Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

A facile approach for hierarchical architectures of enzyme-metal-organic framework biocatalyst with high activity and stability

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1. Experimental

1.1 Materials

5-(ethylthio)-1H-tetrazole (98%), copper acetate monohydrate (99.95% metals basis), catalase (from bovine live, lyophilized, ≥3000 units/mg protein), proteinase K (*tritirachium album limber*), bovine serum albumin, and dopamine hydrochloride were purchased from Aladdin Industrial Inc., (Shanghai, China). Hemoglobin (from bovine red cells, lyophilized powder) was purchased from Sangon Biotech (Shanghai, China). Laccase (from *trametes versicolor*, ≥0.5U/mg), lysozyme (from chicken egg white, ≥4000 units/mg protein), norepinephrine (98%), (±)-epinephrine hydrochloride (≥98%), and dopamine hydrochloride were bought from Sigma-Aldrich Co. All the above-mentioned chemicals were used without further purification.

1.2 Synthesis of hierarchical [Cu(ett)] MOF and laccase-MOF

For the synthesis of hierarchical [Cu(ett)] MOF: 5-(ethylthio)-1H-tetrazole (ett), copper acetate, and laccase were dissolved in water and the concentrations of them were 0.02M, 0.1M and 30.3 μM respectively. The copper ion (100 μl) and the ett (100 μl) were mixed, and then keeping it for 24 hours at room temperature. Ultimately, the MOF product was centrifuged at 10000 rcf for 10 mins and washed three times with water. Then the sample was sealed and heated at 70℃ for 24 hours for further characterization experiment.

For the synthesis of hierarchical laccase-MOF: hierarchical laccase-MOF is synthesized in the similar way of hierarchical MOF, and the only difference is that 0.1M copper acetate (50 μl) is pre-mixed with 30.3 μM laccase (50 μl).

1.3 Activity Investigation

Laccase content in hierarchical laccase-MOF was determined via the element analysis using oxygen content as a reference and the assay of laccase activity is performed using dopamine (21.1 mM) as substrate. The concentration of laccase in free and hierarchical MOF solution was 1.5 μM based on quantitative results (Supplementary table 2). The total volume of the reaction system is

100 μl and consists of three components, hierarchical laccase-MOF or free laccase (10 μl), substrate solutions (80 μl) and citric acid buffer solution (pH 5.5,10 μl).

1.4 Stability of hierarchical laccase-MOF and free laccase

To determine the effects of temperature on hierarchical laccase-MOF and free laccase, dopamine substrate solutions (21.1 mM) were prepared with 0.1M citric acid buffer solution at different pH values (4, 4.5, 5, 5.5, 6, 6.5). The same amount (1.5 μM, 10 μl) of free laccase and hierarchical laccase-MOF were added to substrate solution at room temperature for 1 hour, then the supernatant was measured at 485 nm by Multiskan Spectrum (MultiskanSky_1530-800537C). To determine the effects of temperature on hierarchical laccase-MOF, hierarchical laccase-MOF (50 μl) and free laccase (50 μl) were treated at different temperatures (20, 30, 40, 50, and 60 ℃) for1 hour. To determine the effects of organic solvent on hierarchical laccase-MOF, hierarchical laccase-MOF and free laccase were treated at different organic solvent (30%) for1 hour. The storage stability of the hierarchical laccase-MOF and free laccase was assessed by storing it at 4℃ for 20 days, then the laccase activity was determined every two days.

1.5 Formation kinetics test of [Cu(ett)] MOF:

The kinetic analysis of hierarchical MOF (100 μl 0.06 M $Cu^{2+}$and 100 μl 0.01 M 5-(ethylthio)-1H-tetrazole) and MOF nano-aggregates (100 μl 0.2 M $Cu^{2+}$and 100 μl 0.015 M 5-(ethylthio)-1H-tetrazole) formation was determined by measuring the optical density at 600nm (OD600). The material synthesis method and the concentration of each mother solution are the same as above.

