

Electronic Supplementary Information

An AIE-active fluorescence turn-on bioprobe mediated by hydrogen-bonding interaction for highly sensitive detection of hydrogen peroxide and glucose

Zhegang Song, ‡^a Ryan T. K. Kwok, ‡^a Dan Ding,^b Han Nie,^a Jacky W. Y. Lam,^a Bin Liu^c

and Ben Zhong Tang*^a

^a *Department of Chemistry, Hong Kong Branch of Chinese National Engineering Research Center for Tissue Restoration and Reconstruction, Institute for Advanced Study, Division of Biomedical Engineering, Division of Life Science, State Key Laboratory of Molecular Neuroscience and Institute of Molecular Functional Materials, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China. Email: tangbenz@ust.hk.*

^b *State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Bioactive Materials, Ministry of Education and College of Life Sciences, Nankai University, Tianjin 300071, China*

^c *Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117585.*

‡ These authors contributed equally to this work.

*Corresponding author: tangbenz@ust.hk (B.Z.T)

Table of Contents

Experimental section	S3-S4
Scheme S1 Synthetic route to TPE-HPro and 1	S4
Fig. S1 ¹ H NMR and ¹³ C NMR spectra of TPE-HPro	S5
Fig. S2 High resolution mass spectrum of TPE-HPro	S5
Fig. S3 ¹ H NMR and ¹³ C NMR spectra of compound 1	S6
Fig. S4 High resolution mass spectrum of compound 1	S6
Fig. S5 Optimization of pH for H ₂ O ₂ detection	S7
Fig. S6 PL detection of H ₂ O ₂ at pH = 9.0	S7
Fig. S7 ¹ H NMR spectrum of TPE-HPro after reaction with H ₂ O ₂ at pH 10.0	S8
Fig. S8 HRMS spectrum of TPE-HPro after reaction with H ₂ O ₂ at pH 10.0	S8
Fig. S9 Selectivity test of TPE-HPro for ROS detection	S9
Fig. S10 PL spectra for glucose assay	S9
Fig. S11 PL spectra of TPE-HPro supplemented with GOx incubated in Tris-HCl buffer (pH 10.0), buffer mixture with 1% FBS and buffer mixture with 1% FBS and glucose for 0 h and 1 h. Solution concentration: 40 μM; incubation temperature: 37 °C; [GOx]: 2 U/mL; [Glu]: 1 mM.....	S10

Experimental Section

Materials and instrumentation

All chemicals and reagents were commercially available and used as received without further purification. Acetonitrile (ACN) and diethyl ether (Et₂O) purified by Innovative Solvent Purification System. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen immediately prior to use. Ethanol (EtOH) was dried by anhydrous magnesium sulphate and filtered prior to use. Benzophenone, 4-aminobenzophenone, salicylaldehyde, zinc powder, pinacol, benzoyl peroxide (BPO) and N-bromosuccinimide (NBS) were purchased from Sigma-Aldrich. Benzaldehyde, anhydrous magnesium sulphate, D(+)-glucose, potassium carbonate were obtained from Merck Chemicals Co. Hydrogen peroxide, glucose oxidase (GOx) from *Aspergillus niger* and saccharides used in the selectivity test were purchased from Sigma-Aldrich. Fetal bovine serum (FBS) was purchased from Gibco. Tris-HCl buffer (pH = 10.0, 10 mM) were prepared with pure water from a Millipore filtration system. Buffers (pH = 7–13) were commercially available from Aldrich. ¹H and ¹³C spectra were measured on a Bruker ARX 400 NMR spectrometer using CDCl₃ and CD₂Cl₂ as solvents and tetramethylsilane (TMS; δ = 0 ppm) was chosen as internal reference. UV absorption spectra and photoluminescence (PL) spectra were recorded on a Biochrom spectrophotometer and a Perkin-Elmer LS 55 spectrofluorometer, respectively. High-resolution mass spectra (HRMS) were obtained on a GCT Premier CAB 048 mass spectrometer operated in MALDI-TOF mode. Fluorescence images were taken on an FL microscope (BX41 Microscope).

