

Supplementary information

Aldehyde N,N-dimethylhydrazone-based fluorescent substrate for peroxidase-mediated assays

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Supplementary results

NMR spectra

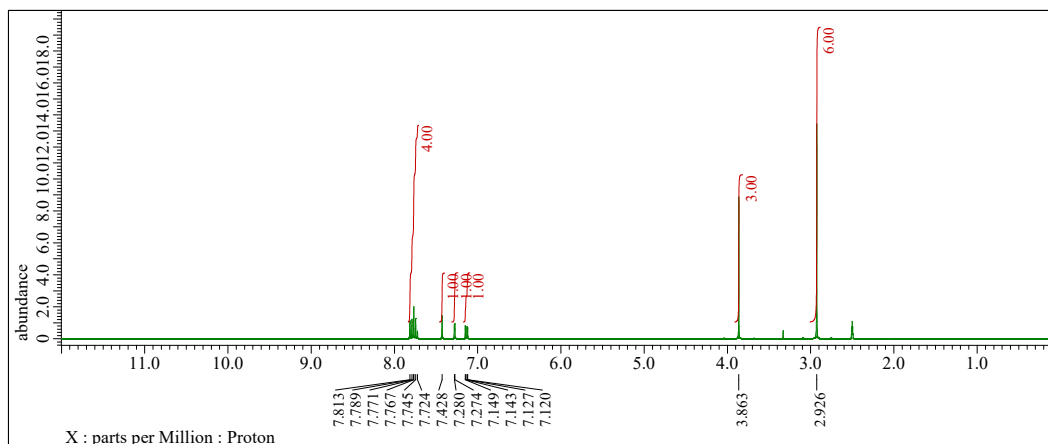


Fig. S1 ¹H NMR (400 MHz, DMSO-d₆) of MNDH.

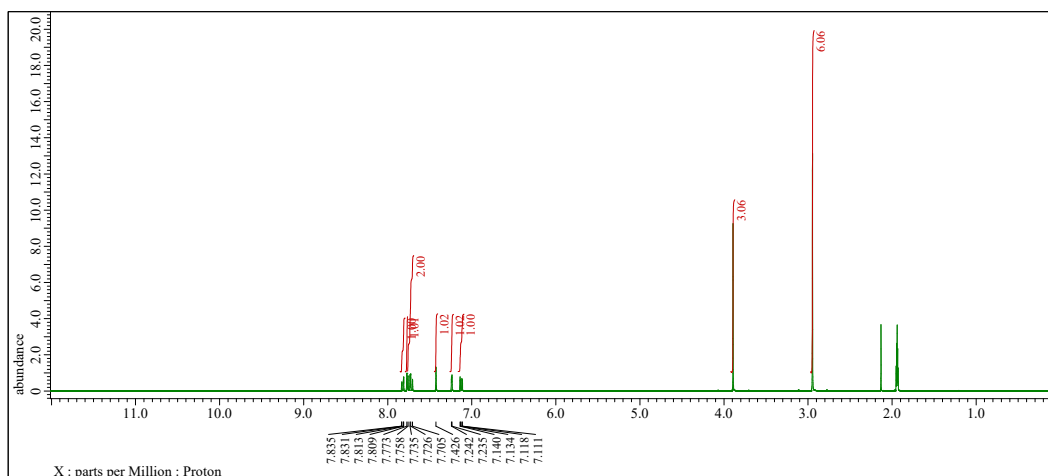


Fig. S2 ¹H NMR (400 MHz, ACN-d₃) of MNDH.

