Supporting information

Integrated and insulated boronate-based fluorescent probes for the detection of hydrogen peroxide

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1. General Methods:

2-Naphthylboronic acid (1), 2-naphthol, D-fructose and hydrogen peroxide were purchased from Sigma-Aldrich Chemical Co. and used as received. Probe 2 was prepared according to described methods. The concentration of \( \text{H}_2\text{O}_2 \) was determined from the absorption at 240 nm with \( E = 43.6 \text{ M}^{-1}\text{cm}^{-1} \). The fluorescence titrations with \( \text{H}_2\text{O}_2 \) were carried out at 25 °C in a pH 7.20 buffer (52.1% methanol in water with KCl, 10 mM; KH₂PO₄, 2.752 mM; Na₂HPO₄, 2.757 mM) and pH 9.70 buffer (10.0% methanol in water with Na₂CO₃, 50 mM; NaHCO₃, 50 mM). Photographs were taken using a Canon camera and images were processed with Bitmap Image. Spectral data were processed in OriginPro 8.1 and non-linear fitted for Figure S3, Figure S6, and Figure S7.

2. Supplementary Spectral Data:

![Figure S1](image1.png) **Figure S1.** Plots of \( \frac{F_T}{F_0} \) for probe 1 (10 μM) with input of D-fructose (100 mM) and \( \text{H}_2\text{O}_2 \) (1 mM). Pink – Blank; Black – (+ \( \text{H}_2\text{O}_2 \)); Red – (+ D-fructose); Blue – (+ D-fructose + \( \text{H}_2\text{O}_2 \)). The mixture was incubated in pH 7.20 PBS buffer at 25 °C. Fluorescence intensities at 340 nm were measured with excitation at 290 nm.

![Figure S2](image2.png) **Figure S2.** Column spectral and truth table with D-fructose (100 mM) and \( \text{H}_2\text{O}_2 \) (0.10 mM) as inputs. The mixture was incubated in pH 9.70 NaCO₃/NaHCO₃ buffer at 25 °C. Fluorescence intensities at 340 nm were measured with excitation at 290 nm.
**Figure S3.** Time curve of fluorescent spectral changes for probe 1 (10 μM) in various concentrations of H$_2$O$_2$ (Red - 0.10 mM, Pink - 0.20 mM, Blue - 0.50 mM, Black - 1.00 mM). The mixture was incubated in pH 7.20 PBS buffer at 25 °C. Fluorescence intensities at 340 nm were measured with excitation at 290 nm.

**Figure S4.** Fluorescence spectral of 2-naphthol (10 μM) with excitation at 290 nm. The mixture was incubated in pH 7.20 PBS buffer and pH 9.70 NaCO$_3$/NaHCO$_3$ buffer at 25 °C.
**Figure S5.** (a) Fluorescent spectral changes for probe 2 (10 μM) – black line and 2-d-fructose complex – red line; (b) Time curve of fluorescent spectral changes for 2-d-fructose complex with addition of H₂O₂ (0.50 mM, 0, 1, 6, 12, 18, 30, 42, 54, 84, 114, 144, 174 min). The mixture was incubated in pH 7.20 PBS buffer at 25 °C with excitation at 370 nm.

**Figure S6.** (a) Time curve of fluorescent spectral changes for probe 2 (10 μM) upon adding H₂O₂ (0.50 mM, 0, 1, 6, 12, 18, 30, 42, 54, 84, 114, 144, 174 min); (b) Plots of F/F₀ upon addition of H₂O₂ (Red - 0.05 mM, Black - 0.10 mM, Blue - 0.50 mM). The mixture was incubated in pH 7.20 PBS buffer at 25 °C. Fluorescence intensities at 410 nm were measured with excitation at 370 nm.
Figure S7. Time curve of fluorescent spectral changes for 2-d-fructose complex in various concentrations of H$_2$O$_2$ (Black - 0.05 mM, Red - 0.50 mM). The mixture was incubated in pH 7.20 PBS buffer at 25 °C. Fluorescence intensities at 410 nm were measured with excitation at 370 nm.

Figure S8. Time curve of fluorescent spectral changes for probe 2 (10 μM) with addition of H$_2$O$_2$ (0.05 mM, 0, 0.5, 1, 6, 10 min). The mixture was incubated in pH 9.70 NaCO$_3$/NaHCO$_3$ buffer at 25 °C with excitation at 370 nm.
Figure S9. (a) Fluorescent spectral changes for probe 2 (10 μM) – black line and 2-D-fructose complex – blue line; (b) Time curve of fluorescent spectral changes for 2-D-fructose complex with addition of H₂O₂ (0.05 mM, 0, 1, 6, 12, 18, 30, 42, 54, 84, 114, 144, 174 min). The mixture was incubated in pH 9.70 NaCO₃/NaHCO₃ buffer at 25 °C with excitation at 370 nm.

Figure S10. Time curve of fluorescence intensity changes with probe 2 (10 μM) and D-fructose (100 mM) in aqueous H₂O₂ (0.05 mM). (Black circle – H₂O₂ in pH 9.70 buffer that was left for 1 min before adding probe 2, Red circle – H₂O₂ in pH 9.70 buffer that was left for 1 h before adding probe 2, Pink circle – H₂O₂ in pH 9.70 buffer that was left for 2 h before adding probe 2). The mixture was incubated in pH 9.70 NaCO₃/NaHCO₃ buffer at 25 °C. Fluorescence intensities at 418 nm were measured with excitation at 370 nm.

3. References