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Electronic supplementary information

Highly Efficient Synergistic Biocatalysis Driven by Stably Loaded Enzymes within Hierarchically Porous Iron/Cobalt Metal-Organic Framework via Biomimetic Mineralization

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Experimental Section

Characterization

The morphological characteristics of synthetic materials were observed by transmission electron microscopy (TEM; JEM-2010HR, Tokyo, Japan) operating at 200 kV. Powder X-ray diffraction (PXRD) patterns were investigated using an X-ray diffractometer (Bruker D8 Advance, Karlsruhe, Germany). The fluorescence measurements were confirmed by confocal laser scanning microscopy (CLSM; Leica SP8 X, Buffalo Grove, USA). Fourier-transform infrared (FT-IR) spectra were obtained by an infrared spectrometer (Thermo Fisher Nicolet iS20, Waltham, USA). Specific surface area was determined and pore volume and size analyzed using the Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) methods, respectively, on a TR2 Star 3020 Surface Area & Pore Size Analyzer (Micromeritics, Norcross, USA). Water contact angle was determined using an optical contact angle tension measuring instrument (LAUDA Scientific LSA100, Hamburg, Germany). X-ray photoelectron spectra (XPS) were recorded on a Thermo VG Escalab 250 X-ray photoelectron spectrometer (Waltham, USA) at a pressure of approximately 2×10^{-9} Pa with Al Kα X-rays as the excitation source. The electron spin resonance (ESR) signal diagram was based on the measurements made by a Bruker A300 (X-band) instrument (Karlsruhe, Germany). Electrochemical measurements were made by an Autolab PGSTAT302N electrochemical workstation (Metrohm, Netherland). Enzymatic assays were carried out with a UV-Vis spectrophotometer (Hitachi UH5300, Tokyo, Japan). The glucose concentrations of the serum samples were measured by a glucose meter (Omron HEA-215, Kyoto, Japan). Lactose levels were measured in serum and milk samples using an HPLC system (Thermo Fisher Dionex Ultimate 3000, Waltham, USA) with an evaporative light-scattering detector (ELSD).

Fluorescence Labeling of Enzymes

FITC and RhB labeling were based on the conjugation of the amino of lysine residues of enzymes and the thiocarbmide of fluorescent dyes. Briefly, 20 mg of enzyme was dispersed into 10 mL carbonate buffer solution (pH 9.0, 0.5 M) and 1 mg of fluorescence dye (FITC or RhB) added. The mixed solution was then stirred for 12 h in the dark. Finally, the dye-labeled enzymes were obtained through ultrafiltration three times to remove excess reaction reagents and salts.

PVP Exchange and SDS Washing

GOx@FCM-TA (5.0 mg) and GOx/FCM-TA were dispersed in 2 mL of 5% PVP water solution, shaken for 10 min, and then centrifuged to obtain the supernatant. The methylene blue (MB) released into the supernatant was detected using the BCA Protein Assay Kit (Beyotime Biotechnology, China). This PVP exchange was repeated five times.

For the SDS washing experiment, GOx@FCM-TA (5.0 mg) and GOx/FCM-TA were dispersed in 2 mL of SDS solution (0.1 g/mL in water), shaken for 10 min, and then centrifuged to obtain the supernatant. The MB released into the supernatant was detected using the BCA Protein Assay Kit. This SDS wash was repeated five times.

Electrochemical Measurements

Typically, the modified electrodes were fabricated as follows. The shiny and smooth Au electrode was acquired first by lapping with 0.05 μm α-Al_2O_3 powder and sonicated in 5% H_2SO_4 solution, ethanol, and water for 1 min to clean the surface. Next, FCM (5 μL, 0.1 mg/mL) was dropped onto the Au electrode surface and dried under an infrared lamp, and Nafion (5% wt in ethanol, 2 μL) was added to the surface as glue. After drying, the electrode denoted as FCM/Au was obtained. For chronoamperometry measurements, pure nitrogen was used to remove the dissolved oxygen in $K_3Fe(CN)_6$ (3 mM) electrolyte solution for 10 min. The Q-$t^{1/2}$ curve was recorded by the work potential at +2 V.

