Electronic Supplementary Information for

# Electrochemiluminescent Detection of Glucose in Human Serum by BODIPY-Based Chemodosimeters for Hydrogen Peroxide using accelerated self-immolation of boronates

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## Contents

# Experimental

## 1. Materials

All materials were used as received from commercial suppliers including Sigma-Aldrich, Tokyo Chemical Industry (TCI), and Acros Organics without further purification. All samples for the measurements were freshly prepared before experiments from corresponding stock solutions (2 mM probe in acetonitrile and 40 mM $H_2O_2$ in water). Photophysical experiments were conducted using V-730 spectrophotometer (JASCO, Tokyo, Japan) and FP-8300 spectrofluorometer (JASCO, Tokyo, Japan). Density functional theory (DFT) calculations were carried out over B3LYP/6-31+G(d) level and CPCM for acetonitrile with Gaussian '09 program (Gaussian, Inc.).

## 2. ECL experiments

The ECL intensity was measured by an H-6780 photomultiplier tube (PMT) module (Hamamatsu Photonics K. K., Tokyo, Japan) on which a home-made ECL cell was directly mounted. All experimental conditions for PL were maintained for ECL experiments except adding 0.1 M tetrabutylammonium perchlorate (TBAP) as an electrolyte and using tri-*n*-propylamine (TPrA) pre-containing potassium phosphate buffer (100 mM, pH 8).

The Pt working electrode was polished with 0.05 mm alumina (Buehler, IL, USA) on a felt pad and sonicated in a 1:1 (v/v) mixture of deionized water and absolute ethanol for 10 min. The electrode was fully dried with ultra-pure $N_2$ gas. All ECL experiments were simultaneously conducted with cyclic voltammetry (CV) by CH Instruments 650B Electrochemical Analyzer (CH Instruments, Inc., TX, USA) using a three-electrode setup. Pt was employed as working and counter electrodes and Ag/AgCl as a reference electrode. Otherwise mentioned, all ECL signals were recorded in the potential range of -0.1 and 1.9 V at a scan rate of 1.0 V/s. All ECL data was the average of the first-scan values from three repeated experiments.

3. pH dependency

50 mM of citrate/potassium phosphate buffer solutions of pH 5.0, 6.0, 7.0, 7.4, 7.8, 8.0, and 9.0 were prepared at 25°C. The total amount of sample in a quartz cuvette cell was 2.0 mL containing 10 μM probe and 200 μM $H_2O_2$ in a 1:1 (v/v) mixture of acetonitrile and phosphate buffer. The maximum fluorescence intensity of each sample was recorded for 2 h at excitation wavelength ($\lambda_{ex}$) of 625 nm.

4. Sensitivity and selectivity

To a solution of 10 μM probe in a 1:1 (v/v) mixture of acetonitrile and potassium phosphate buffer (50 mM, pH 8) were added aliquots of micromolar concentrations of $H_2O_2$ (0-200 μM). Fluorescence and UV-vis spectra of the sample were recorded after incubation at 25°C. The limit of detection (LOD) was determined as three times the standard deviation of blank samples divided by the slope of the titration curve (3σ/m). The standard deviation of blank was obtained by at least 5 measurements of samples containing probe without an analyte. Maximum fluorescence intensity of probe was measured after reaction with 20 equivalent of reactive oxygen species (ROS) and reactive nitrogen species (RNS).

*tert*-Butyl hydroperoxide (TBHP, 70% solution in water) was purchased from ACROS. Hydroxy radical (·OH) and *tert*-butoxy radical (·OtBu) were produced via the Fenton reaction.[1] Superoxide ($O_2^{\cdot -}$) was generated from potassium superoxide ($KO_2$). Nitric oxide (·NO) was generated from sodium nitroprusside (SNP, Fluka). Hypochlorite ($ClO^-$), nitrite ($NO_2^-$) and nitrate ($NO_3^-$) were produced from aqueous solutions of their sodium salts, respectively. Peroxynitrite ($ONOO^-$) was synthesized as follows: 0.6 M $H_2O_2$ was mixed with 0.6 M $NaNO_2$ at 0°C. 0.9 M NaOH was added right after the mixture was acidified with 0.7 M HCl. Remaining $H_2O_2$ was removed with manganese dioxide ($MnO_2$). The $ONOO^-$ concentration was determined by Beer-Lambert law (ε = 1670 $cm^{-1}M^{-1}$ at 302 nm).