Synthesis of *N*-2-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)benzylidene)-4-(1,2,2-triphenylvinyl)aniline (TPE-HPro)

4-(1,2,2-triphenylvinyl)aniline (**2**) and 2-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)benzaldehyde (**3**) was synthesized according to our previous report. Compound **2** (0.5 mmol, 174 mg) and compound **3** (0.55 mmol, 186 mg) were dissolved in anhydrous ethanol and heated to reflux under N₂ atmosphere for 12h. After the resulting reaction mixture cooled to room temperature, the precipitates were collected by suction filtration and washed by ethanol and hexane for three times. The target product was obtained as pale yellow powder after dried in vacuum. Yield = 43%. ¹H NMR (400 MHz, CDCl₃): δ (TMS, ppm) 8.95 (s, 1H), 8.13–8.11 (d, 1H), 7.81–7.79 (d, 2H), 7.46–7.40 (m, 3H), 7.17–6.96 (m, 21H), 5.21 (s, 2H), 1.35 (s, 12H). ¹³C NMR (100 MHz, CDCl₃): δ (TMS, ppm) 157.98, 154.89, 150.06, 143.18, 140.74, 140.26, 139.98, 139.15, 134.27, 131.92, 131.63, 131.39, 130.63, 130.56, 127.50, 127.09, 126.99, 126.95, 126.87, 126.83, 126.73, 125.81, 125.75, 125.67, 125.37, 124.52, 120.45, 119.70, 113.28, 112.07, 83.22, 69.63, 24.02. HRMS (MALDI-TOF), *m/z* calcd. for C₄₆H₄₂BNO₃: 667.3258. Found 667.3268 (M⁺).

Synthesis of 2-(((4-(1,2,2-triphenylvinyl)phenyl)imino)methyl)phenol (**1**)

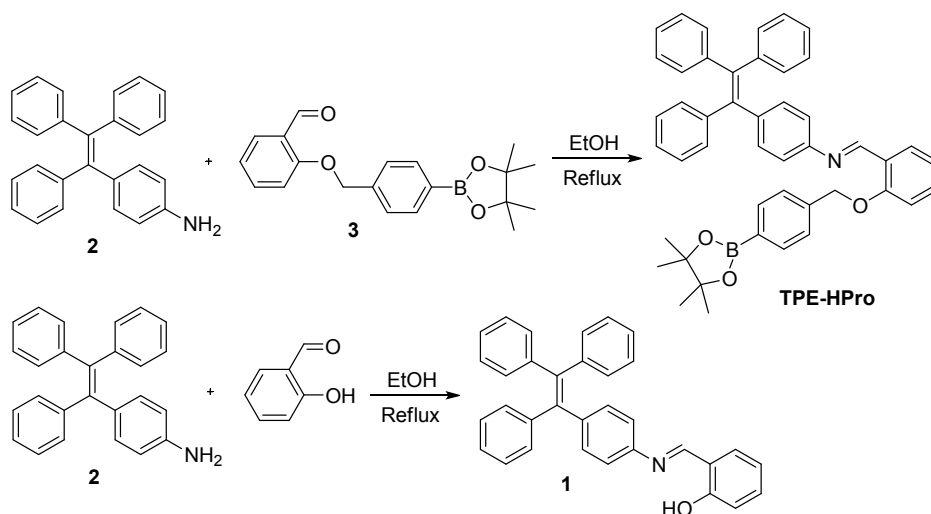
Compound **2** (0.4 mmol, 139 mg) and salicylaldehyde (0.44 mmol, 54 mg) were dissolved in 10 mL anhydrous ethanol and the reaction mixture was heated to reflux overnight. After the resulting solution cooled to room temperature, the precipitates were collected by suction filtration and washed with ethanol and hexane for three times. The desired product was obtained as yellow powder after dried in vacuum. Yield = 83%. ¹H NMR (400 MHz, CDCl₃): δ (TMS, ppm) 13.29 (s, 1H), 8.59 (s, 1H), 7.36 (t, 2H), 7.15–6.92 (m, 21H). ¹³C NMR (100 MHz, CDCl₃): δ (TMS, ppm) 161.30, 160.51, 145.65, 142.98, 142.90, 142.10, 140.76, 139.51, 132.39, 131.80, 131.52, 130.74, 130.70, 130.68, 127.20, 127.12, 127.03, 125.99, 125.95, 125.88, 119.90, 118.60. HRMS (MALDI-TOF), *m/z* calcd. for C₃₃H₂₅NO: 451.1936. Found 451.2018 (M⁺).

