Electronic Supplementary Material (ESI) for ChemComm.


# Customized Hierarchically Porous Metal Organic Frameworks Engender Stable Enzymatic Nanoreactors

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**Experimental Details:**

**Reagents and chemicals**

Catalase (CAT), Iron (III) chloride hexahydrate, Ammonium iron(II) sulfate hexahydrate, D-sorbitol, Xylenol orange disodium salt, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride, N-Hydroxysuccinimide, coomassie brilliant blue and hydrogen peroxide (H2O2, 30%) were obtained from SIGMA-ALDRICH (UK). Tris-HCl buffer (1M, pH 8.0) and Trypsin-EDTA were purchased from ThermoFisher.

**Characterization methods**

Transmission electron microscopy (TEM) images were obtained on a FEI Tecnai Twin (Bruker, Germany) at an acceleration voltage of 120 kV. Scanning electron microscope (SEM) images were obtained (ZEISS Merlin, Germany). Zeta potentials of the nanoparticles were recorded on a Malvern Zetasizer ZS. Thermal gravimetric analysis (TGA) was performed on METTLER SDTA851. $N_2$ adsorption-desorption isotherms were measured on Micromeritics ASAP 2420 analyzer. Bruker D8 Advance was applied to characterize the wide-angle X-ray diffraction (XRD) patterns of the prepared samples.

**Synthesis of MIL-101**

In a typical experiment, $Fe_2Cl_3.6H_2O$ (675 mg, 2.45 mmol) and $H_2BDC$ (206 mg, 1.24 mmol) were dissolved in DMF (15 mL) solution and stirred for 5 minutes. The mixture was placed in a Teflon-lined autoclave and kept in an oven at 110 °C for 20 h without stirring. The products were harvested by centrifugation and then washed at least three times with DMF. Finally, the products were dried at 110 °C under vacuum.

**Synthesis of Big Pore MIL-101**

MIL-101 (Fe) (100 mg) was dispersed in the distilled water (10 mL) and glacial acetic acid (0.25 mL) using magnetic stirring for 20 minutes. The suspension was added into a Teflon-lined autoclave, reacting at 80 °C for 30, 60 and 90 min under static conditions. After the reaction, the products were collected by centrifugation and washed three times with DMF and distilled water.

**Synthesis of CAT@BP-MIL-101**

CAT (10 mg) was dissolved in 10 mL DI water and 9 mg BP-MIL-101 was dissolved in 9 mL DI water. The CAT solution (1 mL) was then slowly added to the BP-MIL-101 solution, stirring for 24 hours at 4 $^oC$. After incubation, the mixed solution was collected by centrifugation (6000 rpm, 10 min) and washed three times with distilled water. The precipitate was then incubated in a Trypsin solution at 37 $^oC$ for 10 min. The mixed solution was collected by centrifugation (6000 rpm, 10 min) and washed with water three times. 10% CAT@BP-MIL-101 was obtained after lyophilization. Based on this method, 20% and 40% CAT@BP-MIL-101 were also achieved.

**FITC labelled CAT**

CAT (10 mg) was dispersed in 10 mL of PBS (pH 7.4) under 4 °C. EDC (0.03 mmol) and NHS (0.02 mmol) were added into the CAT solution, stirring for 1 h at 4 °C. FITC (0.025 μmol) was then dissolved in 500 μL PBS solution and added into the CAT solution. The whole solution was stirred for 4 h at 4 °C in the dark. Unreacted EDC, NHS and FITC in the FITC-CAT containing solution were removed by dialysis (MWCO: 12k-14k) for one day.

The synthesis of FCAT@BP-MIL-101 and FCAT@MIL-101 were similar with that of CAT@BP-MIL-101. The fluorescent signal was observed by Confocal Microscope (Excitation: 490 nm and Emission: 525 nm).

**Loading capacity and efficiency of CAT@BP-MIL-101**

In our research, Bradford reagent was used to measure different concentrations of CAT (0- 500 μg/mL), drawing the standard curve at 595 nm. Then, 1 mg of CAT@BP-MIL-101 at different time (0, 30, 90 and 150 min) was dissolved into 1 mL of DI water, respectively. The CAT in BP-MIL-101 was determined by the Bradford assay. The loading efficiency and capacity of CAT in BP-MIL-101 were calculated accordingly.

