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Electronic Supplementary Information

Metal-nucleobase hybrid nanoparticles for enhancing the activity and stability of metal-active enzymes

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Materials

Adenine, zinc chloride, zinc nitrate hexahydrate, 2-methylimidazole, sodium chloride, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), ammonium persulfate, sodium hydrogen sulfite, bovine serum albumin (BSA) and methionine were purchased from Beijing Chemical Works (Beijing, China). Kanamycin, isopropyl β-D-1-thiogalactopyranoside (IPTG) and adenosine triphosphate (ATP) were obtained from Beijing QXTD-Biotechnology Co.,Ltd. (Beijing, China). All other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and used without additional purification. Milli-Q water was used to prepare all the buffers and solutions.

Expression and Purification

T. thermophilus HB27 cells were grown in LB-medium at 65 °C for 48 h. The cells were harvested by centrifugation. The genomic DNA was isolated using a Bacterial Genomic DNA Isolation Kit (Beijing Biomed Co., Ltd.) according to the manufacturer's instructions. The Tt MAT gene (GI: 46197207 in the Gen Bank database) was amplified from the genomic DNA of *T. thermophilus HB27* by PCR using the following primers: 5'-ggaattc CATATGCGCGCGTTGAGGCTGGTCA' and 5'-cccaagctt AAGCCCGCTTCCCGCCT-3'. The PCR product was purified and digested with Nde I and Hind III restriction endonucleases. The digested PCR product was purified and cloned into pET22b vector (Novagen, Madison, WI, USA). The resulting expression plasmid was subsequently transformed into *Escherichia coli BL21* (DE3) cells. The cells were grown at 37 °C in LB medium with Kanamycin (1‰) to $OD_{600\ nm}$ of 1.0, and overexpression of Tt MAT was induced with 0.5 mM

isopropyl-β-D-thiogalactopyranoside (IPTG) at 30 °C for 6 h. The cells were harvested and suspended in the lysis buffer (50 mM Tris–HCl, pH 8.0, 300 mM NaCl, 10 mM imidazole), and lysed by sonication on ice. The protein was purified with affinity chromatography using a Ni-NTA column (Qiagen) pre-equilibrated with the lysis buffer and eluted with the lysis buffer supplemented with 300 mM imidazole, which was followed by purification with gel filtration using a Superdex-200 (GE Healthcare) column equilibrated with a buffer containing 50 mM Tris-HCl, pH 8.0. The purified protein was analyzed by SDS-PAGE. The fractions containing the target protein were pooled and concentrated to 20 mg/ml.

Enzymatic Activity Determination

The standard assay mixture of 0.5 mL contained 50 mmol/L Tris-HCl buffer (pH 8.0), 50 mmol/L KCl, 20 mmol/L $MgCl_2$, 10 mmol/L ATP, 10 mmol/L methionine, and 0.1 mg/mL enzyme. The stock solutions of ATP or methionine were prepared at concentration of 100 mmol/L using 50 mmol/L Tris-HCl buffer (pH 8.0) containing 50 mmol/L KCl and 20 mmol/L $MgCl_2$, respectively. MAT activity was assayed at 70 °C for 30 min in a water bath without agitation. After incubation, 20% (v/v) perchloric acid solution was added to stop the reaction. One unit of MAT activity was defined as the amount of enzyme activity that produces 1 mmol of SAM per minute. HPLC Shimadzu 2010 equipped with a C18 column (Kromasil, φ4.6 ×250 mm, 5 mm, 100 Å) was used for detection of produced SAM. Mobile phase was at flow rate of 1 mL/min containing 0.01 mol/L sodium 1-hexanesulfonate and 10% acetic acid. SAM was monitored under UV-light at 260 nm. The standard curve was generated using

authentic SAM in a concentration range from 0.025 mmol/L to 0.75 mmol/L.

When testing the relative activity of the immobilized MAT by Zn_2(adenine) nanoparticles compared to the free MAT, we added different metal ions in reaction buffer. A: Tris-HCl buffer (50 mM, pH 8.0) containing 50 mmol/L KCl and 20 mmol/L $MgCl_2$; B: Tris-HCl buffer (50 mM, pH 8.0) containing 50 mmol/L KCl and 20 mmol/L $ZnCl_2$; C: Tris-HCl buffer (50 mM, pH 8.0) containing 50 mmol/L KCl; D: Tris-HCl buffer (50 mM, pH 8.0).

