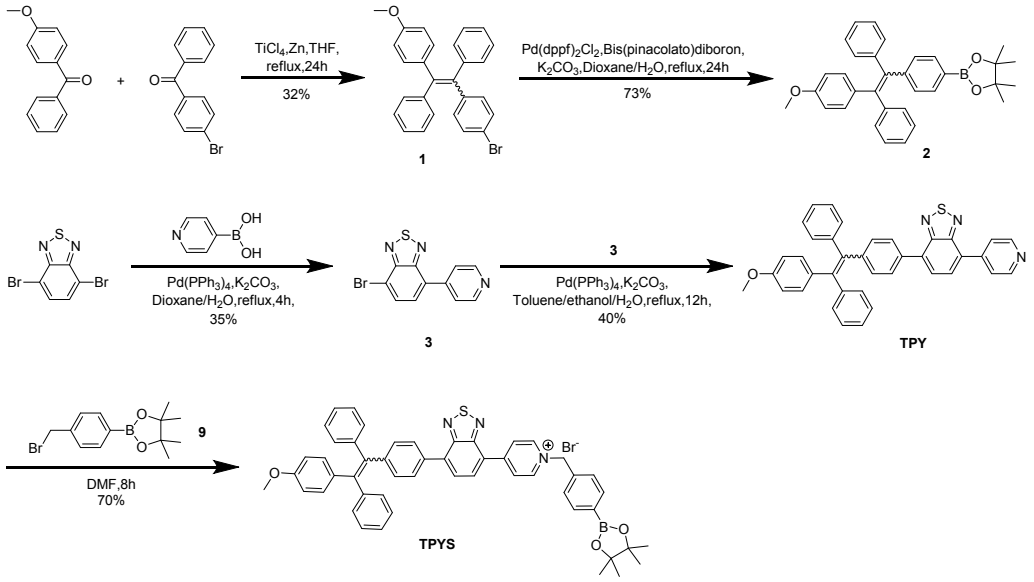
*Synthesis of compound **TPYS***

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.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 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). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 162.56, 158.27, 153.74, 152.73, 144.44, 143.74, 139.28, 135.92, 132.57, 131.83, 131.43, 128.81,

128.76, 127.91, 127.87, 126.76, 126.57, 126.50, 113.09, 84.11, 63.76, 55.12, 24.63.

HRMS (ESI): m/z $[M\text{-}Br]^+$ calcd for $C_{51}H_{45}BN_3O_3S$: 790.3278; found: 790.3288.



Scheme S1. Synthetic of compound **TPYS**.

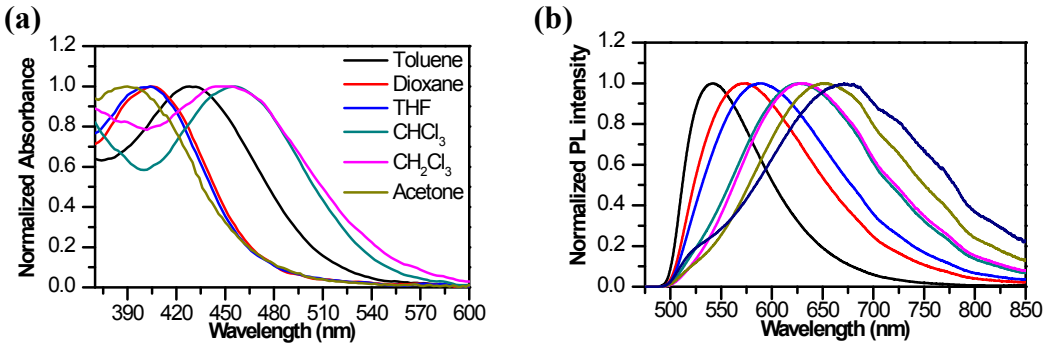


Figure S1 (a) Normalized absorbance spectra of **TPYS** in solvents with different polarity. (b) Normalized emission spectra of **TPYS** in solvents with different polarity: hexane (black line), toluene (red line), dioxane (blue line), tetrahydrofuran (green line), chloroform (magenta line), dichloromethane (olive line), and acetone (navy line).

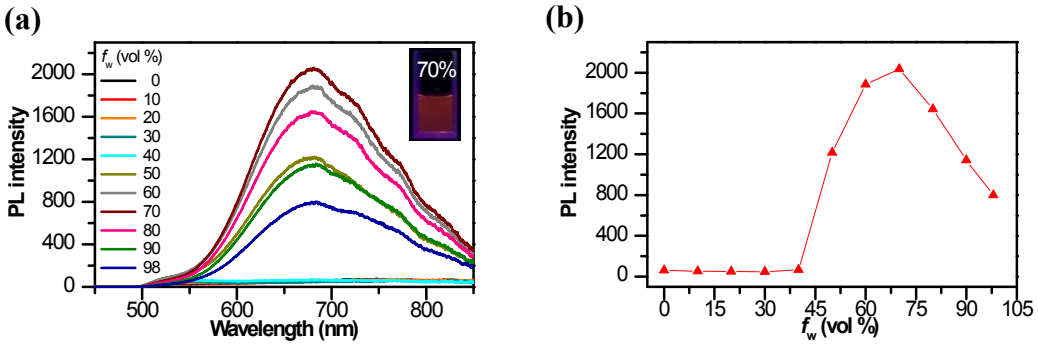


Figure S2 (a) Emission spectra and (b) changes in the fluorescent intensities of **TPYS** in DMSO/PBS buffer mixtures with different PBS buffer fractions.

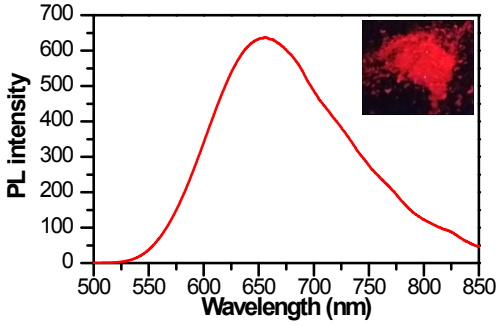


Figure S3 Solid-state emission spectrum **TPYS**. Inset: photo images of the powder solids for **TPYS** (λ_{ex}= 420 nm).