**The measurement of $H_2O_2$ based on the FOX assay**

FOX assay was used for the measurement of hydrogen peroxide concentration. The FOX reagent was composed of 100 μM xylenol orange, 250 μM Ammonium iron(II) sulfate hexahydrate, 100 mM D-sorbitol in 25 mM $H_2SO_4$. The concentration of H2O2 was calibrated spectrophotometrically at 240 nm with ε= 39.4 $M^{-1}$ $cm^{-1}$. The corresponding calibration line of the FOX assay was obtained from the absorbance at 560 nm using $H_2O_2$ standards in concentrations from 0 to 200.

CAT@BP-MIL-101 (2.3 mg of 20%, 0.3 mg/mL of free CAT), CAT@MIL-101 and 0.3 mg CAT were added respectively into 1000 μL Tris buffer (pH 8.0, 0.05 M), incubating for 30 min at room temperature. After incubation, samples were added to 1 mL 0.2 mM hydrogen peroxide to give the initial $H_2O2$ concentration of 0.1 mM. 100 μL of this solution was respectively removed at different time intervals and centrifuged (12 000 rmp 5 min) at 4 °C. Then 50 μL of the supernatant was mixed with 950 mL of FOX reagent and incubated for 30 min at room temperature. The absorbance of the mixed solution at 560 nm was recorded and the final concentration of hydrogen peroxide was calculated by the standard curve.

In order to compare differences between CAT@BP-MIL-101 and CAT at high temperature, 20% CAT@BP-MIL-101 and CAT were incubated at 80 °C for 3 min, respectively. After incubation, the $H_2O_2$ was measured following the same method.

The kinetic parameters of CAT in CAT@BP-MIL-101

The Michealis-Menten equation was used for enzyme kinetics.

$$V_0 = \frac{V_{\max}[S]}{(K_M + [S])}$$

Here, $V_0$ is the initial catalytic rate; Vmax is the maximum rate conversion, which is obtained when the catalytic sites on the enzyme are saturated with substrate. [S] is the initial substrate concentration, and KM is the Michealis-Menten constant. The kinetic parameters, KM and Vmax, can be obtained by measuring the initial rates of the reaction with different initial substrate concentration using a Lineweaver–Burk plot. The initial reaction rates of CAT can be obtained in time course mode by monitoring the rate of substrate (hydrogen peroxide) decomposition spectrophotometrically at 560 nm using the indicator xylenol orange in the FOX reagent.

