

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.¹ The concentration of H₂O₂ was determined from the absorption at 240 nm with $\epsilon = 43.6 \text{ M}^{-1}\text{cm}^{-1}$. The fluorescence titrations with H₂O₂ 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)² 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:

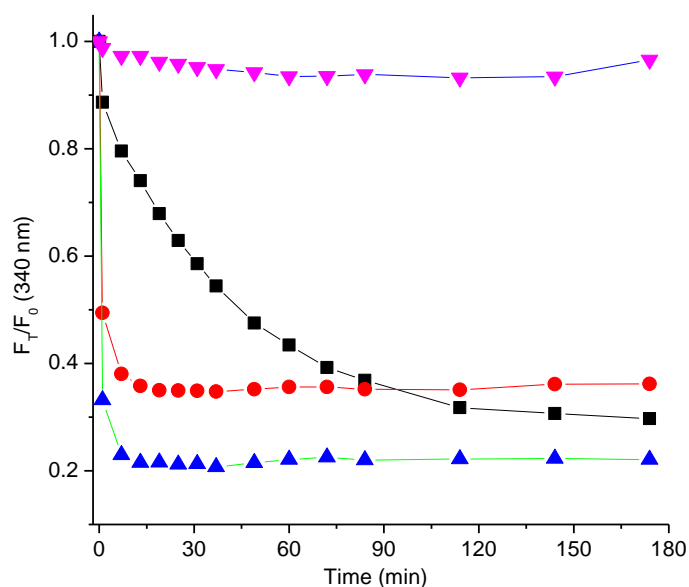


Figure S1. Plots of F_T/F_0 for probe **1** (10 μM) with input of D-fructose (100 mM) and H₂O₂ (1 mM). Pink – Blank; Black – (+ H₂O₂); Red – (+ D-fructose); Blue – (+ D-fructose + H₂O₂). The mixture was incubated in pH 7.20 PBS buffer at 25 °C. Fluorescence intensities at 340 nm were measured with excitation at 290 nm.

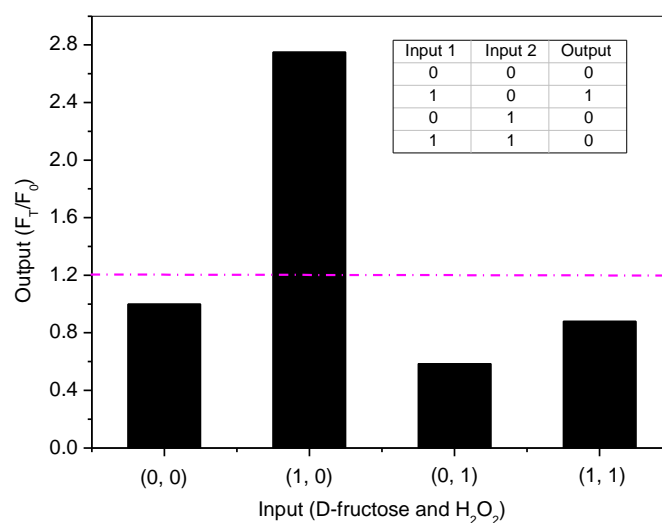


Figure S2. Column spectral and truth table with D-fructose (100 mM) and H₂O₂ (0.10 mM) as inputs. The mixture was incubated in pH 9.70 NaCO₃/NaHCO₃ buffer at 25 °C. Fluorescence intensities at 340 nm were measured with excitation at 290 nm.