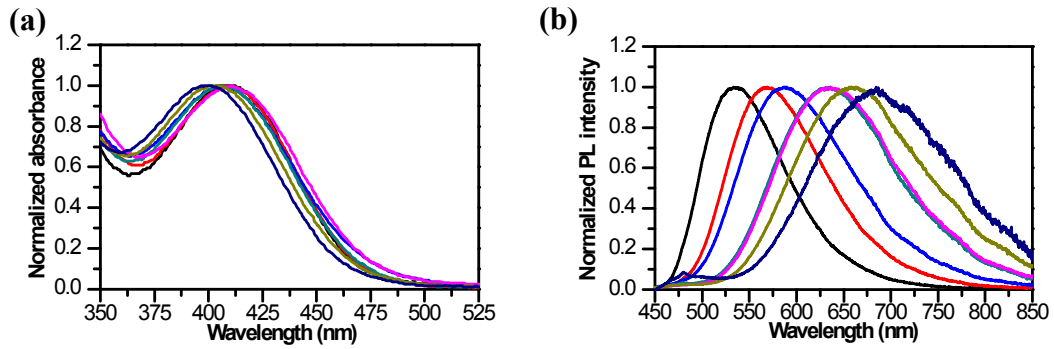


Figure S4 (a) Normalized absorbance and (b) emission spectra of **TPY** in solvents with different polarity: hexane (black line), toluene (red line), dioxane (blue line), tetrahydrofuran (green line), chloroform (magenta line), dichloromethane (olive line), and acetone (navy line).

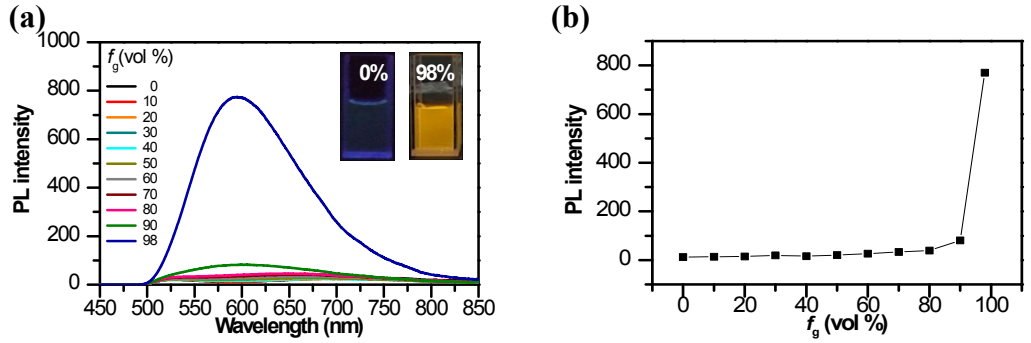


Figure S5 (a) Emission spectra and (b) intensity at 590 nm changes of **TPY** in DMSO/Glycerol mixtures with different glycerin fractions (f_g).

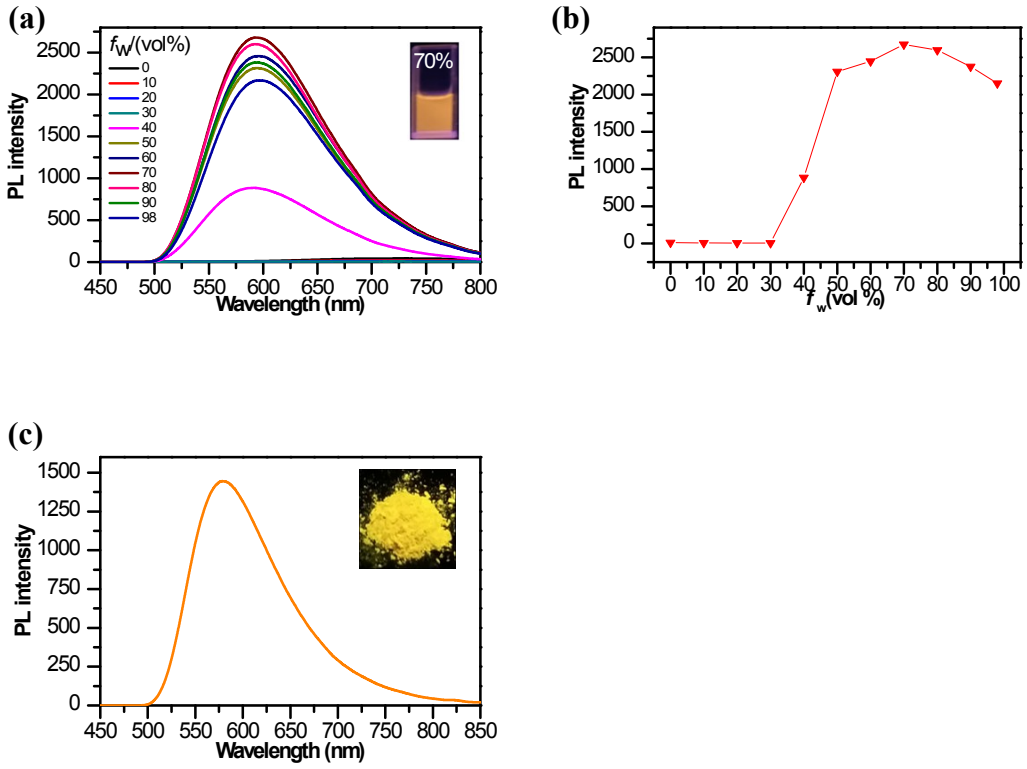


Figure S6 (a) Emission spectra (b) Changes in the fluorescent intensities at 590 nm of **TPY** in DMSO/PBS buffer mixtures with different PBS buffer fractions (f_w). (c) Solid-state emission spectrum **TPYS**. Inset: photoimages of the powder solids for **TPY** (λ_{ex}= 365 nm).

