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Electronic Supplementary Information

$CoFe_2O_4$ magnetic nanoparticles as peroxidase mimic mediated chemiluminescence for hydrogen peroxide and glucose

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Materials and methods

Reagents and materials

All chemicals used in this work were of analytical grade and used as received without further purification. A 0.01 M luminol solution was prepared. Horseradish peroxidase (EC1.11.1.17, 300 U/mg), glucose oxidase (GOx, EC 1.1.3.4. 47, 200 U/mg), 3,3',5,5'-tetramethylbenzidine (TMB) and *o*-phenylenediamine (OPD) were purchased from Sigma-Aldrich (Shanghai, China). H_2O_2 and other reagents were obtained from Chongqing Chemical Reagents Company (Chongqing, China). Doubly deionized water was used to prepare all aqueous solutions and related cleaning purposes. Before use, the doubly deionized water was pretreated by adding MnO_2 to eliminate H_2O_2. All glassware was soaked in 10% nitric acid and cleaned before use. Na_2CO_3–$NaHCO_3$ buffer (0.1 M, pH 10.15) was made by mixing 60 mL of Na_2CO_3 (0.1 M) and 40 mL of $NaHCO_3$ (0.1 M).

Instrumentation

CL measurements were performed on a MCFL-A Multifunction Chemiluminescence/Bioluminescence Analyzer (Ruike Electronic Equipment Company Ltd., Xi'an, China). Absorption measurements were performed on a UV-2400 UV-Vis spectrophotometer (Shimadzu, Japan). The CL spectra were obtained with an F-4500 spectrofluorimeter (Hitachi, Japan) under the model of fluorescence scan by turning off the excitation light. The pH of the solutions were detected by a PHS-3D precision pH meter (Shanghai Precision Scientific Instruments Co., Ltd., China); The X-ray diffraction (XRD) patterns of the as-prepared products were measured by XD-3 X-ray diffractometer (PuXi, Beijing, China) under the conditions of nickel filtered CuKα radiation (λ=0.15406 nm) at current of 20 mA and a voltage of 36 KV. The scanning rate was 4°/min in the angular range of 10~70° (2θ). The size of the nanoparticles was characterized by transmission electron microscope (TEM) analyses with Philips Tecnai 10 (Philips, The Netherlands).

Synthesis of $CoFe_2O_4$ nanoparticles

The detailed method for $CoFe_2O_4$ preparation was described elsewhere.[1] Briefly, an aqueous mixture of ferric chloride (40 mL, 1 M) and cobalt nitrate (10 mL, 2 M, in HC1 2 M) was added to sodium hydroxide (500 mL, 0.7 M) under agitation. The obtained solutions were heated to boiling point for 30 min and then allowed to cool

down to room temperature. Then, the products were washed with dilute nitric acid, and then washed with doubly deionized water until the pH value of supernatant was about 7-8. The as-prepared product was azeotroped with 0.5 M $FeCl_3$ for 30 min, then centrifuged and washed with doubly deionized water until the supernatant became neutral. After the supernatant was discarded, the obtained solid products were further washed with acetone for removal of the residual water, and were air-dry at room temperature for 2 h. Finally, the obtained nanoparticles were treated with 0.05 M nitric acid and stored at room temperature for use (referred to as the $CoFe_2O_4$ nanoparticles stock solution with concentration of 80.0 g/L $CoFe_2O_4$). According to Tourinho et al.,[1] the as-prepared $CoFe_2O_4$ nanoparticles showed much more stable than that obtained by coprecipitation.

Characterization of $CoFe_2O_4$

The XRD pattern of the prepared products is shown in Fig. S1). Seven characteristic peaks can be indexed as the cubic structure $CoFe_2O_4$, which is accordance with the reported data (JCPDS File No. 22-1086). The XRD pattern indicates the nanomaterials obtained *via* our current synthetic methods consist of pure phases. The morphology and microstructure of the $CoFe_2O_4$ products were further examined with transmission electron microscopy (TEM). Fig. S2 shows a typical TEM image of the $CoFe_2O_4$ products. The average size of the prepared $CoFe_2O_4$ nanoparticles is about 14±2 nm.