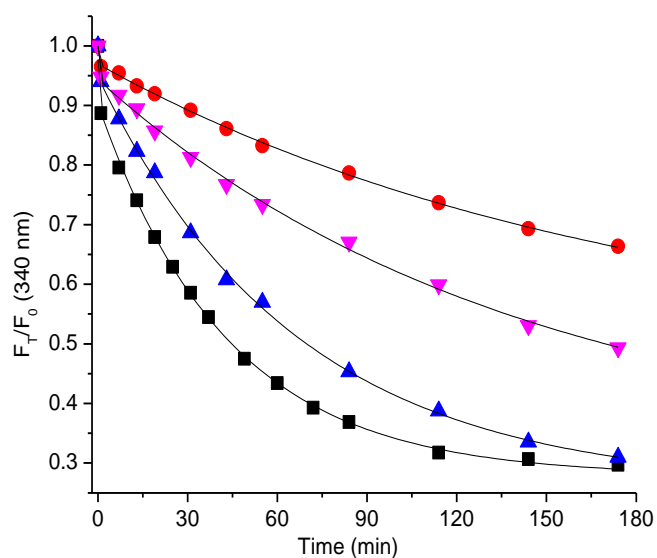


Figure S3. Time curve of fluorescent spectral changes for probe **1** (10 μ M) in various concentrations of H_2O_2 (Red - 0.10 mM, Pink - 0.20 mM, Blue - 0.50 mM, Black - 1.00 mM). The mixture was incubated in pH 7.20 PBS buffer at 25 $^\circ\text{C}$. Fluorescence intensities at 340 nm were measured with excitation at 290 nm.

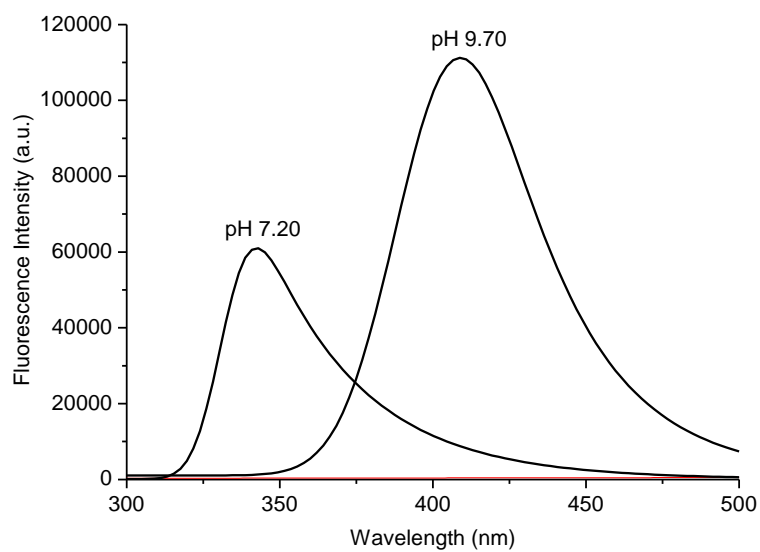


Figure S4. Fluorescence spectral of 2-naphthol (10 μ M) with excitation at 290 nm. The mixture was incubated in pH 7.20 PBS buffer and pH 9.70 $\text{NaCO}_3/\text{NaHCO}_3$ buffer at 25 $^\circ\text{C}$.