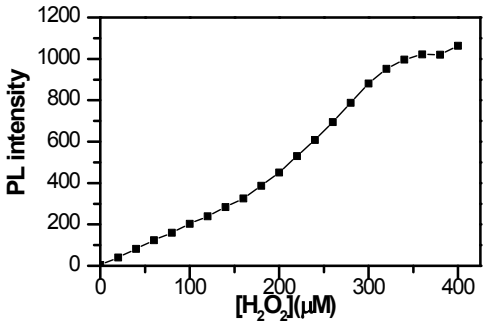


Figure S7 Plot of the intensity at 590 nm of compound **TPYS** with different amount of H_2O_2.

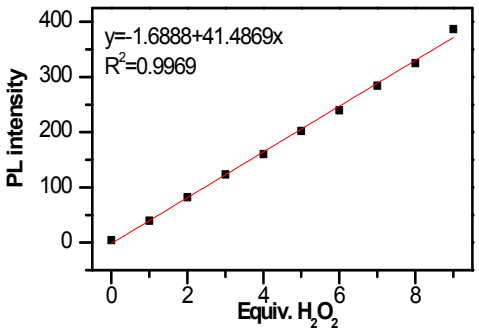


Figure S8 Plot of the intensity at 590 nm for a mixture of the sensor **TPYS** (20 μM) and H_2O_2 in DMSO/PBS buffer (1:1, v/v, pH 7.0) solution at 37 °C in the range of 0-9 equivalents. Fluorescence intensity at 590 nm was measured with excitation at 420 nm.

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

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

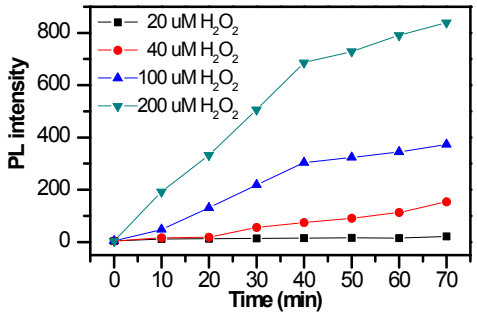


Figure S9 Time-dependent fluorescence intensity at 590 nm of **TPYS** (20 μM) after incubation with different concentration of hydrogen peroxide. The reaction was performed at 37 ˚C in DMSO/PBS buffer (1:1, v/v, pH 7.0). Fluorescence intensity at 590 nm was measured with excitation at 420 nm.

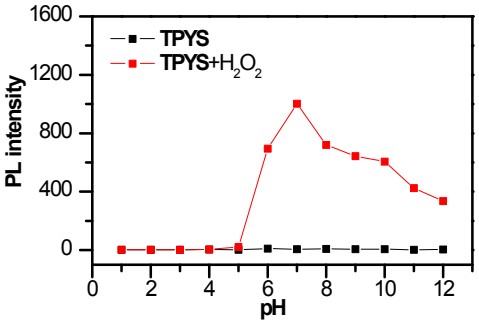


Figure S10 Effect of different pH from 2 to 14 on the fluorescence intensity at 590 nm of **TPYS**, **TPYS**+H_2O_2.

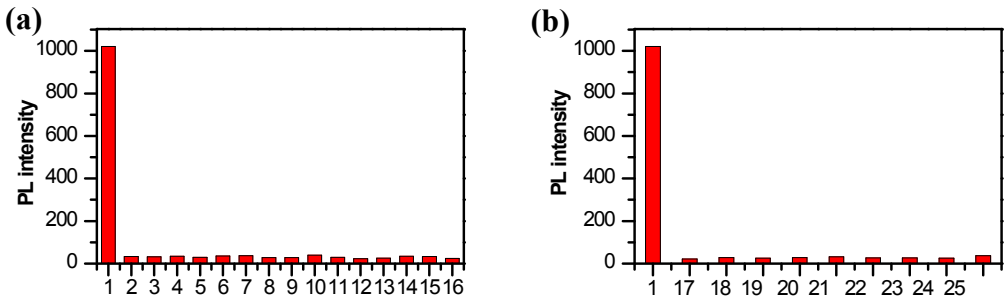


Figure S11 (a) Fluorescence changes of **TPYS** (20 μM) in DMSO/PBS buffer (1:1, v/v, pH 7.0) upon addition of 400 μM various kinds of ions, amino acid and ROS/RNS (1. H_2O_2, 2. Fe^{2+}, 3. Fe^{3+}, 4. Zn^{2+}, 5. Ca^{2+}, 6. NH_4^+, 7. Na^+, 8. F^-, 9. Cl^-, 10. I^-, 11. $C_6H_5O_7^{3-}$, 12. ClO_3^-, 13. NO_3^-, 14. HCO_3^-, 15. CO_3^{2-}, 16. $S_2O_3^{2-}$, 17. NO_2^-, 18. GSH, 19. Cys, 20. Glu, 21. TBHP, 22. ^{t}BuOOH$^\bullet$, 23. HO$^\bullet$, 24. $ONOO^-$, 25. $^1O_2$). All data were obtained after 60 min of incubation with the analytes.