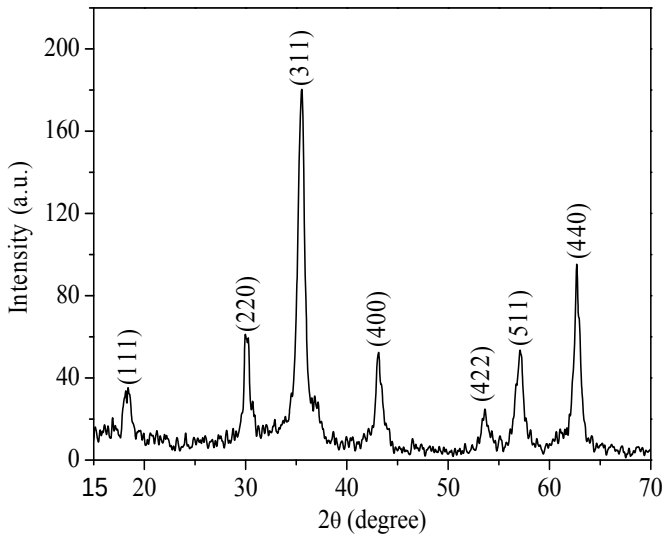

Figure S1. XRD patterns of $CoFe_2O_4$

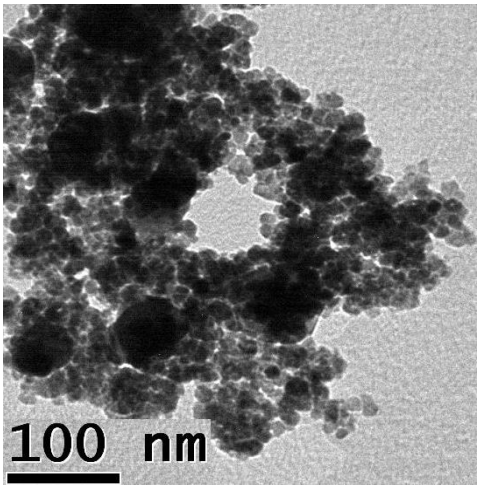

Figure S2. TEM image of $CoFe_2O_4$

General procedure for CL analysis

A schematic diagram of flow system used in this work is shown in Fig. S3. Two peristaltic pumps were used to deliver all solutions; one at a flow rate of 3 mL/min (per tube, pump1) for delivering the catalyst solution ($CoFe_2O_4$ or HRP) and water carrier stream; the other for delivering CL reaction reagents (luminol and hydrogen peroxide) at a flow rate of 3 mL/min (per tube, pump2). PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. Injection was made by using an eight-way injection valve equipped with a 160 μL sample loop. The CL signal produced in the flow cell was detected by a photomultiplier tube (operated at −600 V) of the Type MCFL-A Multifunction Chemiluminescence/ Bioluminescence Analyzer. The signal was recorded by a computer, equipped with a data acquisition interface. Data acquisition and processing were performed with REMAX software running under Windows XP.

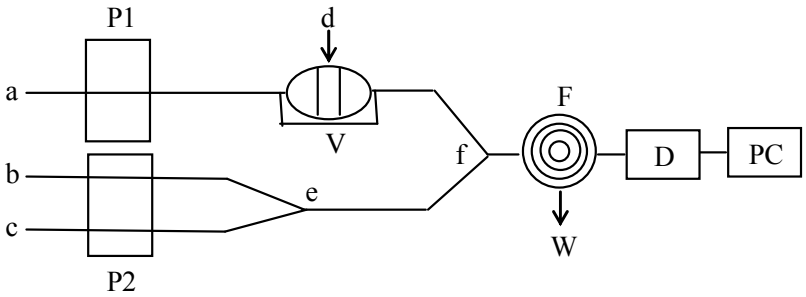

Figure S3. Schematic for flow-injection analysis system. (P1) and (P2) Peristaltic pumps; (a) Carrier; (d) $CoFe_2O_4$ or HRP; (V) Injection valve; (e) and (f) Tee; (b) Hydrogen peroxide; (c) Luminol； (F) Flow cell; (W) Waste liquid; (D) Detector; (PC) Personal computer.