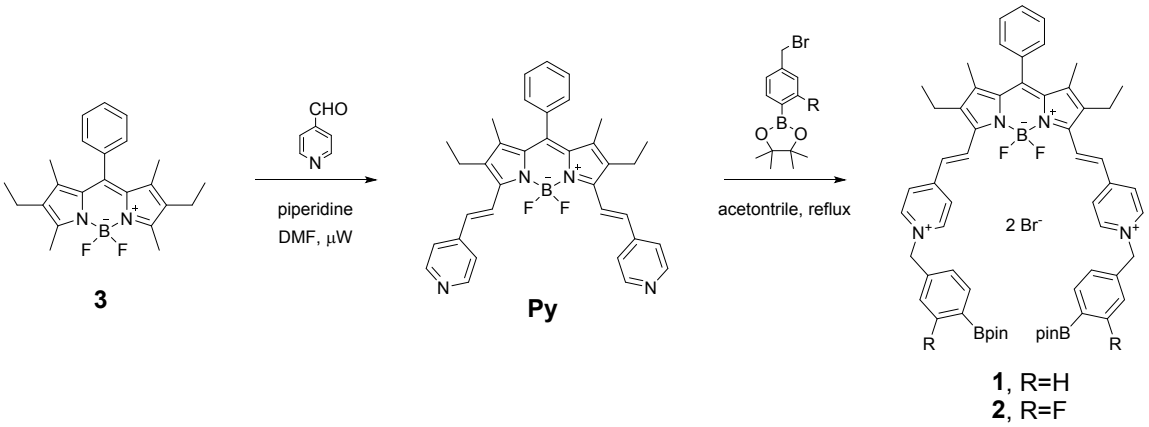
[1]K. Setsukinai, Y. Urano, K. Kakinuma, H. J. Majima and T. Nagano, *J. Biol. Chem.* 2003, **278**, 3170-3175.

5. Serum sample preparation

Human serum (from human male AB plasma, USA origin, sterile-filtered), GOx (from Aspergillus Niger), and D-(+)-glucose (Sigma Aldrich) were stored at -80℃ in small aliquots. The serum was deproteinized by centrifugation (13000 RPM, 2 minutes) after precipitation with acetonitrile. After acetonitrile was removed, the supernatant was diluted (×200) with a 1:1 (v/v) mixture of potassium phosphate buffer (50 mM, pH 8) and acetonitrile. 10 μM of probe, 6.5 U/mL of GOx, and 0–200 μM of D-(+)-glucose were added, and the mixture was incubated at 37℃ for 30 min.

## Synthesis of probes

Thin layer chromatography (TLC) was performed on aluminum plates precoated with silica gel 60 $F_{254}$ (Merck, Germany). Flash column chromatography was conducted with SilicaFlash® P60 (230-400 mesh) from SILICYCLE (Canada). $^{1}H$ and $^{13}C$ NMR spectra were obtained using Bruker Avance DPX-300 (Germany), Agilent 400-MR DD2 Magnetic Resonance System (USA), and Varian 500 MHz NMR System (USA). **3** was prepared according to a previous report.[2]




**Scheme S1.** Synthetic procedure for **1** and **2**.

**Synthesis of Py**

Compound **3** (95 mg, 0.25 mmol) in dry N,N-dimethylformamide (5 ml) was mixed with 4-pyridine carboxaldehyde (108 mg, 1 mmol) and piperidine (188 mg, 1.7 mmol) in a 10 ml microwave tube. The tube was sealed and purged with $N_2$ for 15 min. The mixture was heated with stirring in a microwave oven at 100°C for 10 min. After the solvent was removed under low pressure, the purple crude product was diluted with ethyl acetate and washed with brine (×3). The organic layer was dried over sodium sulfate and concentrated. Dark blue solid (51 mg, 0.091 mmol) was obtained by silica gel column

[2] A. Coskun and E. U. Akkaya, *J. Am. Chem. Soc.*, 2005, **127**, 10464-10465.

chromatography (ethyl acetate:methanol = 10:1) and repeated recrystallization (dichloromethane/*n*-hexane). Yield = 40%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, J = 5.7 Hz, 4H), 7.91 (d, J = 16.8 Hz, 2H), 7.55 – 7.48 (m, J = 2.3 Hz, 3H), 7.45 (d, J = 5.7 Hz, 4H), 7.34 – 7.28 (m, 2H), 7.14 (d, J = 16.8 Hz, 2H), 2.59 (q, J = 7.2 Hz, 4H), 1.33 (s, 6H), 1.15 (t, J = 7.5 Hz, 6H); $^{13}$C NMR (100 MHz, CDCl3) δ 149.90, 149.53, 144.61, 140.80, 140.12, 135.31, 134.76, 133.77, 132.79, 129.32, 129.25, 128.15, 124.09, 121.33, 18.24, 13.98, 11.55; HRMS (FAB) m/z: $[M + H]^+$ calculated for $C_{35}H_{34}BF_2N_4$ 559.2845, observed 559.2851