1.6 Characterization of MOF and hierarchical laccase-MOF

The synthesized materials were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), fourier transform infrared spectrometer (FT-IR), thermogravimetric analysis (TGA), and brunauer–emmett–teller (BET). SEM (ZEISS, Germany) imaging was employed to characterize the morphology of the MOF crystals. Briefly, samples were dispersed into water and then deposited on a silicon wafer above which a 5 nm gold layer was sputtered over 30 s and imaging was carried out at 10 kV. XRD (Rigaku, Japan) measurements were carried out employing X-ray

diffractometer with θ-2θ geometry using Cu Kα radiation (λ= 1.54 Å). Each measurement was recorded using a PSD detector (MBraun) in the range of 2θ = 5°- 50°at a scan step of 0.2°/min at 40 kV and 30mA. FT-IR (Nicolet iS10, USA) was conducted in the 400–4000 cm^{-1} range at a resolution of 4 cm^{-1}. TGA (STA449F5, German) was performed under a nitrogen atmosphere at a rate of 10 ℃/min from 30 to 600 ℃. BET (ASAP2460, USA) test was based on N_2 adsorption–desorption curves operating at 77 K with 5 s equilibration interval. The pore size distribution was obtained from the N_2 adsorption isotherm through the Barrett–Joyner–Halenda (BJH) approach. Elemental contents of oxygen in [Cu(ett)]MOF and laccase-[Cu(ett)]MOF were measured via an Elementar Vario EL Cube elemental analyzer (Elementar Analysensysteme GmbH, Germany)

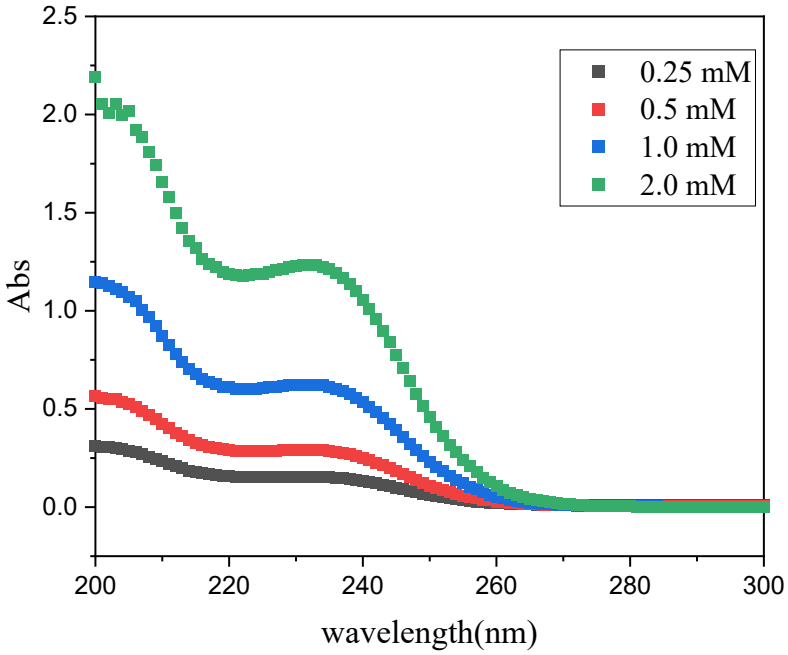

Figure S1. UV absorption spectrum of 5-(ethylthio)-1H-tetrazole aqueous solution at different concentration.