Preparation of MAT@Zn_2(adenine) hybrid nanoparticles.

In a typical experiment, the MAT@Zn_2(adenine) hybrid nanoparticles were prepared by mixing 100 μL $ZnCl_2$ (50 mM), 100 μL adenine (15 mM), 50 μL MAT (1 mg/mL) and 500 μL HEPES buffer (100 mM, pH 7.4). The volume of the system was 1 mL, and water was added to make up. After 2 hours at room temperature, the samples were centrifuged at 10,000 rpm for 5 min and washed with Milli-Q water to remove remaining chemicals. The amounts of protein incorporated into the Zn_2(adenine) complexes were measured by the Bradford assay.[1]

Assay of Kinetics of MAT.

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 0.2 to 1.0 mM, and the other's concentration was 5 mM. The activity of both free and immobilized MAT was calculated by the initial reaction rate from the slope of changes in absorbance versus time.

The kinetic parameters of K_m and V_{max} were calculated using the Lineweaver-Bruke plot:

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

where [S] is the concentration of substrate and V and V_{max} represent the initial and maximum rates of reactions, respectively. The K_m and V_{max} values of free and immobilized MAT were shown in Table S4.

Preparation of MAT@ZIF-8 composites.

In a typical experiment, the MAT@ZIF-8 nanoparticles were prepared by adding 50 μL MAT (1 mg/mL) into water solutions of zinc nitrate hexahydrate (0.31 M, 200 μL) and 2-methylimidazole (2.5 M, 1 mL). Then, the mixture was stirred at room temperature for 30 minutes. The product was obtained by centrifugation at 10,000 rpm for 5 minutes and washed 3 times with Milli-Q water to remove remaining chemicals. The amounts of protein incorporated into the Zn_2(adenine) complexes were measured by the Bradford's method.

Raman spectroscopy of MAT

The Raman spectrum of each sample was measured on a Raman spectrometer (QE65-Pro-Raman spectrometer, Ocean Optics Inc., Florida, USA). Raman spectra were recorded at 20-25 °C under the following conditions: laser power, 5 mW; laser excitation wavelength, 633 nm; grating, 600 lines/mm; slit, 200 mm; spectral resolution, 35.0 cm^{-1}; Raman shift, 0-2500 cm^{-1}, scanning time, 60 s; integration times,

10 times; and integration time, 500 ms. Phenylalanine (1600 cm^{-1}) was set as a normalization factor. The samples for the Raman studies were prepared simply by drop casting the liquid onto the glass slide. Each experiment was performed in triplicates. The data transformation and deconvolution were conducted by OMNIC software (Thermo Scientific, Madison, WI, USA). Peak-separation analysis at amide I band (1600–1700 cm^{-1}) was conducted using PeakFit V4.12 software (SPSS Inc., Chicago, IL, USA).

Enzyme stability test.

For stability test at different pH values, the free MAT and the suspension of MAT@Zn_2(adenine) complexes were added into 1 mL of various pH solutions for 4 h respectively. Then the enzymatic activity was measured by the above method. To test stability at different temperatures, free and immobilized enzymes were incubated at 25-90 °C for 30 min. To test stability against protease degradation, the suspension of the MAT@Zn_2(adenine) complexes and free MAT in Tris buffer (50 mM, pH 8.0) was incubated with 1 mg mL^{-1} of trypsin at 37 °C for 2 h. For solvent stability, the suspensions of the nanofibers in Tris buffer (50 mM, pH 8.0) were incubated with 50 wt% ethanol or isopropanol at room temperature for 2 h. To test its long-term stability, the samples were stored in Tris buffer (50 mM, pH 8.0) at 4 °C. To test the recycling of the MAT@Zn_2(adenine) complexes, the reaction was performed for 30 min, and the immobilized enzyme was separated by centrifugation. The enzyme activity was measured by HPLC. Then new substrate and other solution were added to start the

new cycle of the reaction. Repeat the above steps several times to observe the change of the activity. In all the experiments, the error bars were calculated based on the standard deviation from three independent measurements.