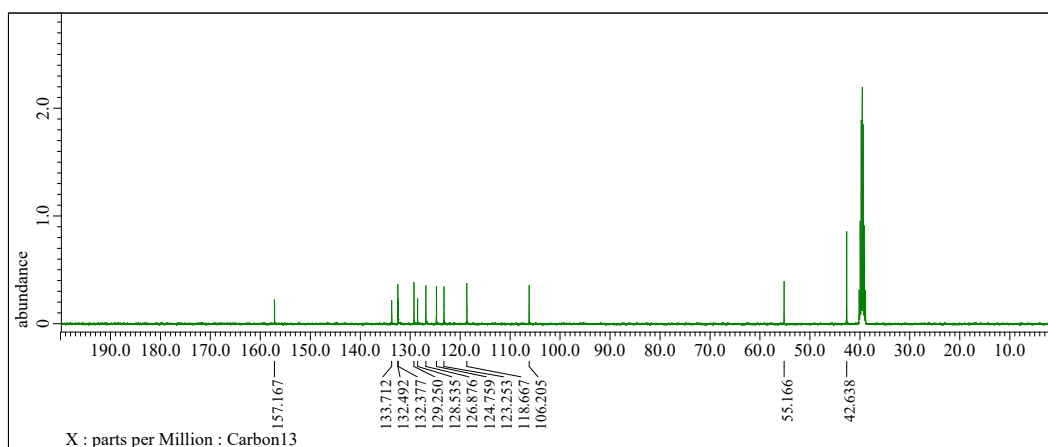


Fig. S3 ¹³C NMR (101 MHz, DMSO-d₆) of MNDH.

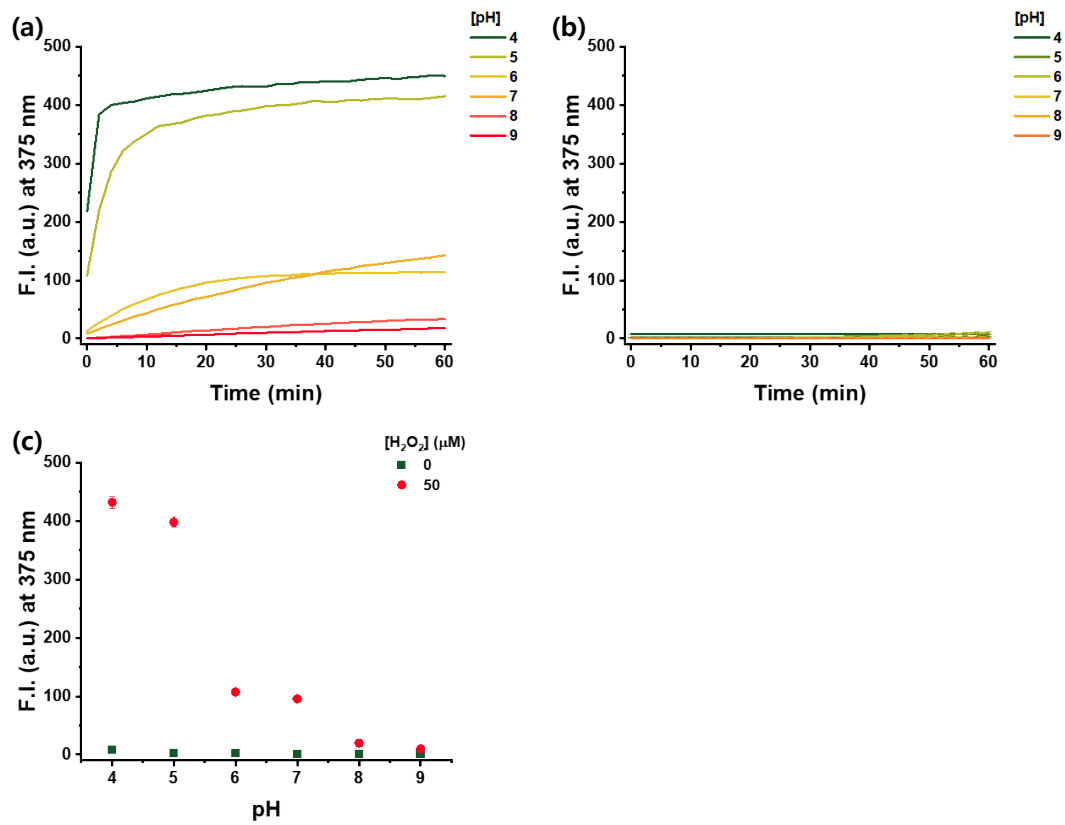


Fig. S4 Fluorescence intensity versus time for **MNDH** in the various pH buffer solution containing the HRP and (a) with or (b) without H₂O₂. (c) Plot of fluorescence intensity for assay solutions versus pH in the present or absent of H₂O₂. [**MNDH**] = 20 μ M, [H₂O₂] = 50 μ M, [HRP] = 50 mU/mL, [Acetate pH 4-5, MES pH 6 and Tris-HCl pH 7-9] = 20 mM.