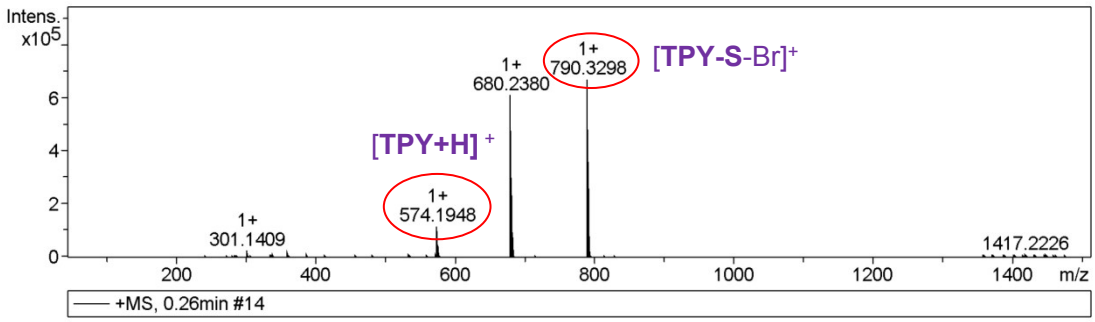


Figure S12 HRMS (ESI$^+$) of **TPYS**+H_2O_2

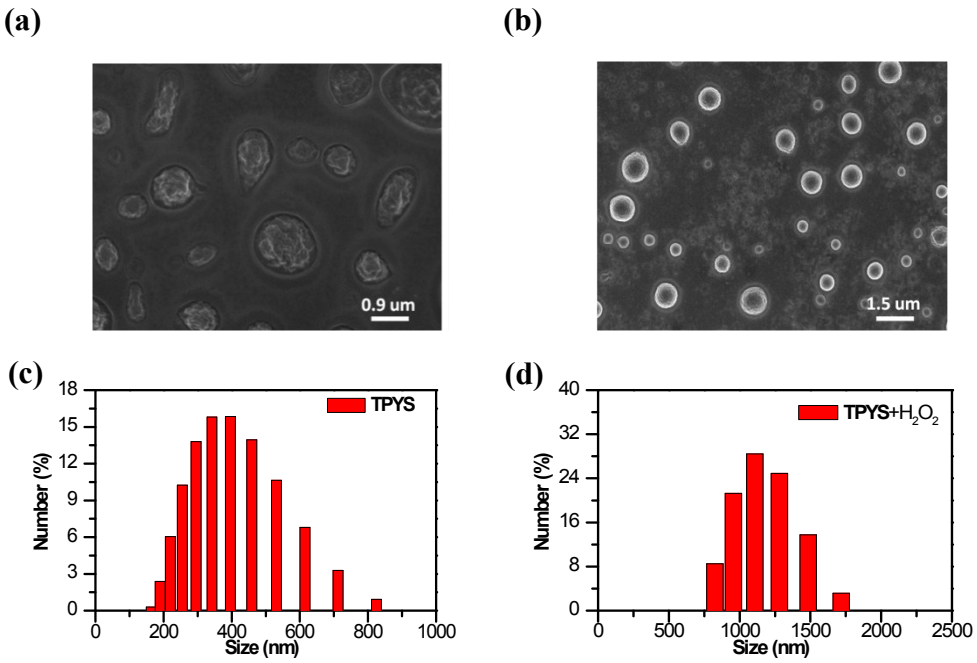


Figure S13 TEM images of **TPYS** (20.0 μM) (a) before and after incubation with H_2O_2 (400.0 μM) (b); DLS data of **TPYS** (20.0 μM) (c) before and after incubation with H_2O_2 (400.0 μM) (d). The reaction between **TPYS** and H_2O_2 was performed at 37 ℃ for 30 min in DMSO/PBS buffer (1:1, v/v, pH 7.0) before the collection of DLS data.

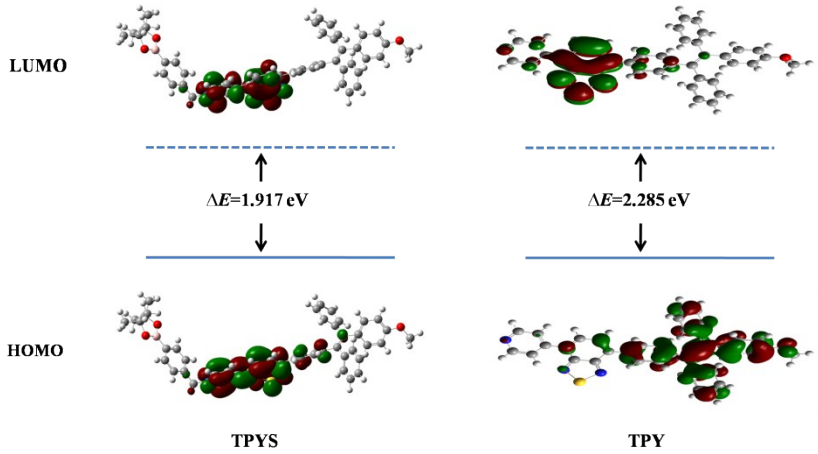


Figure S14 Calculated the energy gap between HOMO and LUMO of compound **TPYS** and compound **TPY**.

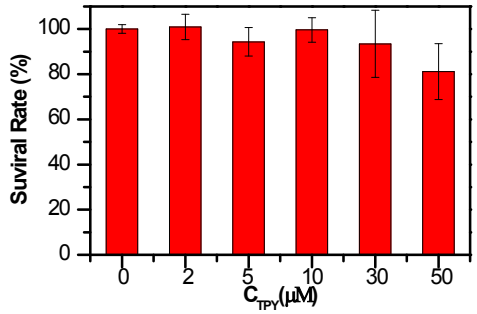


Figure S15 Relative cell viability of HeLa cells in vitro after incubation with (b) **TPY** at various concentrations for 24 h .

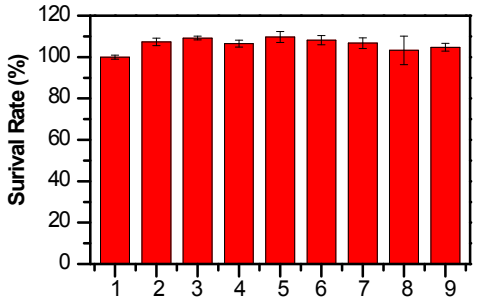


Figure S16 Relative cell viability of HeLa cells in vitro after incubation with different concentrations of **TPYS** and H_2O_2 (1. **TPYS** (0 uM) and H_2O_2 (0 uM), 2. **TPYS** (10 uM) and H_2O_2 (10 uM), 3. **TPYS** (10 uM) and H_2O_2 (20 uM), 4. **TPYS** (10 uM) and H_2O_2 (30 uM), 5. **TPYS** (10 uM) and H_2O_2 (50 uM), 6. **TPYS** (10 uM) and H_2O_2 (100 uM), 7. **TPYS** (10 uM) and H_2O_2 (150 uM), 8. **TPYS** (10 uM) and H_2O_2 (200 uM), 9. **TPYS** (10 uM) and H_2O_2 (300 uM)) for 24 h.