A three-electrode system was used to record the Mott-Schottky curves at a frequency of 1000 Hz: the working electrode is an FCM-coated Au electrode, the counter electrode is a Pt wire, and the reference electrode is Ag/AgCl. The electrolyte was Na_2SO_4 solution (0.5 M) deoxygenated by N_2. All tests were carried out at room temperature.

Preparation of Other MOF-based Nanozymes

Fe-MIL-88NH_2, MIL-53(Fe), and MIL-101(Fe) were prepared according to previous reports.[1-3] For Fe-MIL-88NH_2, 0.126 g of 2-aminoterephthalic acid and 0.187 g of $FeCl_3 \cdot 6H_2O$ were dissolved in 15 mL of DMF, and then 0.207 g acetic acid was added into this mixed solution. The mixed solution was placed in an oil bath at 120°C for 4 h. After cooling to room temperature, the particles were

isolated by centrifugation and washed with DMF and ethanol to remove the excess reactants. Finally, the Fe-MIL-88NH_2 was dried in a vacuum oven.

Metal-organic framework MIL-53(Fe) was prepared by mixing 0.272 g of $FeCl_3·6H_2O$ and 0.166 mmol of 1,4-BDC slowly into 5 mL of DMF solution. The mixture was stirred for 10 min at room temperature, transferred into a Teflon-lined autoclave with a volume capacity of 20 mL, and heated at 150°C for 6 h. After the heat treatment, the autoclave was allowed to cool naturally to room temperature and the products collected by centrifugation at 6000 rpm for 2 minutes. To remove the solvent, the obtained yellow powder was suspended in 200 mL of distilled water overnight, and then centrifuged in water and vacuum-dried at 60°C for 24 h.

MIL-101(Fe) was synthesized by dissolving 0.41 g $FeCl_3·6H_2O$ and 0.12 g terephthalic acid in 10 mL DMF, transferred to a Teflon-lined autoclave, and heated at 110°C for 24 h. After cooling to room temperature, the precipitate was collected by centrifugation and further purified by repeated washing with water and ethanol. Finally, the obtained product was vacuum-dried at 60°C for 24 h.

Comparison of GOx Activity in Different Biocatalytic System

The activity of natural enzyme GOx is obtained by detecting the concentration of the substrate gluconic acid in the reaction system using HPLC (Agilent 1200). The UPLC system was equipped with an XDB-C_{18} column (4.6 mm × 250 mm, 5 μm) and a photodiode array detector. The mobile phase was sulfuric acid solution (1.25 mM), the flow rate 0.1 mL min^{-1} in isocratic mode, the detector wavelength set to 210 nm, and the injection volume 1 μL.

Calculation of Knudsen Diffusivity

The Knudsen diffusivity is employed to roughly estimate the substrate diffusion within the pore structure. The Knudsen diffusivity for diffusing species A, D_{KA}, can be explained by the equation:

$$D_{KA} = \frac{du}{3} = \frac{d}{3}\sqrt{\frac{8RT}{\pi M_A}}$$

where molar mass MA is expressed as kg/mol, temperature (T) in kelvins, and R is the universal gas constant, a product of Avogadro's number (Na) with Boltzmann constant, K (R = kNa), 8.3144 $J·mol^{-1}·K^{-1}$. Thus, D_{KA} depends on the pore diameter, species molar mass, and temperature.

Stability and Reusability Study

For the stability test, the GOx@FCM-TA stock solution (10 μL) was exposed to high temperature (70°C) for 2 h, ultrasound bath (20 kHz) for 1 h, acetone for 1 h, urea solution (6 M) for 3 h, EDTA (1%) for 3 h, and trypsin solution (2.5 mg/mL) for 3 h. Cascade reactions were then performed to determine the residual reactivity of GOx@FCM-TA. For the reusability test, the GOx@FCM-TA stock solution (10 μL) was centrifuged (10,000 rpm, 5 min) to remove the supernatant before adding glucose (800 μL, 5 mM), TMB (5 mM, 100 μL), and MES buffer (90 μL, 50 mM, pH 5) and incubating at 40°C for 10 min. The reaction solution was collected by centrifugation at 10,000 rpm for 5 min and detected by UV-vis at 652 nm. The centrifugation substrate was washed with 1 mL MES buffer to continue the next reaction. The reaction and washing steps were repeated to achieve reusability of the multienzyme system.

