Electronic supplementary information

Thermostable enzyme-immobilized magnetic responsive Ni-based metal-organic framework nanorods as recyclable biocatalyst for efficient biosynthesis of S-adenosylmethionine

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Additional experimental methods

Adsorption and desorption of His-eGFP and eGFP

To investigate protein adsorption, 100 μL cell lysate of His-eGFP and eGFP were incubated with 2 mg Fe_3O_4/Ni-BTC in 1 mL system (with 900μL Milli-Q water) at room temperature for 30 min with shaking. After separated by a magnet, the supernatants were measured by fluorescence analysis. Then different concentrations of imidazole (5-50 mM) and NaCl (10-200 mM) were used as adsorption buffers. To investigate protein desorption, 100 μL cell lysate of His-eGFP and eGFP were absorbed by 2 mg Fe_3O_4/Ni-BTC. Then, the composites were washed by different concentrations of Tris-HCl (10-50mM). After separated by a magnet, the supernatants were measured by fluorescence analysis.

Assay of Kinetics of the SAMS

For the kinetic analysis, the reaction system is the same as for the enzyme activity determination. The concentrations of methionine or ATP were varied from 1.0 to 8.0 mM, and the other's concentration was 10 mM. The Km and Vmax were calculated by LineweaverBurk plot. The Lineweaver–Burk equation could be written as

$$\frac{1}{v} = \left(\frac{K_m \ 1}{V_{max}[S]}\right) + \frac{1}{V_{max}} \qquad (1)$$

Where [S] is the concentration of substrate and V and Vmax represent the initial and maximum rates of reactions, respectively. Km is the Michaelis–Menten constant (the substrate concentration when the rate is half of Vmax).

Characterization

Transmission electron microscopy (TEM) analysis was performed on a JEM 1200EX transmission electron microscope (Hitachi, Tokyo, Japan). Scanning electron microscopy (SEM) analysis was performed on a Tecnai G2 F20 scanning electron microscope (FEI, USA). The crystal structure of all samples were determined by powder X-ray diffraction (D8 Advance X-Ray diffractometer, Bruker, Karlsruhe, Germany) with a Cu Kα anode (λ = 0.15406 nm) at 40 kV and 40 mA. Fourier transform infrared spectroscopy was recorded on a FTIR spectrometer (8700/Continuum XL Imaging Microscope, Nicolet, Waltham, MA, USA). The spectra were collected between 400 and 4000 cm^{-1}. X-ray photoelectron spectroscopy (XPS) spectra were recorded on a Thermo Scientific K-Alpha X-ray photoelectron spectrometer. Thermogravimetric analysis (TGA) was performed using a thermogravimetric analyzer (DTG-60A, Shimadzu, Japan) in the range of 30-600 °C under a nitrogen flow (heating rate of 10 °C min^{-1}). Nitrogen adsorption-desorption isotherms were obtained using an automated surface area and pore size analyzer (Micromeritics, ASAP2020, USA) at 77 K. The pore size and pore volume were calculated by the Barrett–Joyner–Halenda (BJH) method.

Table S1 Michaelis−Menten kinetics parameters of immobilized and free enzymes

	Met	
	K_m (mmol/L)	V_{max} (mmol/min)
Free enzyme	1.02	0.0053
Immobilized enzyme	1.12	0.004

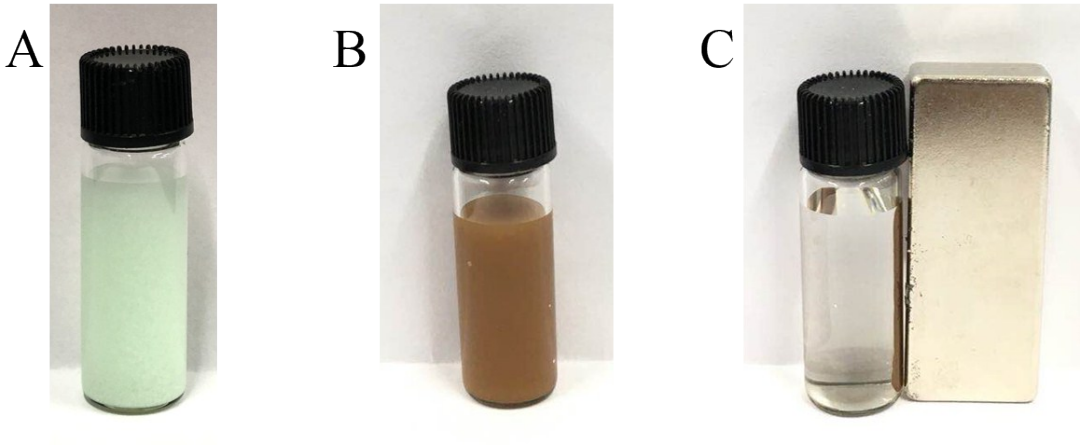

Figure S1. Photographs of (A) Ni-BTC, (B) Fe_3O_4/Ni-BTC, (C) Fe_3O_4/Ni-BTC separated by a magnet.