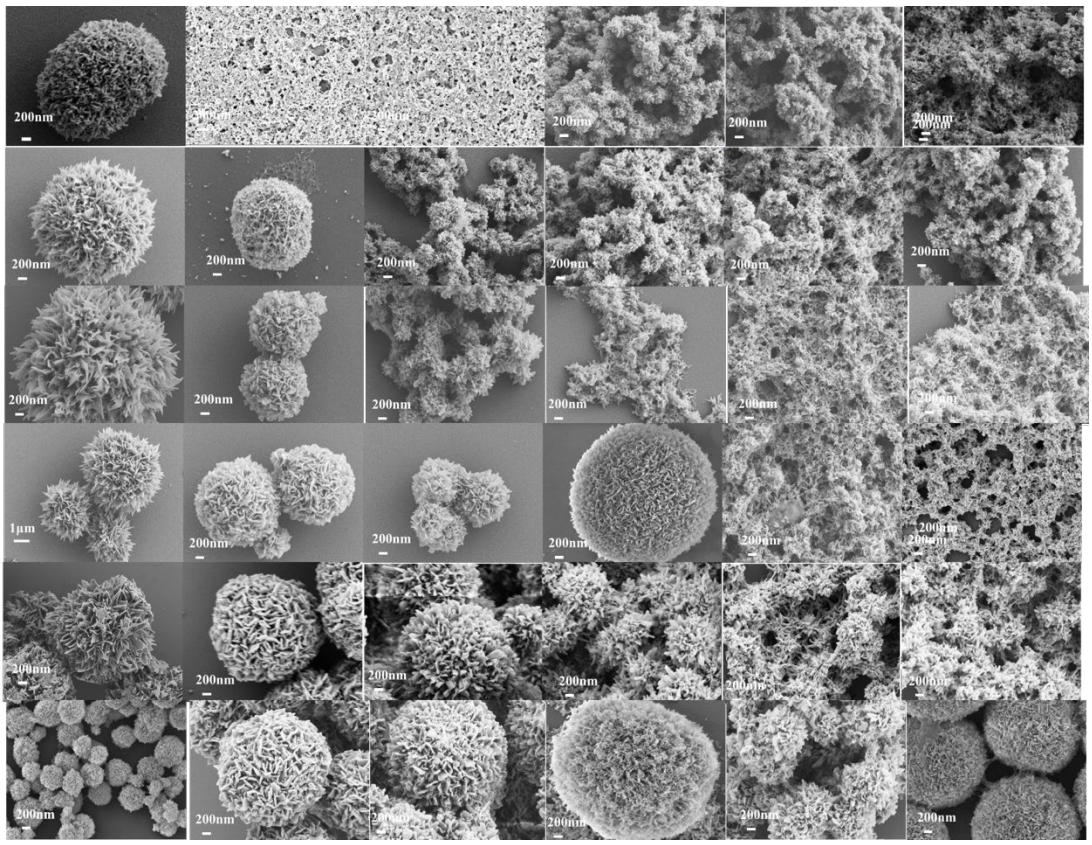

Figure S2. SEM images of [Cu(ett)] MOF which prepared by using orthogonal test method. Corresponding concentration of reactants are as follows: Cu^{2+}(from left to right, the concentration is 0.05 M, 0.1 M, 0.15 M, 0.2 M, 0.25 M, and 0.3 M respectively) and ett ligand (from top to bottom, the concentration is 0.015 M, 0.02 M, 0.025 M, 0.03 M, 0.035 M, and 0.04 M respectively).

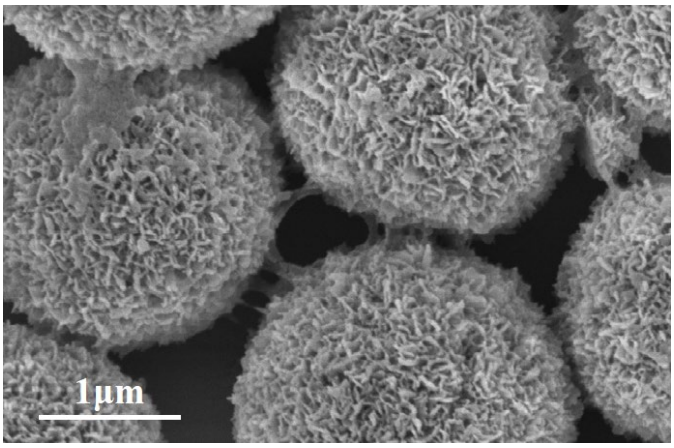

Figure S3. SEM images of laccase-[Cu(ett)] MOF.