Colorimetric Glucose Detection

To establish the standard curve, the GOx@FCM-TA stock solution (10 μL, 1 mg/mL) was added to MES buffer solution (50 mM, pH 6) containing 1 mM TMB and various concentrations of glucose (0, 5, 25, 50, 100, 150, 200, 250, 500, and 750 μM). The above mixture was reacted for 10 min at 40°C, and the absorbance of the supernatant was recorded at 652 nm. To investigate the selectivity of the colorimetric sensor, glucose (100 μM), fructose, xylose, maltose, mannose, galactose, ascorbic acid (1 mM for each), and bovine serum albumin (BSA, 1 mg/mL) were added in MES buffer containing 1 mM TMB. The solutions were then mixed with GOx@FCM-TA for 10 min at 40°C. The absorbance of the supernatant was detected at 652 nm.

For glucose detection in biological samples, GOx@FCM-TA stock solution (10 μL, 1 mg/mL) was mixed with diluted serum sample (800 μL) and TMB solution (1 mM, 190 μL). The above mixture was reacted for 10 min at 40°C and the absorbance of the supernatant recorded at 652 nm. A certain amount of glucose was then added to the serum to make spiked samples. The glucose levels in these spiked samples were detected via the above method. For comparison, the glucose concentration of the serum was measured using the glucose meter.

Colorimetric Lactose Detection

To establish the standard curve, GOx@FCM-TA stock solution (10 μL, 1 mg/mL) was added to

MES buffer solution (50 mM, pH 6) containing 1 mM TMB and various concentrations of lactose (0, 5, 25, 50, 100, 150, 200, 250, 500, and 750 μM). The above mixture was reacted for 10 min at 40°C and the absorbance of the supernatant recorded at 652 nm. To investigate the selectivity of the colorimetric sensor, lactose (100 μM), mannose, galactose, fructose, maltose, arginine, uric acid (1 mM for each), and BSA (1 mg/mL) were added to MES buffer containing 1 mM TMB. The solutions were then mixed with GOx@FCM-TA for 10 min at 40°C. The absorbance of the supernatant was detected at 652 nm.

For lactose detection in biological samples, GOx@FCM-TA stock solution (10 μL, 1 mg/mL) was mixed with diluted serum and milk sample (800 μL) and TMB solution (1 mM, 190 μL). The above mixture was reacted for 10 min at 40°C and the absorbance of the supernatant recorded at 652 nm. For comparison, the lactose levels in samples were measured using HPLC.

Table S1 The enzyme dosage and calculated loading rates in different cascade systems.

Cascade system	Enzyme	Dosage (mg)	Loading (%, w/w)
GOx@FCM-TA	GOx	4	21.6
β-Gal/GOx@FCM-TA	β-Gal	2	16.8
	GOx	2	12.4

Table S2 Nitrogen isothermal sorption profiles of biocatalytic MOF samples.

Sample	BET Surface area (m^2/g)	Total pore volume (cm^3/g)
FCM	102.42	0.10
GOx@FCM	64.58	0.07
GOx@FCM-TA	79.23	0.09

Table S3 Performances of different sensing systems in glucose detection.

Catalyst	Method	Linear range (μM)	LOD (μM)	Ref.
GOx/FeNi-MOF	Colorimetric	300-35,000	1.3	4
GOx@MOF-545(Fe)	Colorimetric	0.5-100	0.28	5
GOx@CuBDC	Colorimetric	10-500	4.1	6
GOx@$Mn_3(PO_4)\cdot 3H_2O$	Colorimetric	50-20,000	10	7
GOx@ZIF-8(NiPd)	Colorimetric	10-300	9.2	8
Au NPs/Cu-TCPP(Co)	Colorimetric	10-300	8.5	9
Cu_2O/GNs	Electrochemical	300-3300	3.3	10
GOx@FCM-TA	Colorimetric	5-750	0.94	This work

Table S4 Performances of different sensing systems in lactose detection.