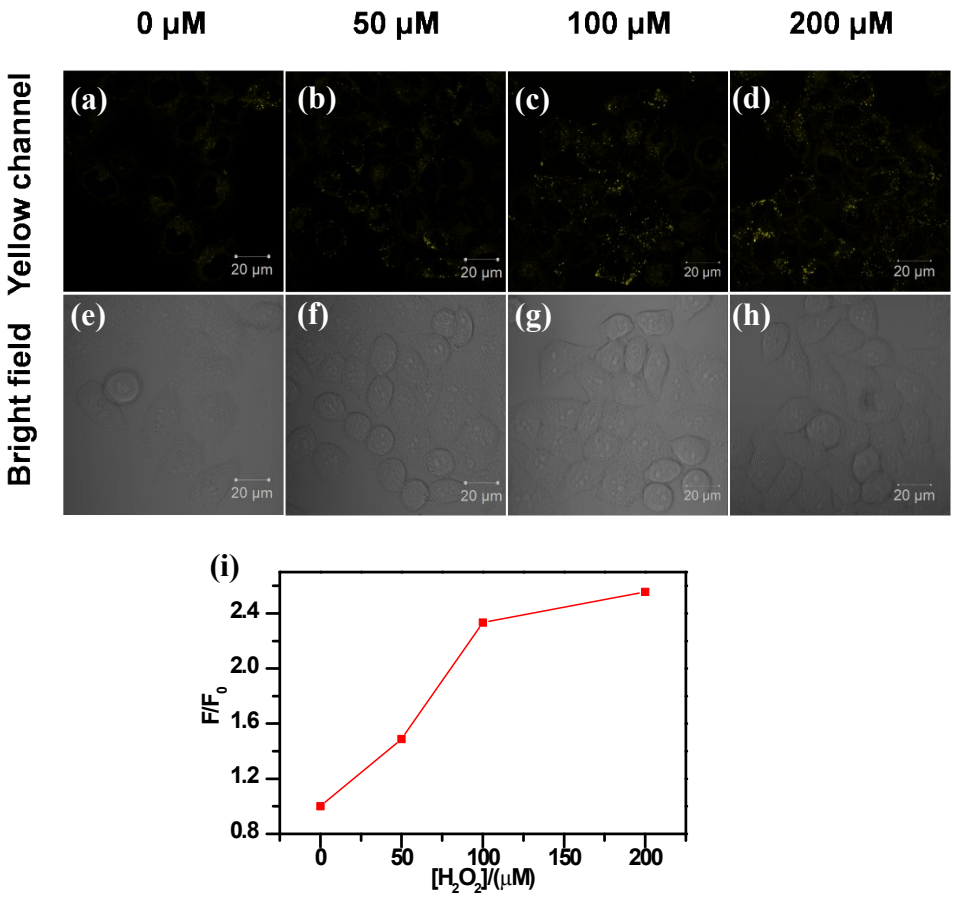


Figure S17 Confocal fluorescence images of Hela cells incubated with probe **TPYS** (10 μM) and different concentrations of (a) 0 μM, (b) 50 μM, (c) 100 μM, (d) 200 μM H_2O_2. Images were collected from yellow (λ_{em} = 550–600 nm) channels. λ_{ex} = 405 nm, scale bar: 20 μm. (e) The fluorescence intensity ratio in the cells treated with H_2O_2 for different concentration to that in untreated cells.

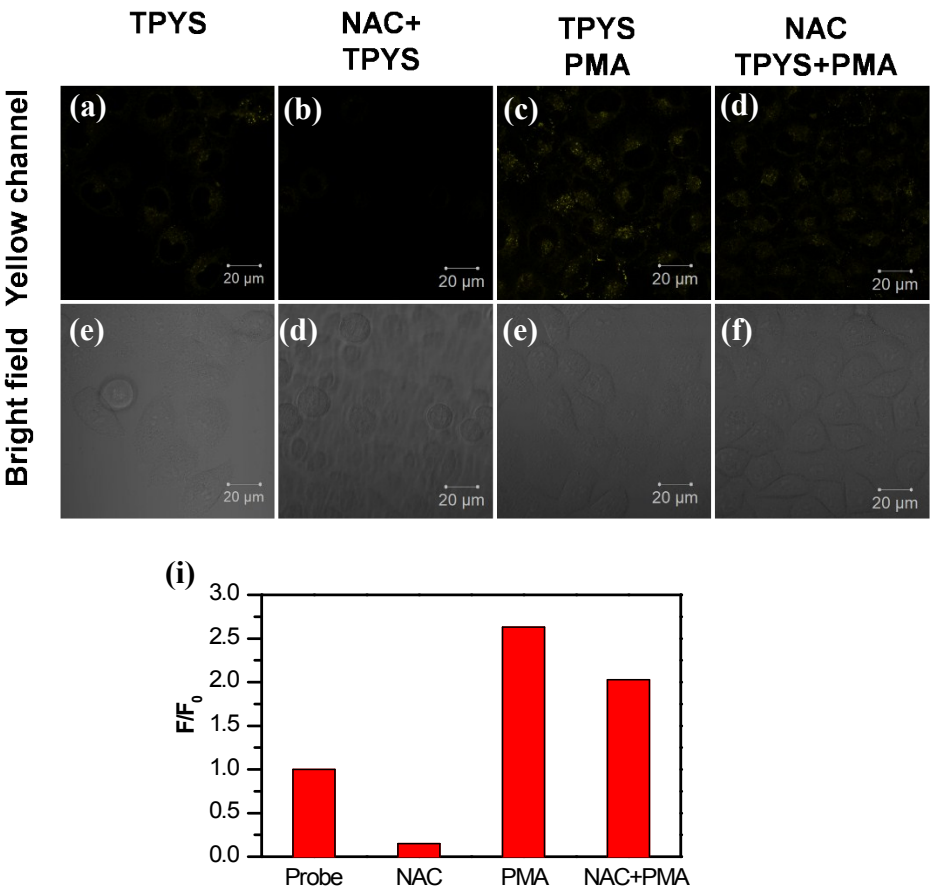


Figure S18 Confocal fluorescence images of Hela cells under different conditions with probe **TPYS**. (a) Cells treated with **TPYS** (10 μM); (b) NAC (10 μM)-pretreated cells further incubated with **TPYS** (10 μM); (c) cells sequentially treated with **TPYS** (10 μM) and PMA (1 mM); (d) cells sequentially 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.