Procedure for catalytic properties assays

Under the reaction conditions of $CoFe_2O_4$ (pH 6.0, 25 ^oC) and HRP (pH 8.6, 25 ^oC), the performance of catalytic properties was evaluated by varying the concentrations of hydrogen peroxide at fixed concentration of $CoFe_2O_4$ (8 mg/L), HRP (0.1 μM) and luminol (1 μM, pH 10.15). As shown in Fig. S3, flow lines were inserted into the luminol solution, hydrogen peroxide solution, doubly deionized water, and $CoFe_2O_4$ or HRP solution, respectively. The pumps were started until a stable baseline was recorded. The CL signal was measured by injecting 160 μL of $CoFe_2O_4$ or HRP into the water carrier stream, which then joined the reagent streams of a mixture of luminol and hydrogen peroxide (in the $0.1–1.0\times10^4$ μM concentration range). At each hydrogen peroxide concentration, the injection was repeated for at least three times, and the average CL signal was obtained. Catalytic properties can be evaluated by CL emission intensities versus concentration of hydrogen peroxide.

Procedure for comparison of robustness of the $CoFe_2O_4$ and HRP

The robustness of the $CoFe_2O_4$ and HRP was evaluated according to method by Gao et. al.[2] Briefly, both $CoFe_2O_4$ and HRP were first incubated at a range of temperature water baths from 5 to 85 ^oC (5, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80 and 85 ^oC) and a range of pH from 1 to 12 (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) for 2 h, respectively. Then their activities were measured under the following conditions: 1 μM luminol in 0.1 M buffer solution of Na_2CO_3-$NaHCO_3$ (pH 10.15), 10 μM hydrogen peroxide, 8 mg/L $CoFe_2O_4$ (pH 6.0) or 0.1 μM HRP (pH 8.6).

Procedure for hydrogen peroxide analysis in real water samples

In this study, the rain water and lake water samples were selected for investigation. Rain water samples were collected from the roof of the located building near our lab. Lake water sample was taken from the ChongDe Lake located in Southwest University. Before experiment, the environmental water samples were filtered through 0.45 μm micropore membrane.

Procedure for glucose analysis in blood samples

For glucose determination in blood, the serum samples from local hospital were firstly treated by centrifugation at 10 000 rpm for 30 min. Then 0.10 mL of the supernatant was diluted into 10 mL using 10 mM PBS buffer (pH 7.0) for the

following work. Glucose determination was carried out by adding 0.1 mL of the diluted serum sample and 0.1 mL of 1 mg/mL GOx into a glass tube, then the mixture was diluted to 10 mL by 10 mM phosphate buffer solution (pH 7.0), and incubated at 37 °C for 30 min to yield the testing sample solutions. The calibration curve for glucose detection was realized as follows: a) 0.1 mL of 1 mg/mL GOx and 0.1 mL of glucose of different concentrations in 9.80 mL of 10 mM PBS buffer (pH 7.0) were incubated at 37 °C for 30 min (with final glucose concentration of 0.1–10 μM); b) the produced mixed solution was used to prepare calibration curve for glucose by the proposed CL method. The results were compared with those by the conventional method. The comparison study was carried out by an OneTouch Ultra glucose meter (Johnson and Johnson Medical Ltd., Shanghai, China).

References

1. F.A. Tourinho, R. Franck, R. Massart, *J Mater Sci* 1990, **25**, 3249-3254.
2. L. Z. Gao, J. Zhuang, L. Nie, J. B. Zhang, Y. Zhang, N. Gu, T. H. Wang, J. Feng, D. L. Yang, S. Perrett and X. Yan, *Nat Nanotechnol*, 2007, **2**, 577-583.