Catalyst	Method	Linear range (μM)	LOD (μM)	Ref.
β-Gal/GOD/HRP	Colorimetric	200-1800	100	11
$(PEI/\beta\text{-Gal})_n$	Electrochemical	1.4×10^5-8×10^5	1130	12
NH_2-PD/SWCNTs	Electrochemical	1-150	0.5	13
CDH	Electrochemical	1-100	1	14
Co-hemin MOF/chitosan	Electrochemical	10^3-10^5	4000	15
β-Gal/GOx@FCM-TA	Colorimetric	5-250	1.56	This work

Table S5 Comparison of β-Gal/GOx@FCM-TA biosensors with HPLC.

Sample	Result of HPLC (μM)	Proposed method (μM)	RSD (%, n=3)
Serum-1	13.2	13.8	5.63
Serum-2	9.4	9.0	2.56
Serum-3	11.8	11.9	4.58
Pure milk	117.6	115.8	9.17
Banana milk	84.4	86.1	5.18
Drinking yogurt	18.7	17.7	1.99

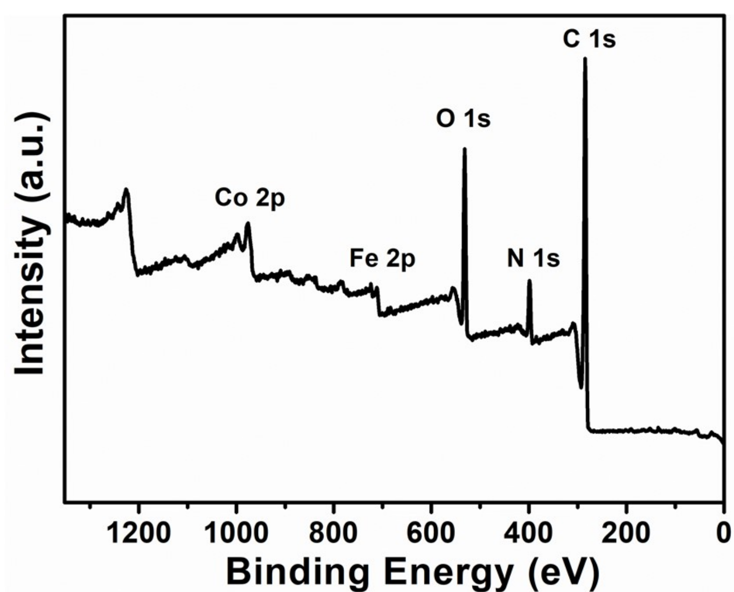

Fig. S1 XPS survey of GOx@FCM-TA.

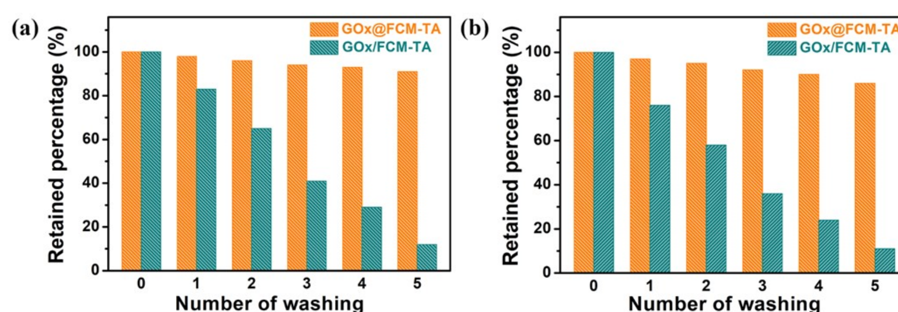

Fig. S2 (a) PVP exchange results for GOx@FCM-TA and GOx/FCM-TA. (b) SDS washing results for GOx@FCM-TA and GOx/FCM-TA.

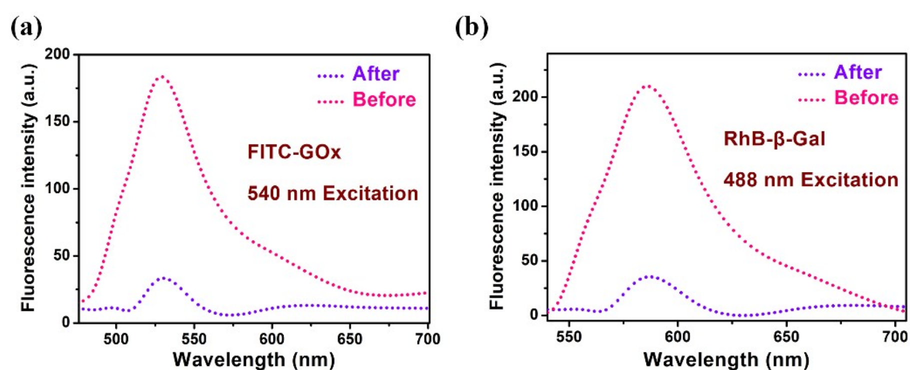

Fig. S3 The change in FITC-GOx (a) and RhB-β-Gal (b) fluorescence in the supernatants before and after assembly of FCM.

