Electronic Supplementary Information

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

Xiaolin Pei*[a,b], Yifeng Wu[a], Jiapao Wang[a], Zhiji Chen[a], Wen Liu[a], Weike Su[b], Fangming Liu*[a]

[a] College of Material, Chemistry and Chemical Engineering, Hangzhou Normal University, Hangzhou, 310012, PR China

[b] Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou, 310032, PR China

E-mail: pxl@hznu.edu.cn (X. Pei), fmliu859@sohu.com (F. Liu)

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 $CoCl_2$. After 3 h of culture at 37 °C to the cell density of OD_{600} = 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, Misonicx, 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 $Co(NO_3)2$, 0.039 M or 0.052 M mIM, and

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):

$$\text{Activity recovery}\ (\%) = \frac{A_{im}}{A_{in}} \times 100\% \qquad (1)$$

Where A_{im} and A_{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.

Figures

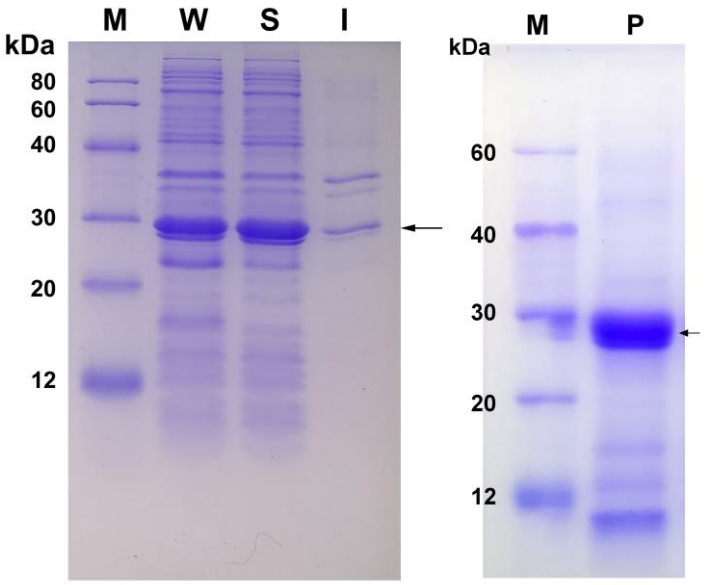

Fig. S1 The expression of recombinant NHase1229 in *E. coli* BL21(DE3) analyzed by SDS-PAGE. M: protein molecular mass markers (ProteinRuler I, 12-80 kDa, TransGen Biotech, China), W: whole cell proteins, S: soluble region, I: insoluble region and P: purified NHase1229.