Preparation of AM coated MAT@Zn_2(adenine) composites

The precipitation of MAT@Zn_2(adenine) nanoparticles was re-dispersed into water solution (500 μL), then adding 200 μL of acrylamide (AM) solution (10%, w/w), 10 μL of ammonium persulfate (APS) solution (10%, w/w) and 50 μL of sodium hydrogen sulfite solution (10% w/w). The mixture was incubated at room temperature. After 4 h, the solution was centrifuged at 10,000 rpm for 5 minutes to obtain AM coated composites. The activity assay and recycling of the AM-coated MAT@Zn_2(adenine) nanoparticles was measured by the above method.

Characterization

The Powder X-ray diffraction (XRD) patterns of MAT@Zn_2(adenine) hybrid nanoparticles and Zn_2(adenine) complexes were recorded using a D8 Advance X-Ray diffractometer with a Cu Kα anode (λ= 0.15406 nm) at 40 kV and 40 mA. Scanning electron microscope (SEM) images of samples were taken on an S-4700 scanning electron microscope at an accelerating voltage of 10.0 kV. Samples for SEM measurements were prepared by pipetting a drop of the solution of the sample onto a cover glass and drying on a filter paper. Transmission electron microscope (TEM) was performed on a Hitachi H-800 transmission electron microscope. The sample was prepared by pipetting a drop of the solution of the Zn_2(adenine) complexes onto a 230

mesh holy carbon copper grid and drying on a filter paper. Fourier transform infrared spectroscopy (FTIR) spectra of adenine, Zn_2(adenine) complexes and MAT@Zn_2(adenine) hybrid nanoparticles were performed on a Nicolet 8700/Continuum XL Imaging Microscopy with measuring wavelength range from 4000 to 550 cm^{-1}. Nitrogen adsorption-desorption isotherms were obtained using an automated surface area and pore size analyzer (Micromeritics, ASAP2020, USA) at 77 K.

Figure S1. SDS-PAGE of purified the recombinant MAT from the culture supernatant (Lane 1: molecular weight marker. Lane 2: His6-tagged MAT purified using Ni-NTA column).

Table S1. Purification of the Recombinant MAT from BL21 Culture Supernatant.

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/g)	Recovery of total activity (%)
Culture supernatant	87.5	43.06	492.1	100
300 mM imidazole	50	35.27	705.3	81.9

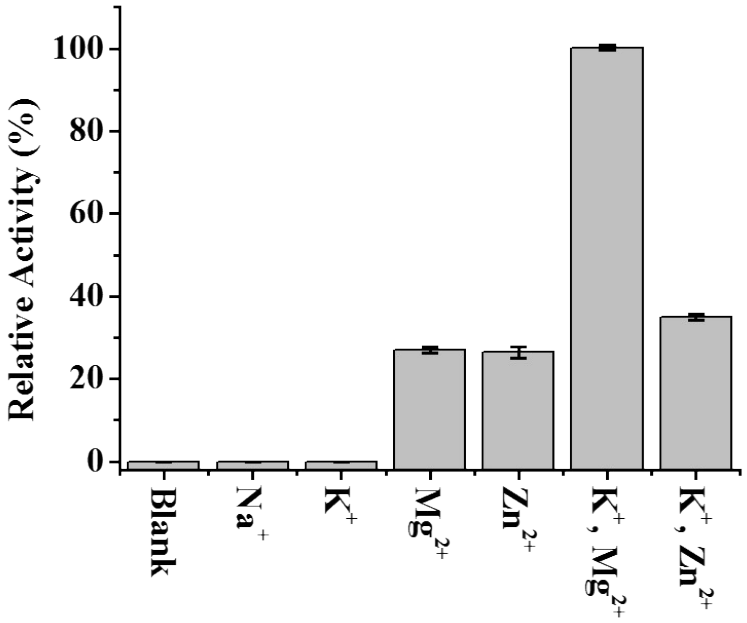

Figure S2. The relative bioactivity of free MAT in Tris-HCl buffer (50 mM, pH 8.0) containing different metal ions (Na^+, K^+: 50 mM, Mg^{2+}: 20 mM, Zn^{2+}: 20 mM).