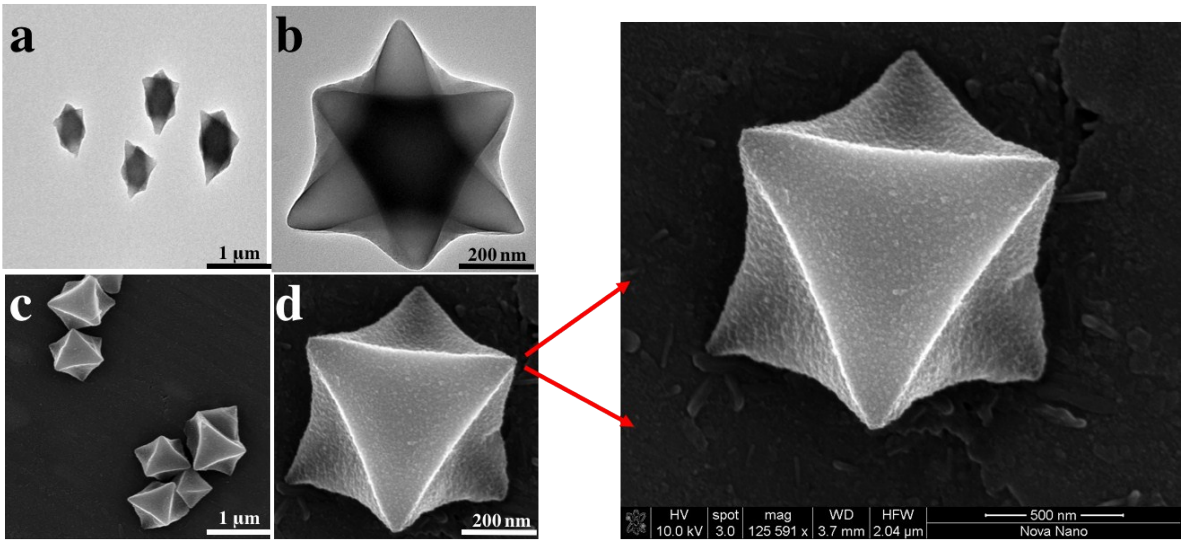


**Figure S1.** TEM images of MIL-101 (a and b) and SEM images of MIL-101 (c and d). The 3D TEM videos are included as separate .avi files.