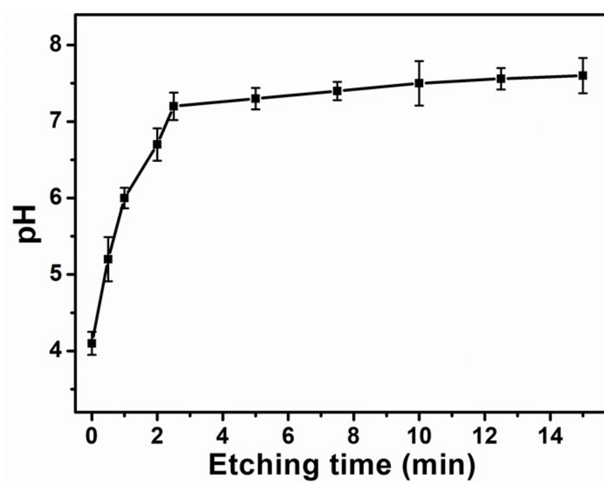

Fig. S4 pH of the reaction system as a function of etching time.

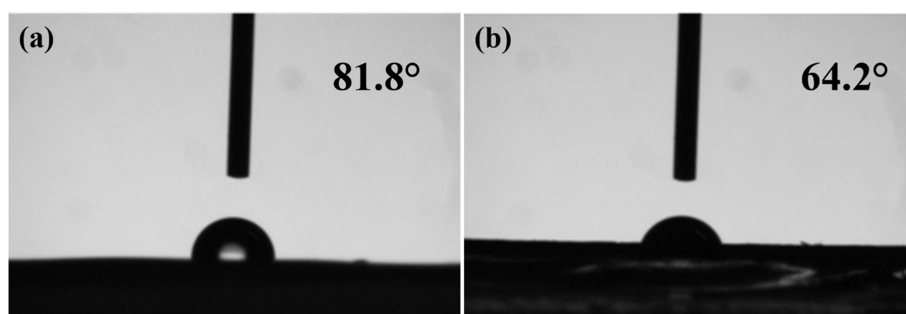

Fig. S5 Water contact angle images of GOx@FCM (a) and GOx@FCM-TA (b).

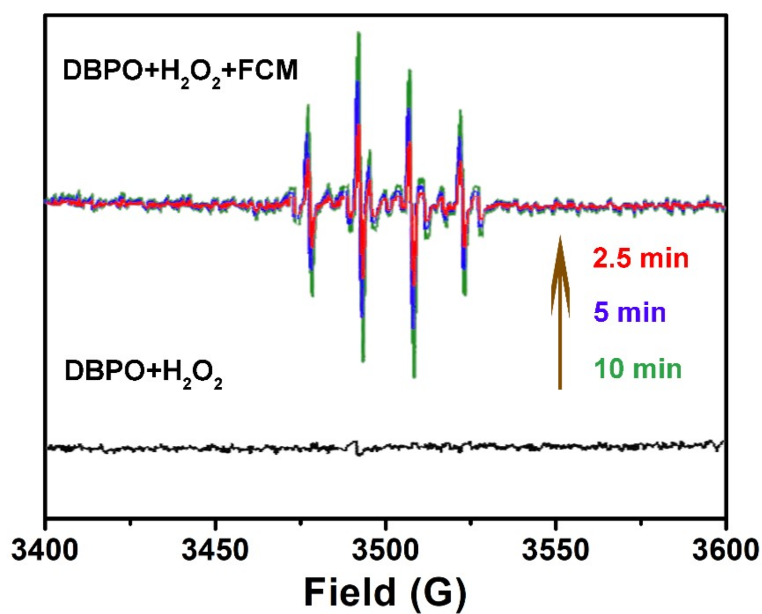

Fig. S6 ESR spectra of H_2O_2/DMPO spin adduct produced in HAc-NaAc buffer.