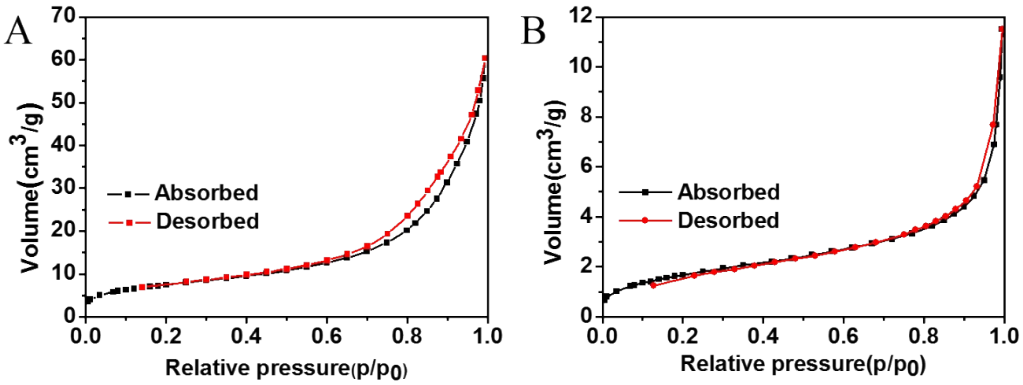

Fig.S2 (A) N_2 sorption–desorption isotherms of Fe_3O_4/Ni-BTC, (B) N_2 sorption–desorption isotherms of Ni-BTC.

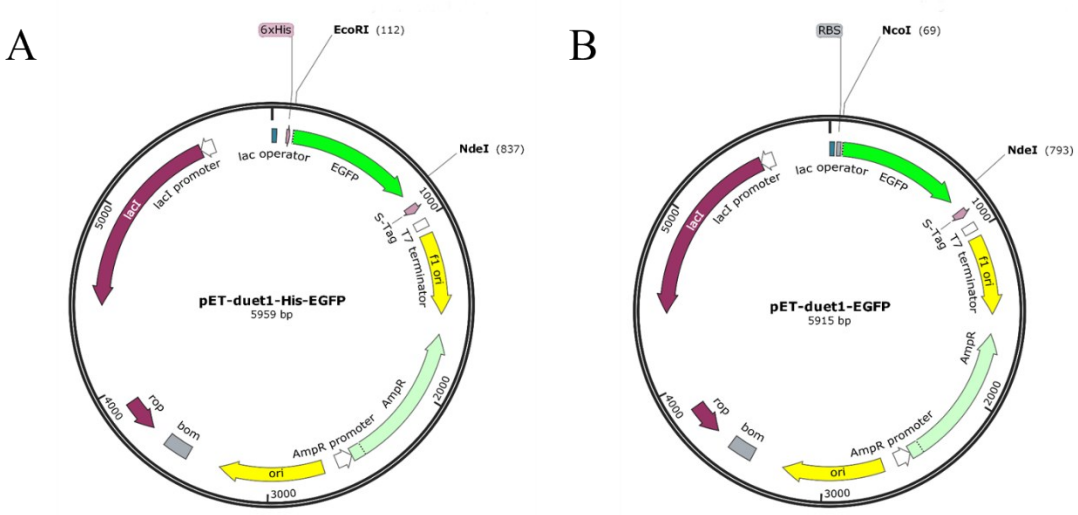

Fig.S3 The plasmid maps of pETDuet-1-His-eGFP (A) and pETDuet-1-eGFP (B).