Fluorescence detection of hydrogen peroxide and dynamic monitoring of reaction process

A 40 μM TPE-HPro probe solution was prepared by mixing 200 μL of the stock solution (400 μM , in ACN) with 1.8 mL of Tris-HCl buffer (10 mM, pH 10.0). Different amounts of H_2O_2 stock solutions (1 M) were added into the Tris-HCl buffer solution to yield final concentrations of H_2O_2 from 0 to 200 μM . PL measurements were carried out after the prepared solutions upon incubation at 37 $^\circ\text{C}$ for 40 min. The experimental conditions for other PL measurements (pH optimization, selectivity test, etc.) were the same unless otherwise specified. The reaction process was monitored by the fluorescence spectral measurements which scanned at intervals of 2 min. Various ROS were prepared according to previous report.

Fluorescence detection of glucose

The probe solutions for fluorescence detection of glucose was prepared by blending 1.78 mL of Tris-HCl buffer (10 mM, pH 10.0), 20 μL GOx stock solutions (200 U/mL) and 200 μL TPE-HPro stock solution (400 μM , in ACN). Then different amounts of glucose were added to pre-blended solutions of probes and GOx and carefully mixed, with the concentration of glucose ranging from 0 to 400 μM . PL measurements were carried out after the prepared solutions upon incubation at 37 $^\circ\text{C}$ for 1 h. For the experiments of glucose detection with FBS, all the conditions are exactly the same as those in buffers except for extra addition of 20 μL FBS (1%).



Scheme S1 Synthetic route to TPE-HPro and compound 1.

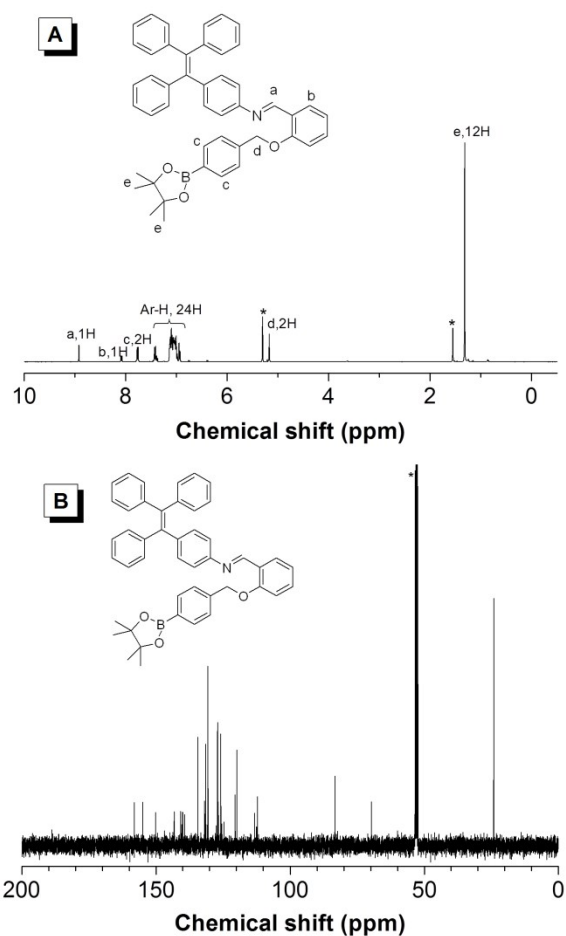


Fig. S1 ^1H NMR (A) and ^{13}C NMR (B) of TPE-HPro in CD_2Cl_2 .