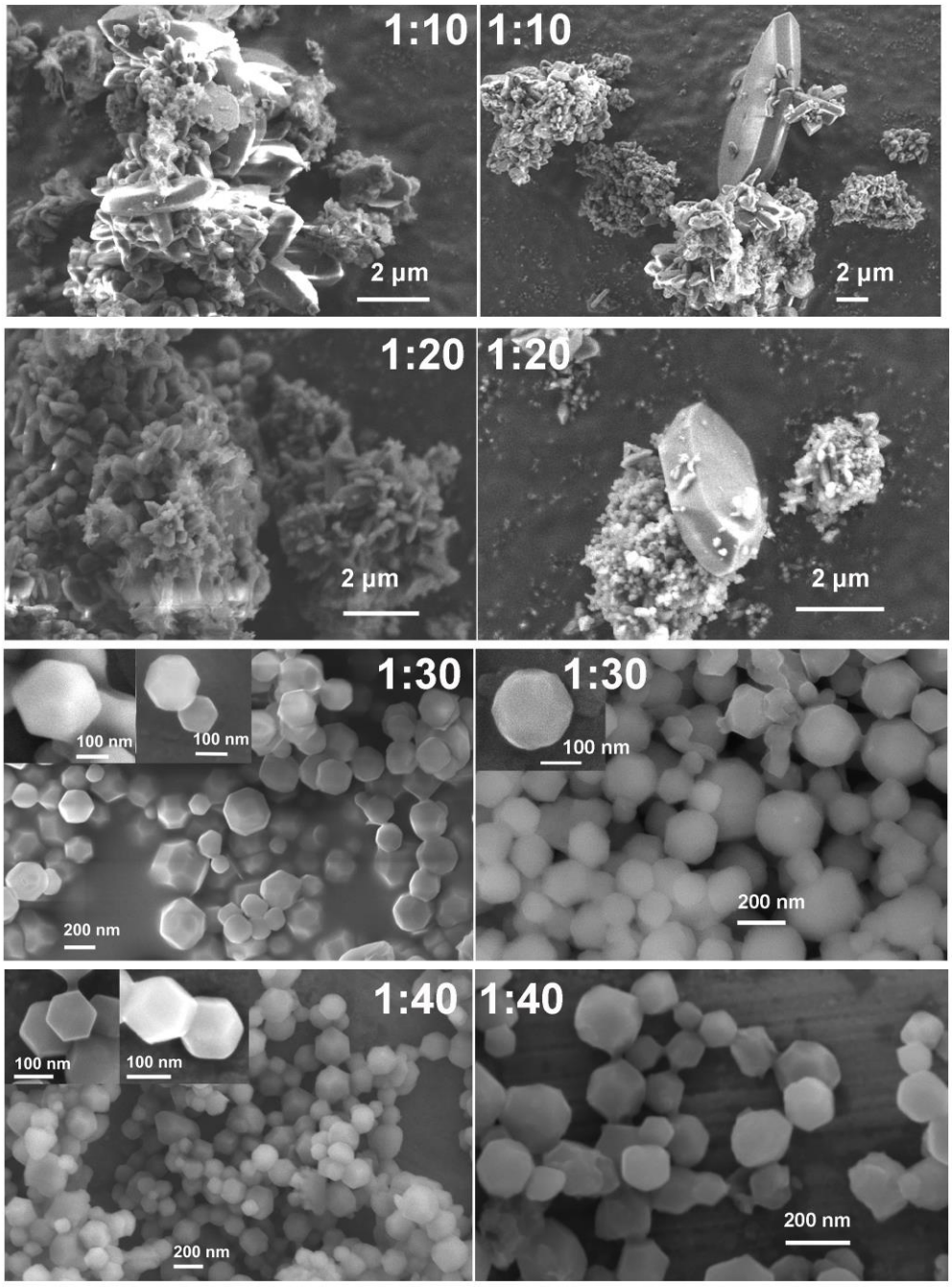

Fig. S2 SEM images of synthesized ZIF-67 with different mole ratio of Co^{2+}/mIM in pure aqueous solution

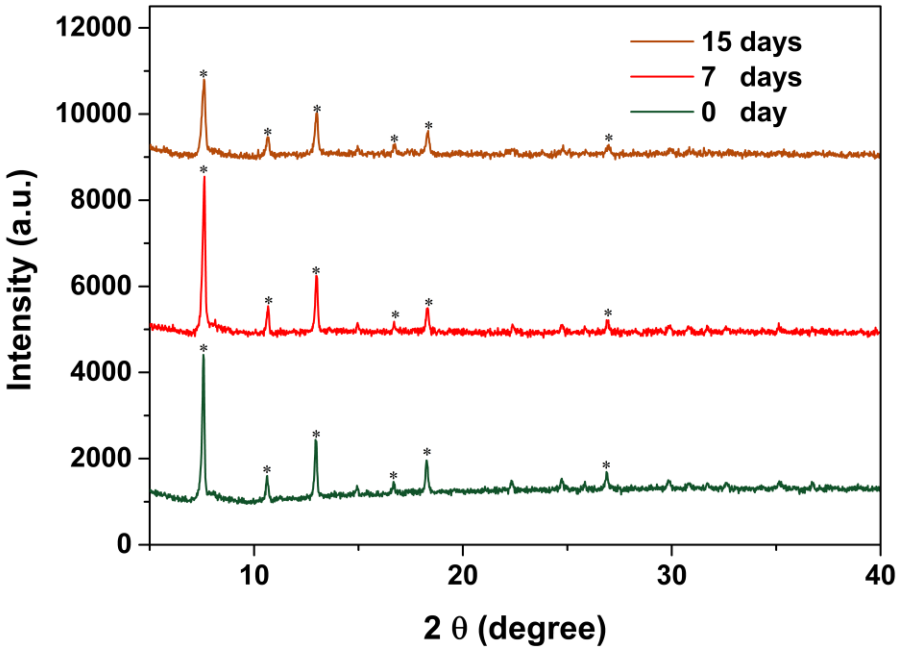

Fig. S3 XRD patterns of ZIF-67 after stored in pH 8.0 phosphate buffer solution for different time