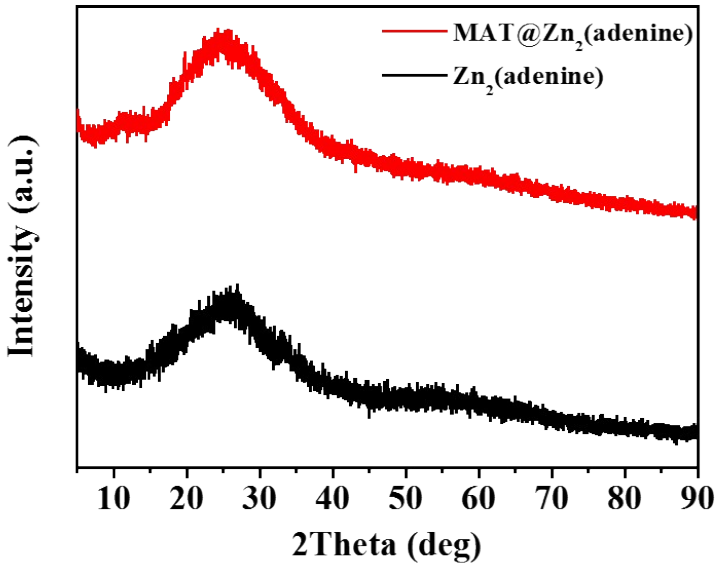

Figure S3. X-ray diffraction (XRD) patterns of powdery Zn_2(adenine) nanoparticles and MAT@Zn_2(adenine) composites.

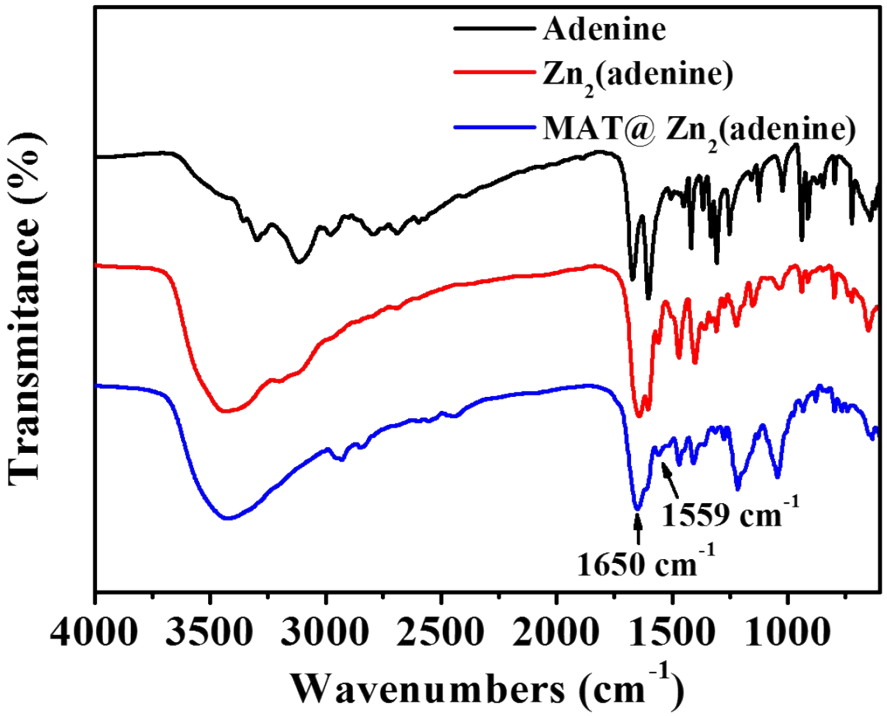

Figure S4. The FTIR spectra of Adenine, Zn_2(adenine) complexes and MAT@Zn_2(adenine) composites.

Table S2. Changes in wavenumber of stretching vibrations in Figure S4.

	$-N_3$	$-N_9$	amide I	amide II
Adenine	1450.7	1418.4	-	-
Zn_2(adenine)	1470.6	1401.2	-	-
MAT@Zn_2(adenine)	1469.5	1407.4	1650.1	1559.6

After coordination with Zn^{2+}, the N3 stretching vibrations of adenine was considered to arise from 1450.7 cm^{-1} to 1470.6 cm^{-1}, and the N9 stretching vibrations

of adenine shifted to lower wavenumbers (from 1418.4 cm^{-1} in adenine to 1401.2 cm^{-1} in the coordination polymer),[2-4] suggesting that the adenine is involved in metal coordination. For the Zn_2(adenine) nanoparticles with MAT, two IR absorption bands centered at 1650 and 1559 cm^{-1} were observed, attributable to the typical amide I and II absorption bands in protein, respectively.[5, 6].This also confirmed that MAT was successfully encapsulated into the Zn_2(adenine) nanoparticles.