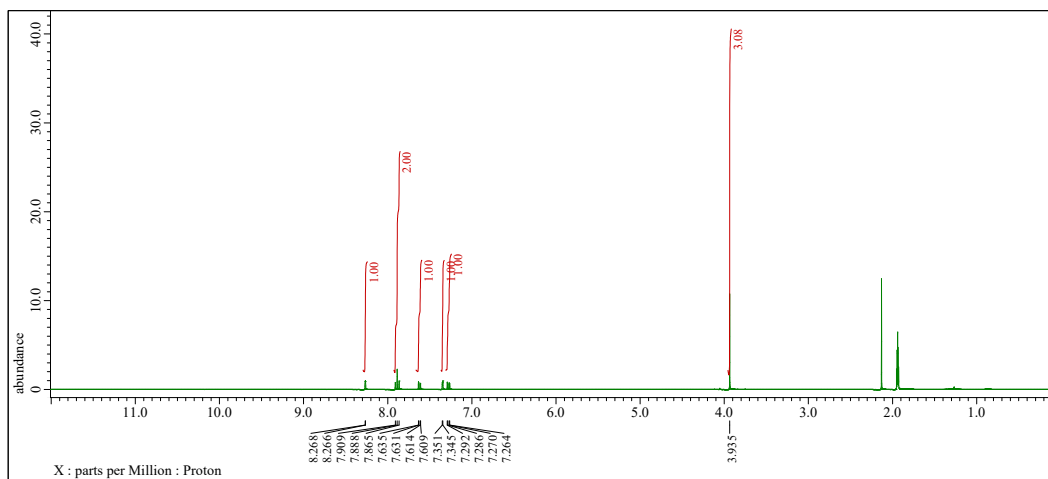


Fig. S5 ^1H NMR (400 MHz, ACN- d_3) of oxMNDH.

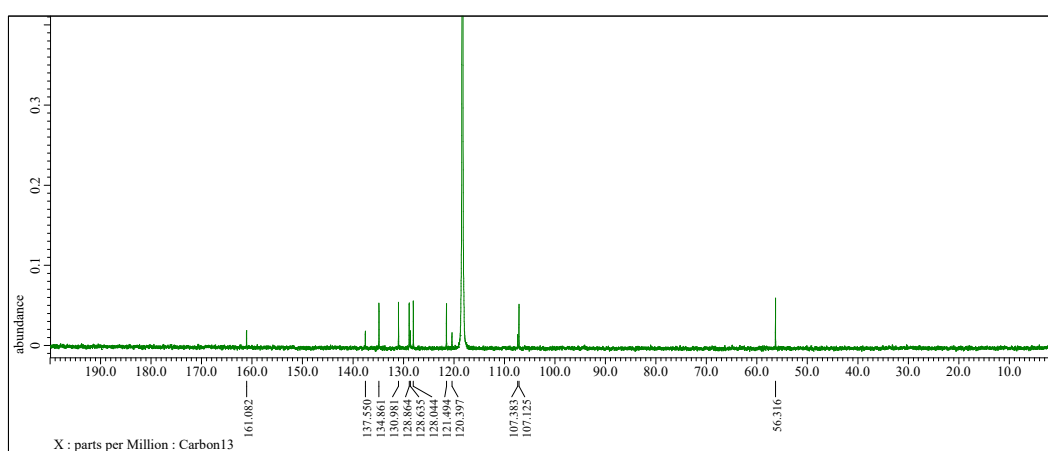


Fig. S6 ^{13}C NMR (101 MHz, ACN- d_3) of oxMNDH.