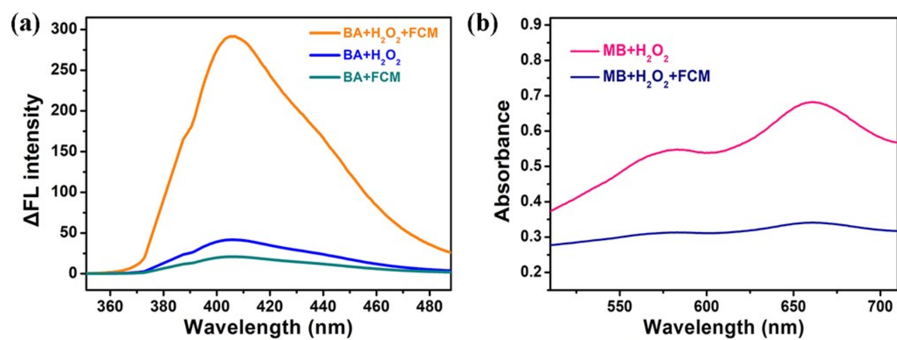

Fig. S7 (a) Fluorescence spectra in various reaction systems. (b) Absorbance spectra of MB before and after addition of FCM.

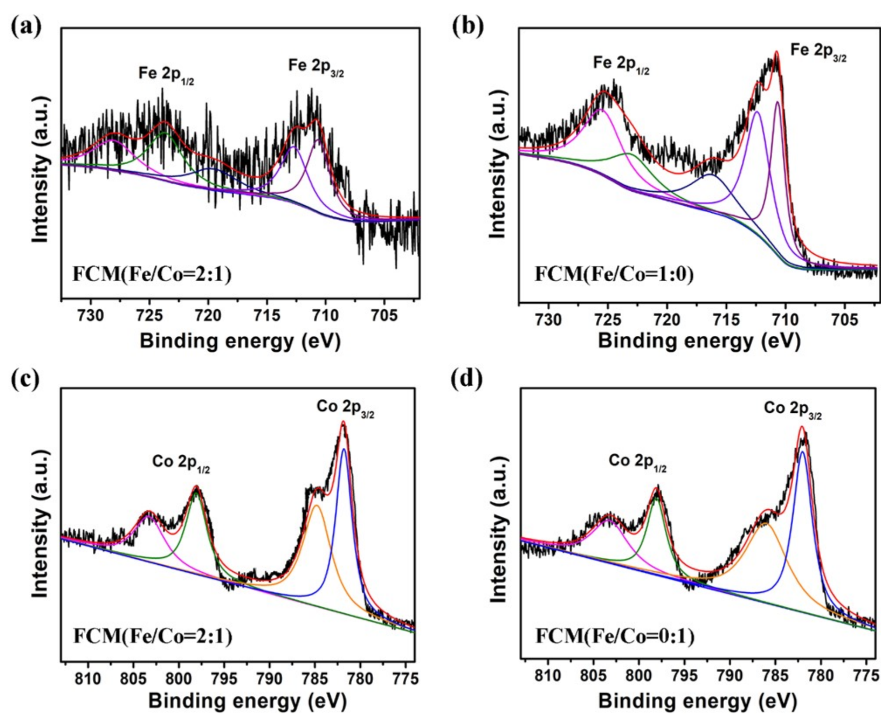

Fig. S8 XPS spectra of Fe (a, b) and Co (c, d) in FCM(Fe/Co=2:1), FCM(Fe/Co=1:0), and FCM(Fe/Co=0:1).