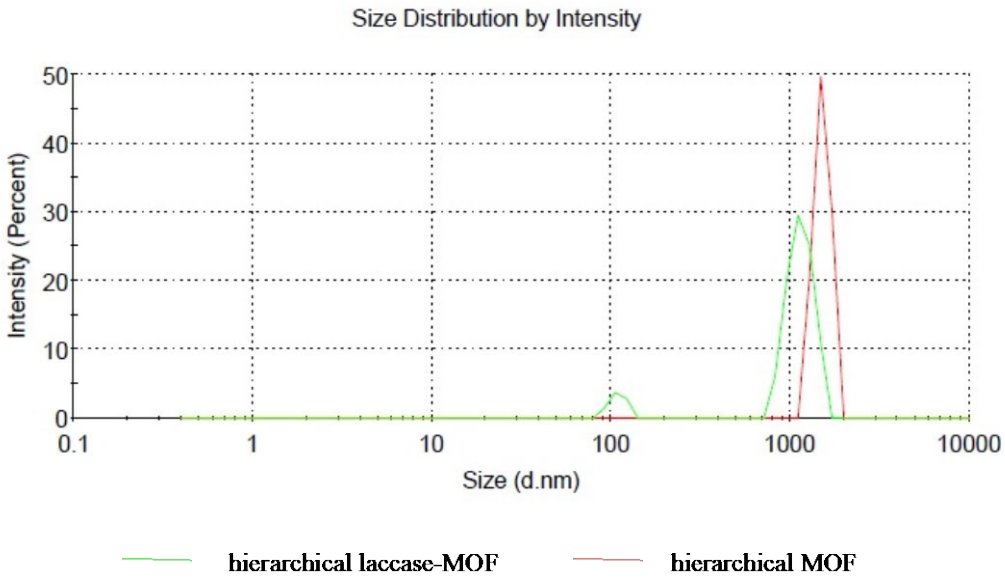

Figure S4. Dynamic light scattering (DLS) results for the size distribution of hierarchical laccase-[Cu(ett)] MOF and [Cu(ett)] MOF obtained at aqueous suspension.

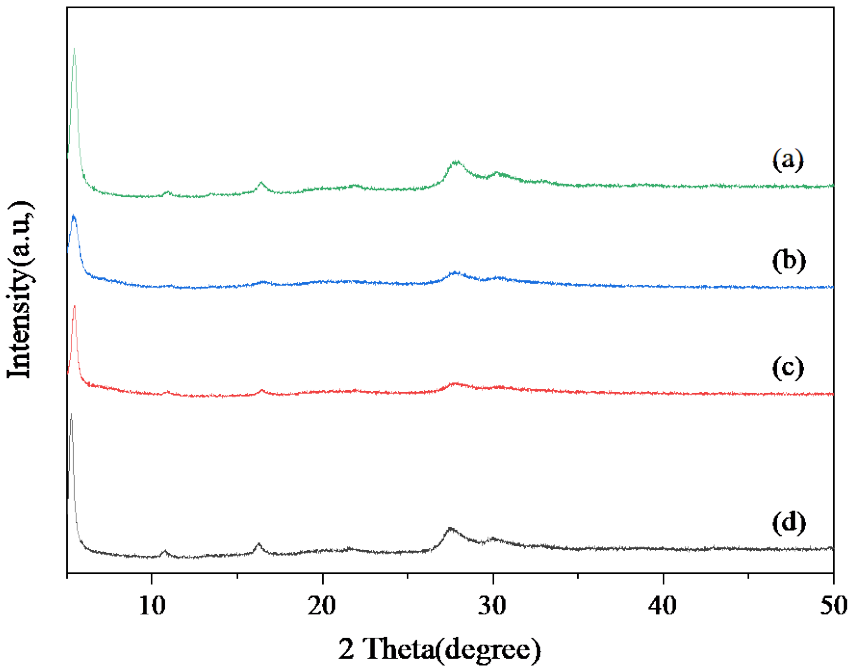

Figure S5. XRD patterns of different enzyme-[Cu(ett)] MOF composites; the corresponding enzymes are as follows: (a) proteinase K, (b) lysozyme, (c) hemoglobin, (d) catalase.