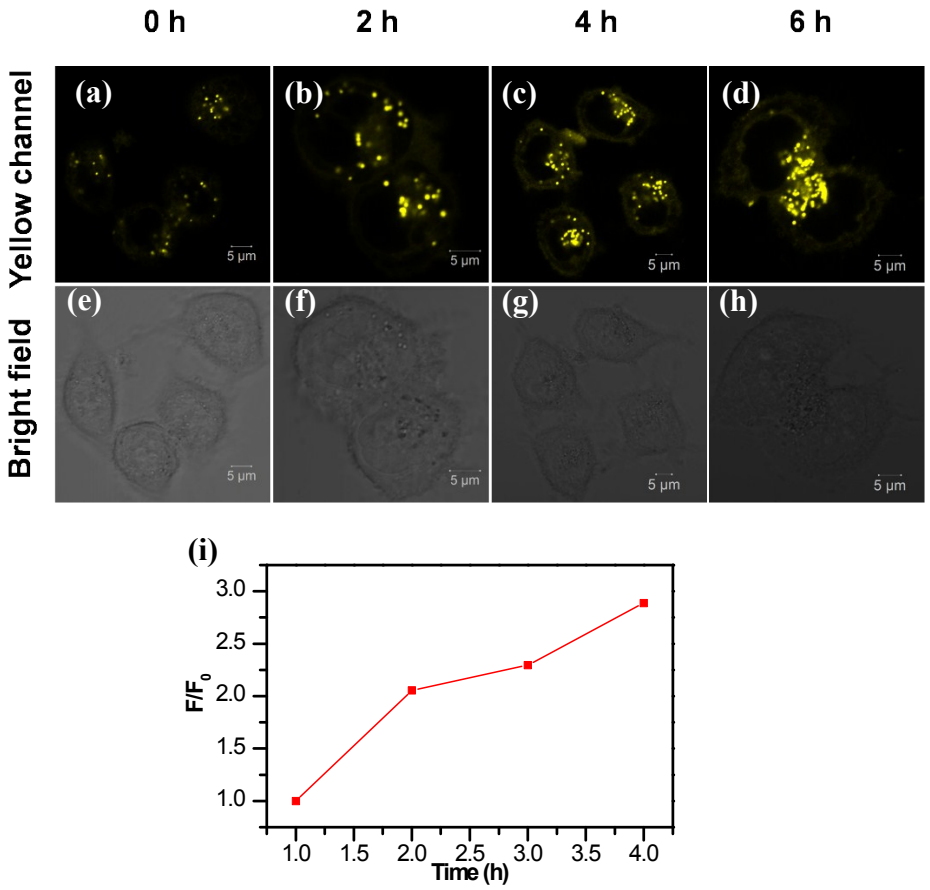


Figure S19 Confocal fluorescence images of HeLa cells untreated and treated with 0.1 mM oleic acid for different time intervals of (a) 0, (b) 2, (c) 4, and (d) 6 h and then stained with probe **TPYS** (10 μM) and H_2O_2 (200 μM). λ_{ex} = 405 nm, scale bar: 20 μm. (i) The ratio of fluorescence intensity 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 H_2O_2 (200 μM).

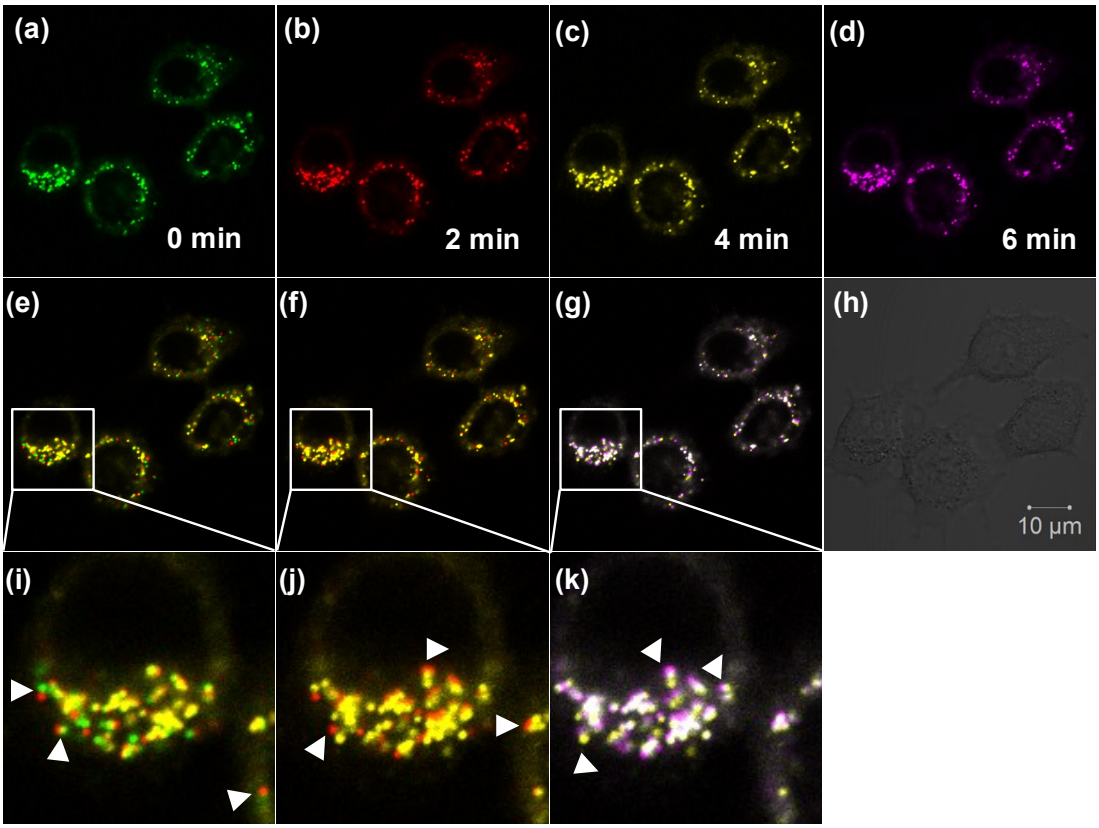


Figure S20 CLSM images of HeLa cells stained with **TPYS** (20 μM) +H_2O_2 system. (a–d) Different pseudo-colors are used to illustrate the fluorescence images at different times of 0, 2, 4, and 6 min. Merging images at two different times:(e) 0 and 2 min, (f) 2 and 4 min, (g) 4 and 6 min, and (h) bright-field image. Scale bar = 10 μm. i – k: Amplified fluorescence images of white rectangular areas in e – g.