Synthesis of 6-Methoxy-2-naphthonitrile

A 6-Methoxy-2-naphthonitrile was synthesized following the previous literature.¹ The methyltrioxorhenium (0.004 mmol, 0.001 g) and H_2O_2 (1.85 mmol, 0.16 mL) were added to the ethanol (1 mL) and cooled at $-30\text{ }^\circ\text{C}$. After MNDH (0.87 mmol, 0.20 g) dissolved in dichloromethane (1 mL) was added dropwise to above the mixture, the mixture was stirred at $-30\text{ }^\circ\text{C}$ for 2 hr. The product was extracted with ethyl acetate. The organic layer was dried using sodium sulfate and concentrated under reduced pressure. The mixture was then purified by column chromatography (CHCl_3) to yield a white crystalline solid. ^1H -NMR (400 MHz, ACN- d_3) δ 8.26 (d, $J = 0.9\text{ Hz}$, 1H), 7.88 (t, $J = 9.0\text{ Hz}$, 2H), 7.62 (dd, $J = 8.5, 1.8\text{ Hz}$, 1H), 7.34 (d, $J = 2.4\text{ Hz}$, 1H), 7.27 (dd, $J = 9.0, 2.6\text{ Hz}$, 1H), 3.93 (s, 3H). ^{13}C -NMR (101 MHz, ACN- d_3) δ 161.0, 137.5, 134.8, 131.0, 128.8, 128.6, 128.0, 121.4, 120.4, 107.3, 107.1, 56.3.

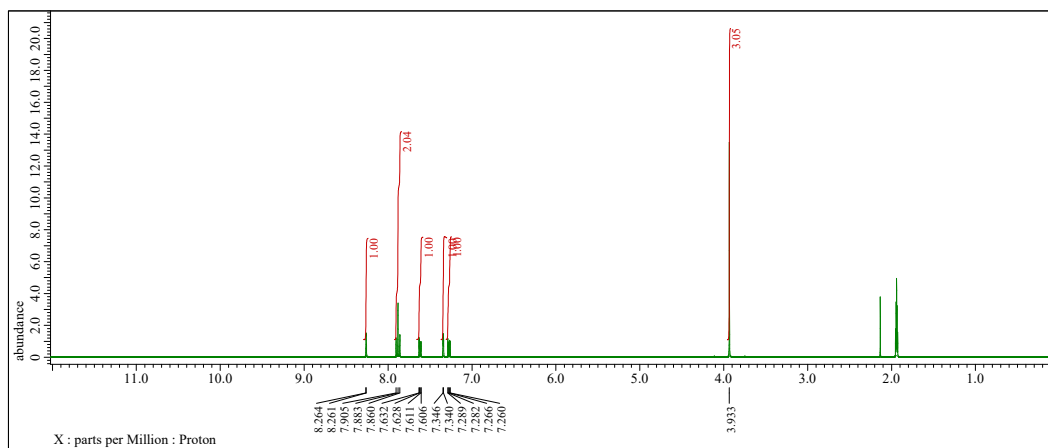


Fig. S7 ^1H NMR (400 MHz, ACN-d_3) of 6-methoxy-2-naphthonitrile.