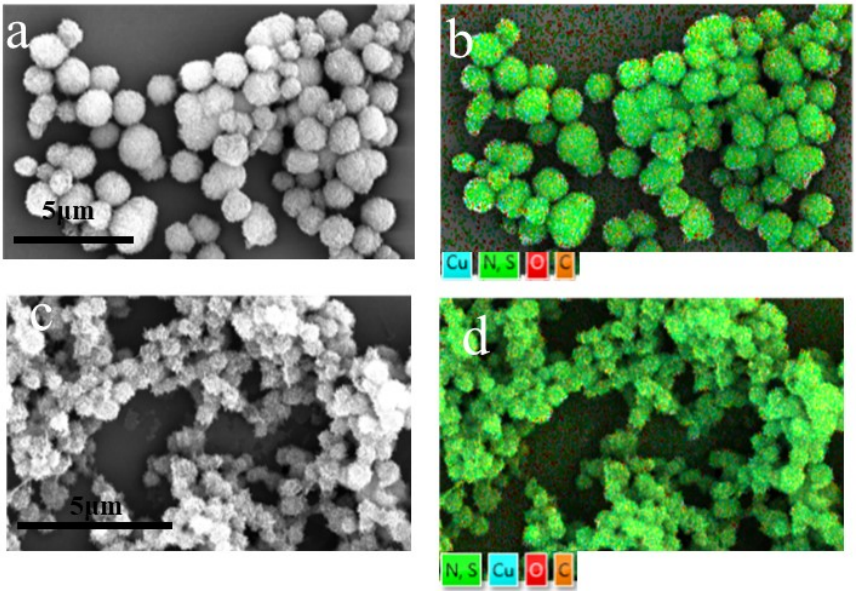

Figure S6. (a) SEM image of laccase-[Cu(ett)] MOF (a) and [Cu(ett)] MOF (c). The corresponding elemental distributions of laccase-[Cu(ett)] MOF (b) and [Cu(ett)] MOF (d). Scale bar: 5 μm.

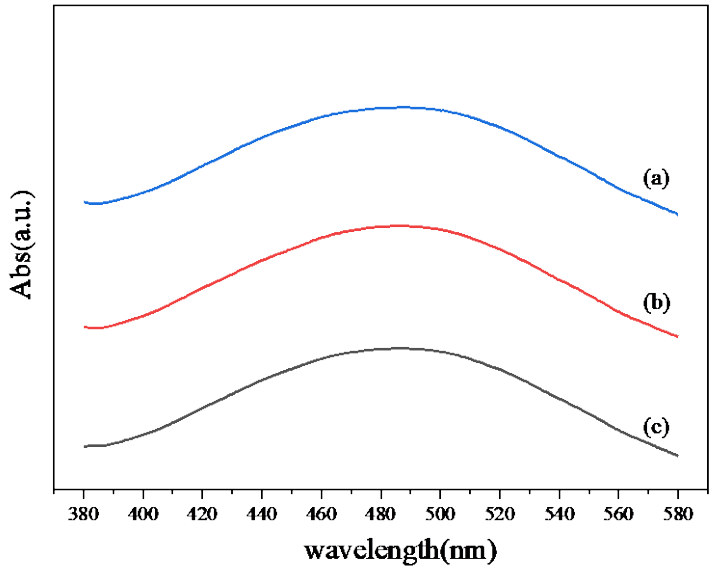

Figure S7. UV-vis absorption spectrum of different enzyme-substrate in aqueous solution; (a)norepinephrine, (b) (±)-epinephrine, and (c) dopamine.