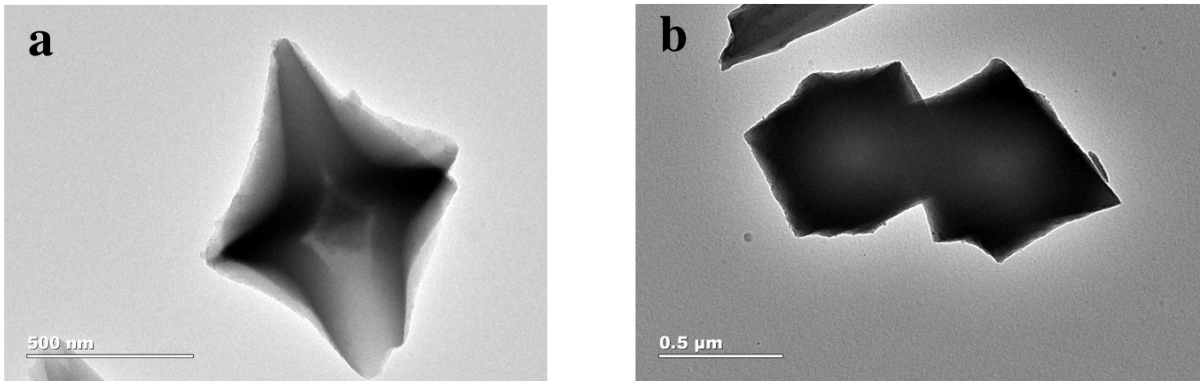


**Figure S2.** TEM images of MIL-101 after 90 min acid etching in (a) water and (b) acetone.

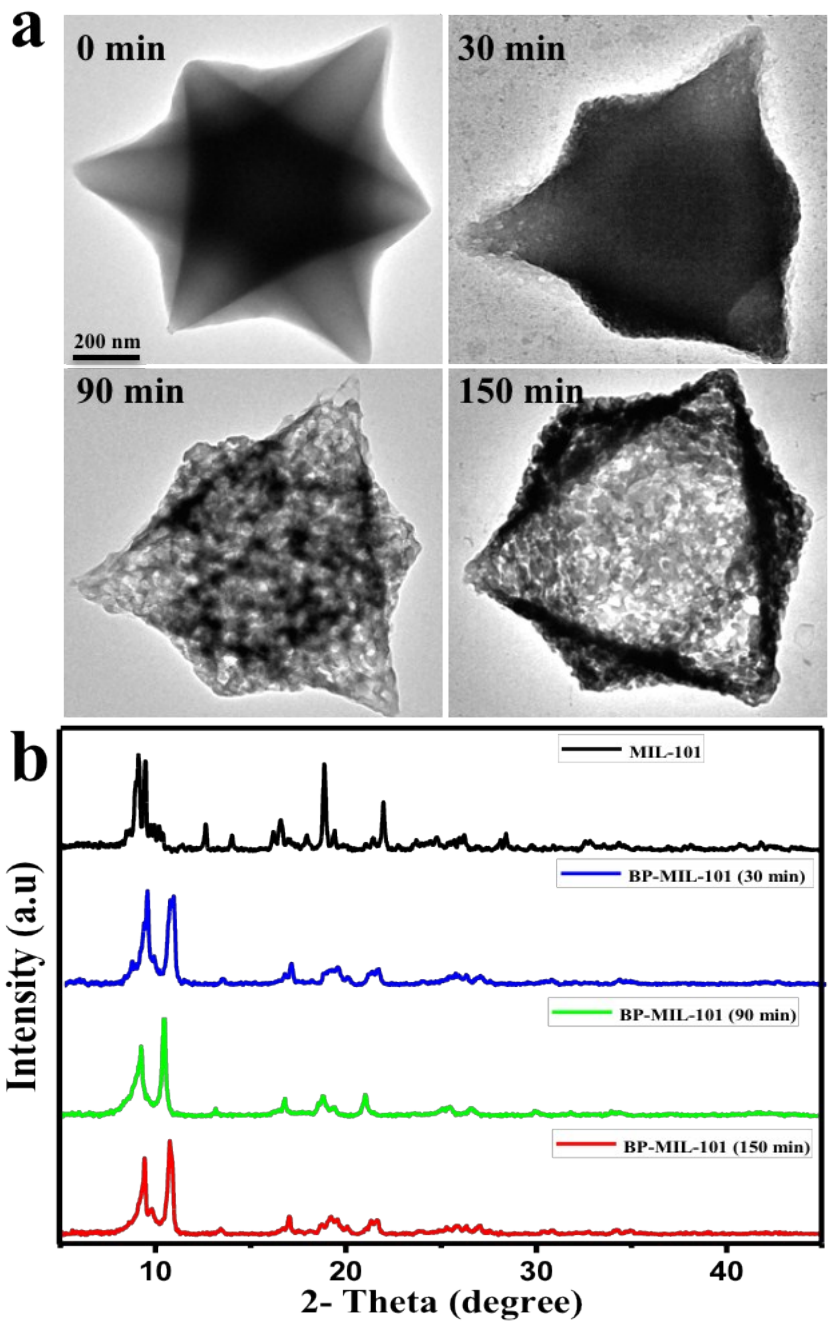


**Figure S3.** (a) TEM images of BP-MIL-101, which are respectively added into an acetic solution for 0, 30, 90 and 150 min. (b) XRD patterns of BP-MIL-101 at different etching time.

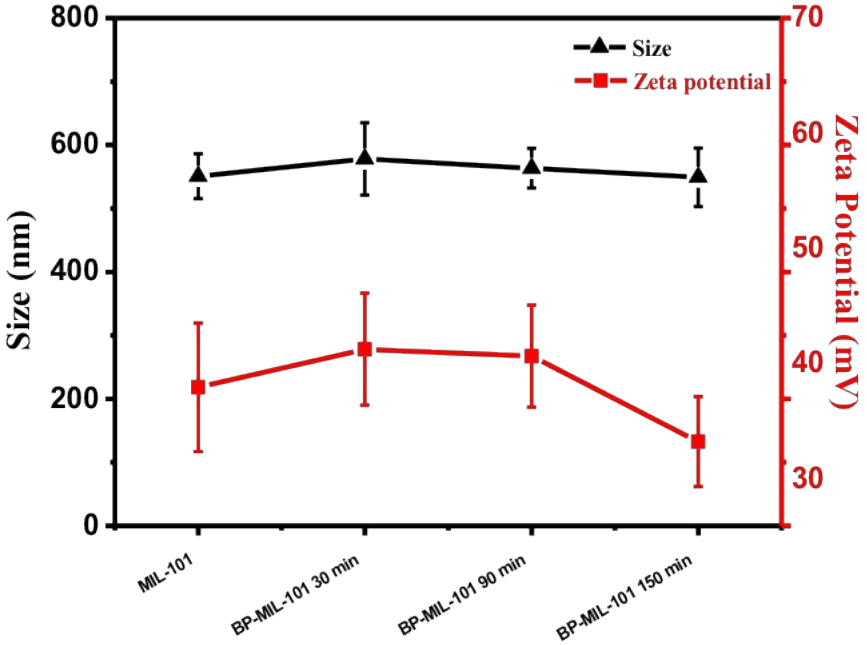


**Figure S4.** Zeta potential and size distributions of BP-MIL-101 samples, which are respectively added into an acetic solution for 0, 30, 90 and 150 min.