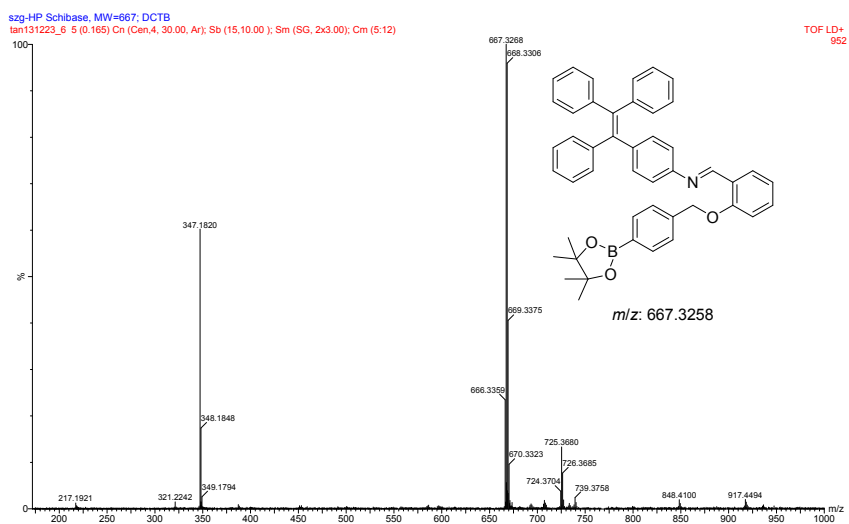


Fig. S2 HRMS spectrum of TPE-HPro.

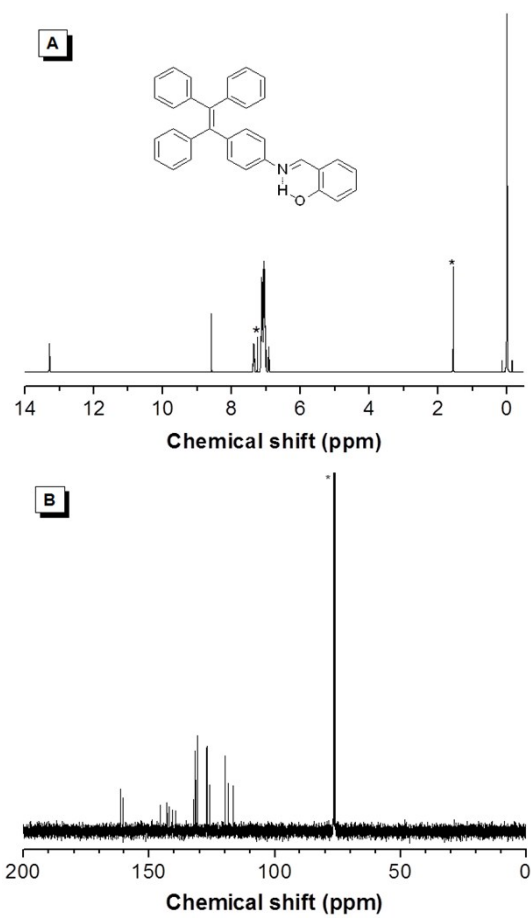


Fig. S3 ^1H NMR (A) and ^{13}C NMR (B) of **1** in CDCl_3 .

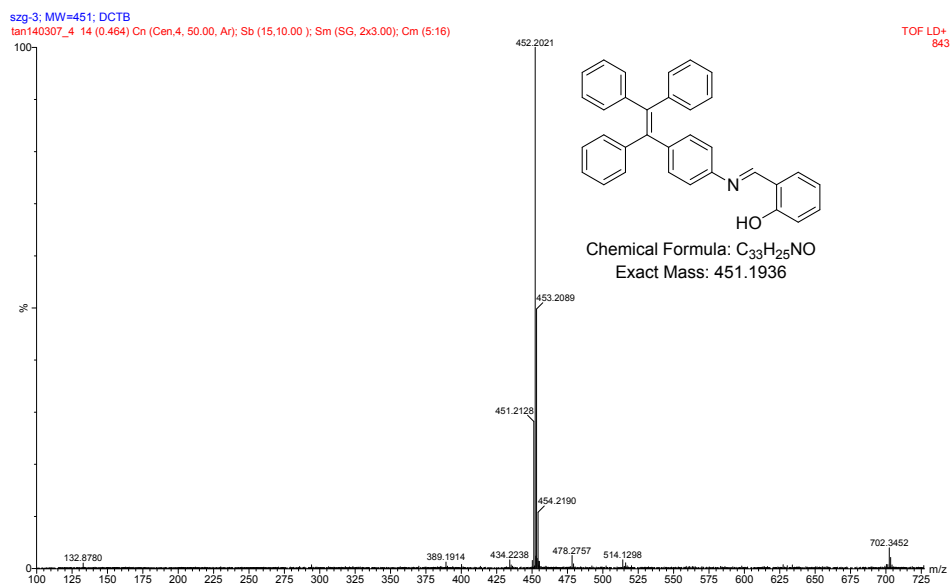


Fig. S4 HRMS spectrum of **1**.