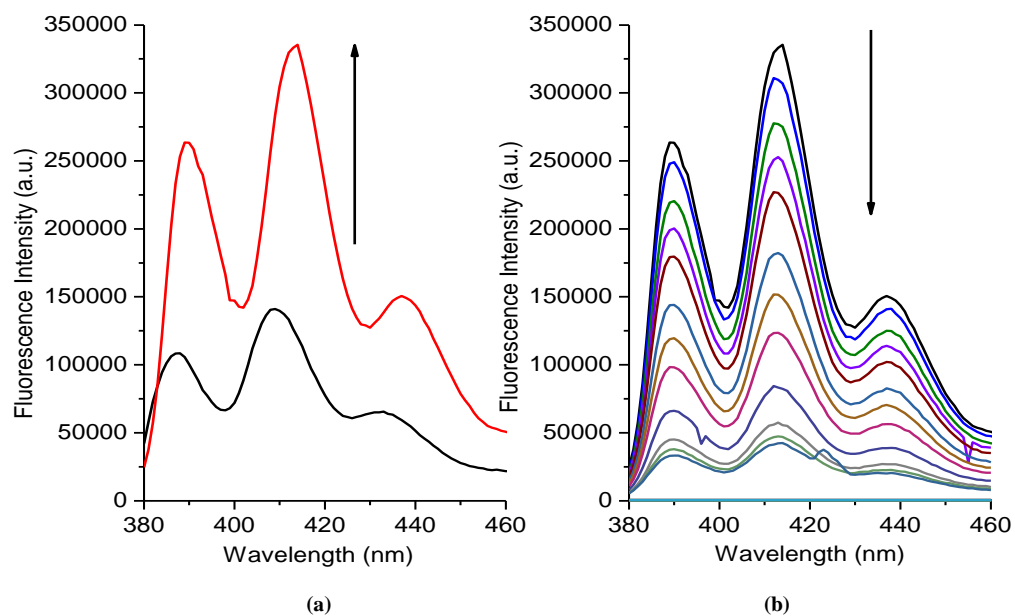


Figure S5. (a) Fluorescent spectral changes for probe **2** (10 μ M) – black line and 2-D-fructose complex – red line; (b) Time curve of fluorescent spectral changes for 2-D-fructose complex with addition of H₂O₂ (0.50 mM, 0, 1, 6, 12, 18, 30, 42, 54, 84, 114, 144, 174 min). The mixture was incubated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

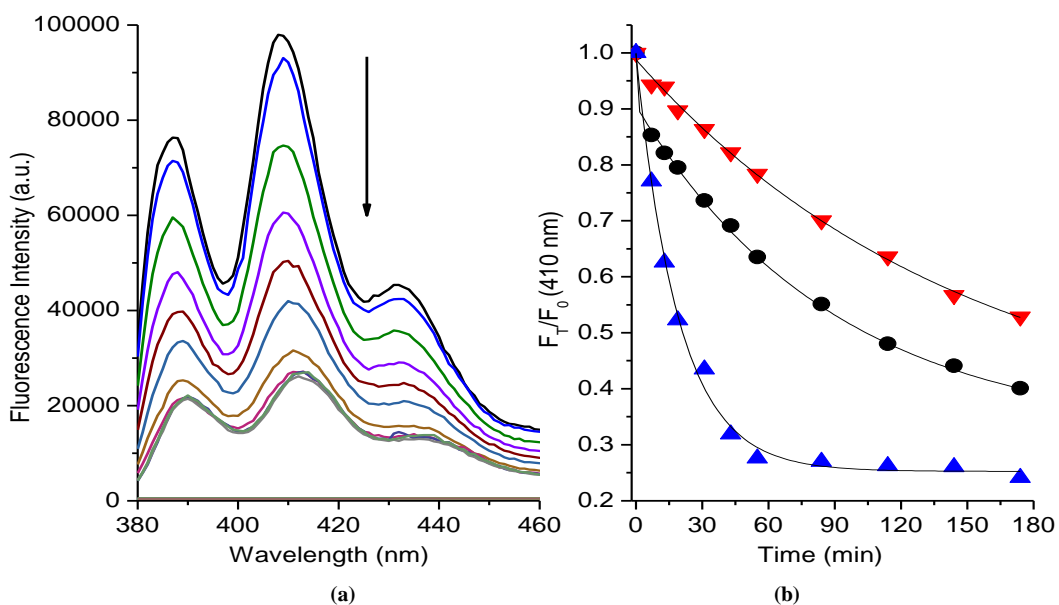


Figure S6. (a) Time curve of fluorescent spectral changes for probe **2** (10 μ M) upon adding H₂O₂ (0.50 mM, 0, 1, 6, 12, 18, 30, 42, 54, 84, 114, 144, 174 min); (b) Plots of F_T/F_0 upon addition of H₂O₂ (Red - 0.05 mM, Black - 0.10 mM, Blue - 0.50 mM). The mixture was incubated in pH 7.20 PBS buffer at 25 °C. Fluorescence intensities at 410 nm were measured with excitation at 370 nm.