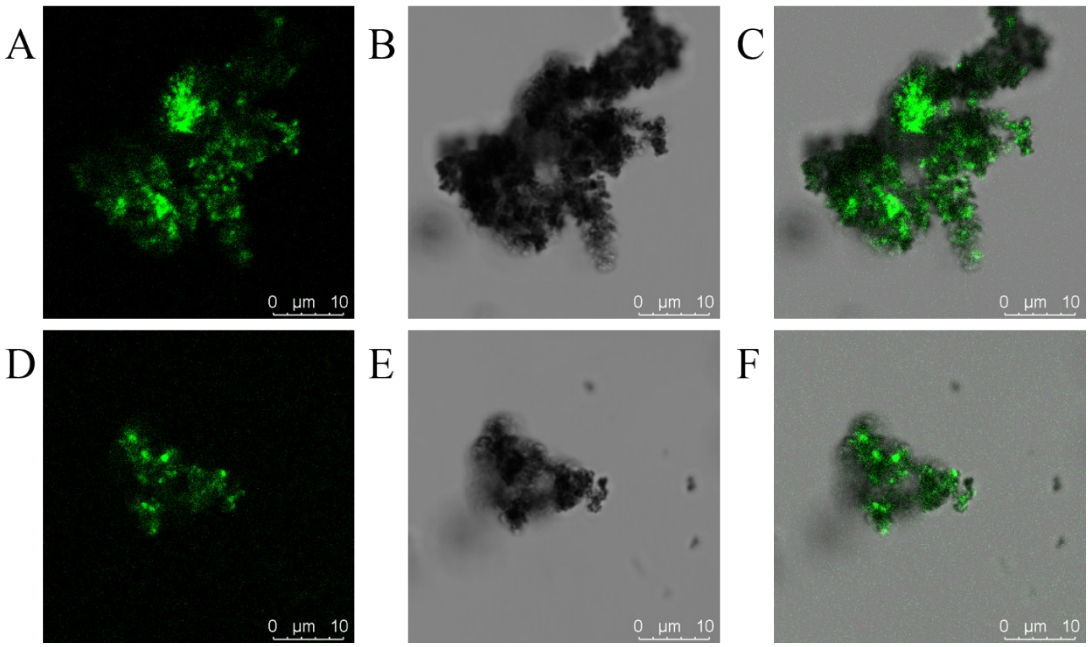

Fig.S4 Confocal laser scanning microscopy images of His-eGFP@Fe_3O_4/Ni-BTC: (A) dark field image; (B) bright field image; (C) overlayer image. Confocal laser scanning microscopy images of eGFP@Fe_3O_4/Ni-BTC: (D) dark field image; (E) bright field image; (F) overlayer image.

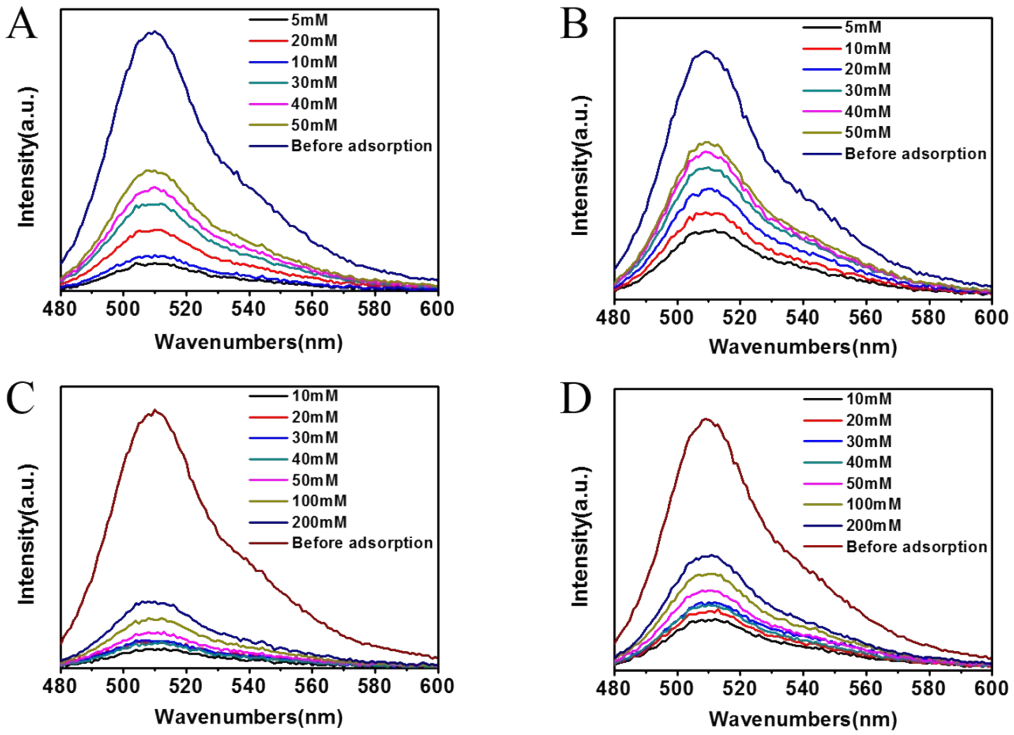

Fig.S5 Fluorescence spectra of His-eGFP (A) and eGFP (B) solution: before and after adsorption with Fe_3O_4/Ni-BTC in different concentration of imidazole. Fluorescence spectra of His-eGFP (C) and eGFP (D) solution: before and after adsorption with Fe_3O_4/Ni-BTC in different concentration of NaCl.