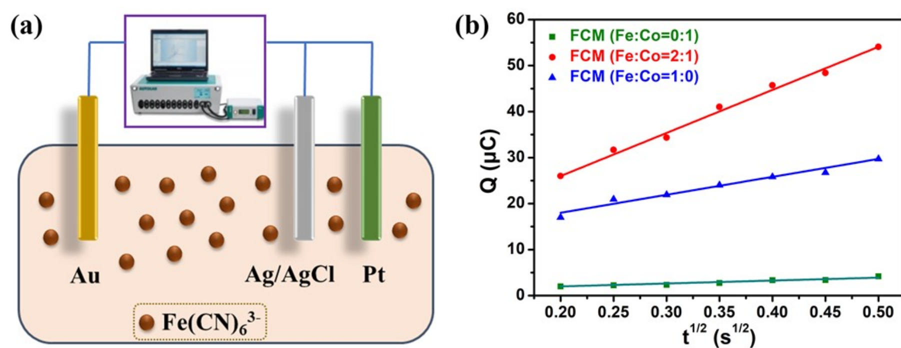

Fig. S9 (a) Scheme of electrochemical detection. FCM samples were modified onto Au electrodes. (b) Linear relationships of Q-$t^{1/2}$ for FCM/Au.

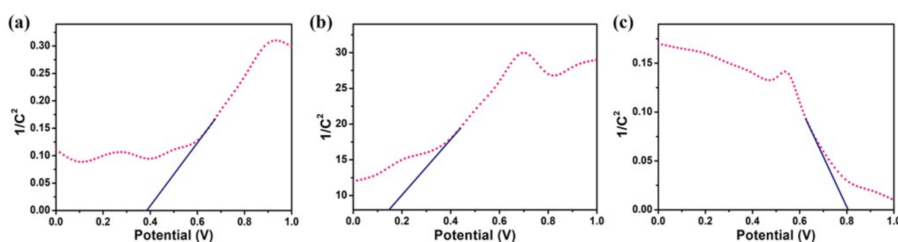

Fig. S10 Mott-Schottky plots of the FCM(Fe/Co=2:1) (a), FCM(Fe/Co=1:0) (b), and FCM(Fe/Co=0:1) (c).

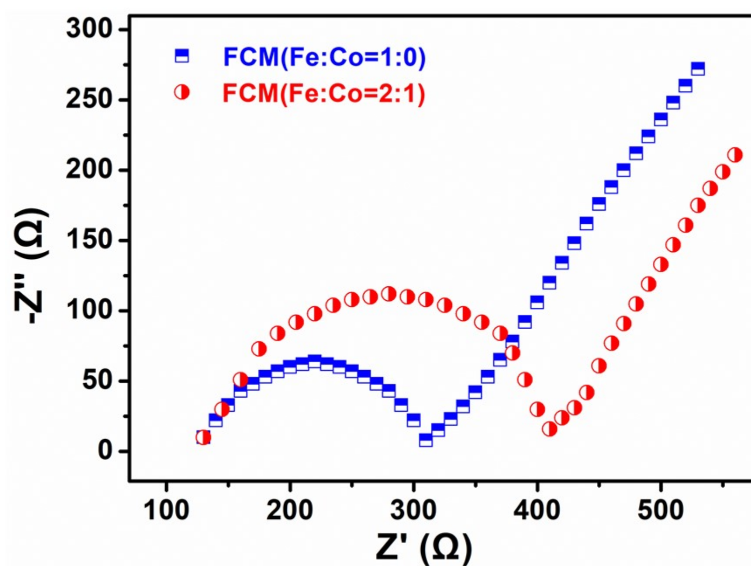

Fig. S11 EIS curves of FCM(Fe:Co=1:0) and FCM(Fe:Co=2:1) acquired in aqueous solution with 0.1 M KCl and 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1).

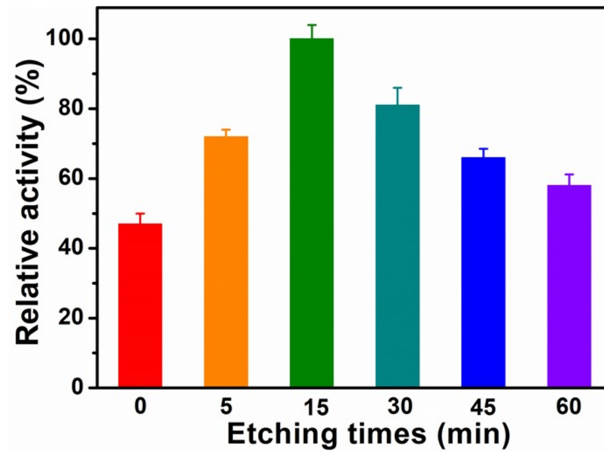

Fig. S12 Relative activities of GOx@FCM-TA with different etching times.