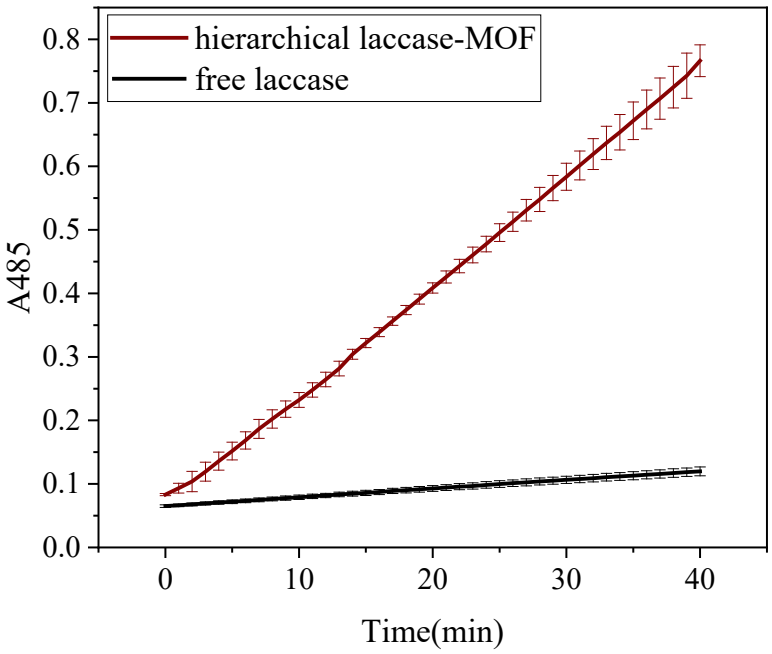

Figure S8. Activity kinetics comparison between hierarchical laccase-[Cu(ett)] MOF (red) and free laccase (black), the substrate is (±)-epinephrine hydrochloride.

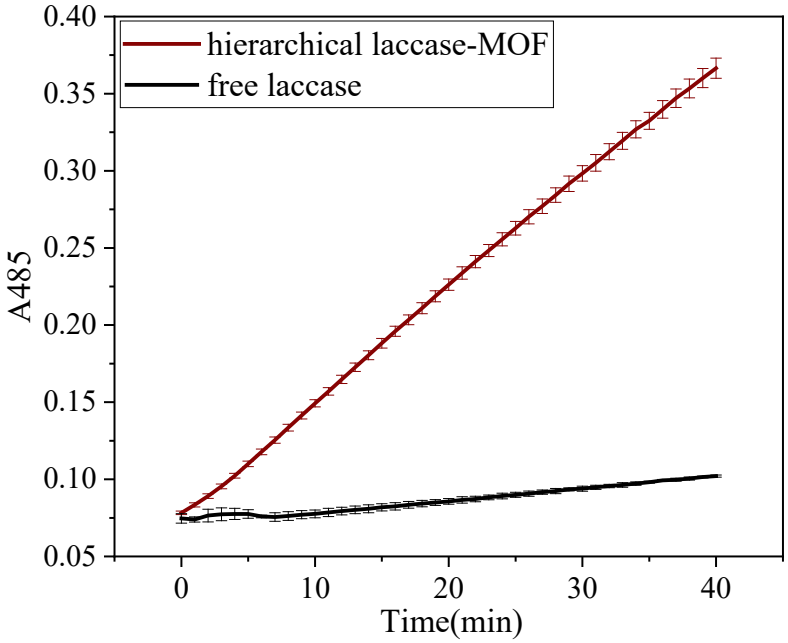

Figure S9. Activity kinetics comparison between hierarchical laccase-[Cu(ett)] MOF (red) and free laccase (black), the substrate is noradrenaline.