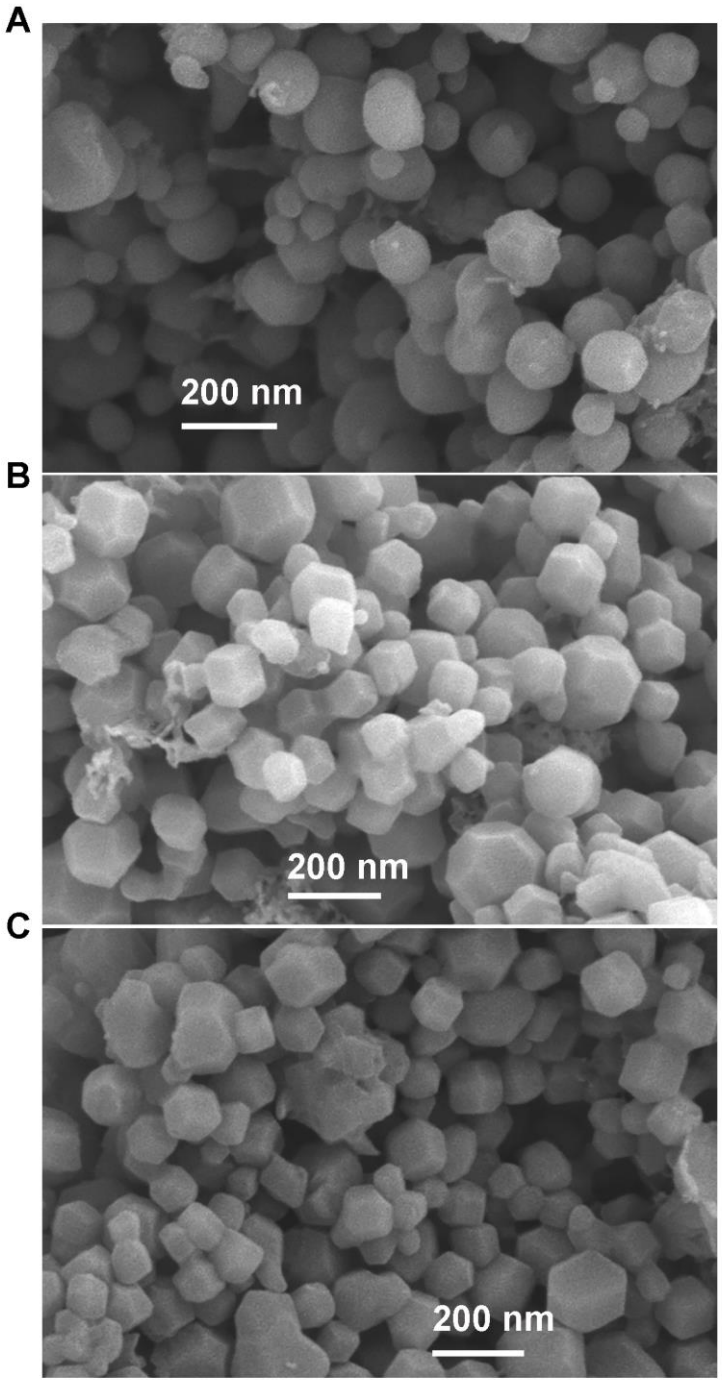

Fig. S4 SEM images of ZIF-67 after stored in pH 8.0 phosphate buffer solution for different time. (A) 0 day, (B) 7 days, (C) 15 days.

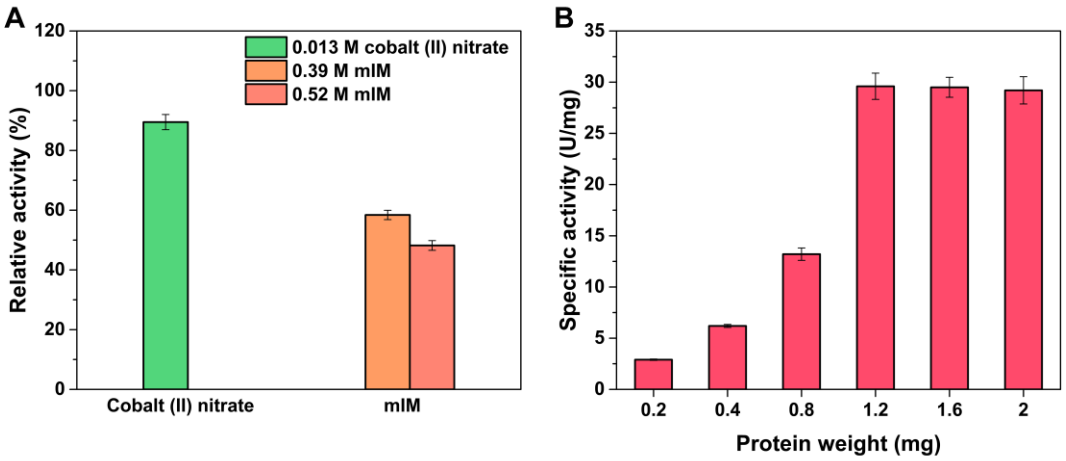

Fig. S5 Synthesis of NHase1229@ZIF-67. (A) The effects of cobalt ions and Mim on the activity of free NHase1229. (B) The effect of the enzyme amount on the activity of NHase1229@ZIF-67