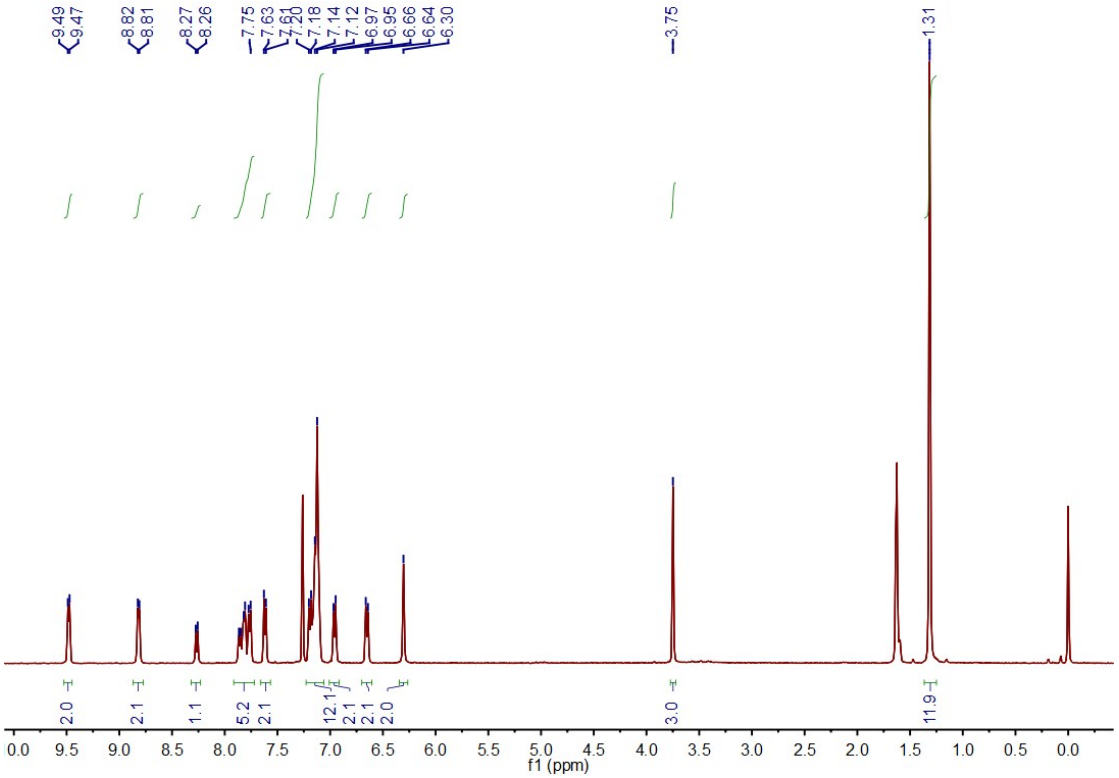


Figure S21 ^{1}H NMR of compound **TPYS**

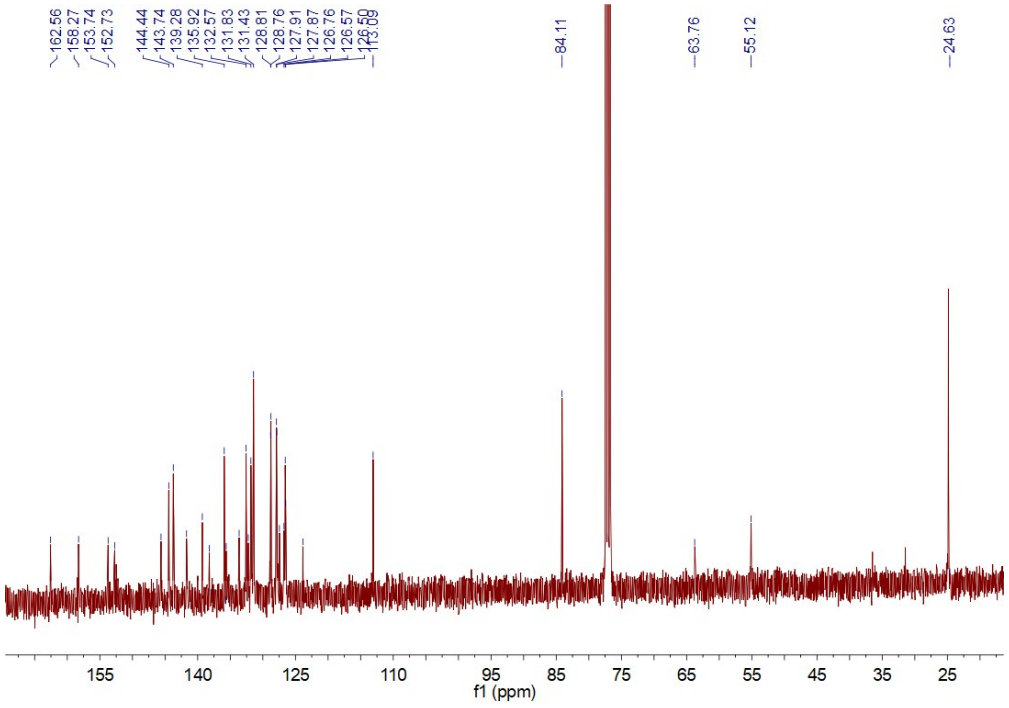


Figure S22 ^{13}C NMR of compound **TPYS**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
Focus	Active	Set Capillary	3500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	0 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