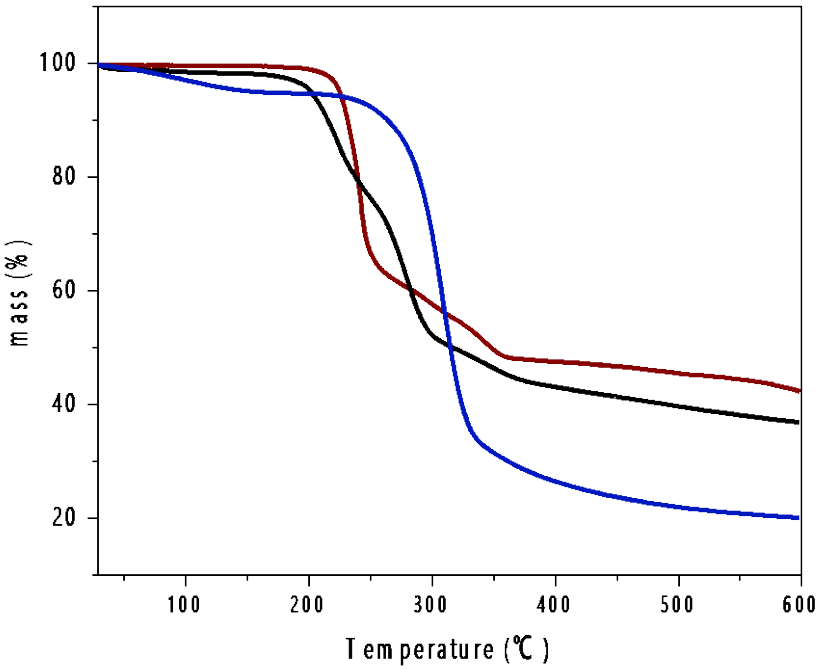

Figure S10. TGA curves of the hierarchical architectures laccase-[Cu(ett)] MOF (black line), [Cu(ett)] MOF (red line) and free laccase (blue line).

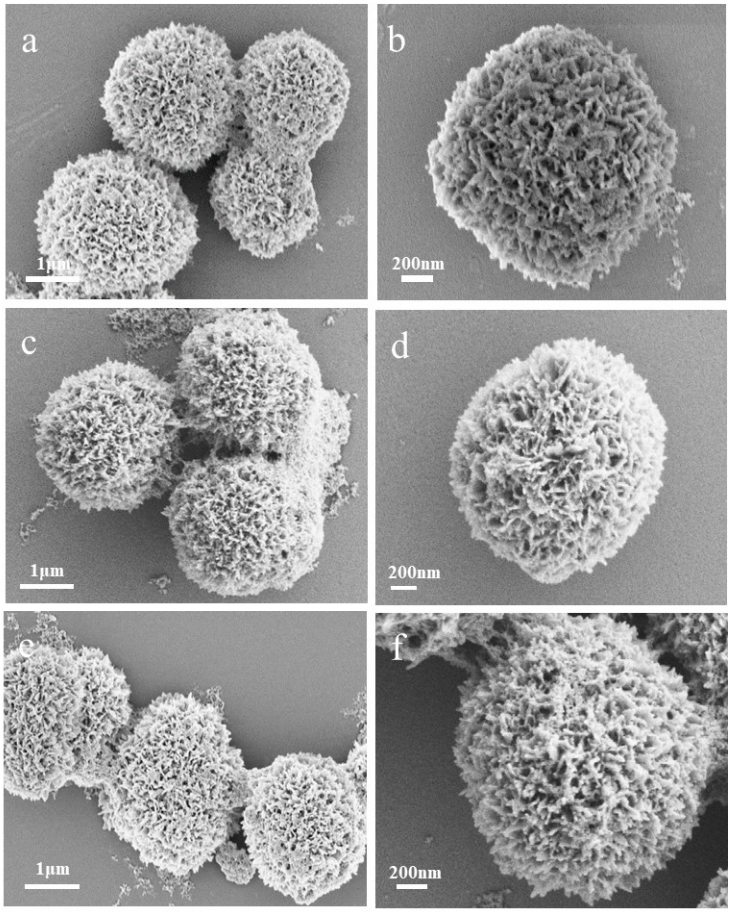

Figure S11. SEM image for laccase-[Cu(ett)] MOF powders as a function of recycle number with 0 (a, b), 1 (c, d) and 3 (e, f).

