Supplementary Information

In situ controllable growth of CoFe₂O₄ ferrite nanocubes on graphene for colorimetric detection of hydrogen peroxide

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Legends

Fig. S1 TEM images of rGO-IHO at low (A) and at high magnification (B).

- Fig. S2 SEM images of rGO-CF at low (A) and at high magnification (B).
- **Fig. S3** TEM images of the production by using GO as precursor (A) and rGO-IHO as precursor (B) in the second step.
- Fig. S4 TEM images of rGO-CF synthesized by varying the volume ratio of ethanol and water added in the reaction system. (A) 0:10, (B) 1:9, (C) 5:5, (D) 6:4, (E) 7:3, and (F) 10:0.
- Fig. S5 (A) UV-vis spectra of TMB solution (black line), TMB-H₂O₂ (blue line), TMB-H₂O₂-CF (green line) and CF solution (red line) in pH 4.0 acetate buffer at room temperature. (B) UV-vis spectra of TMB solution (black line), TMB-H₂O₂ (blue line), TMB-H₂O₂-rGO-CF (green line) and rGO-CF solution (red line) in pH 4.0 acetate buffer at room temperature. Inset: photograph of aqueous dispersions of (a) TMB, (b) TMB-H₂O₂, (c) TMB-H₂O₂-rGO-CF, and (d) rGO-CF.
- Fig. S6 Activity comparison of rGO-CF with different size. The rGO-CF was synthesized from different volume ratio of ethanol and water added in the reaction system. (A) 5:5, (B) 6:4, and (C) 7:3. Particle size: (A)>(B)>(C).
- Fig. S7 Dependence of the peroxidase-like activity of rGO-CF on (A) pH, (B) temperature, and (C) H_2O_2 concentration. Experiments were carried out using 4 μ g mL⁻¹ rGO-CF or 0.5 ng mL⁻¹ HRP in 1 mL of 0.2 M NaAc buffer with 200 μ M TMB as substrate. The H_2O_2 concentration was 20 mM at pH 4.0 and 30 °C unless otherwise stated. The maximum point in each curve was set as 100 %.
- Fig. S8 Steady-state kinetic assay and catalytic mechanism of rGO-CF (A-D). The velocity (v) of the reaction was measured using 4 μ g mL⁻¹ rGO-CF (A, B) or 0.5 ng mL⁻¹ HRP (C, D) in 1 mL of 0.2 M NaAc buffer pH 4.0 at 30 °C. Error bars shown represent the standard error derived from three repeated measurements. (A, C) The concentration of H₂O₂ was 20 mM (rGO-CF) or 0.8 mM (HRP) and the TMB concentration was varied. (B, D) The concentration of TMB was 100 μ M and the H₂O₂ concentration was varied. (E, F)

Double-reciprocal plots of activity of rGO-CF at a fixed concentration of one substrate versus different concentration of the second substrate for H_2O_2 or TMB.

- **Fig. S9** The stability of the rGO-CF: (A) Activity comparison of rGO-CF and HRP after exposing to water/organic mixed solvents for 2 h at 30 °C. The initial activity was set as 100 %. (B) Activity comparison of rGO-CF and HRP after incubating at different temperature for 2 h. The maximum point in each curve was set as 100 %.
- Fig. S10 TEM images of rGO-CF before (A) and after (B) reacting with TMB and H_2O_2 .
- Table S1. Comparison of the apparent Km and Vmax between rGO-CF and HRP.



Fig. S1 TEM images of rGO-IHO at low (A) and at high magnification (B).



Fig. S2 SEM images of rGO-CF at low (A) and at high magnification (B).



Fig. S3 TEM images: (A) GO as precursor. (B) rGO-IHO as precursor.



Fig. S4 TEM images of rGO-CF synthesized by varying the volume ratio of ethanol and water added in the reaction system. (A) 0:10, (B) 1:9, (C) 5:5, (D) 6:4, (E) 7:3, and (F) 10:0.



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Fig. S7 Dependence of the peroxidase-like activity of rGO-CF on (A) pH, (B) temperature, and (C) H_2O_2 concentration. Experiments were carried out using 4 µg mL⁻¹ rGO-CF or 0.5 ng mL⁻¹ HRP in 1 mL of 0.2 M NaAc buffer with 200 µM TMB as substrate. The H_2O_2 concentration was 20 mM at pH 4.0 and 30 °C unless otherwise stated. The maximum point in each curve was set as 100 %.



Fig. S8 Steady-state kinetic assay and catalytic mechanism of rGO-CF (A-D).The velocity (v) of the reaction was measured using 4 μ g mL⁻¹ rGO-CF (A, B) or 0.5 ng mL⁻¹ HRP (C, D) in 1 mL of 0.2 M NaAc buffer pH 4.0 at 30 °C. Error bars shown represent the standard error derived from three repeated measurements. (A, C) The concentration of H₂O₂ was 20 mM (rGO-CF) or 0.8 mM (HRP) and the TMB concentration was varied. (B, D) The concentration of TMB was 100 μ M and the H₂O₂ concentration was varied. (E, F) Double-reciprocal plots of activity of rGO-CF at a fixed concentration of one substrate versus different concentration of the second substrate for H₂O₂ or TMB.



Fig. S9 The stability of the rGO-CF: (A) Activity comparison of rGO-CF and HRP after exposing to water/organic mixed solvents for 2 h at 30 $^{\circ}$ C. The initial activity was set as 100 %. (B) Activity comparison of rGO-CF and HRP after incubating at different temperature for 2 h. The maximum point in each curve was set as 100 %.



Fig. S10 TEM images of rGO-CF before (A) and after (B) reacting with TMB and H_2O_2 .

Catalyst	Substance	$K_m [mM]^a$	$V_{max} [10^{-8} M \cdot s^{-1}]^{a}$
rGO-CF	TMB	0.046±0.001	1.121±0.007
rGO-CF	H_2O_2	14.72±2.332	21.71±3.445
HRP	TMB	0.204±0.003	0.929±0.012
HRP	H_2O_2	0.171±0.048	0.356±0.004

Table S1. Comparison of the apparent K_{m} and V_{max} between rGO-CF and HRP.

^aMean value \pm standard deviation (n=3).