**Table S1. Porosity data by Micromeritics ASAP 2420 analyzer**

| | Total Pore Volume ($cm^3/g$) | BJH adsorption average pore size (nm) |
|---|---|---|
| **Mil 101** | 0.33 | 3.6 |
| **30 min** | 0.19 | 15.6 |
| **90 min** | 0.2 | 30.5 |

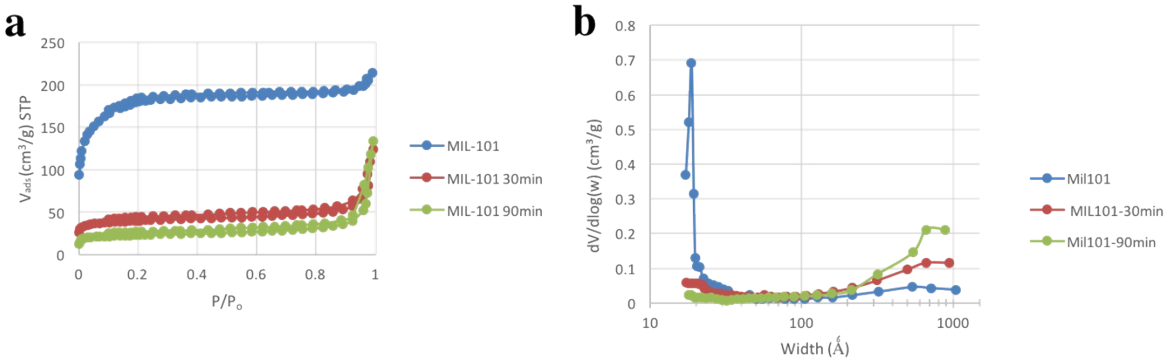


**Figure S5.** (a) $N_2$ absorption/desorption isotherms before and after etching. (b) Pore size distribution before and after etching.

