Supporting information for

Facile Synthesis of Multiple Enzyme-Contained Metal-Organic Frameworks in Biomolecule-Friendly Environment

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Materials

Glucose oxidase (GOx) from *Aspergillus niger*, peroxidase from horseradish (reagent grade) (HRP), phosphate buffer saline (1x), 2-methylimidazole, glucose and 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich. Zinc nitrate hexahydrate (99.998%) was purchased from Alfa Aesar. All the other reagents were purchased from Sigma-Aldrich and used without further purification.

Methods

Synthesis of the GOx&HRP/ZIF-8, GOx/ZIF-8 and HRP/ZIF-8 composite.

A water solution (4 mL) of GOx (5 mg/mL) and HRP (7.5 mg/mL), and $Zn(NO_3)_2$ water solution (0.31 M, 4 mL) were mixed with 2-methylimidazole water solution (1.25 M, 40 mL) under stirring at 25 ^oC. The mixture then turned milky almost instantly after mixing. After stirring for about 30 min, the product was collected by

centrifuging at 6 000 rpm for 10 min, and washed with DI water for three times. A small part of the product was re-dispersed in methanol to prepare samples for TEM and SEM characterizations. The rest part of the product was re-dispersed in DI water for lyophilization and used for other characterizations.

Synthesis of GOx/ZIF-8 and HRP/ZIF-8 composite was following the same protocol by replacing the multi-enzyme solution with GOx water solution (5 mg/mL) and HRP water solution (7.5 mg/mL), respectively

Synthesis of the ZIF-8 crystals.

The synthesis of pure ZIF-8 nanocrystals followed the same procedure to the preparation of GOx&HRP/ZIF-8 but in the absence of enzyme solution. $Zn(NO_3)_2$ water solution (0.31 M, 4 mL) was added into 2-methylimidazole water solution (1.25 M, 40 mL) under stirring at 25 ^oC. After stirring for about 30 min, the product was collected by centrifuging at 6 000 rpm for 10 min, and washed with DI water for three times.

Detection of glucose by GOx&HRP/ZIF-8 composite, the mixture of GOx/ZIF-8 and HRP/ZIF-8 and pure ZIF-8.

Glucose with varied concentrations was added to 1 mL of potassium phosphate buffer (10 mM, pH 7.4) containing 0.75 mg of GOx&HRP/ZIF-8 composite and 532 mM ABTS, followed by incubation at room temperature for 10 min. Then, the reaction solution was centrifuged at 10 000 rpm for 3 min to remove the GOx&HRP/ZIF-8 composite and the absorbance at 415 nm was recorded on a Shimadzu UV-2450 UV-VIS Spectrophotometer. Glucose detection using the mixture of GOx/ZIF-8 and

HRP/ZIF-8 was carried out by premixing the powder of GOx/ZIF-8 and HRP/ZIF-8 to achieve the same protein content with the multi-enzyme system.

Selectivity of the GOx&HRP/ZIF-8 composite.

The selectivity of the GOx&HRP/ZIF-8 composite for glucose detection was evaluated by monitoring the absorbance increase at 415 nm in the presence of various saccharides and other interferes. The experiments were taken by using 1.0 mM fructose, 1.0 mM mannose, 1.0 mM galactose, 1.0 mM lactose, 1 mg/mL BSA and 100 μM glucose.

Stability of the GOx&HRP/ZIF-8 composite.

The suspension of GOx&HRP/ZIF-8 composite or in PBS was incubated with 1 mg/mL of trypsin at 37 ^{o}C for 30 min, followed by the measurement of the residual overall enzymatic activity using the standard method. For EDTA treatment, the suspension of GOx&HRP/ZIF-8 composite was incubated in PBS in the presence of EDTA (1 wt%) at room temperature for 30 min, followed by the measurement of the residual overall enzymatic activity using the standard method.

TEM analysis for MOF and enzyme/MOF composites.

A drop of methanol suspension containing the synthesized MOF or enzyme/MOF composites was added on a carbon grid and dried at room temperature. TEM images were taken on a JEOL JEM-2010 high-resolution TEM with an accelerating voltage of 120 kV.

SEM analysis for MOF and enzyme/MOF composites.

Scanning electron microscope (SEM) images of samples were taken on a Sirion 200 SEM at an accelerating voltage of 10.0 kV. Samples for SEM measurements were prepared by first suspending the composites in methanol and then 1-10 microliters of the sample solution was dropped onto a silica wafer. After all methanol was evaporated, the silica wafer was attached to a carbon paste and then sputter-coated with a thin layer of conductive gold to improve the electrical conductivity.

XRD analysis of MOF and enzyme/MOF composites.

Powder X-ray diffraction (XRD) patterns were recorded using a Bruker D8 Advance X-Ray diffractometer with a Cu Kα anode (λ= 0.15406 nm) at 40 kV and 40 mA.

Thermogravimetric analysis of MOF and enzyme/MOF composites.

Thermal gravimetric analyses (TGA) in air were performed on a TA Instruments TGA 2050 Thermogravimetric Analyzer. The sample was heated from room temperature to 600 ^{o}C at a rate of 20 ^{o}C/min under air atmosphere.

Laser scanning confocal microscope.

8 mg of fluorescein isothiocyanate (FITC) or rhodamine B isothiocyanate (RhB) dissolved in DMSO (2 mg/mL) was slowly added in to 1 mL of GOx or HRP solution (5 mg/mL of enzyme in 0.5 M, pH 9.5 carbonate buffer). The solution was shaken for 6 h at 300 rpm at ambient temperature in dark. Free FITC or RhB was removed via dialysis against DI water. The fluorescent molecule-labeled enzymes were freeze dried and then dissolved in water for the subsequent synthesis of fluorescently labeled GOx&HRP/ZIF-8 composite. Laser scanning confocal microscope images were taken

on a Zeiss LSM 780 confocal microscope. The detection wavelengths were 488 nm and 543 nm for FITC and RhB, respectively.