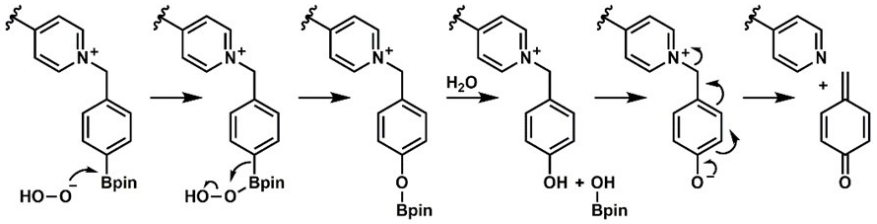
**Synthesis of 1**

Compound **Py** (20 mg, 35.8 μmol) in acetonitrile (10 mL) was mixed with 4-bromomethylphenylboronic acid pinacol ester (23.4 mg, 78.8 μmol) with stirring. The reaction mixture was refluxed overnight and monitored by thin-layer chromatography (ethyl acetate). The mixture was cooled and evaporated when the reaction was completed. Green powder (28 mg, 24.2 μmol) was obtained by recrystallization (×3, dichloromethane/n-hexane). Yield = 68%. $^{1}$H NMR (500 MHz, $CDCl_3$) δ 9.51 – 9.40 (m, 4H), 8.23 (d, *J* = 6.3 Hz, 4H), 8.18 (s, 2H), 7.81 (d, *J* = 7.6 Hz, 4H), 7.63 (d, *J* = 7.9 Hz, 4H), 7.59 – 7.51 (m, *J* = 3.0 Hz, 5H), 7.35 (s, 2H), 7.33 – 7.29 (m, 4H), 5.99 (s, 4H), 2.62 (q, 4H), 1.35 (s, 6H), 1.30 (s, 24H), 1.12 (t, *J* = 7.3 Hz, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 152.82, 148.55, 148.36, 144.80, 141.24, 141.23, 137.30, 135.95, 135.67, 135.65, 129.73, 129.59, 128.58, 128.58, 127.81, 126.08, 125.03, 83.99, 63.78, 30.84, 24.83, 20.39, 13.95, 11.60; HRMS (FAB) m/z: $[M-2Br]^+$ calculated for $C_{61}H_{69}B_3F_2N_4O_4$ 992.5566, observed 992.5593

**Synthesis of 2**

The same method was applied for the synthesis of **1** using compound **Py** (16.2 mg, 29 μmol) and 4-bromomethyl-2-fluorophenylboronic acid pinacol ester (22.85 mg, 72.5 μmol) as starting materials. Yield = 57 % (19.6 mg) $^{1}$H NMR (500 MHz, $CDCl_3$) δ 9.60 (d, 4H), 8.21 (d, *J* = 5.3 Hz, 4H), 8.16 (s, 2H), 7.78 – 7.73 (m, 2H), 7.55 (s, 4H), 7.52 (s, 2H), 7.35 (d, *J* = 10.8 Hz, 4H), 7.29 (d, *J* = 3.7 Hz, 2H), 6.07 (s, 4H), 2.60 (s, 4H), 1.34 (s, 6H), 1.30 (s, 24H), 1.13 – 1.09 (m, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 153.08, 152.36, 149.49, 148.28, 145.04, 141.33, 139.75, 138.39, 138.27, 137.32, 135.70, 134.46, 130.63, 129.65, 128.49, 127.76, 124.99, 124.67, 115.84, 84.10, 62.68, 29.71, 24.79, 18.26, 14.01, 11.64; HRMS (FAB) m/z: $[M-2Br]^+$ calculated for $C_{61}H_{67}B_3F_4N_4O_4$ 1028.5378, observed 1028.5404

## Proposed detection mechanism




**Scheme S2.** Proposed mechanism of the reaction unit in response to $H_2O_2$.

## Optimization for $H_2O_2$ detection

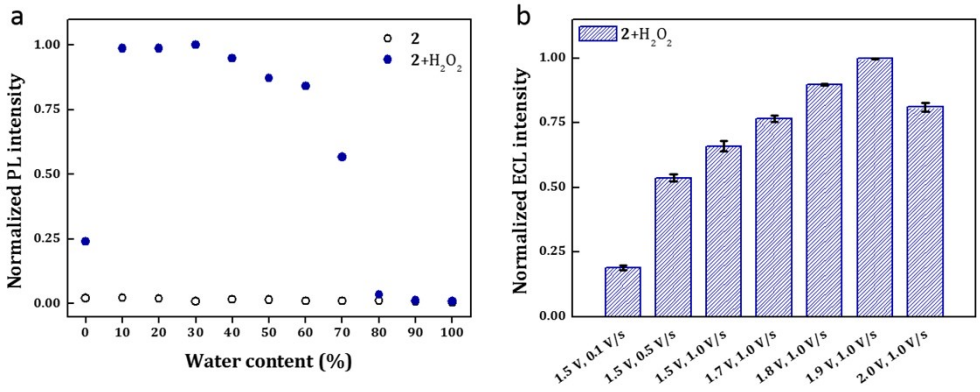



**Fig. S1.** Optimization of experimental conditions;

(a) Normalized PL intensity of probe **2** at 640 nm before (hollow circle) and after (blue circle) the reaction with $H_2O_2$ in a mixture of acetonitrile and 50 mM phosphate buffer (pH 8) with different water contents.

(b) Normalized ECL intensities of probe **2** + $H_2O_2$ mixtures under various applied potentials and scan rates. 0.1 M TBAP, 67.5 mM TPrA in a 1:1 (v/v) mixture of acetonitrile and 50 mM phosphate buffer (pH 8.0) at 25°C. Error bars represent the standard deviations obtained from three independent measurements.