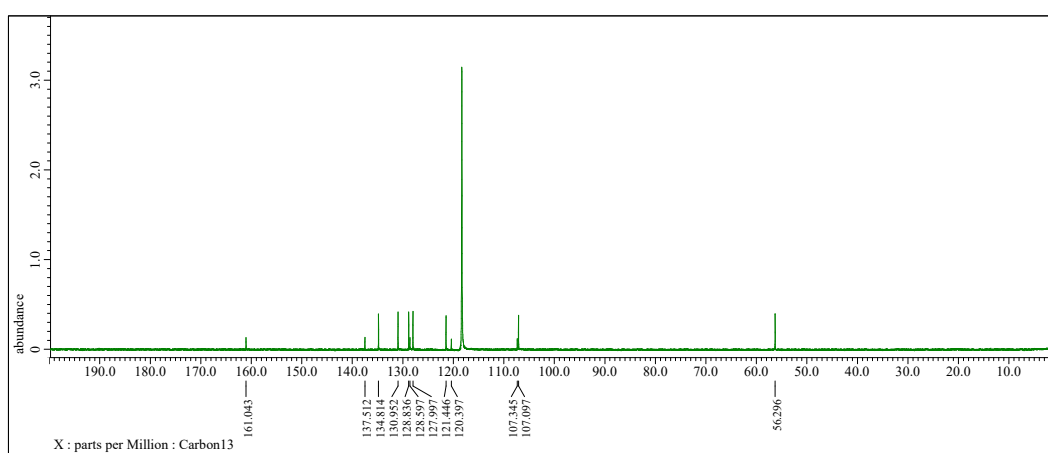


Fig. S8 ^{13}C NMR (400 MHz, ACN-d_3) of 6-methoxy-2-naphthonitrile.

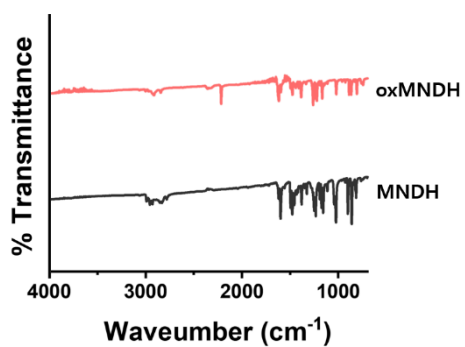


Fig. S9 FT-IR spectra of MNDH and oxMNDH.

