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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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 PageRulerTM 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 cupper belt.

3. Supplementary Figures

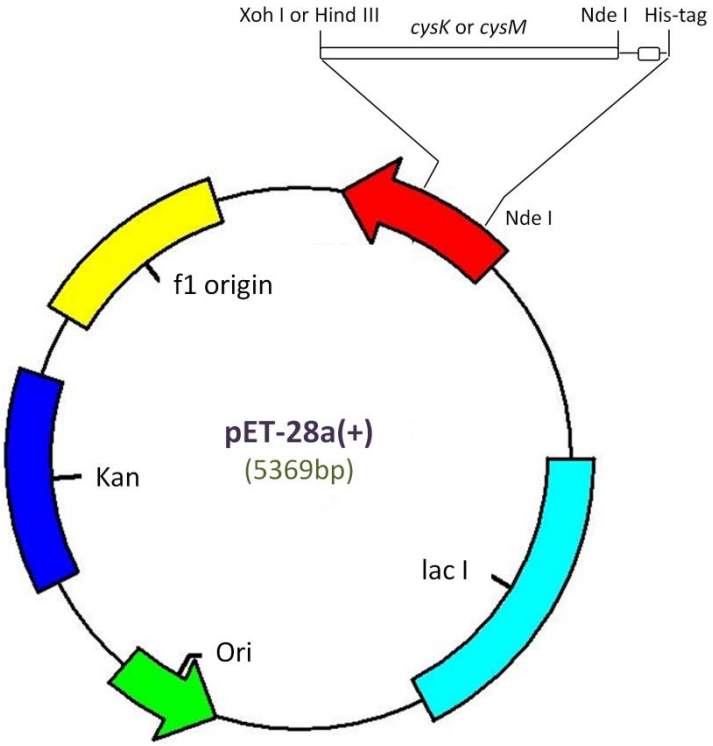

Figure S1. Engineered plasmid pET28a-CysK and pET28a-CysM.

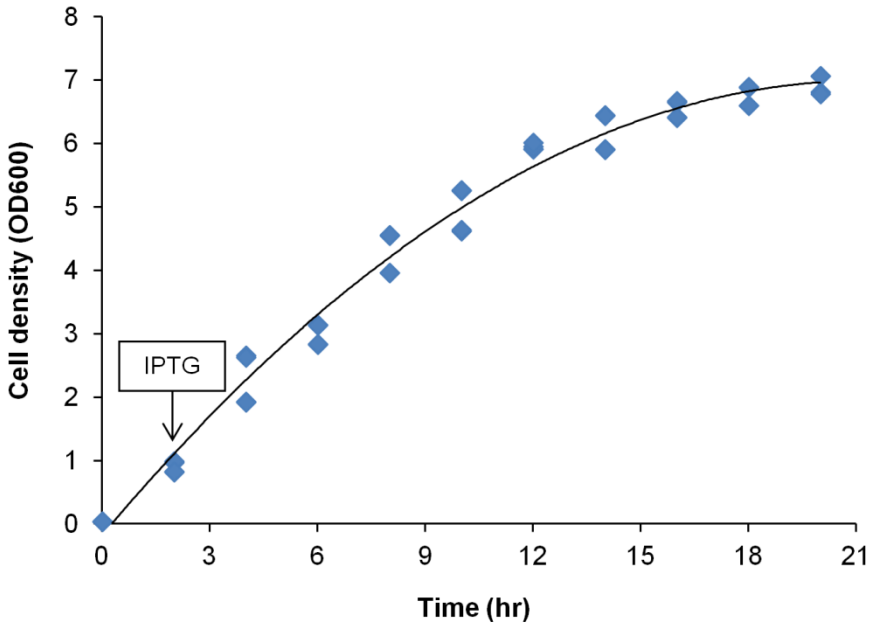

Figure S2. Growth curve of recombinant *E. coli* (His-CysM) in LB medium. Protein induction was started by the addition of IPTG to a final concentration of 1 mM at 2 h.