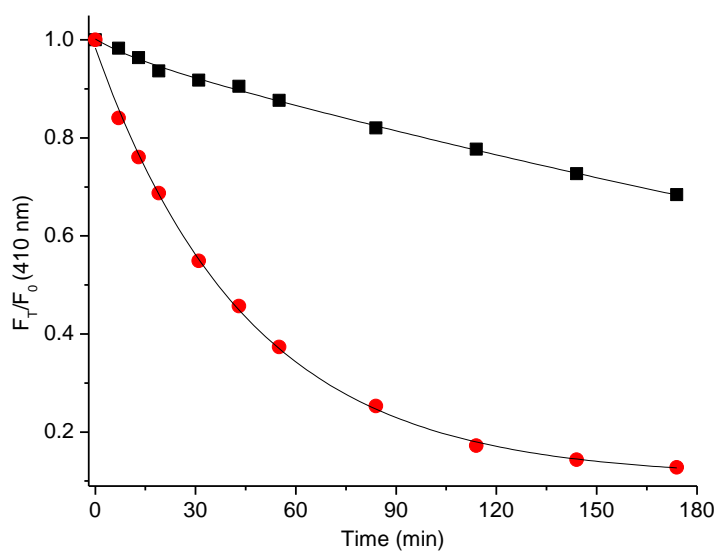


Figure S7. Time curve of fluorescent spectral changes for 2-D-fructose complex in various concentrations of H_2O_2 (Black - 0.05 mM, Red - 0.50 mM). The mixture was incubated in pH 7.20 PBS buffer at 25 °C. Fluorescence intensities at 410 nm were measured with excitation at 370 nm.

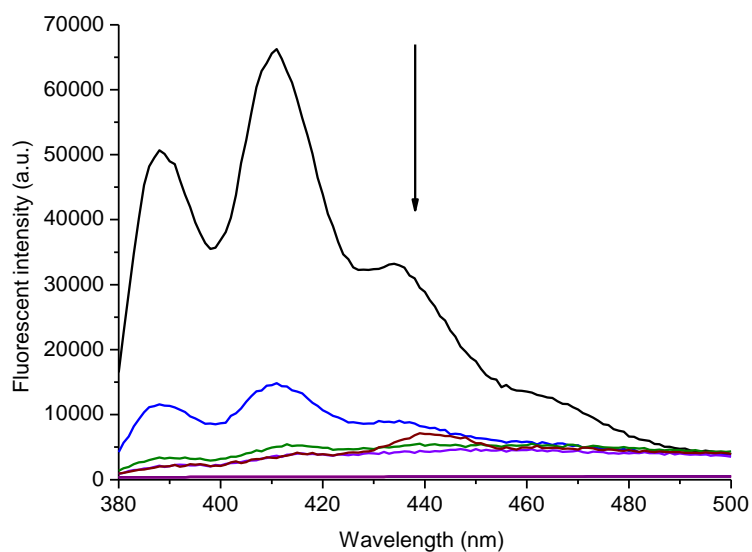


Figure S8. Time curve of fluorescent spectral changes for probe 2 (10 μM) with addition of H_2O_2 (0.05 mM, 0, 0.5, 1, 6, 10 min). The mixture was incubated in pH 9.70 $NaCO_3/NaHCO_3$ buffer at 25 °C with excitation at 370 nm.