Determination of protein concentration in the supernatant by SEC.

In the synthesis of GOx&HRP/ZIF-8 composite, the concentrations of unencapsulated HRP and GOx in the supernatant after synthesis were determined by size exclusion chromatography (SEC) on a SHIMADZU HPLC system equipped with a TSK-GEL G2000SWxL column. 20 μL of sample solution was injected and eluted by using phosphate buffer solution (0.1 M) containing 0.1M Na_2SO_4, 0.1% NaN_3 at an elution speed of 1 mL/min. The detection of protein was carried out by UV/Vis absorbance at 280 nm.

Optimization of enzyme ratio for the GOx&HRP system.

To construct a multi-enzyme system that involves two enzymes working together in a cascade reaction, the optimization of the ratio of free enzymes is a key step. At the fixed total amount of enzyme, we varied the mass ratio of GOx to HRP as 1:4, 2:3, 3:2, 4:1. The overall activity of the bi-enzyme system determined by using 100 μM glucose as the substrate indicated that the best mass ratio of GOx to HRP was 2:3 as shown in Figure S2, which agreed well with the previous study.[1]

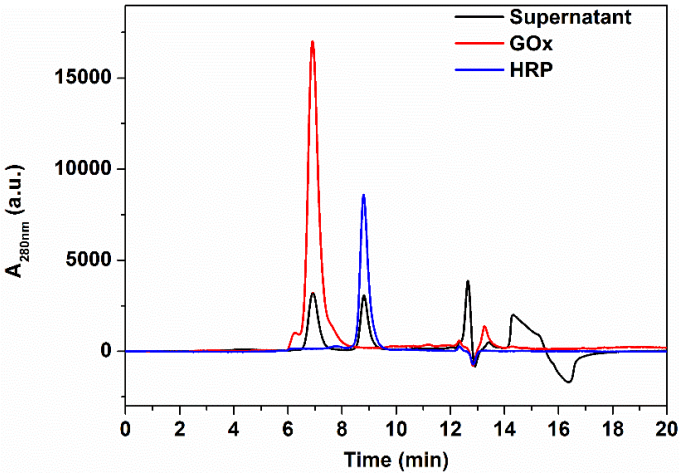

Figure S1. Determination of GOx and HRP concentration in the supernatant by SEC.

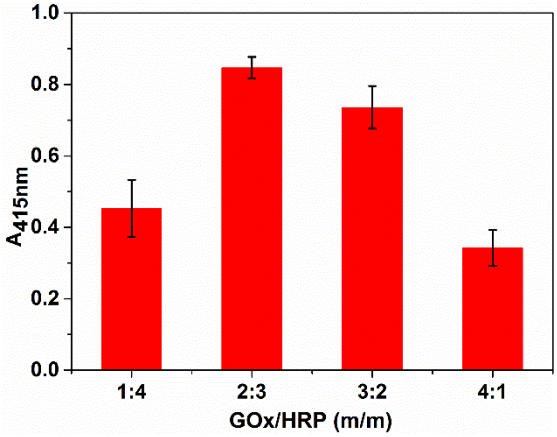

Figure S2. Comparison of overall activities of GOx/HRP free enzyme system with different mass ratios of GOx and HRP[1].

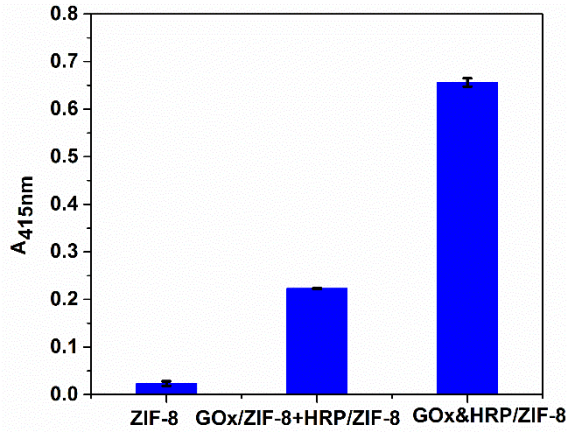

Figure S3. The glucose detection of pure ZIF-8, the mixture of GOx/ZIF-8 and HRP/ZIF-8, and GOx&HRP/ZIF-8.

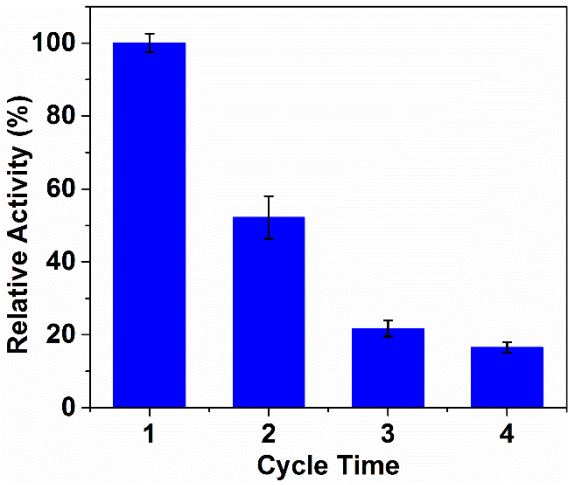

Figure S4. Relative activity of GOx&HRP/ZIF-8 after reusing for several cycles.

References

1. Zhang, Y.; Lyu, F.; Ge, J.; Liu, Z. *Chem. Commun.* 2014, ***50*****,** 12919.