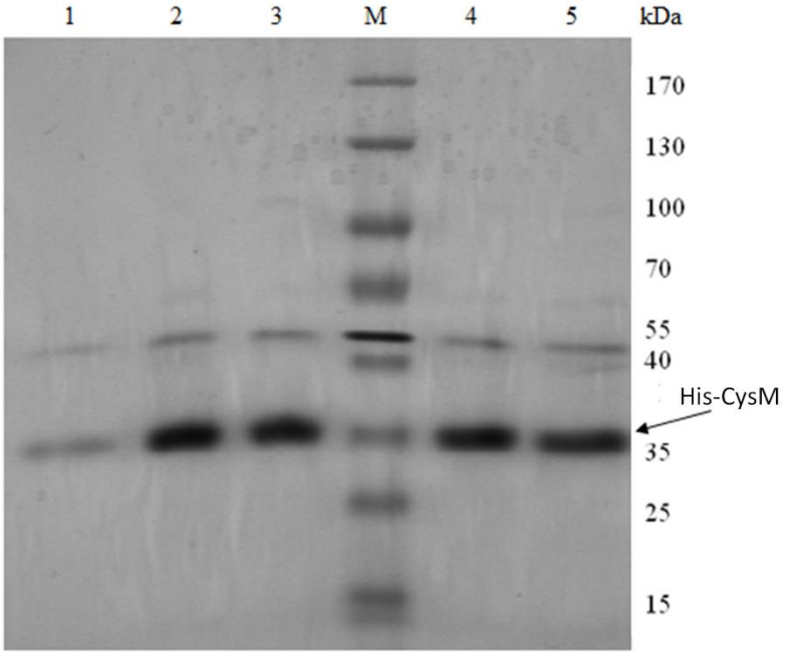

Figure S3. SDS-PAGE. Lane: 1, 2, 3, 4, and 5 correspond to cell-free extract of the cells harvested at 4, 6, 8, 10 and 12 h, respectively, during the growth of recombinant *E. coli* (His-CysM). Lane M is marker. Approximately 5 μg protein was loaded in each lane.

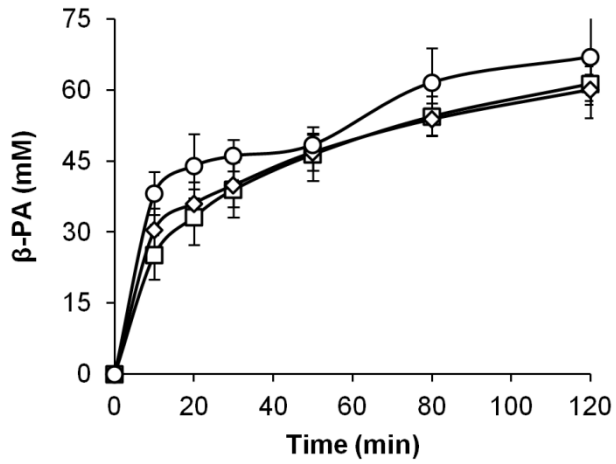

Figure S4. Time courses of the biotransformation of OAS (122 mM) and pyrazole (244 mM) to synthesize *β*-PA with OASS CysM (1.5 g protein/L): CFE containing untagged CysM (□), CFE containing His-CysM (◇), and purified His-CysM (○). Reactions were performed in KP-buffer (300 mM, pH 7.0, 1.1 mM PLP) at 35 °C. Data are the mean values of triplicated experiments.

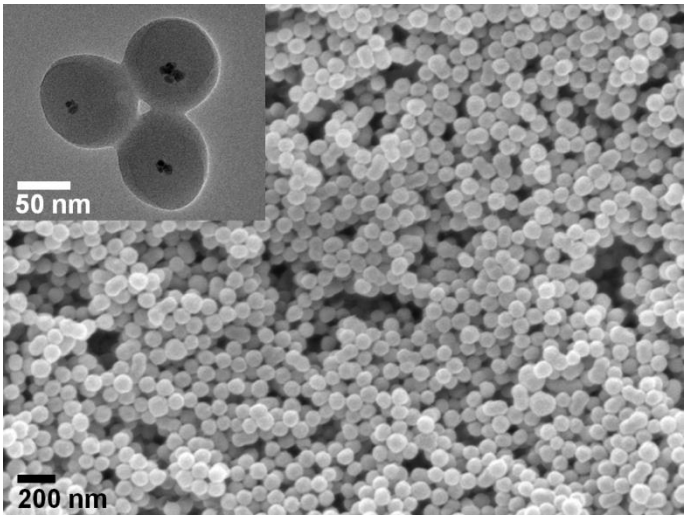

Figure S5. FESEM and TEM (inset) images of His-CysK-MNPs.