Table S3. Secondary structure contents of MAT with different metal ions (K^+: 50 mM, Mg^{2+}: 20 mM, Zn^{2+}: 20 mM) and immobilized MAT.

Samples	α-helix (%)	β-sheet (%)	β-turn (%)	Random coil (%)
MAT	12.2	43.0	10.2	34.6
MAT+K^+	17.8	41.6	8.4	32.2
MAT+K^+, Mg^{2+}	27.0	29.1	18.9	25
MAT+K^+, Zn^{2+}	26.4	35.9	8.6	29.1
MAT@Zn_2(adenine)	26.7	33.6	8.2	31.5

Table S4 Kinetic parameters of free and immobilized MAT with adding different metal ions in reaction buffer: A: Tris, K^+, Mg^{2+}; B: Tris, K^+, Zn^{2+}; C: Tris, K^+; D: Tris (Tris: 50 mM, K^+: 50 mM, Mg^{2+}: 20 mM, Zn^{2+}: 20 mM).

	Buffer Solutions	Met		ATP	
		V_{max}	K_m	V_{max}	K_m
		mmol/min/mg	mmol/L	mmol/min/mg	mmol/L
A	Free MAT	0.012±0.0005	0.636±0.0064	0.016±0.0006	0.952±0.0058
	MAT@Zn_2(adenine)	0.014±0.0014	0.514±0.0182	0.020±0.0006	0.793±0.0023
B	Free MAT	0.007±0.0002	1.282±0.0021	0.008±0.004	1.226±0.0064
	MAT@Zn_2(adenine)	0.010±0.0003	0.925±0.0027	0.015±0.0002	1.038±0.0139
C	Free MAT [a]	-	-	-	-
	MAT@Zn_2(adenine)	0.011±0.0007	0.870±0.0058	0.016±0.0008	0.994±0.0049
D	Free MAT [b]	-	-	-	-
	MAT@Zn_2(adenine)	0.007±0.0005	1.182±0.0078	0.008±0.0010	1.160±0.0145

[a, b] Free MAT has no catalytic activity in these buffer solutions (C and D).

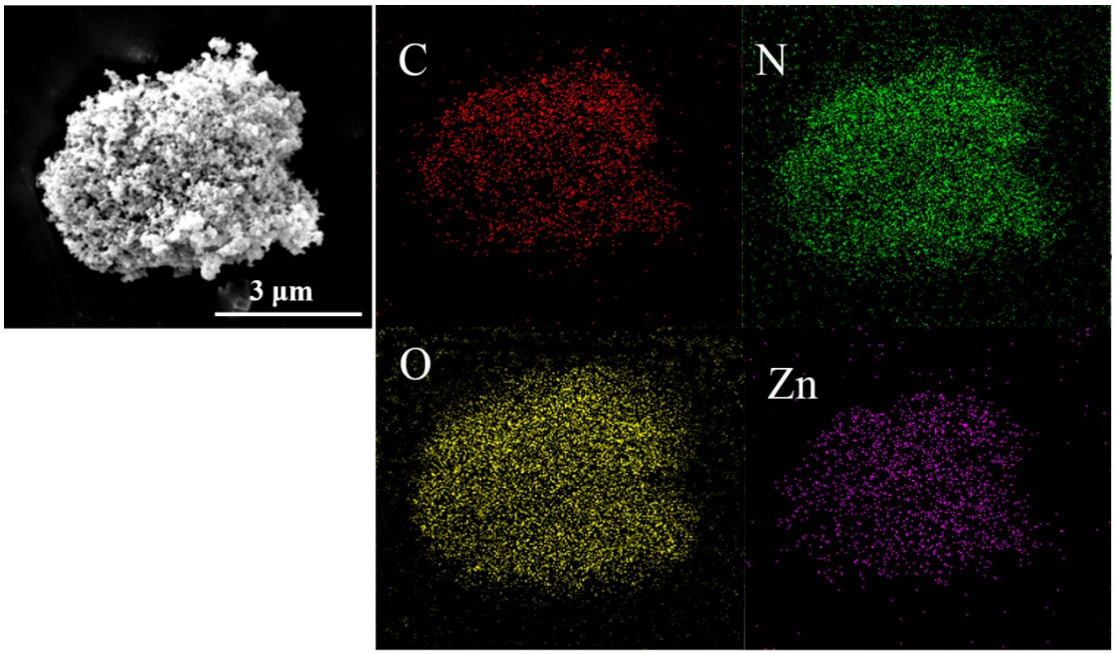

Figure S5 SEM image and corresponding carbon, nitrogen, oxygen and zinc elemental mapping of MAT@Zn_2(adenine).