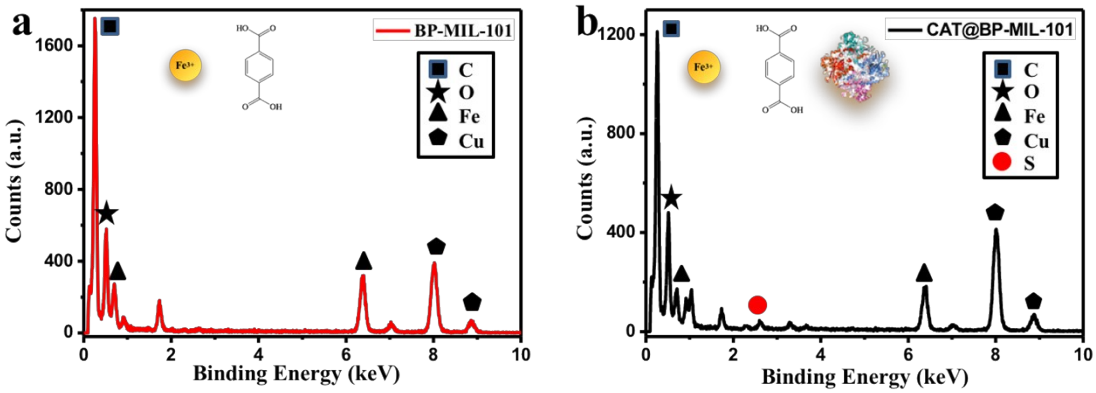


**Figure S6.** EDS analysis of a) BP-MIL-101 and b) CAT@BP-MIL-101 on Cu grid.

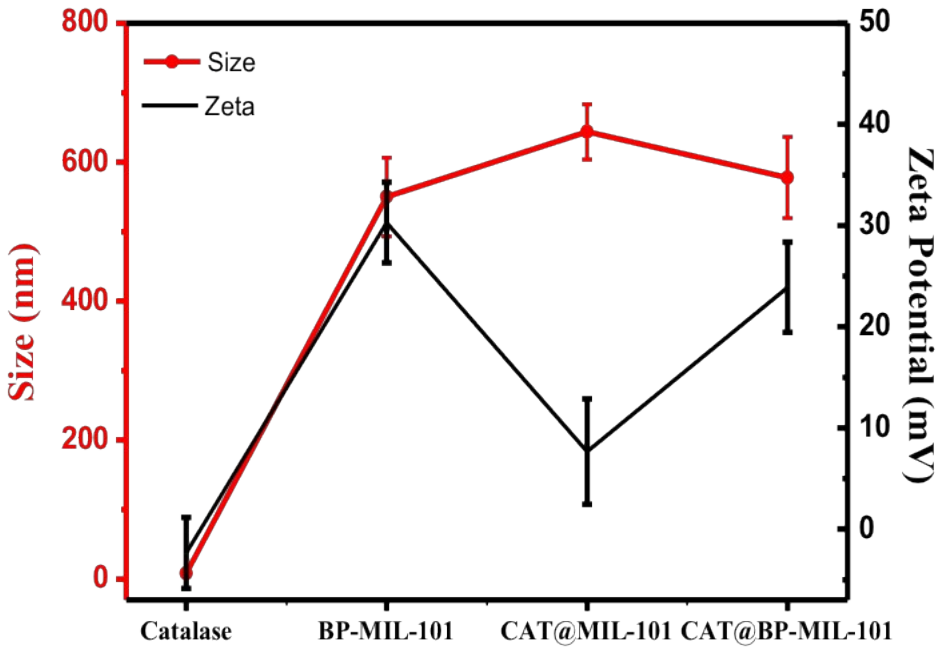


**Figure S7.** Zeta potential and size distribution of CAT, BP-MIL-101, CAT@MIL-101 and CAT@BP-MIL-101.

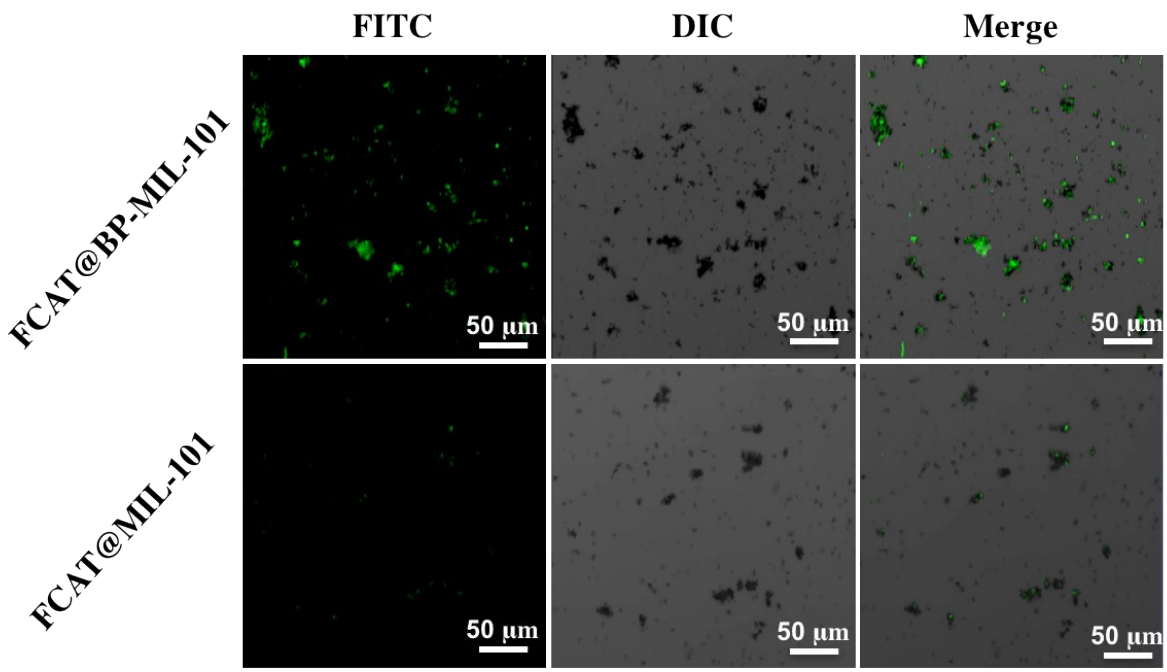