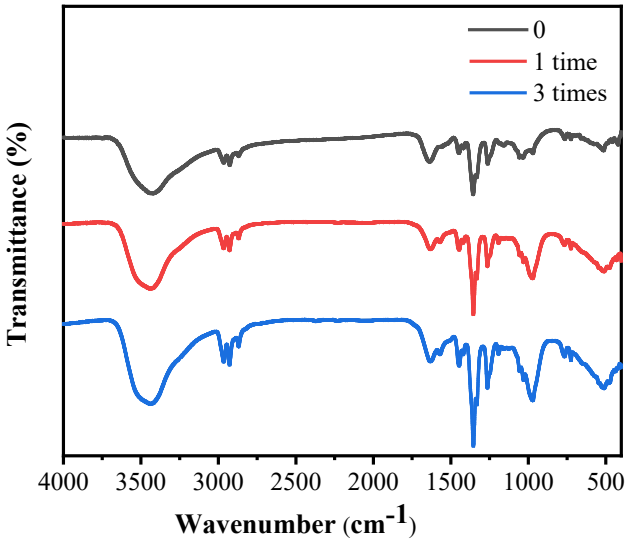

Figure S12. FT-IR spectra for laccase-[Cu(ett)] MOF powders as a function of recycle number with 0 (red line) and 3 (black line).

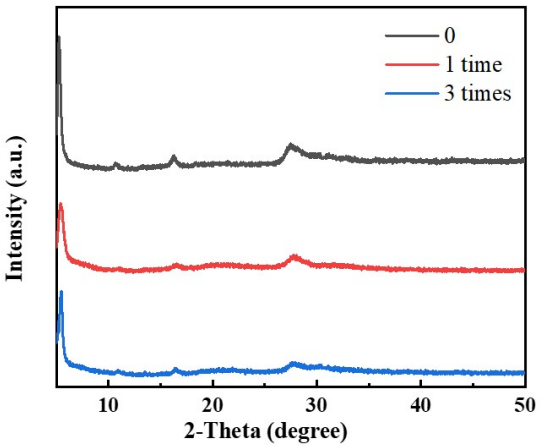

Figure S13. XRD spectra for laccase-[Cu(ett)] MOF powders as a function of recycle number with 0 (red line) and 3 (black line).

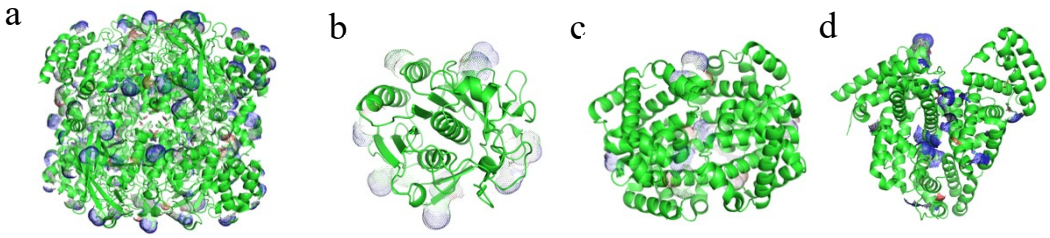

Figure S14. Crystal structures of (a) catalase (PDB: 1TH3), (b) proteinase K (PDB: 2PKC), (c) hemoglobin (PDB: 2QSP), and bovine serum albumin (PDB: 4F5S) with the solvent accessible surface area of N-atoms in the protein structures marked in blue.

Table S1. Chemical composition of the [Cu(ett)] MOF and laccase-[Cu(ett)] MOF (wt%, based on nergy-dispersive X-ray spectroscopy)

Materials	C	N	O	S	Cu
laccase-[Cu(ett)] MOF	28.47	17.04	5.36	13.14	35.99
[Cu(ett)] MOF	13.38	7.12	1.57	17.48	60.44

Table S2. Elemental analysis of hierarchical MOF, hierarchical laccase-[Cu(ett)] MOF and free laccase.

Sample name	Oxygen content (%)	Enzyme loading
Hierarchical [Cu(ett)] MOF	15.731	3.3 ± 0.5%
	14.621	
	15.125	
Hierarchical laccase-[Cu(ett)] MOF	16.257	
	16.008	
	16.361	
Laccase	45.943	
	49.059	
	46.538	