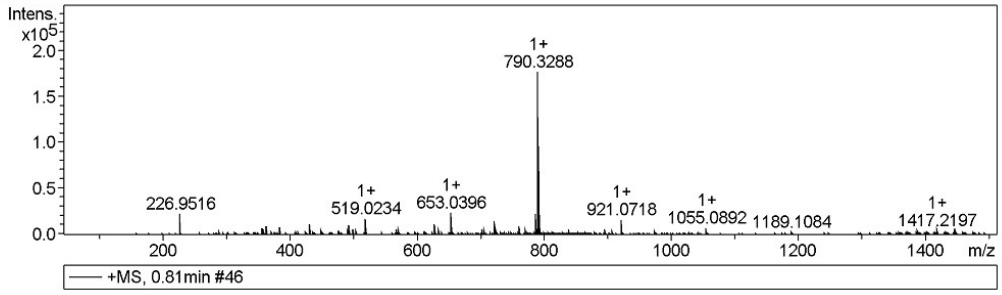
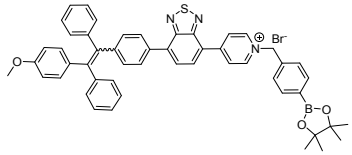
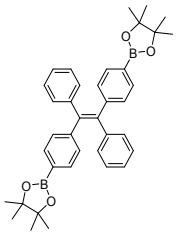
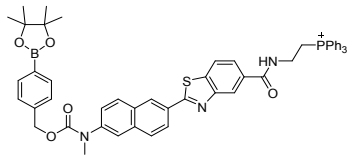
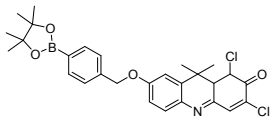
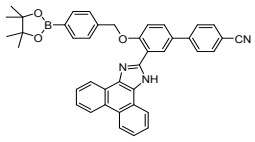
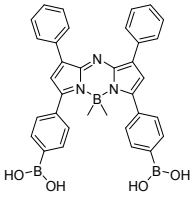
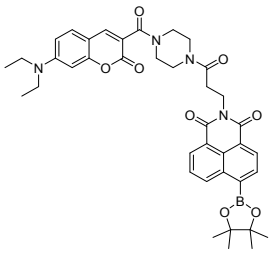
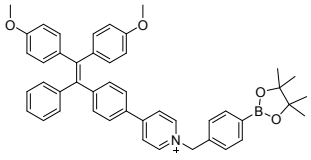
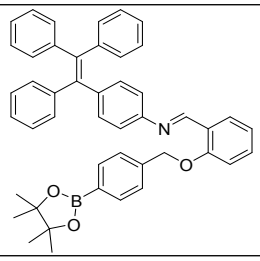
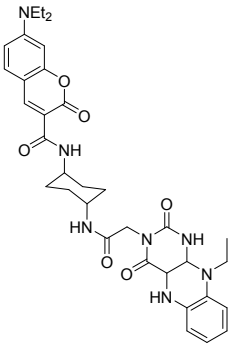
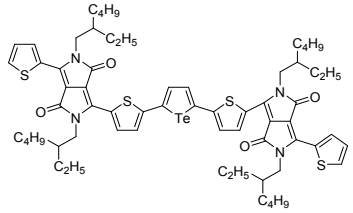


Figure S23 HRMS(ESI$^+$) of compound **TPYS**.

Table S1. Comparison of detection performance for H_2O_2 by our work and those reported in literature

Structure	Strategy	Limit of detection	Reference
	Fluorescence "Turn on" at 580 nm, specific lipid droplet (LD)-targeting,	0.083 μM for H_2O_2	This work
	Fluorescence "Turn on" at 500 nm	0.52 μM	1
	Ratiometric fluorescence, mitochondria imaging	4.6 μM	2
	Ratiometric fluorescence	0.42 μM	3
	Fluorescence "Turn on" at 500 nm	200 μM	4
	Emission red-shifted from 682 to 724 nm	0.2 mM	5
	Emission red-shifted from 485 to 558 nm	0.28 μM	6
	Fluorescence "Turn on" at 510 nm	180.0 nM for H_2O_2	7

	Fluorescence “Turn on” at 540 nm	100 nM for H_2O_2	8
	Emission red-shifted from 475 nm to 520 nm.	—	9
	Emission red-shifted from 565 nm to 579 nm.	6 μM	10

References

[1] Zhang, W.; Liu, W.; Li, P.; Huang, F.; Wang, H.; Tang, B. *Anal. Chem.* **2015**, 87, 9825-9828.

[2] Masanta, G. C. Heo, H. C. Lim, S. Bae, S. K. Cho, B. R. Kim, H. M. *Chem. Commun*. **2012**, 48, 3518–3520

[3] Hou, J. L.; Qian, M.; Zhao, H. H.; Li, Y. C.; Liao, Y. F.; Han, G. F.; Xu, Z. L.; Wang, F.; Song, Y. G.; Liu, Y. P. *Anal. Chim. Acta* **2018**, 1024, 169-176.

[4] Chen, Y. Z.; Shi, X. M.; Lu, Z. L.; Wang, X. F.; Wang, Z. *Anal. Chem.* **2017**, 89, 5278-5284.

[5] Xu, J. J.; Zhai, J. Y.; Xu, Y. M.; Zhu, J. W.; Qin, Y.; Jiang, D. C. *Analyst* **2016**, 141, 2380–2383.

[6] Chena, J. Q. Xue S. F.; Chen, Z. H.; Zhang, S. Q. Shi, G. Y. Zhang, M. *Biosens. Bioelectron.* **2018**, 100, 526–532.

[7] Hu, F. Huang, Y. Zhang, G. Zhao, R. Zhang, D. *Tetrahedron Lett.* 55 (2014) 1471–1474.

[8] Song, Z. Kwok, R. T. K. Ding, D. Nie, H. Lam, J. W. Y. Liu, B. Tang, B. Z. *Chem. Commun.* 2016, 52, 10076-10079.

[9] Kaur, A. Haghighatbin, M. A. Hogan, C. F. New, E. J. *Chem. Commun.* **2015**, 51, 10510–10513.

[10] Kaur, M. Yang, D. Choi, S. K. Cho, M. J. Choi, D. H. *Dyes Pigm.* **2014**, 100, 118–126.