## Electrochemical and computational studies

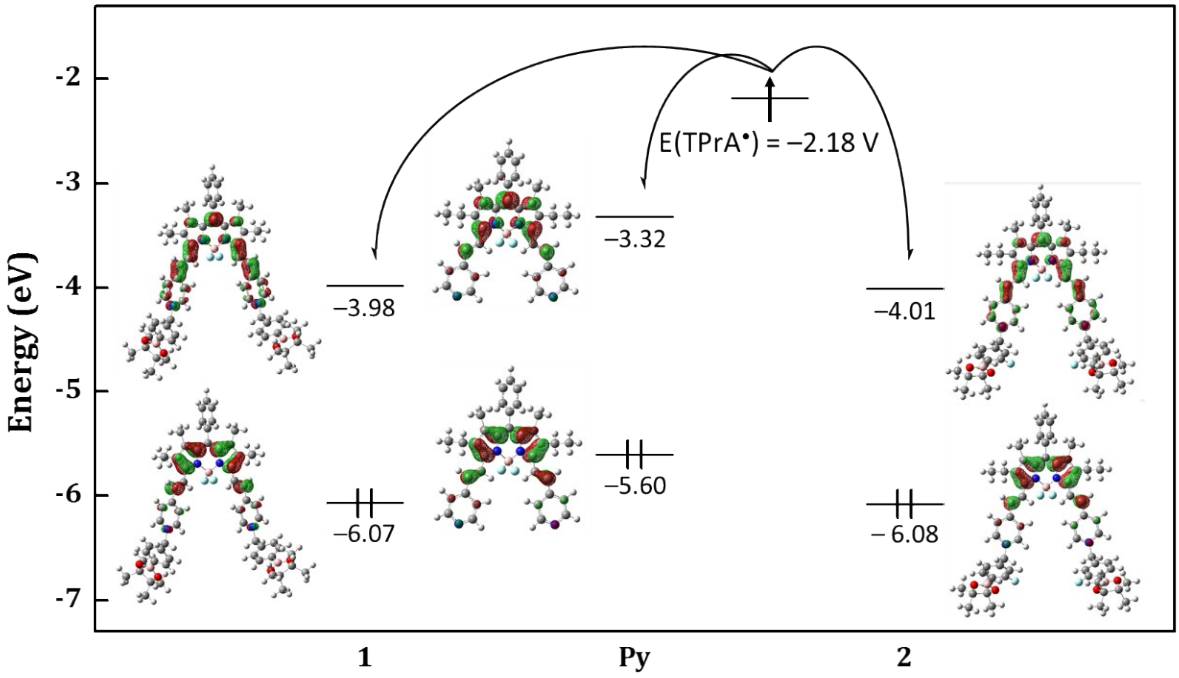



**Fig. S2.** Plausible electron transfer pathways (half arrows) between DFT-calculated SOMO of TPrA radical (TPrA•)[3] and HOMO/LUMOs of **1**, **2**, and **Py** (Gaussian '09, B3LYP/6-31G+(d) level).

[3] H. J. Kim, K –S. Lee, Y.–J. Jeon, I.–S. Shin, and J.–I. Hong, *Biosens. Bioelectron.* 2017, **91**, 15, 497-503.

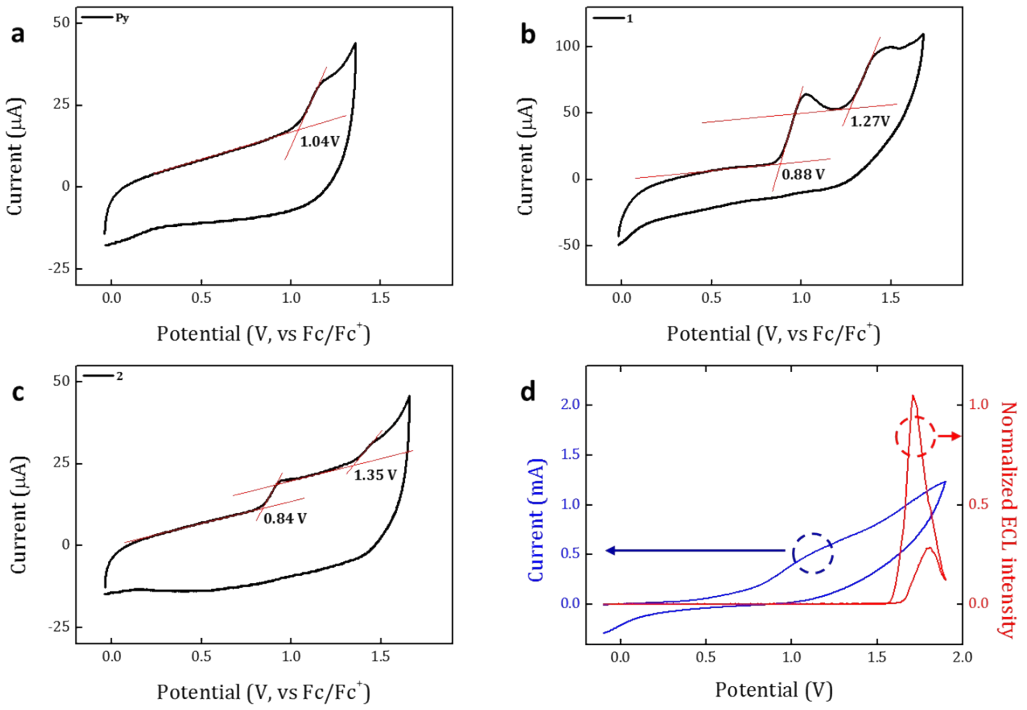



**Fig. S3**. Cyclic voltammograms of (a) **Py**, (b) **1**, and (c) **2** with 0.1 M TBAP in acetonitrile solution and (d) a representative current-ECL intensity trace of **2** (10 μM) + $H_2O_2$ (150 μM) with 0.1 M TBAP, 67.5 mM TPrA in a 1:1 (v/v) acetonitrile and phosphate buffer (50 mM, pH 8.0).