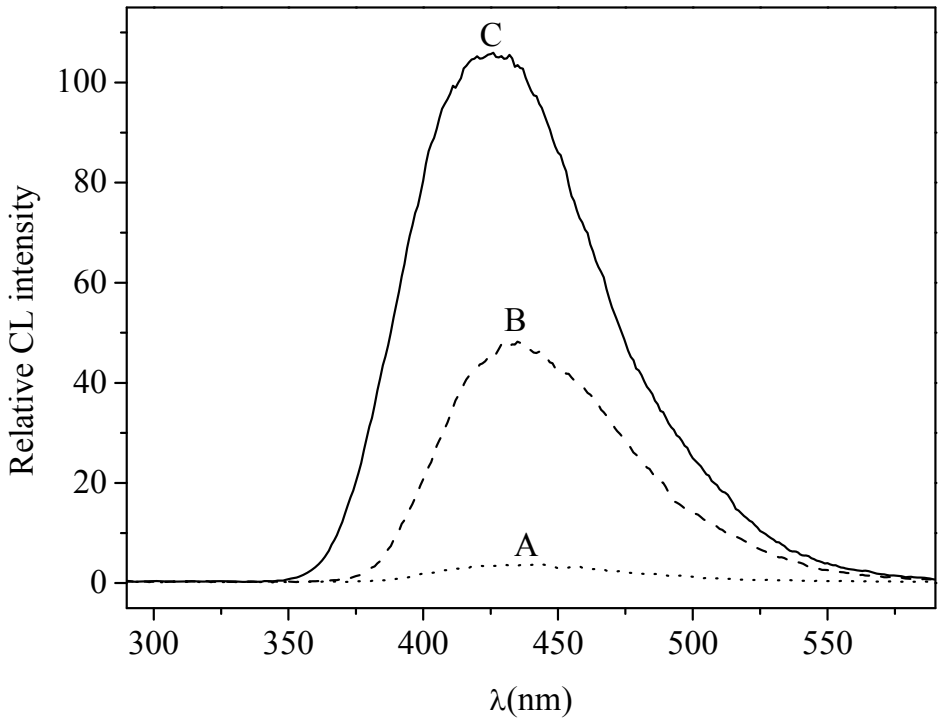

Figure S4. The CL spectra of 100 μM hydrogen peroxide–100 μM luminol reaction product catalyzed by $CoFe_2O_4$ or HRP; (A): luminol–hydrogen peroxide; (B): luminol–hydrogen peroxide–0.1 μM HRP; (C): luminol–hydrogen peroxide–8 mg/L $CoFe_2O_4$.

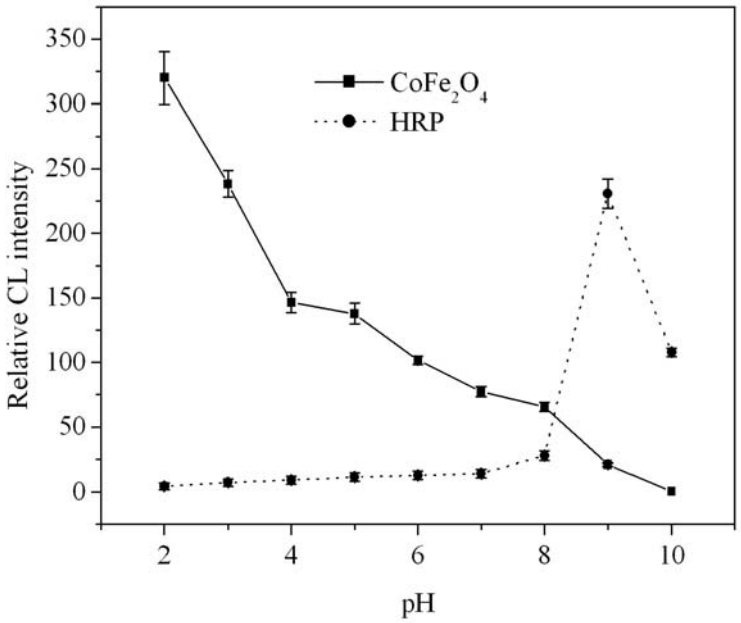

Figure S5. Effect of pH on catalytic activity of $CoFe_2O_4$ and HRP. Experimental conditions: 1 μM luminol in 0.1 M buffer solution of Na_2CO_3-$NaHCO_3$ (pH 10.15), 10 μM hydrogen peroxide, 8 mg/L $CoFe_2O_4$ or 0.1 μM HRP at 25 °C. Error bars represent one standard deviation for five measurements.