**Figure S8.** Confocal images of FCAT@BP-MIL-101 and FCAT@MIL-101.

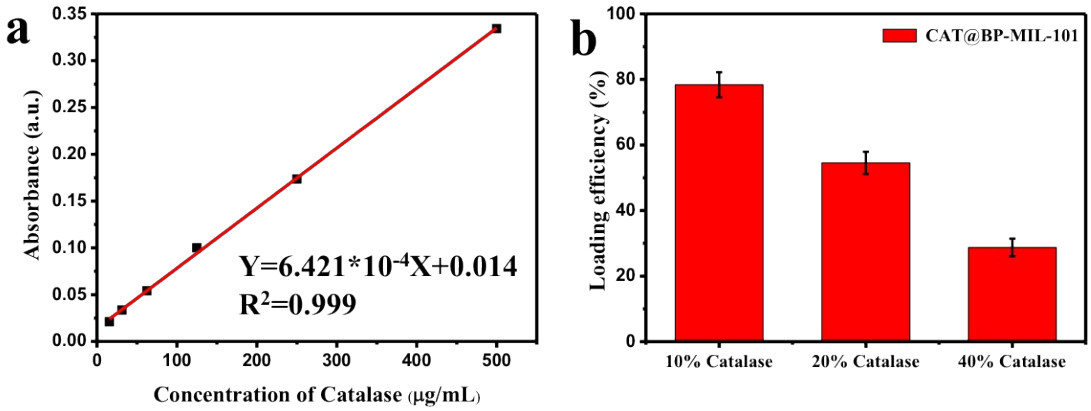


**Figure S9.** (a) The calibration curve of CAT using the Bradford assay. (b) The loading efficiency of CAT@BP-MIL-101.

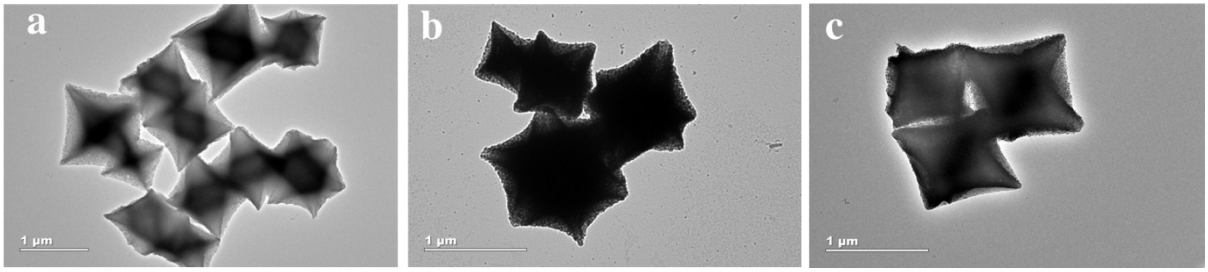


**Figure S10.** pH stability of CAT@BP-MIL-101 at (a) pH 3, (b) pH 6, and (c) pH10.

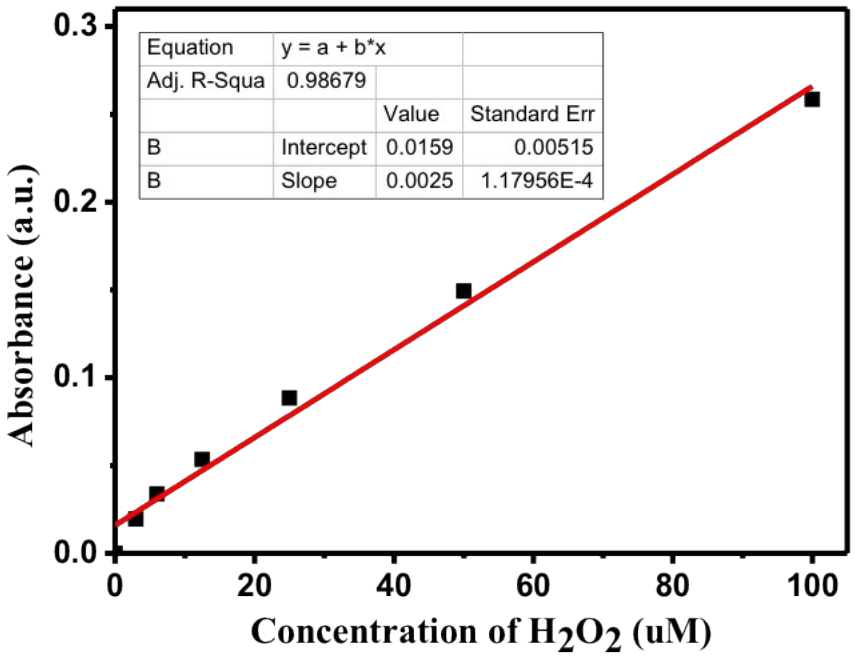