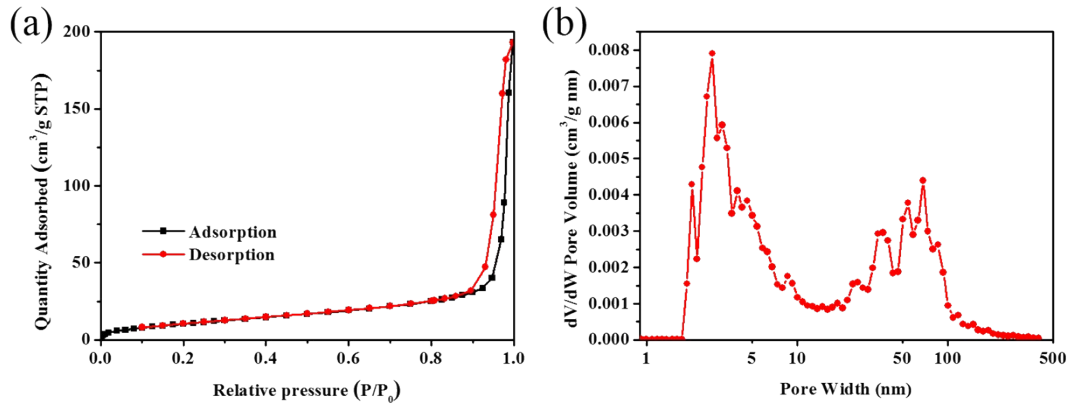

Figure S6 (a) N_2 sorption–desorption isotherms of MAT@Zn_2(adenine), (b) pore size distribution curve of MAT@Zn_2(adenine) analyzed by the NL-DFT method.

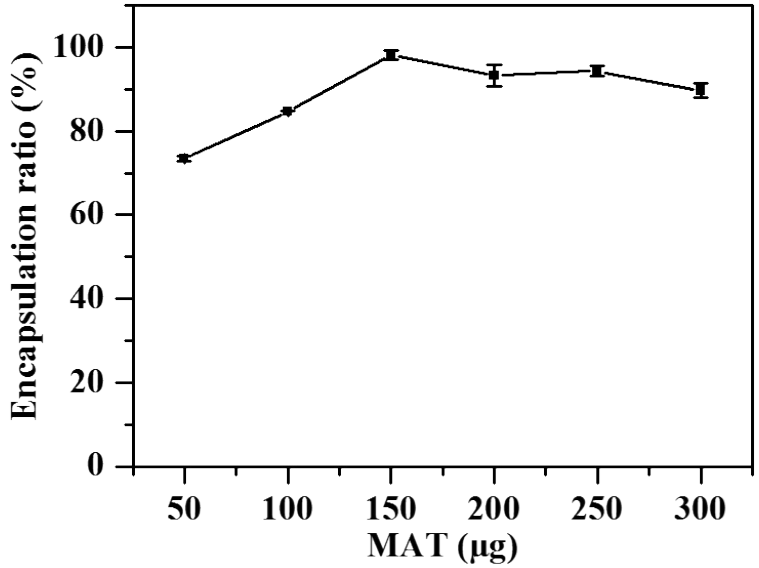

Figure S7. The encapsulation efficiency at the different amount of MAT (50-350 μg) entrapped by Zn/Adenine.