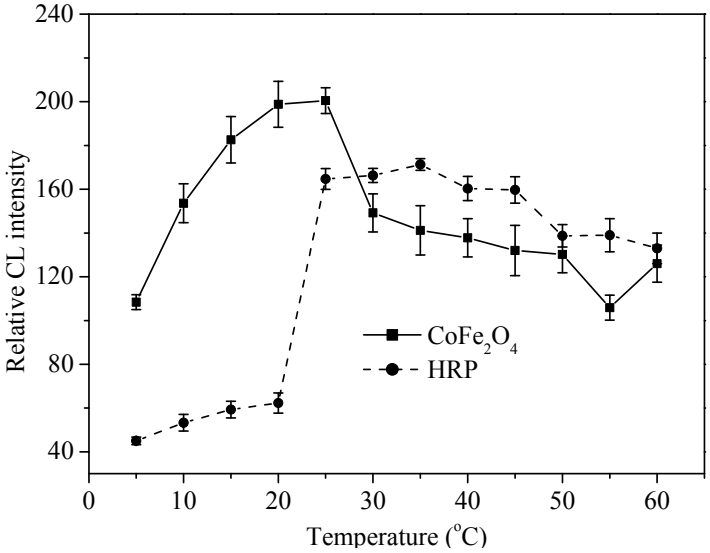

Figure S6. Effect of temperature on catalytic activity of $CoFe_2O_4$ and HRP. Experimental conditions: 1 μM luminol in 0.1 M buffer solution of Na_2CO_3-$NaHCO_3$ (pH 10.15), 10 μM hydrogen peroxide, 8 mg/L $CoFe_2O_4$ (pH 6.0) or 0.1 μM HRP (pH 8.6). Error bars represent one standard deviation for five measurements.

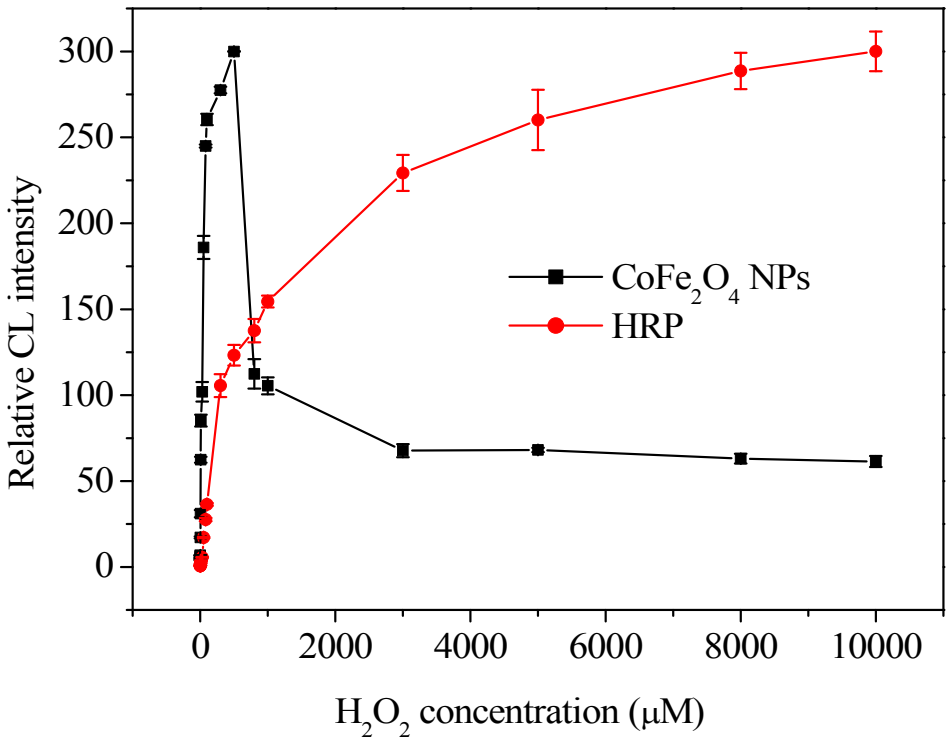

Figure S7. Effect of the hydrogen peroxide concentration on catalytic activity of $CoFe_2O_4$ and HRP. Experimental conditions: 1 μM luminol in 0.1 M buffer solution of Na_2CO_3-$NaHCO_3$ (pH 10.15), 8 mg/L $CoFe_2O_4$ (pH 6.0) or 0.1 μM HRP (pH 8.6), the temperature is 25 °C. Error bars represent one standard deviation for three measurements.