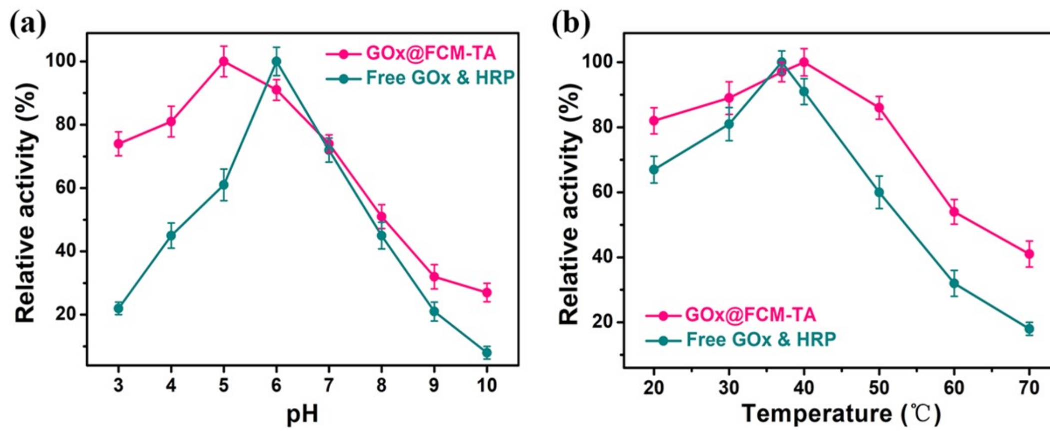

Fig. S13 Effect of pH (a) and temperature (b) on the cascade activity of GOx@FCM-TA and free GOx and HRP.

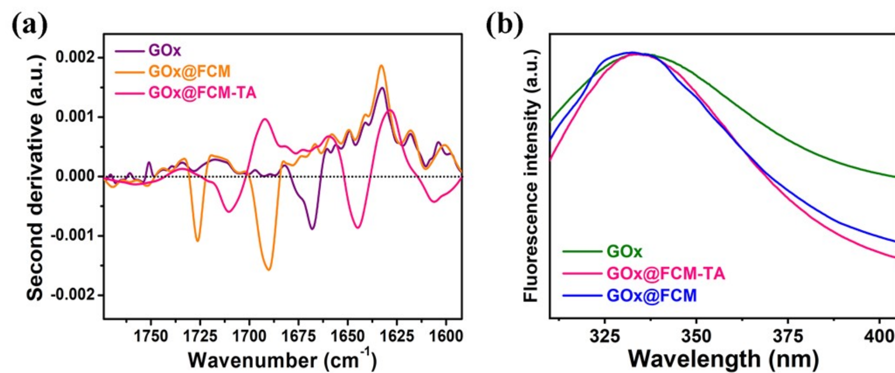

Fig. S14 The second-derivative FT-IR (a) and fluorescence (b) spectra of GOx, GOx@FCM, and GOx@FCM-TA.

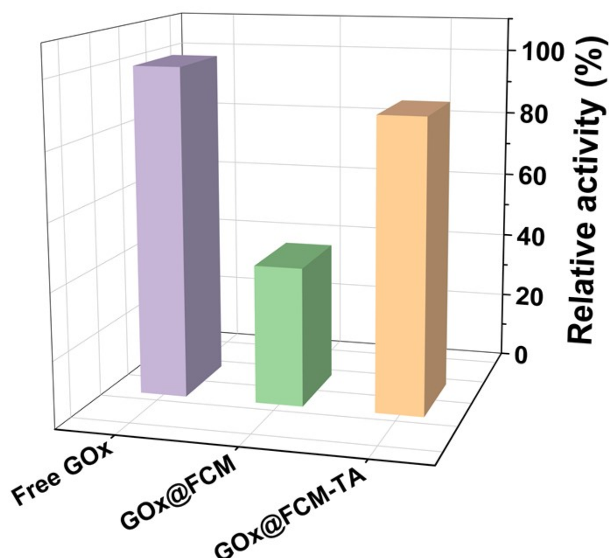

Fig. S15 Comparison of GOx bioactivity in free GOx, GOx@FCM, and GOx@FCM-TA.

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