Electronic Supplementary Information

Immobilization of O-Acetylserine Sulfhydrylase as Highly Active and Recyclable Nanobiocatalyst: Efficient Synthesis of β-Pyrazol-1-yl-L-alanine

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**Figure S5.** FESEM and TEM (inset) images of His-CysK-MNPs.

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1. Chemicals, strains, and plasmids

O-acetylserine hydrochloride (97%), pyrazole (98%), β-pyrazol-1-yl-L-alanine (95%), pyridoxal 5’-phosphate (98%), and hydrochloric acid (37%) were purchased from Sigma Aldrich. Monopotassium phosphate (99%), dipotassium phosphate (98%), phosphoric acid (85%), and acetic acid (100%) were obtained from Merck. Kanamycin and Isopropyl β-D-1-thiogalactopyranoside (IPTG) (97%) were purchased from Apollo Scientific and Gold Biotechnology, respectively. Gel electrophoresis equipment was obtained from Biorad. Loading dye GelPilot and PageRuler™ prestained protein ladder were purchased from Qiagen and Thermo Scientific, respectively. Ni-NTA Agarose Resin was obtained from Roche Diagnostics GmbH. Amicon ultra-15 centrifugal tube (10 kDa) was purchased from Millipore.

E. coli T 7 was bought from New England Biolab and Plasmid pET 28a was obtained from Novagen.

2. Analytical methods

Transmission electron microscope (TEM). JEOL:JEM-2010 TEM was used to analyze the morphology and size of magnetic nanoparticles (MNPs).

Field emission scanning electron microscopy (FESEM). FESEM was conducted using JEOL:JSM-6700F and dried particles on a copper belt.
3. Supplementary Figures

Figure S1. Engineered plasmid pET28a-CysK and pET28a-CysM.
Figure S2. Growth curve of recombinant *E. coli* (His-CysM) in LB medium. Protein induction was started by the addition of IPTG to a final concentration of 1 mM at 2 h.
**Figure S3.** SDS-PAGE. Lane: 1, 2, 3, 4, and 5 correspond to cell-free extract of the cells harvested at 4, 6, 8, 10 and 12 h, respectively, during the growth of recombinant *E. coli* (His-CysM). Lane M is marker. Approximately 5 µg protein was loaded in each lane.
Figure S4. Time courses of the biotransformation of OAS (122 mM) and pyrazole (244 mM) to synthesize β-PA with OASS CysM (1.5 g protein/L): CFE containing untagged CysM (□), CFE containing His-CysM (◇), and purified His-CysM (○). Reactions were performed in KP-buffer (300 mM, pH 7.0, 1.1 mM PLP) at 35 °C. Data are the mean values of triplicated experiments.
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