**Table S1.** Electrochemical properties of **Py**, **1**, and **2**.

| | $E_{ox}$(V vs Fc/Fc$^+$)[a] | HO/SOMO (eV)[b] | LUMO (eV)[c] | $E_{g,opt}$ (eV)[d] | $E_{g,cal}$ (eV)[e] |
|---|---|---|---|---|---|
| **Py** | 1.04 | –5.84 (HOMO) | –3.88 | 1.96 | 2.28 |
| **1** | 0.88, 1.27 | –5.68 (HOMO) | –3.88 | 1.80 | 2.09 |
| **2** | 0.84, 1.35 | –5.64 (HOMO) | –3.81 | 1.83 | 2.07 |
| **TPrA•** | –1.7 (vs SCE)[4] | –2.7 (SOMO) | n.d. | n.d. | n.d. |

[a]The onset oxidation potential determined by cyclic voltammetry. [b]HO/SOMO (eV) = –($E_{ox,\ vsFc/Fc+}$) – 4.80. [c]LUMO (eV) = HOMO + $E_{g,opt}$ (optical band gap). [d]The cross-point of normalized UV and PL spectra. [e]DFT calculations. n.d. = not determined

[4] R. Y. Lai and A. J. Bard, *J. Phys. Chem. A*, 2003, **107**, 3335-3340.

## pH dependency

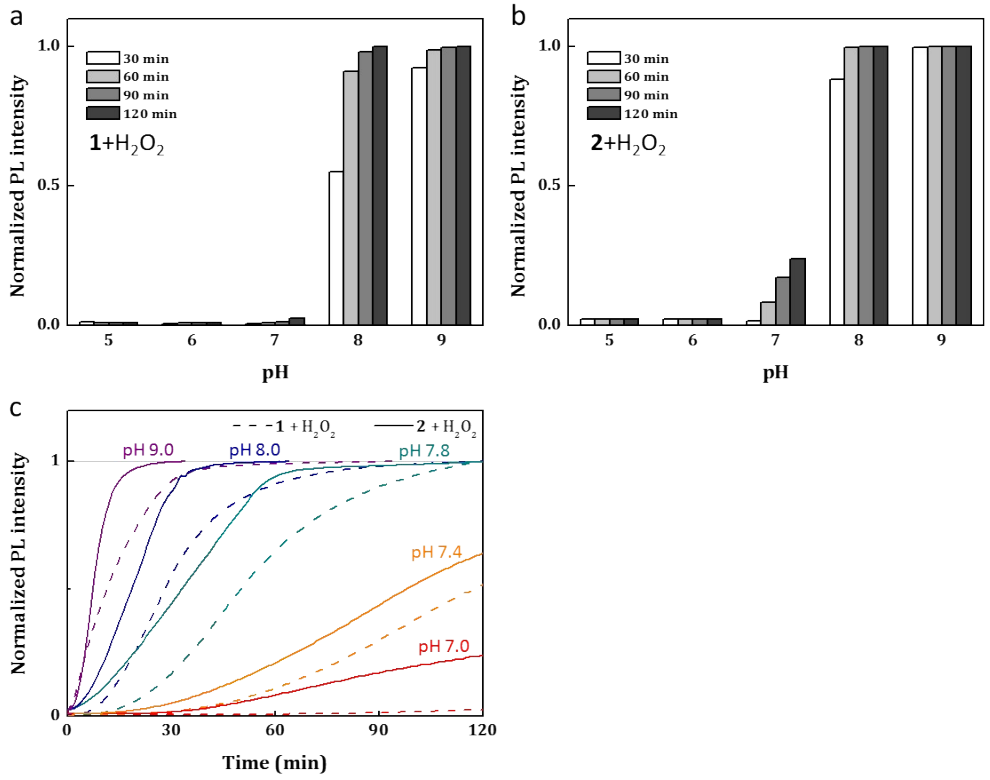



**Fig. S4.** Relative PL intensities at 640 nm of 10 μM **1** (a) and **2** (b) in the presence of 200 μM $H_2O_2$ measured with 30 min intervals at various pH conditions (pH 5.0 to 9.0) in a 1:1 (v/v) mixture of acetonitrile and citrate/potassium phosphate buffer (50 mM) at 25°C. (c) Normalized PL intensity changes at 640 nm of probes **1** (dashed line) and **2** (solid line) at different pH values (pH 7.0, red; pH 7.4, orange; pH 7.8, teal; pH 8.0, blue; pH 9.0, purple).