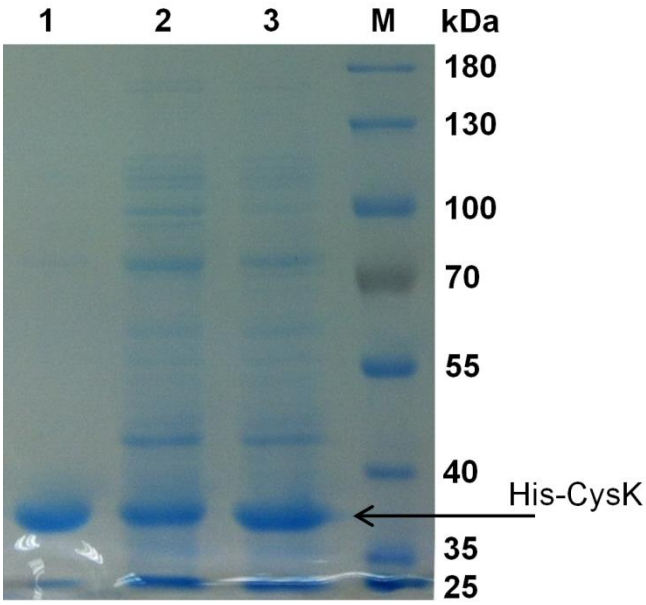

Figure S6. SDS-PAGE analysis. Lane M: protein marker; Lane 1: eluate of washing His-CysK-MNPs with 300 mM imidazole; Lane 2: CFE containing His-CysK after immobilization; Lane 3: CFE containing His-CysK before immobilization.