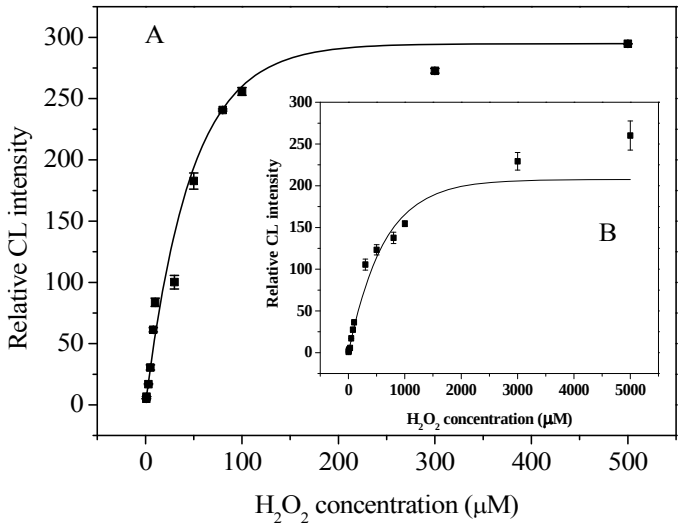

Figure S8. Steady-state kinetic assay of $CoFe_2O_4$ (A) and HRP (B) as catalysts. Conditions: (A) 1 μM luminol in 0.1 M Na_2CO_3-$NaHCO_3$ buffer solution (pH 10.15), 8 mg/L $CoFe_2O_4$ (pH 6.0); (B) 1 μM luminol in 0.1 M Na_2CO_3-$NaHCO_3$ buffer solution (pH 10.15), 0.1 μM HRP (pH=8.6) at 25 °C. Error bars represent one standard deviation for three measurements.

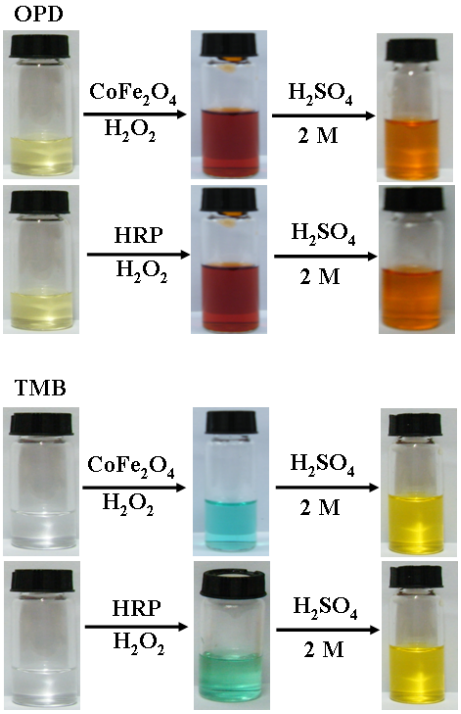

Figure S9. Images of oxidation color reaction of OPD or TMB by H_2O_2 after catalyzing by $CoFe_2O_4$ or HRP, and subsequently being quenched by H_2SO_4.

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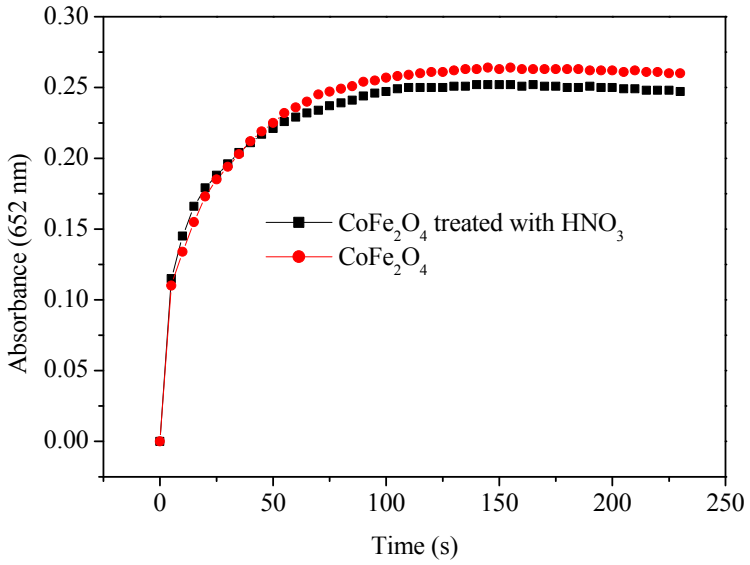

Figure S10. Illustration that $CoFe_2O_4$ activity does not result from metal leaching. The as-prepared $CoFe_2O_4$ nanoparticle was treated with (square) and without nitric acid (circle). $CoFe_2O_4$ concentration: 8 mg/L, TMB: 0.4 mM; H_2O_2: 0.1 mM; pH=6.0.