PL selectivity and sensitivity

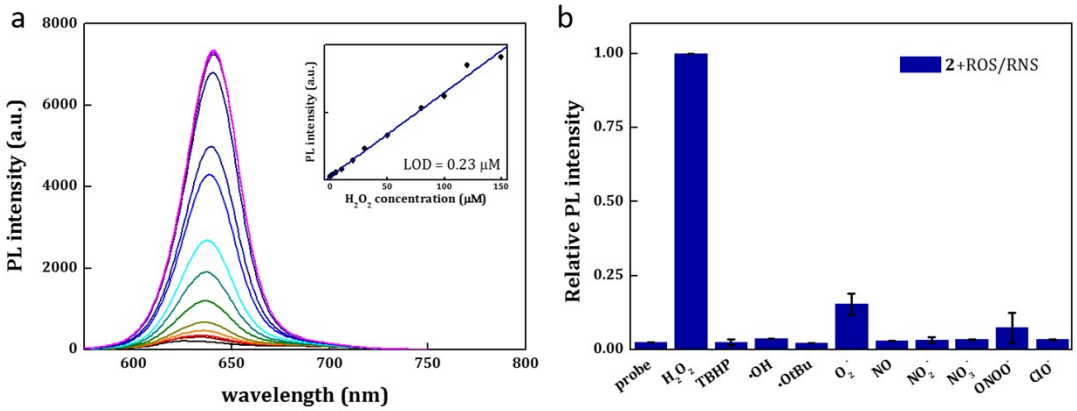



**Fig. S5.** (a) Fluorescence spectra of **2** with increasing $H_2O_2$ concentrations (0–400 μM). Inset: linear calibration curve of the fluorescence intensity at 640 nm of **2** for $H_2O_2$ concentration (0-150 μM). (b) Relative fluorescence intensity of **2** in the presence of various ROS and RNS (200 μM each). Error bars represent the standard deviations obtained from three independent measurements.

## Preliminary experiments for real sample monitoring

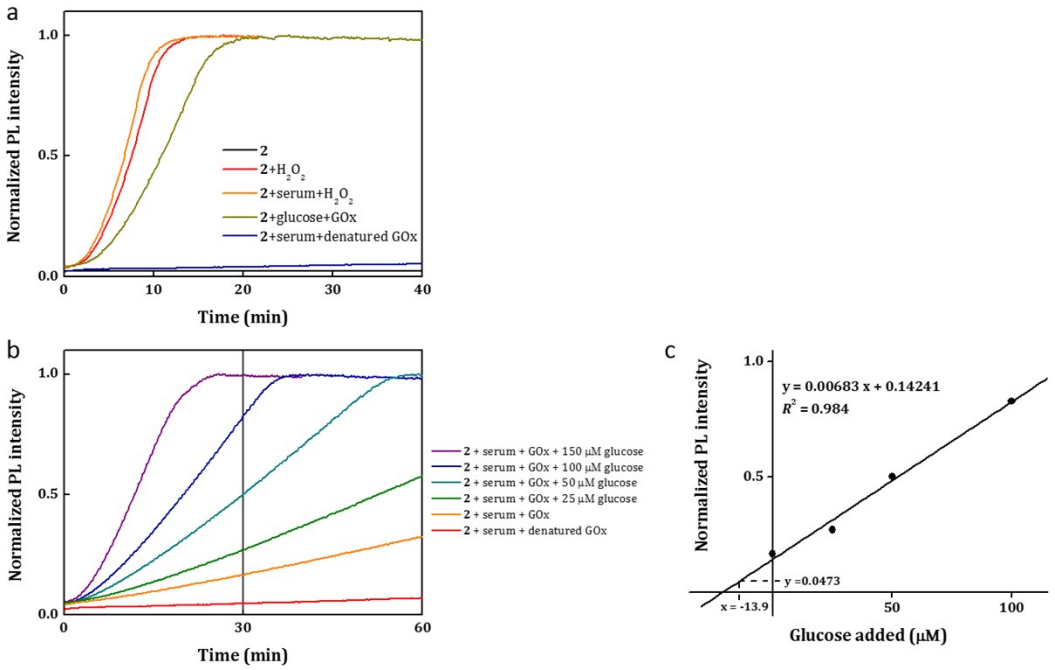
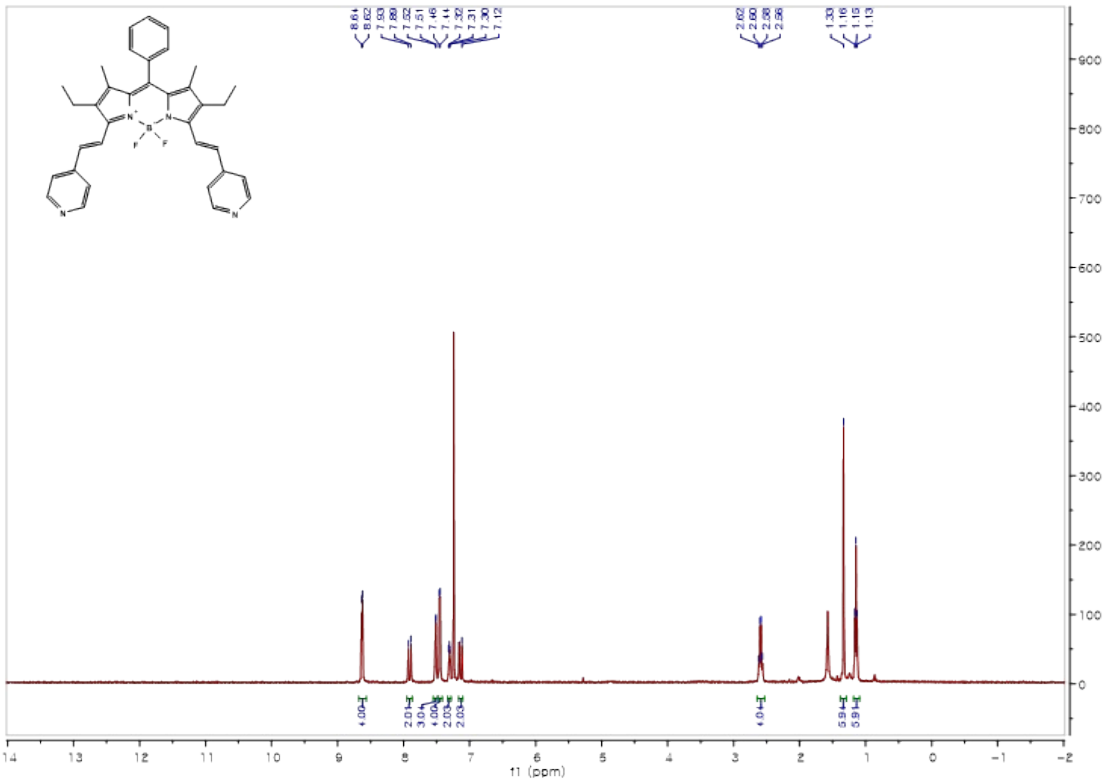