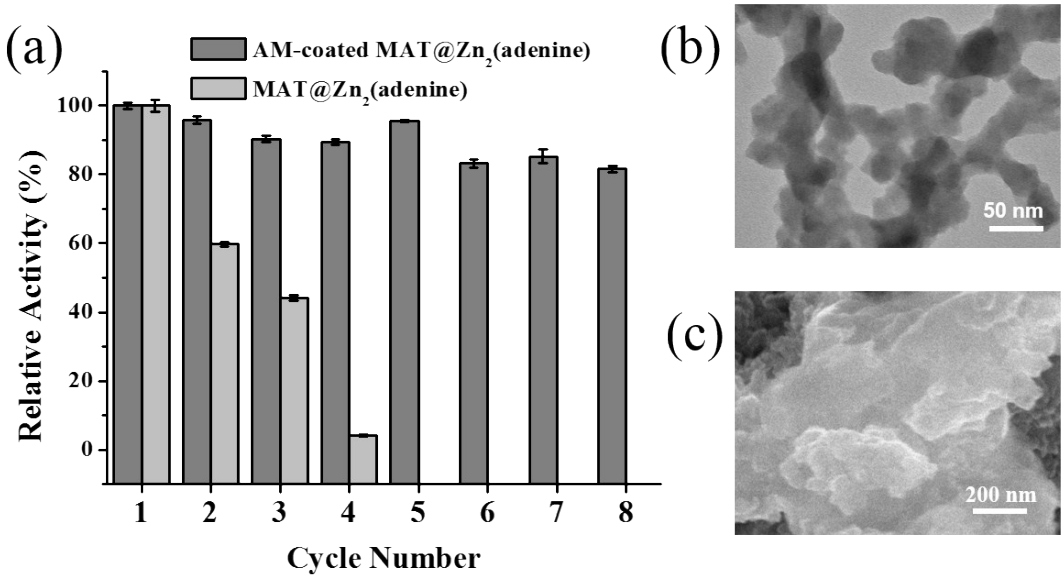

Figure S8 (a) reusability of MAT@Zn_2(adenine) and AM-coated MAT@Zn_2(adenine), TEM image (b) and SEM image (c) of AM-coated MAT@Zn_2(adenine).

