Electronic Supplementary Information

**Biomimetic mineralization of nitrile hydratase into a mesoporous cobalt-based metal-organic framework for efficient biocatalysis**

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Experimental

Protein expression and purification

Luria-Bertani (LB) medium comprised 10 g/L of tryptone, 5 g/L of yeast extract and 10 g/L of NaCl. The pH was adjusted to 7.0 by 3 M NaOH. Recombinant E. coli harboring pENh1229 was grown in LB medium supplemented with 50 μg/mL kanamycin and 20 mg/L CoCl₂. After 3 h of culture at 37 °C to the cell density of OD₆₀₀ = 0.6-0.8, isopropyl β-D-1-thiogalactopyranoside (IPTG, 100 μM) was added to induce the expression of recombinant NHase1229 at 18 °C for 4 h. The collected cells were collected and disrupted through sonication (Sonicator 400, Misonix, USA) in an ice bath. The recombinant NHase1229 was purified by a nickel chelate affinity column.

Recombinant NHase1229 expression and activity assays

The expression level of recombinant NHase1229 was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 16%) with 4% stacking gel. The concentrations of 3-cyanopyridine and nicotinamide were determined by reversed-phase high-performance liquid chromatography (Agilent 1100, Santa Clara, CA, USA).

The activity was assayed in a standard reaction mixture (0.5 ml) containing 50 mM phosphate buffer (pH7.0), 100 mM 3-cyanopyridine and an appropriate amount of free NHase1229 solution or immobilized NHase1229. The reaction was incubated at 25 °C for 2 min and stopped by adding 0.5 mL acetonitrile. One unit (U) of NHase activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol nicotinamide in 1 min at 25 °C. The protein concentrations were determined by a Bradford Protein Assay Kit (Quick Start, Bio-Rad, USA).

Structural stability of ZIF-67 in aqueous solution

To investigate the structural stability of ZIF-67 in aqueous solution, ZIF-67 crystals were suspended in pH 8.0 phosphate buffer solution (PBS) and stored at 4 °C for different time. Samples were collected, and characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM) to obtain their structural information.

Effects of cobalt ions and 2-methylimidazole (mIM) on the activity of NHase1229

To test the effects of cobalt ions and mIM on the activity of NHase1229, NHase1229 was suspended in pH 8.0 PBS (1.0 mg/mL protein) containing 0.013 M Co(NO₃)₂, 0.039 M or 0.052 M mIM, and 2
stored at 4 °C overnight. Residual activity was measured by standard assay. All experiments were performed in duplicate.

**Optimization of the enzyme amount for preparing NHase1229@ZIF-67**

Different amount of NHase1229 was dissolved in 6.5 mL 0.026 M Co(NO3)2 solution, and then mixed with 0.78 M mIM solution and stirred at room temperature for 30 min. The mixture was further stored at 4 °C overnight. The purple precipitation was separated by membrane filtration and further wash with pre-cooling deionized water three times. The activity was measured by standard assay. The percent activity recovery of immobilized NHase1229 was determined as the following equation (1):

\[
\text{Activity recovery (\%) = } \frac{A_{\text{im}}}{A_{\text{in}}} \times 100\%
\]  

(1)

Where \( A_{\text{im}} \) and \( A_{\text{in}} \) are the total activity of the immobilized NHase1229@ZIF-67 and the initial supernatant before immobilization, respectively.

**The effect of buffer pH on the structural stability of NHase1229@ZIF-67**

Equivalent NHase1229@ZIF-67 crystals were suspended in different pH PBS and stored at 4 °C overnight. The color was visually compared.
Fig. S1 The expression of recombinant NHase1229 in *E. coli* BL21(DE3) analyzed by SDS-PAGE. M: protein molecular mass markers (ProteinRuler I, 12-80 kDa, TransGen Biotech, China), W: whole cell proteins, S: soluble region, I: insoluble region and P: purified NHase1229.
Fig. S2 SEM images of synthesized ZIF-67 with different mole ratio of Co^{2+}/mIM in pure aqueous solution
Fig. S3 XRD patterns of ZIF-67 after stored in pH 8.0 phosphate buffer solution for different time
**Fig. S4** SEM images of ZIF-67 after stored in pH 8.0 phosphate buffer solution for different time. (A) 0 day, (B) 7 days, (C) 15 days.
Fig. S5 Synthesis of NHase1229@ZIF-67. (A) The effects of cobalt ions and Mim on the activity of free NHase1229. (B) The effect of the enzyme amount on the activity of NHase1229@ZIF-67
Fig. S6 The effect of pH on the structural stability of NHase1229@ZIF-67
Fig. S7 The influence of the 3-cyanopyridine concentration on the catalytic efficiency using NHase1229@ZIF-67 as catalyst.