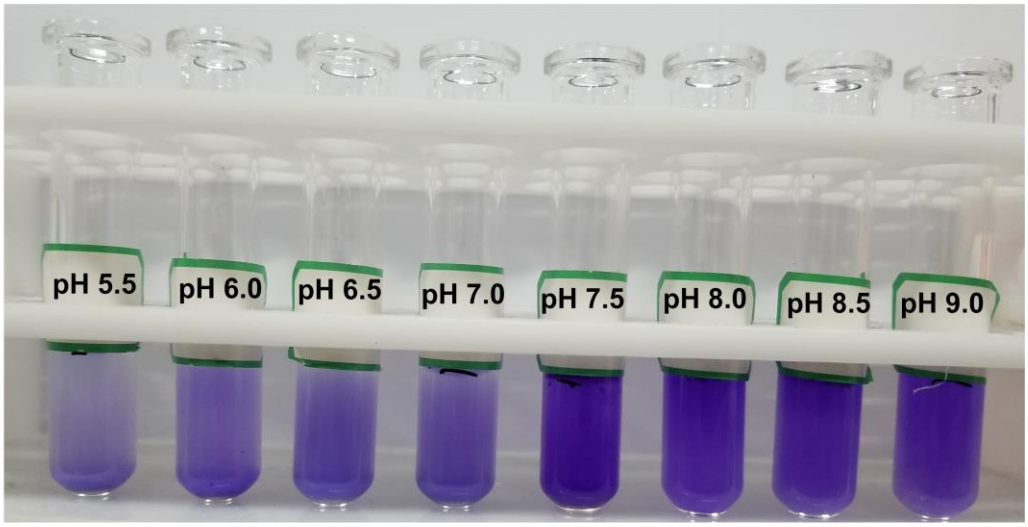

Fig. S6 The effect of pH on the structural stability of NHase1229@ZIF-67

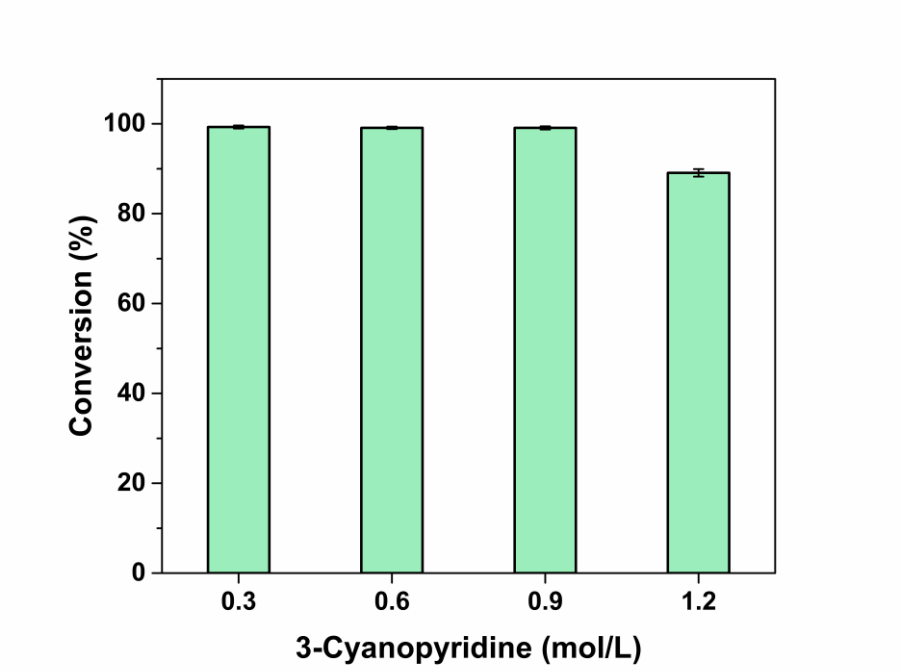

Fig. S7 The influence of the 3-cyanopyridine concentration on the catalytic efficiency using NHase1229@ZIF-67 as catalyst.