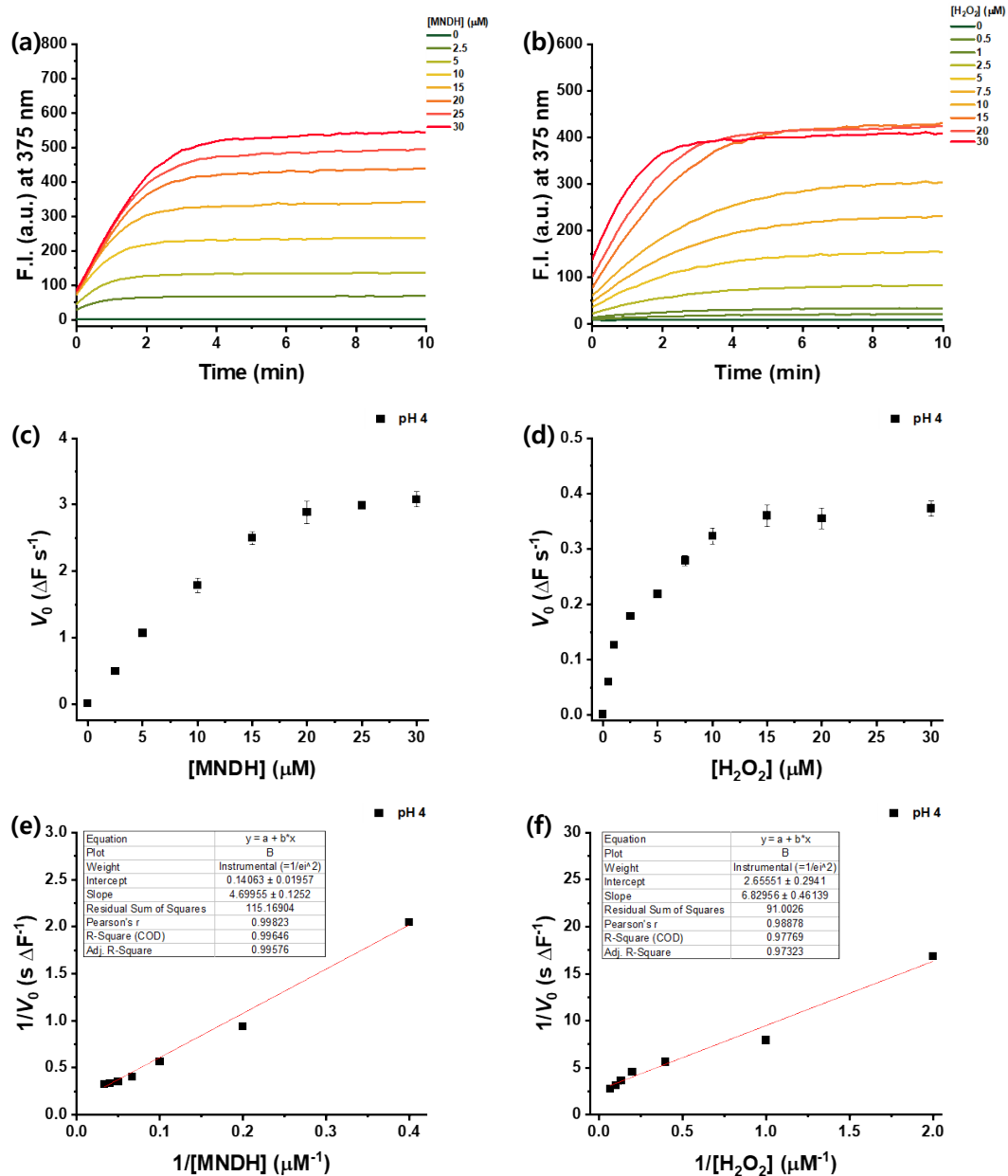


Fig. S10 Fluorescence intensity versus time for the assay solution containing the various concentration of (a) MNDH or (b) H_2O_2 at a fixed concentration of the other, in the presence of HRP, at pH 4. Michaelis-Menten plots of the assay solution versus the various concentrations of (c) MNDH or (d) H_2O_2 . Double reciprocal plots of the peroxidase-like activity of assay solution versus concentration of (e) MNDH or (f) H_2O_2 . $[\text{MNDH}] = 20 \mu\text{M}$, $[\text{H}_2\text{O}_2] = 50 \mu\text{M}$, $[\text{HRP}] = 50 \text{ mU/mL}$, $[\text{Acetate pH 4.0}] = 20 \text{ mM}$.

