Supplementary Information for

Synthesis of enzyme-embedded metal-organic framework nanocrystals in reverse micelles

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Materials

Horseradish peroxidase (reagent grade) (HRP), phosphate buffer saline (1x), 2-methylimidazole, Brij C10, 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 enzyme/MOF nanocrystals in reverse micelles

The reverse micelle solution A was prepared by mixing Brij C10 (3.420 g), cyclohexane (15.0 mL) and 1 mL of an aqueous solution containing $Zn(NO_3)_2 \cdot 6H_2O$ at different concentrations (1.0 mol/L, 0.5 mol/L, 0.25mol/L) under magnetic stirring at 37 °C. The reverse micelle solution B was prepared under the same condition as preparing solution A, but replacing $Zn(NO_3)_2 \cdot 6H_2O$ with 2-methylimidazodole (4mol/L, 2mol/L, and 1mol/L) and HRP (1 mg/mL). Then, rapidly

adding the solution B to the solution A under stirring resulted in the turbidity of the mixture. Followed by incubation at 37 °C for 2 h, the product was separated out by precipitation in isopropanol, centrifugation and washing with isopropanol for three times.

TEM analysis for enzyme/MOF nanocrystals

A drop of methanol suspension containing t 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 enzyme/MOF nanocrystals

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 enzyme/MOF nanocrystals

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.

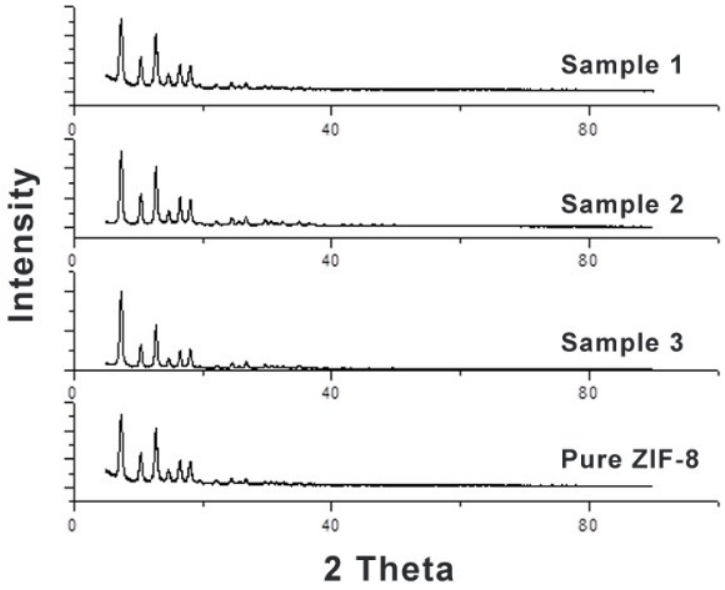

Figure S1. X-ray diffraction (XRD) patterns HRP/ZIF-8 nanocrystals and pure ZIF-8.

Thermogravimetric analysis of enzyme/MOF nanocrystals

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 °C at a rate of 20 °C/min under air atmosphere.

Inductively coupled plasma mass spectrometry (ICP-MS)

Inductively coupled plasma mass spectrometry (ICP-MS) was performed on a X Series ICP-MS, Thermo Fisher Scientific Inc.

Table S1. ICP-MS analysis of the HRP/ZIF-8 nanocrystals

	Fe (μg/g)	Enzyme loading percentage
Pure ZIF-8	43.2	0%
HRP/ZIF-8 **1**	178.9	10.7%
HRP/ZIF-8 **2**	91.88	3.8%
HRP/ZIF-8 **3**	313.1	21.2%

Assay of peroxidase activity of enzyme/MOF nanocrystals

For determining the peroxidase activity of HRP/MOF nanocrystals, 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid)-diammonium salt (ABTS) was used as the substrate. The enzymatic activity was determined by adding 50 μL of HRP/MOF nanocrystals or free HRP solution (containing the same of concentration of HRP of 10 μg /mL, the HRP loading percentage in different HRP/MOF samples was determined by TGA analysis as described in the main text) and 50 μL of 0.3% H_2O_2 to 900 μL of the substrate solution (0.5 mM ABTS in 50 mM phosphate buffer, pH 7.0). The increase of absorbance at 415 nm was recorded for 1 min. The specific activity of free HRP was set as 100% and the relative activities of the HRP/MOF nanocrystals in Figure 5 were calculated based on the activity of free HRP with the same protein content. In a typical experiment, the reaction rates were determined as 0.0064 mM/min, 0.033 mM/min, 0.014 mM/min, and 0.024 mM/min for native HRP, HRP/ZIF-8 **1**, HRP/ZIF-8 **2**, and HRP/ZIF-8 **3** respectively. Pure ZIF-8 didn't show any activity regarding the substrate we utilized in this study.