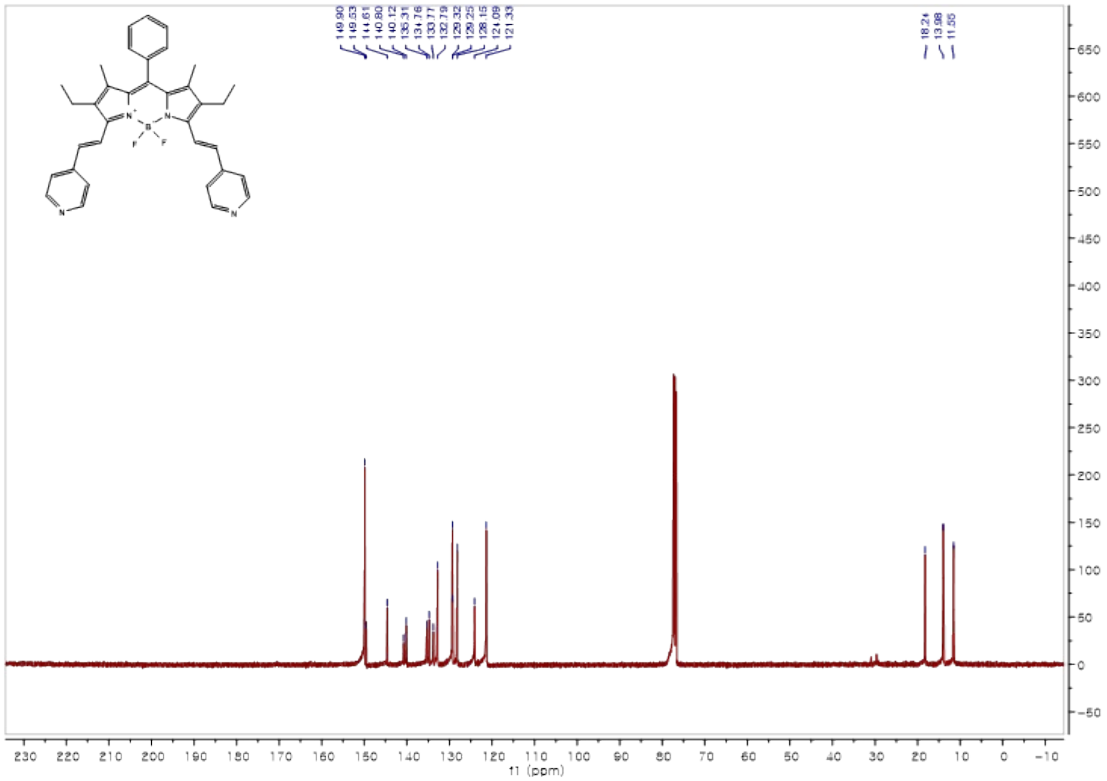

**Fig. S6.** (a) Normalized time-course PL intensity at 640 nm of **2** (10 μM) under various conditions. $H_2O_2$, 150 μM $H_2O_2$; serum, diluted serum (x200); glucose, 150 μM glucose; GOx, 6.5 U/mL GOx; denatured GOx, GOx pre-treated at 60 °C for 1 h.