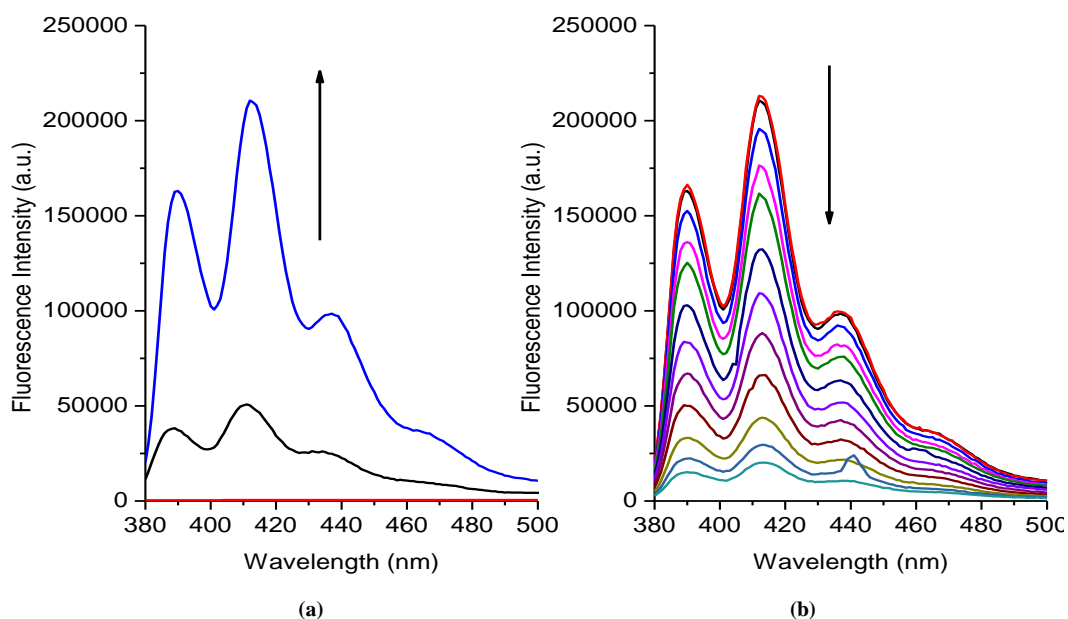


Figure S9. (a) Fluorescent spectral changes for probe **2** (10 μ M) – black line and 2-D-fructose complex – blue line; (b) Time curve of fluorescent spectral changes for 2-D-fructose complex with addition of H₂O₂ (0.05 mM, 0, 1, 6, 12, 18, 30, 42, 54, 84, 114, 144, 174 min). The mixture was incubated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

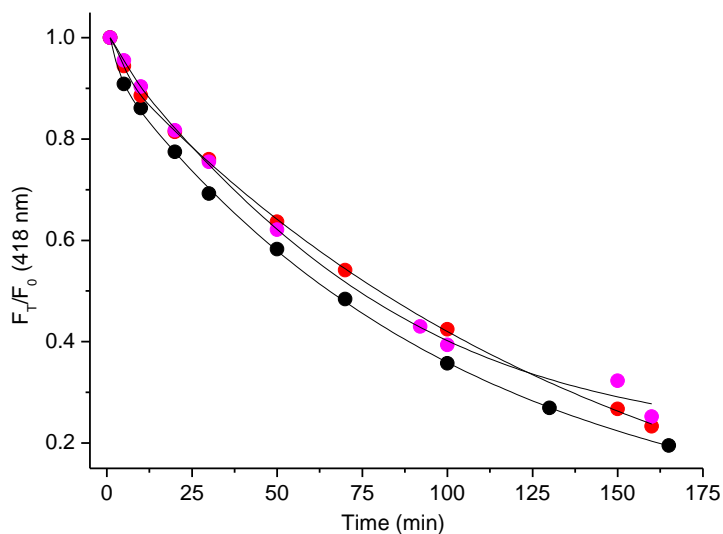


Figure S10. Time curve of fluorescence intensity changes with probe **2** (10 μ M) and D-fructose (100 mM) in aqueous H₂O₂ (0.05 mM). (Black circle – H₂O₂ in pH 9.70 buffer that was left for 1 min before adding probe **2**, Red circle – H₂O₂ in pH 9.70 buffer that was left for 1 h before adding probe **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

1. T. D. James, K. R. A. S. Sandanayake, R. Iguchi and S. Shinkai, *J. Am. Chem. Soc.*, 1995, **117**, 8982-8987.
2. D. D. Perrin and B. Dempsey, *Chapman and Hall: London*, 1974.