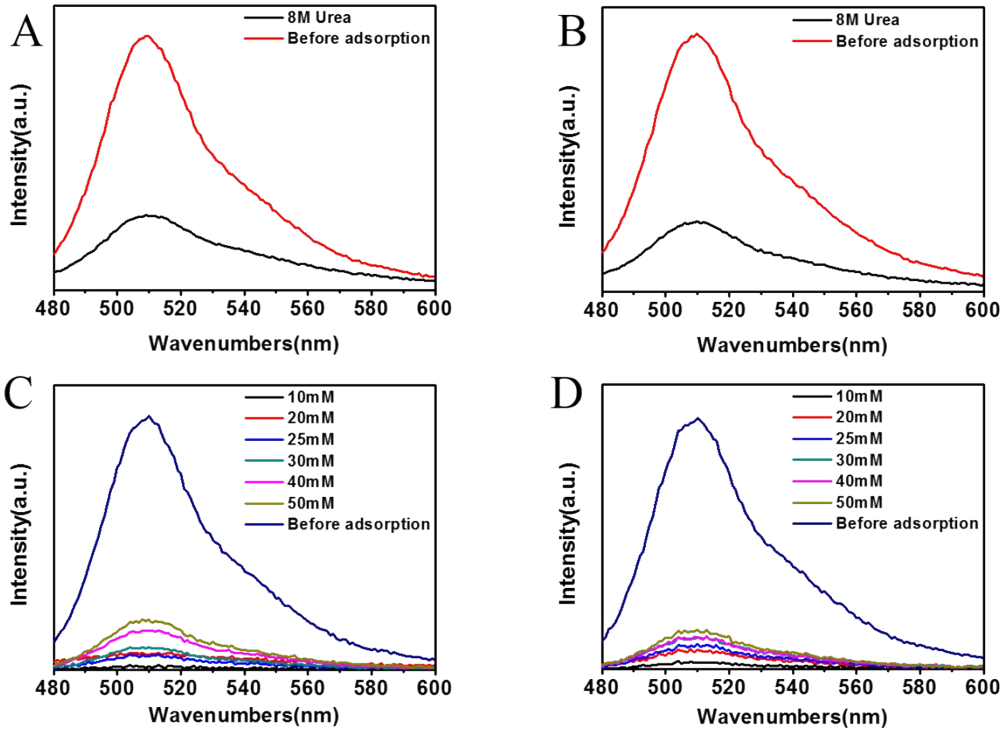

Fig.S6 Fluorescence spectra of His-eGFP (A) and eGFP (B) solution: before reaction with Fe_3O_4/Ni-BTC and after washed by 8M urea. Fluorescence spectra of His-eGFP (C) and eGFP (D) solution: before reaction with Fe_3O_4/Ni-BTC and after washed by different concentration of Tris-HCl.

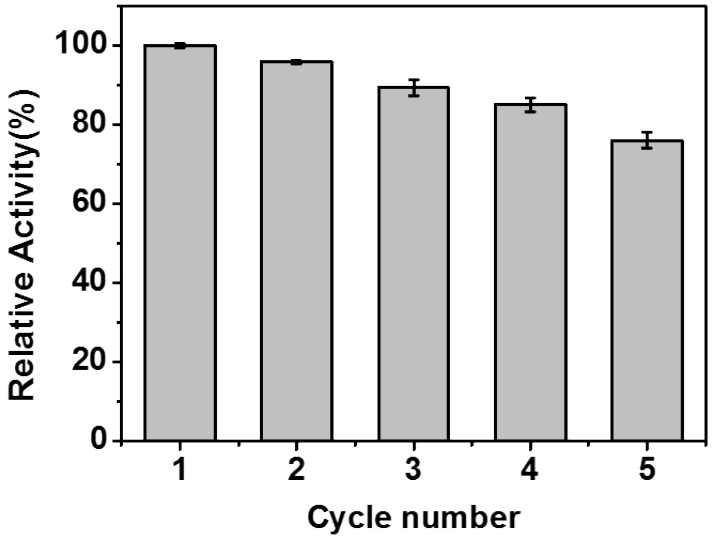

Fig.S7 Reusability of the immobilized SAMS.