**Figure S11.** The calibration curve of $H_2O_2$ using the FOX assay.

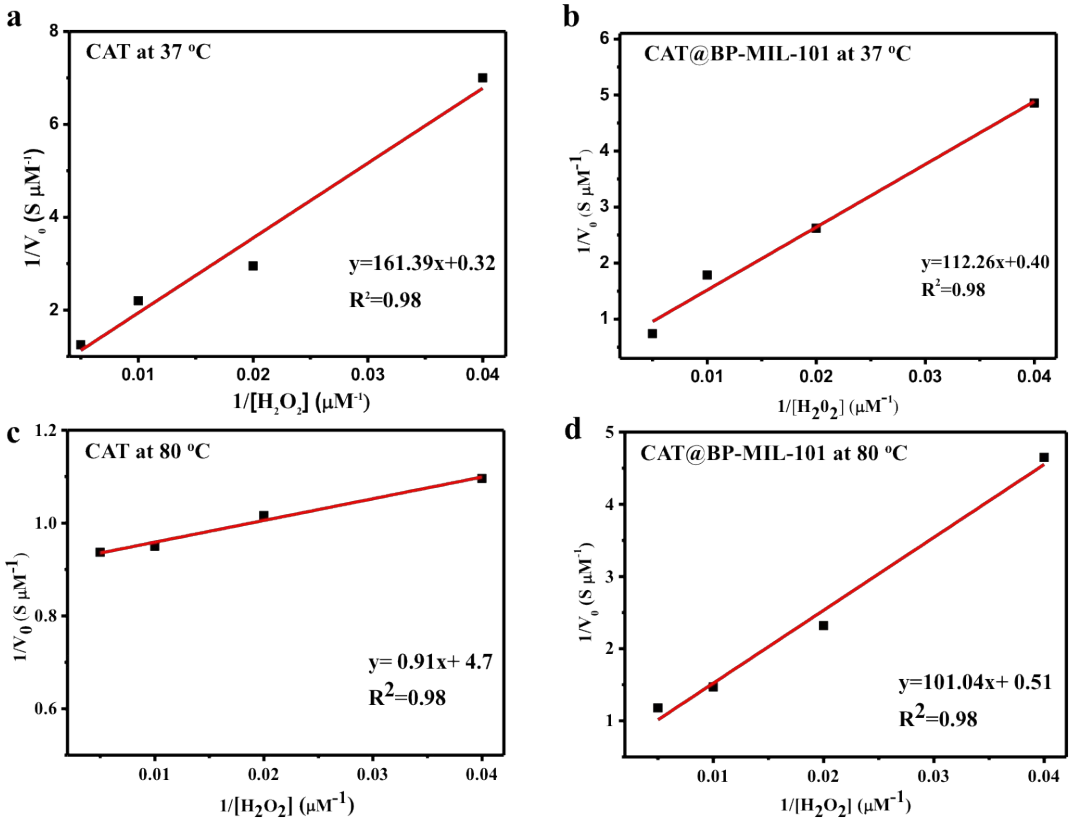


**Figure S12.** Lineweaver–Burk plot for determination of the kinetic parameters of CAT in (a) CAT at 37 °C, (b) CAT@BP-MIL-101 at 37 °C, (c) CAT at 80 °C and (d) CAT@BP-MIL-101 at 80 °C.