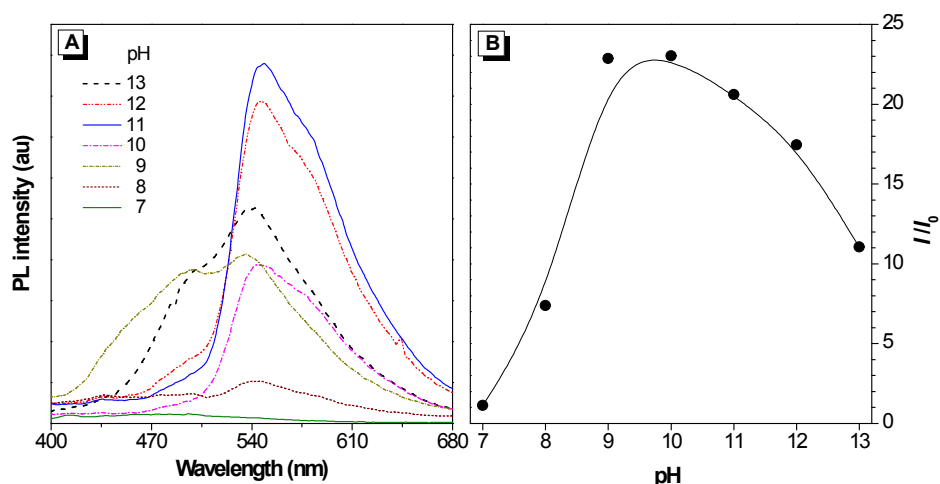


Fig. S5 (A) PL spectra of TPE-HPro in the acetonitrile/water mixtures (1 : 9 v/v) with different pH incubated with H₂O₂ (150 μM) for 60 min. (B) Plot of relative PL intensity (I/I_0) versus pH of acetonitrile/water mixture. I and I_0 are the PL intensities after incubation with and without H₂O₂ at the specific pH value. Solution concentration: 40 μM, excitation wavelength: 373 nm.

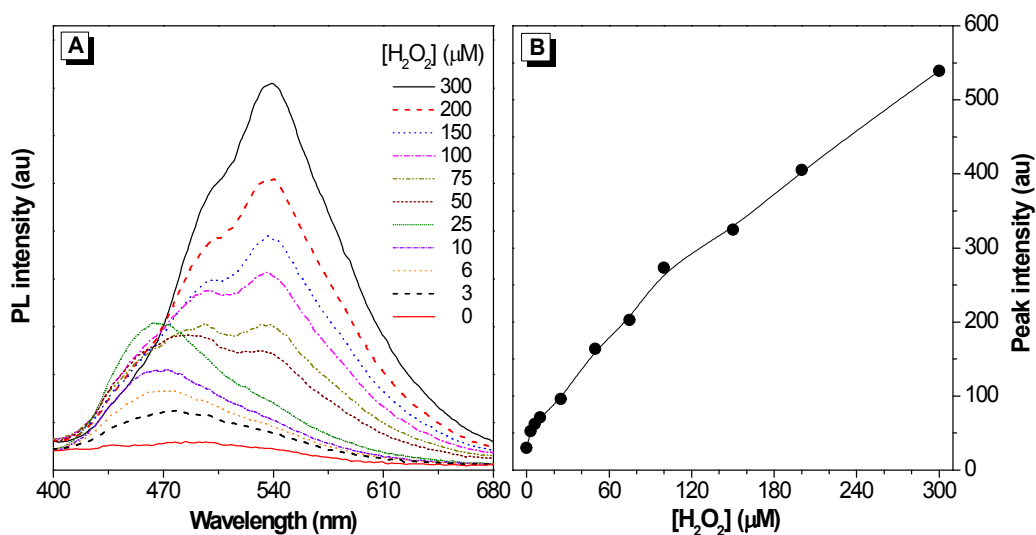


Fig. S6 (A) PL spectra of TPE-HPro in acetonitrile/buffer mixture (1 : 9, v/v; pH 9.0) incubated with different concentrations of H₂O₂ for 40 min at 37 °C. Solution concentration: 40 μM; excitation wavelength: 373 nm. (B) Plot of PL intensity at 540 nm versus the H₂O₂ concentration.