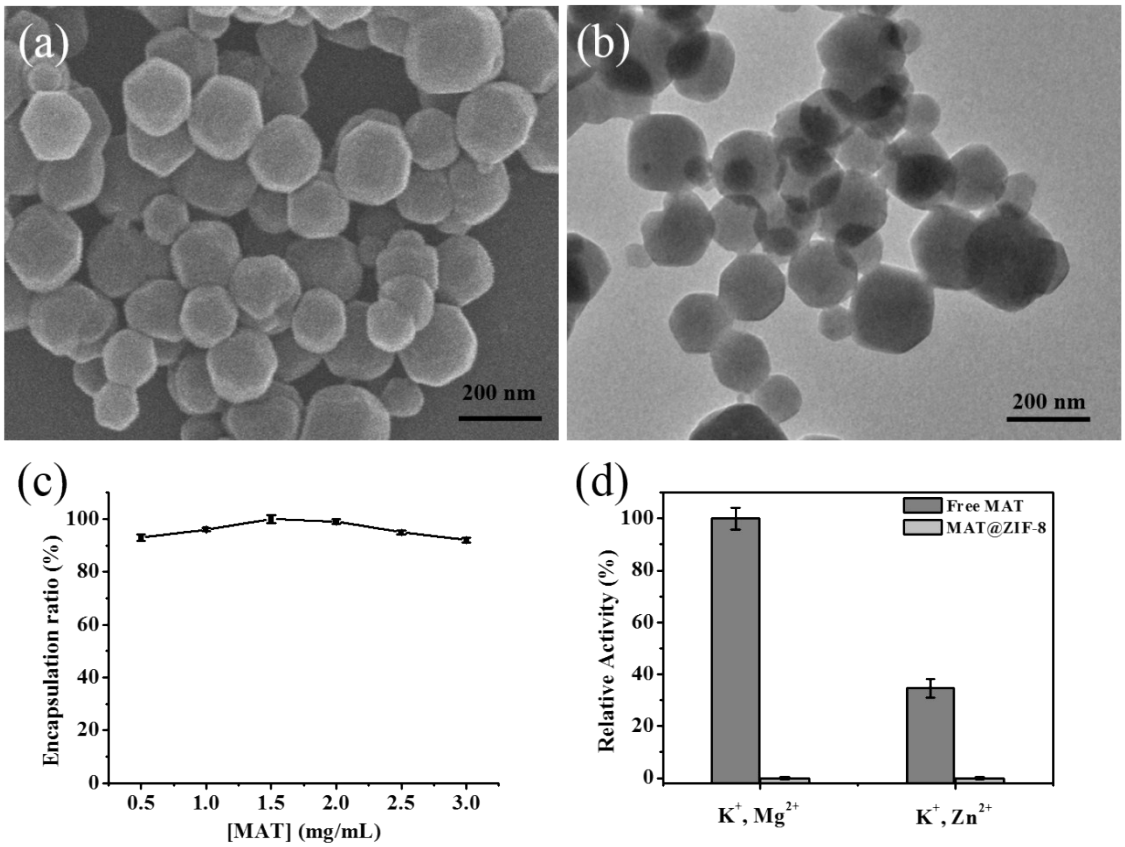

Figure S9. (a) SEM image of MAT@ZIF-8 composites; (b) TEM image of MAT@ZIF-8 composites; (c) The encapsulation efficiency within the range of protein concentration used (0.5-3 mg/mL); (d) The relative activity of the MAT@ZIF-8 compared to the free MAT with adding different metal ions in reaction buffer.

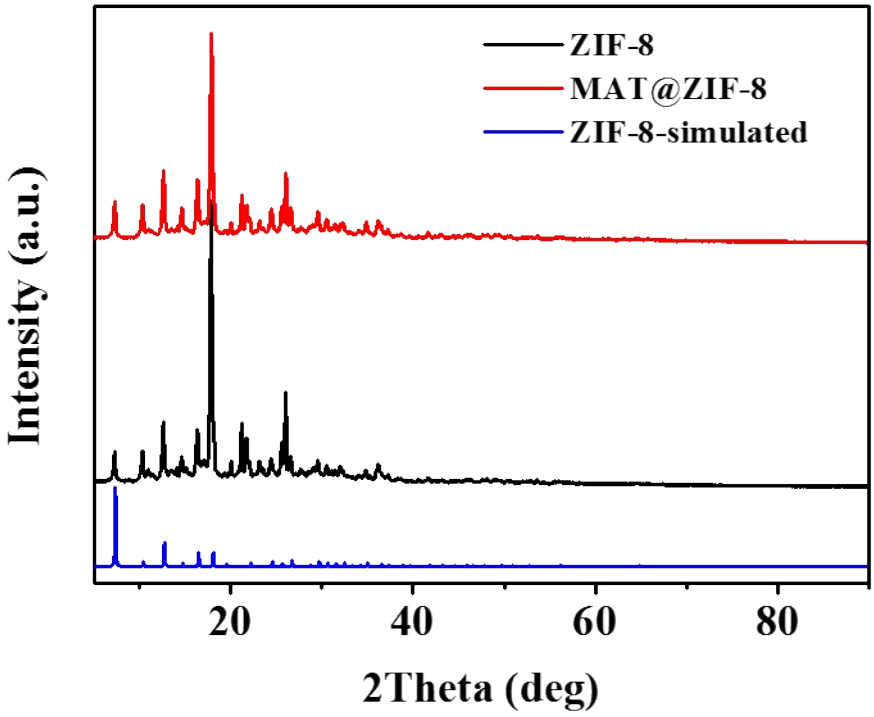

Figure S10. X-ray diffraction (XRD) patterns of powdery ZIF-8 and MAT@ZIF-8.

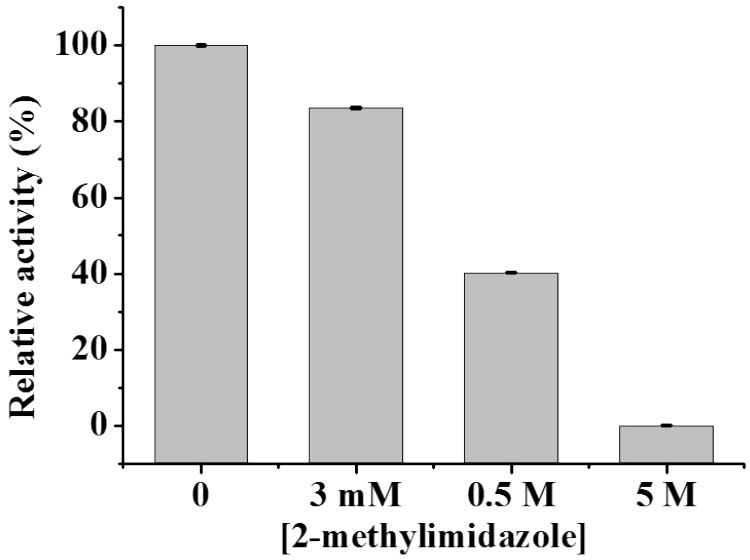

Figure S11. The influence of the different concentration of 2-methylimidazole for MAT activity.

Supplementary Reference

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