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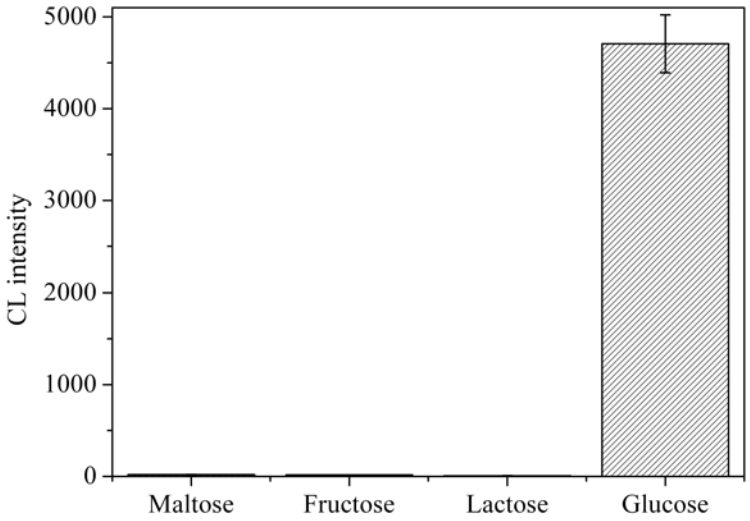

Figure S11. Selectivity analysis for glucose detection by monitoring the relative CL intensity. The analyte concentrations were as follows: 10 mM fructose, 10 mM lactose, 10 mM maltose, and 10 μM glucose. The error bars represent the standard deviation of five measurements.

Table S1 Recoveries of H_2O_2 in the presence of foreign species (H_2O_2: 1.0 μM)

Coexisting species	At concentration (μM)	Recovery (%)[a]	Coexisting species	At concentration (μM)	Recovery (%)[a]
Na^+	1000	99.2±3.2	Ag^+	10	98.3±3.7
K^+	1000	99.6±2.4	Fe^{2+}	1	104±4.9
Ca^{2+}	1000	99.4±2.6	Fe^{3+}	1	103±3.4
Mg^{2+}	1000	98.3±4.2	EDTA	10	96.2±3.6
Al^{3+}	500	97.2±3.1	HPO_4^{2-}	1000	99.6±2.9
Zn^{2+}	100	102.2±1.2	$H_2PO_4^-$	1000	99.1±4.4
Cu^{2+}	100	95.6±4.2	SO_4^{2-}	800	97.2±4.5
Cr^{3+}	100	101.4±5.2	PO_4^{3-}	500	98.2±3.8
Ni^{2+}	5	98.2±4.5	Cl^-	800	99.4±3.9
Pb^{2+}	50	98.2±2.2	NO_3^-	500	99.8±2.6
Cd^{2+}	100	98.4±3.8	NH_4^+	500	99.5±2.9
Hg^{2+}	10	97.2±4.1	NO_2^-	10	95.2±2.4

[a] Mean value±standard deviation (*n*=3).

Table S2 Results of determination of glucose in serum

Sample	Added (μM)	Total found [a] (μM) ±SD	Recovery (%)	RSD (%)	Glucose meter method (mM) ±SD [b]
Serum 1	–	0.46±0.01	–	–	4.62±0.12
	0.50	0.91±0.03	91.0	4.8	
	1.00	1.34±0.03	89.8	3.4	
	5.00	4.76±0.18	86.1	4.0	
Serum 2	–	0.68±0.02	–	–	6.84±0.10
	0.50	1.18±0.05	101.1	5.0	
	1.00	1.55±0.03	86.8	3.8	
	5.00	5.60±0.16	98.4	3.2	
Serum 3	–	0.58±0.04	–	–	5.63±0.10
	0.50	1.15±0.01	114.8	1.4	
	1.00	1.47±0.16	89.2	1.8	
	5.00	5.12±0.13	90.8	2.8	

[a] The blood samples were diluted 10000-fold for glucose determination by the proposed method.

[b] The glucose determination was performed directly without dilution in the laboratory for clinical analysis, The Ninth People's Hospital of Chongqing.

Table S3 Results of the determination of hydrogen peroxide in water samples (n=3)

Sample	Found (μM±SD)	Added (μM)	Total found (μM±SD)	Recovery (%±SD)
Rain water 1	1.59±0.01	2.00	3.65±0.06	102.7±2.76
		5.00	6.21±0.02	92.3±4.67
Rain water 2	0.74±0.01	1.00	1.73±0.01	98.70±0.70
		2.00	2.63±0.03	94.5±1.56
		6.00	7.07±0.01	105.5±0.03
Lake water	nd	0.50	0.49±0.03	98.0±4.29
		1.00	1.07±0.01	107.0±4.93
		5.00	5.82±0.01	116.4±2.88