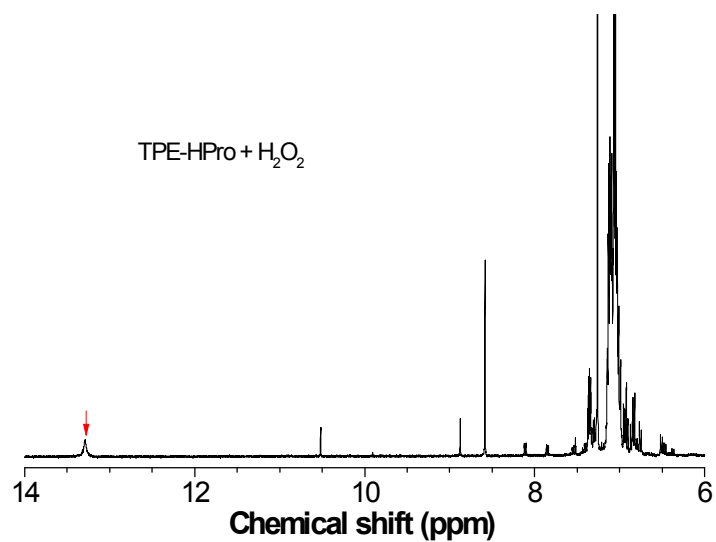


Fig. S7 ¹H NMR spectrum of TPE-HPro after reaction with H₂O₂ at pH 10.0.

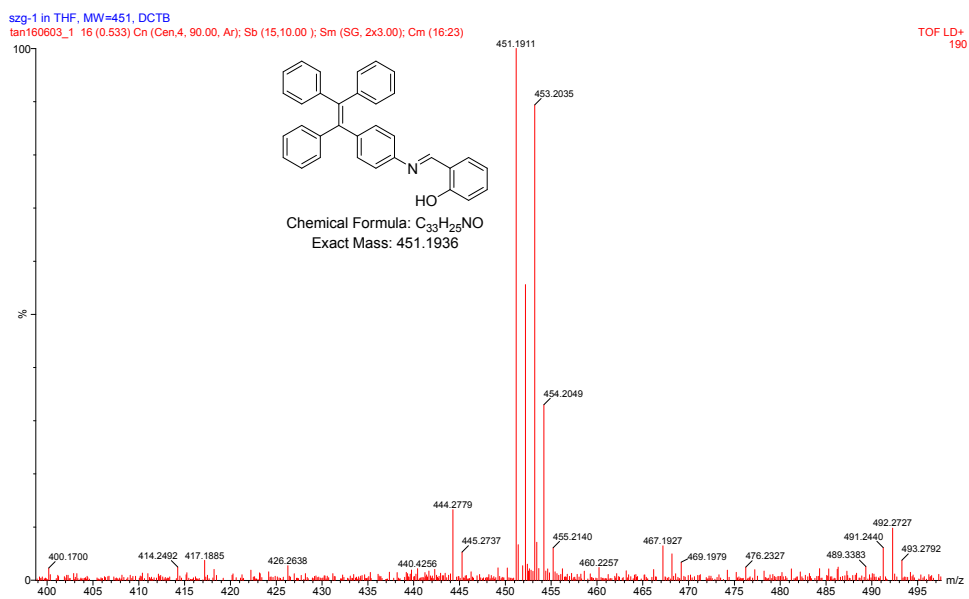


Fig. S8 HRMS spectrum of TPE-HPro after reaction with H₂O₂ at pH 10.0.