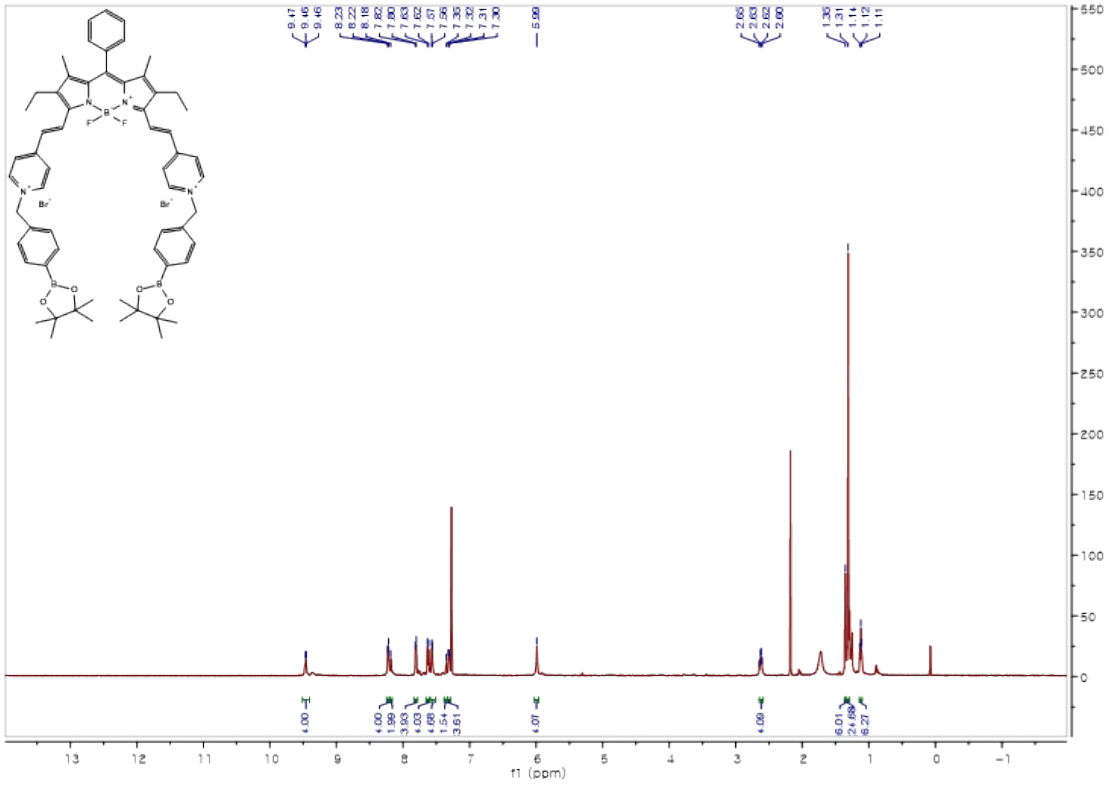
(b) Normalized time-course PL intensity at 640 nm of a mixture of **2** (10 μM), diluted serum (x200), and 6.5 U/mL GOx in the presence of various concentrations of glucose (0–150 μM).

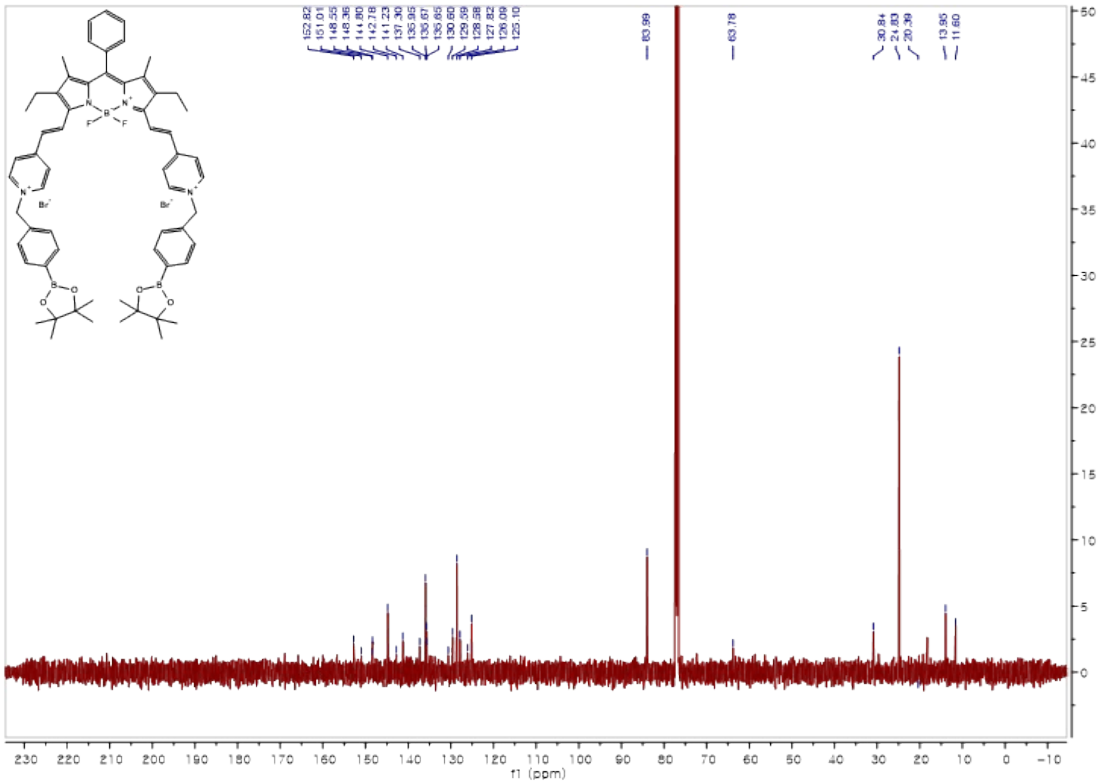
(c) Determination of glucose concentrations in human serum by the standard addition method. $\lambda_{ex}$ = 625 nm. 1:1 (v/v) mixture of acetonitrile and 50 mM phosphate buffer (pH 8.0) at 37°C.

Copies of NMR spectra




f1 (ppm)


f1 (ppm)