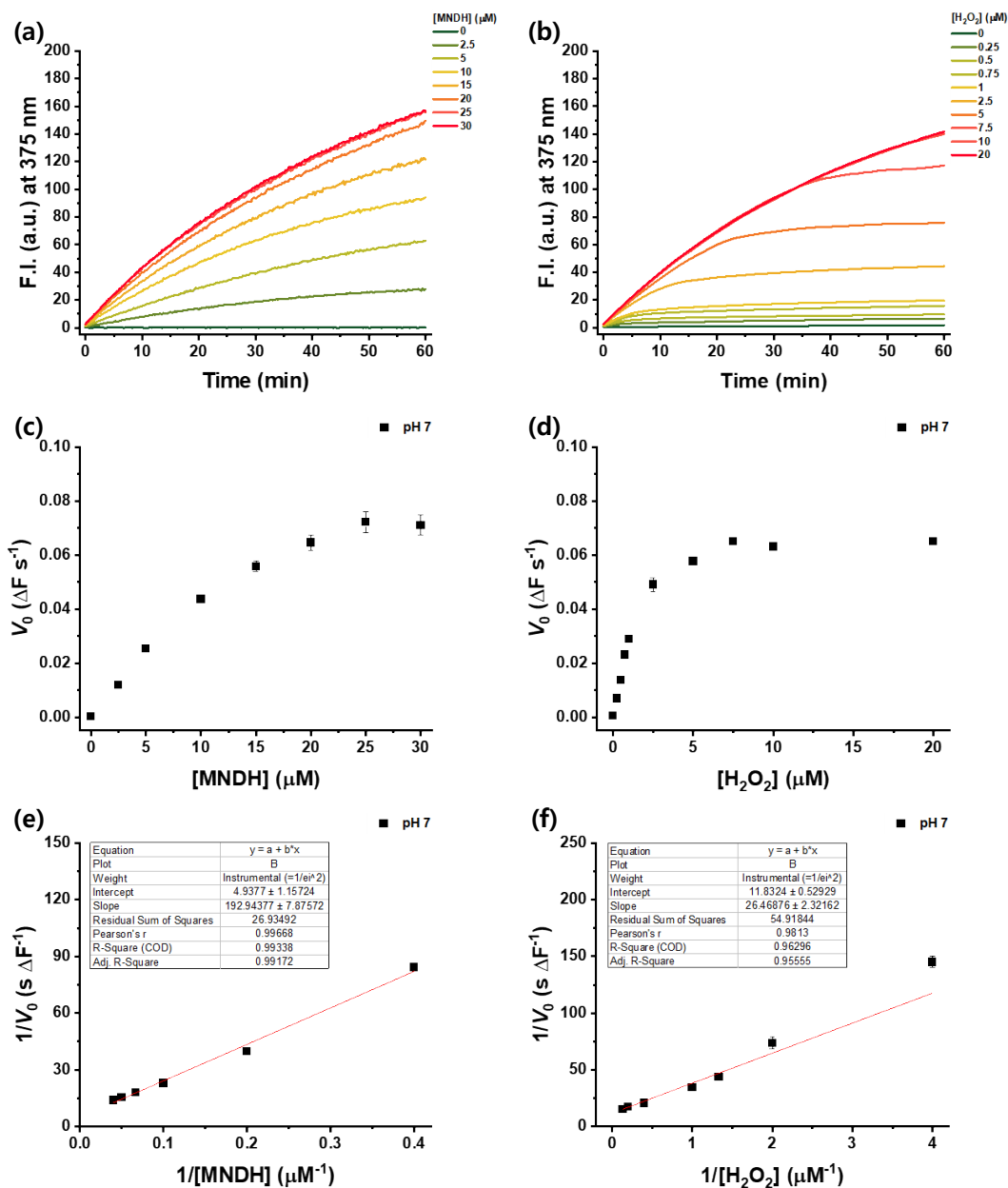


Fig. S11 Fluorescence intensity versus time for the assay solution containing the various concentration of (a) **MNDH** or (b) **H₂O₂** at a fixed concentration of the other, in the presence of **HRP**, at **pH 7**. Michaelis-Menten plots of the assay solution versus the various concentrations of (c) **MNDH** or (d) **H₂O₂**. Double reciprocal plots of the peroxidase-like activity of assay solution versus concentration of (e) **MNDH** or (f) **H₂O₂**. [**MNDH**] = 20 μM, [**H₂O₂**] = 50 μM, [**HRP**] = 50 μU/mL, [Tris-HCl pH 7.0] = 20 mM.

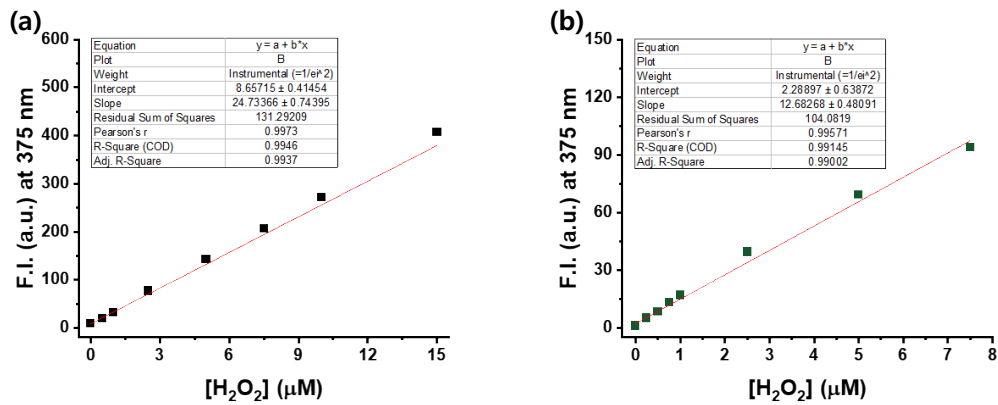


Fig. S12 Linear range of H₂O₂ titration at (a) pH 4 and (b) pH 7. [MNDH] = 20 μM, [H₂O₂] = 50 μM, [HRP] = 50 mU/mL, [Acetate pH 4.0 and Tris-HCl pH 7.0] = 20 mM.