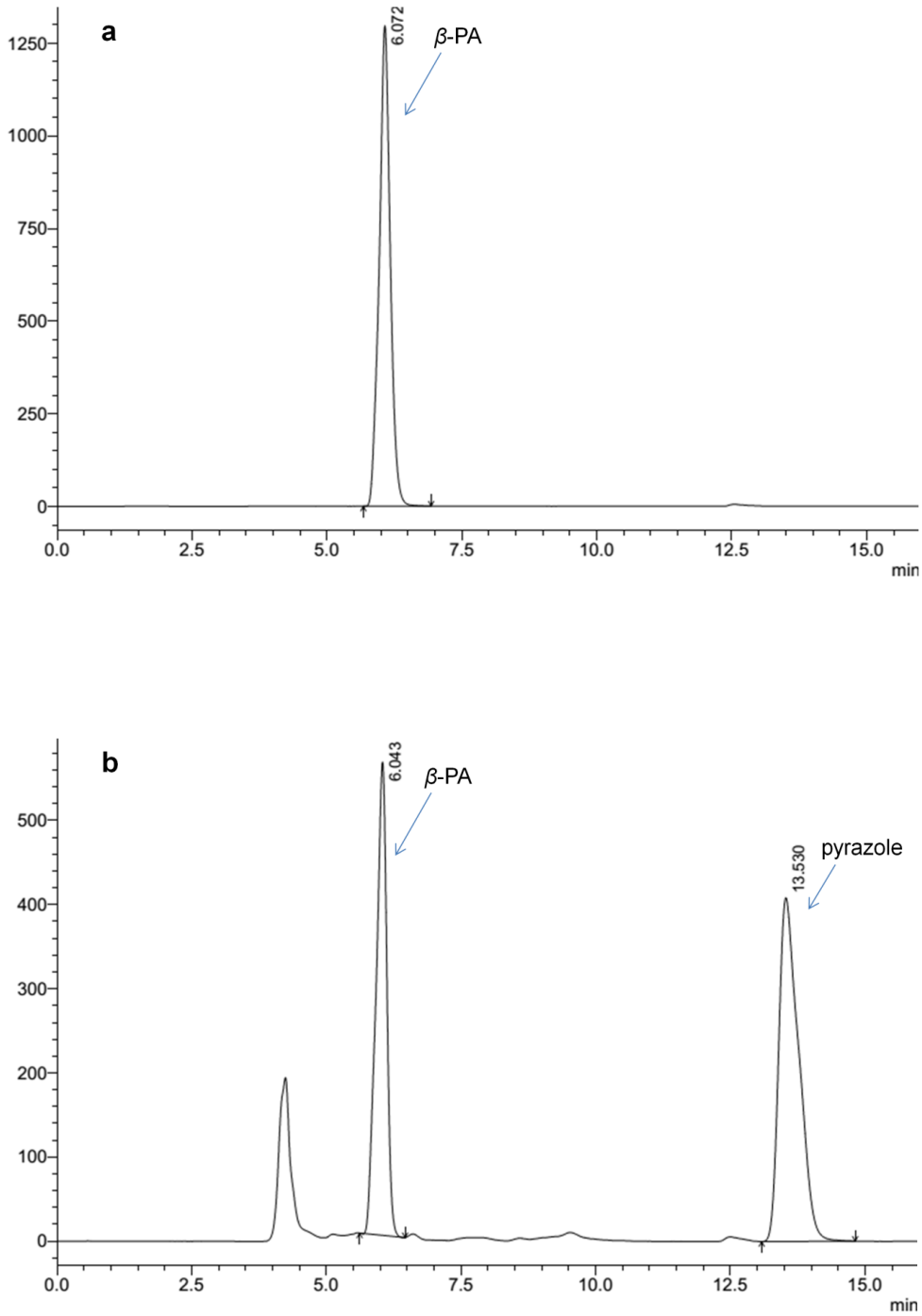

Figure S7. HPLC chromatograms: (a) β-PA standard (10 mM), and (b) Biotransformation mixture from the reaction of 90 mM OAS and 180 mM pyrazole with His-CysK-MNPs cat**4** at 120 min.

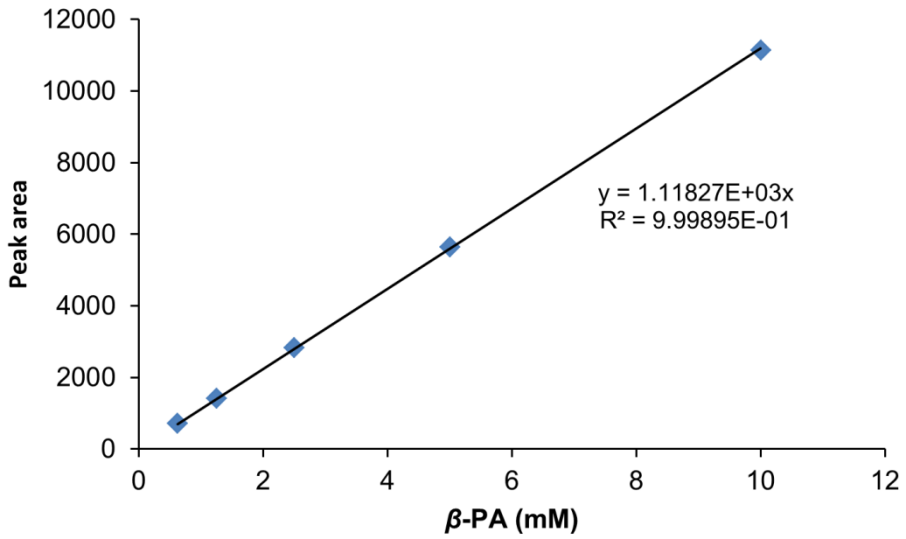

Figure S8. Calibration curve for quantitative HPLC analysis of the concentration of *β*-PA established by using *β*-PA standard samples.