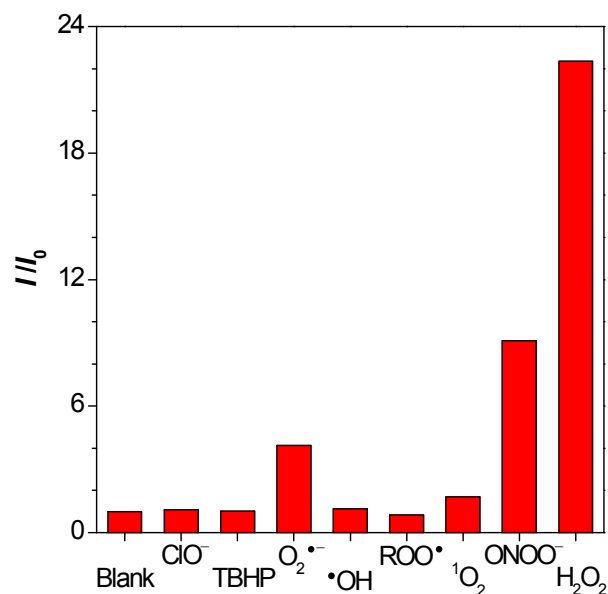


Fig. S9 PL response of TPE-HPro in buffer solution (10 mM, pH = 10.0) buffer solution to different reactive oxygen species. [ClO^-] = 100 μM , [TBHP] = 100 μM , [$\text{O}_2^{\bullet-}$] = 100 μM , $\bullet\text{OH}$: 20 mg FeSO_4 in 0.1 M H_2O_2 , [$\text{ROO}\bullet$] = 100 μM , [$^1\text{O}_2$] = 100 μM , [ONOO^-] = 100 μM , [H_2O_2] = 100 μM).

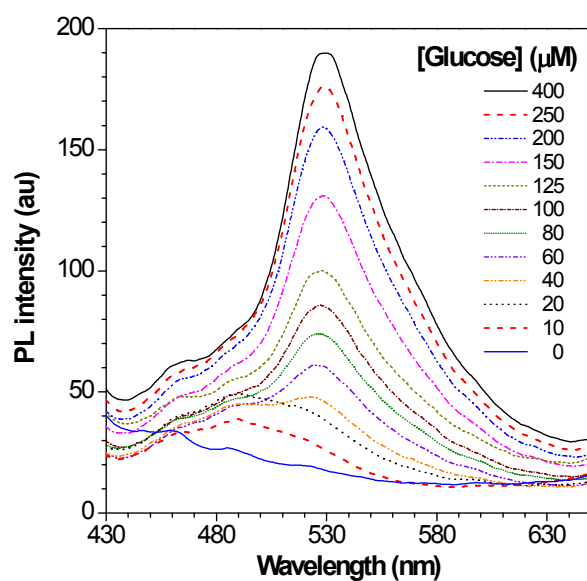


Fig. S10 PL spectra of TPE-HPro in acetonitrile/buffer mixture (1 : 9, v/v; pH 10.0) incubated with GOx (2 U/mL) and different concentrations of glucose for 1h at 37 °C.

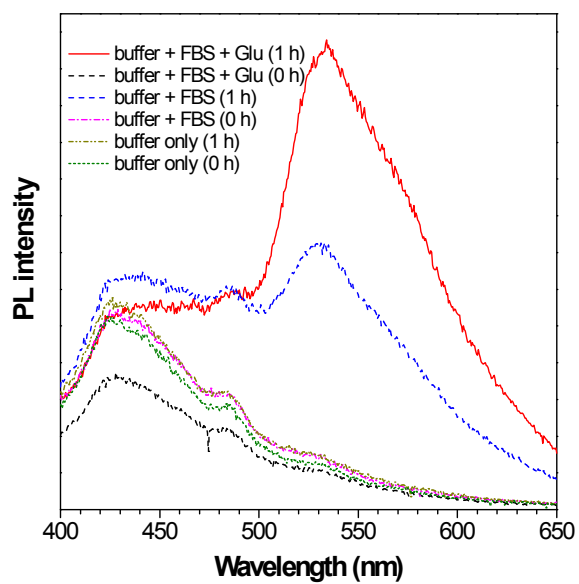


Fig. S11 PL spectra of TPE-HPro supplemented with GOx incubated in Tris-HCl buffer (pH 10.0), buffer mixture with 1% FBS and buffer mixture with 1% FBS and glucose for 0 h and 1 h. Solution concentration: 40 μ M; incubation temperature: 37 oC; [GOx]: 2 U/mL; [Glu]: 1 mM.