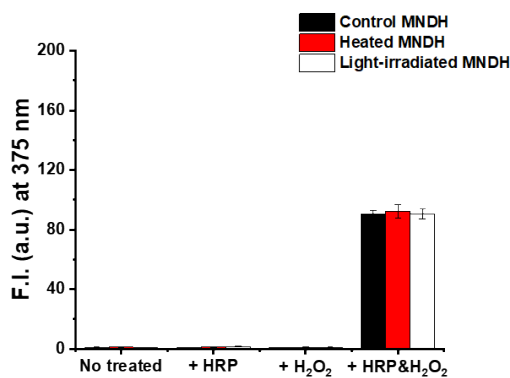


Fig. S13 Plot of fluorescence intensity of MNDH previously exposed to heat and light in buffer solutions containing various combinations of HRP and H₂O₂. [MNDH] = 20 μM, [H₂O₂] = 50 μM, [HRP] = 50 mU/mL, [Tris-HCl pH 7.0] = 20 mM.

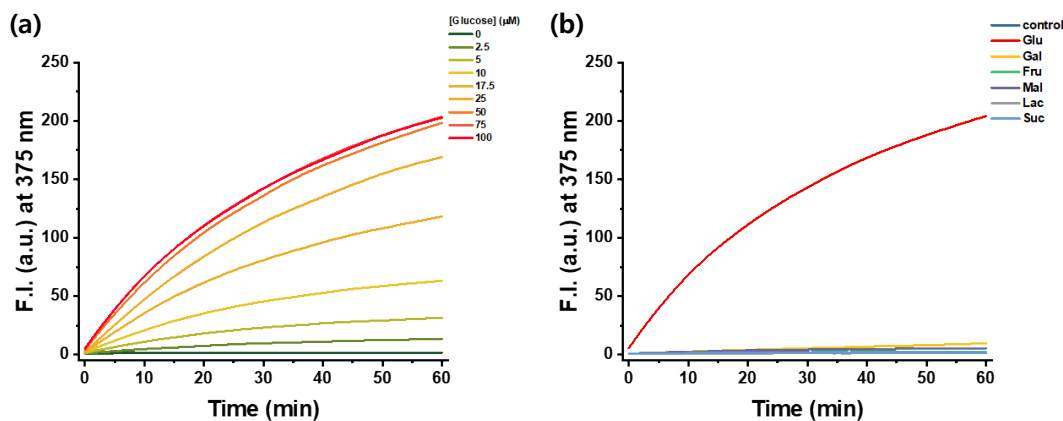


Fig. S14 Fluorescence intensity versus time for assay solutions versus (a) concentration of glucose or (b) type of saccharide. [MNDH] = 20 μM, [glucose] = 50 μM, [other saccharide] = 500 μM, [HRP] = 100 mU/mL, [GOx] = 500 mU/mL, [Tris-HCl pH 7.0] = 20 mM.

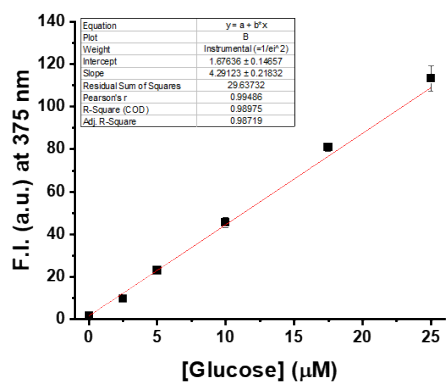


Fig. S15 Linear range of glucose titration. [MNDH] = 20 µM, [HRP] = 100 mU/mL, [GOx] = 500 mU/mL, [Tris-HCl pH 7.0] = 20 mM.

Reference

1. H. Rudler, B. Denise and S. Masi, *C. R. Acad. Sci. Paris, Serie IIc, Chimie/Chemistry*, 2000, **3**, 793-801.