Supporting Information

Facile preparation of MOF-derived $MHCo_3O_4$ &Co/C with hierarchical porous structure for entrapping enzyme: Having both high stability and catalytic activity

Xia Gao,*[a,b] Huibin Pan,[c] Chengfang Qiao,[a] Yongliang Liu,[a] Chunsheng Zhou,*[a] Quanguo Zhai,[b] Mancheng Hu,[b] Shuni Li[b] and Yucheng Jiang*[b]

[a] Shaanxi Key Laboratory of Comprehensive Utilization of Tailings Resources, School of Chemical Engineering & Modern Materials, Shangluo University, Shangluo 726000, P. R. China

[b] School of Chemistry & Chemical Engineering, Shaanxi Normal University, Xi'an 710062, P. R. China

[c] Public Basic Teaching Division, Shangluo Vocational & Technical College, Shangluo 726000, P. R. China

*Corresponding authors, Email: xiagao2007@163.com
jyc@snnu.edu.cn
slzhoucs@126.com

Experimental

Materials

Horseradish peroxidase (HRP, EC 1.11.1.7) was purchased from Shanghai Xueman Biotechnology Co., Ltd and stored at minus 20 °C before use. 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored at minus 4 ℃ before use. Cobalt nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$, 99%) and 2,4-dichlorophenol (99%) were purchased from Beijing Chemical Works (China). Polyvinyl pyrrolidone (PVP, 99%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Trimesic acid (H_3BTC, 98%) was purchased from Alfa Aesar. Dopamine hydrochloride (DA) and hexamethylenetetramine (HMTA) were purchased from Shanghai solarbio Bioscience Technology Co., LTD. Dimethyl formamide (DMF, >99.5%) and pyrazine (>98%) were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Hydrogen peroxide (H_2O_2, 30% in aqueous solution) was obtained from Xi'an Chemical Co. Ltd. All chemicals are of analytical grade unless otherwise indicated.

Measurements

N_2 adsorption-desorption measurements at 77 K were carried out using an automatic physical adsorption instrument (Micromeritics Instrument Corp, Atlanta, USA). The powder X-ray diffraction patterns (PXRD) were recorded on a Rigaku (Woodlands, USA) X-ray Powder Diffractometer equipped with a Cu sealed tube (λ = 1.5404 Å) with voltage at 40 kV, the current at 30 mA, the scanning speed at $1°\cdot min^{-1}$, and the scanning range setting at 5° to 80°. The morphologies of the samples were characterized by a field-emission scanning electron microscope (FESEM, Hitachi SU8220, Tokyo, Japan) and a field-emission transmission

electron microscope (FETEM, Tecnai G2 F20, Hillsborough County, Florida, USA). Fluorescent tags of HRP on PDA@$MHCo_3O_4$ or PDA@Co/C were performed on a FV1200 laser scanning confocal microscope (Olympus, Beijing, China), by using fluorescein isothiocyanate (FITC) as fluorescent marker. TGA data were obtained on a TA Q1000DSC thermoanalyzer systems with a heating rate of 2 °C·min^{-1} under air and nitrogen atmospheres. Ultraviolet-visible (UV-vis) absorption spectra of proteins were recorded using Perkin-Elmer Lambd 950 UV-vis spectrophotometer.

Preparation of $MHCo_3O_4$ &Co/C

In a typical solvothermal preparation procedure, 0.1455 g of $Co(NO_3)_2·6H_2O$, 0.1051 g of H_3BTC, 1.31 g of PVP and a certain amount of pyrazine (0.9~3.0 mmol) were dissolved in 30 mL DMF in a teflon liner. The mixture was sonicated for 30 min to form a homogeneous solution and then heated at 150 °C for 24 h. Subsequently, the resultant purple powders were collected by centrifugation and washed with DMF for 3 times and dried at 60 °C in a vacuum oven for 6 h. The prepared sample was designated as Co-MOF, and the morphology of which was regulated by changing the amount of pyrazine in the preparation process.

The above-treated Co-MOF precursor was put into a ceramic crucible and transferred into the resistance furnace. The furnace was then heated in air from room temperature to 500~600 °C at a heating rate of 1 °C·min^{-1}. After the temperature reached the desired value, it was maintained for 2 h and then cooled to room temperature naturally. The Co-MOF was finally converted into two black glossy crystals at different pyrolysis temperatures (500 ℃, 600 ℃, respectively). The prepared samples were designated as $MHCo_3O_4$-(500 ℃) and $MHCo_3O_4$-(600 ℃).

The above-treated Co-MOF precursor was put into a quartz boat and transferred into the tube furnace. After nitrogen purge for 30 min, the furnace was heated in nitrogen from room temperature to 500~800 °C at a heating rate of 1 °C·min^{-1} and maintained for 2 h, and then cooled to room temperature naturally. The Co-MOF was finally converted into three black glossy crystals at different pyrolysis temperatures (500 ℃, 600 ℃, 800 °C, respectively). The prepared samples were designated as Co/C-(500 ℃), Co/C-(600 ℃) and Co/C-(800 ℃).

Determination of enzymatic kinetic and thermodynamic parameters

Enzymatic kinetic assay was carried out using oxidation of ABTS as model reaction within the concentration range of 0.15 ~ 0.35 mmol·L^{-1}, while the concentration of H_2O_2, the loading capacity of enzyme and the pH value of the phosphate buffer were kept constant.

The changes of enthalpy and entropy of enzyme-catalyzed reactions were determined by NANO ITC (Isothermal Titration Calorimetry). The ABTS and phosphate buffer were degassed for more than 15 min under vacuum to prevent the formation of bubbles in the calorimeter cell. The phosphate buffer was injected into the reference cell. The stirring syringe was filled with ABTS (100 μL, 10 mmo·L^{-1}) as the titrant, while the sample cell was filled with a certain amount of free enzyme or an equivalent amount of enzymatic reactor-H_2O_2-phosphate buffer as the titrant. 18 drops of ABTS (5.5 μL per drop) were discharged sequentially into the sample cell, where the free enzyme or an equivalent amount of enzymatic reactor was mixed with H_2O_2 and phosphate buffer (with constant concentration) ahead, and there was an automatic equilibrate prior the first injection. All ITC experiments were performed at 25 ℃ and the syringe rotation of 250 rpm. Control experiments of the ABTS titrated into phosphatic buffer were subtracted from the heats obtained. Experiments

were performed in triplicate and fitted with TA NanoAnalyze software using an independent site model.

The application of HRP@PDA@MHCo_3O_4 & Co/C.

The degradation of 2,4-dichlorophenol was carried out in simulated wastewater with total volume of 3 mL including enzymatic reactors containing HRP (2.0 μmol·L^{-1}) and 2,4-dichlorophenol in the concentration range of 1-10 mmol·L^{-1}. The reaction was started by adding H_2O_2 (0.3 mmol·L^{-1}). After 15 min, the supernatant of the reaction mixture was extracted three times using ethyl acetate in interval 10 min. The combined organic extract was concentrated by rotary evaporation (0.09 Mpa, 35 ℃) to remove the extractant, and then dissolved in methanol and treated by 0.22 μm organic phase filtration membrane, then measured concentration of 2,4-dichlorophenol by HPLC (LC-20AT, Shimadzu). The mobile phase consisted of a mixture of methanol and water (60:40, v/v, flow-rate gradient elution), at a flow rate of 1.0 mL·min^{-1}. The detector was set at 284 nm, and the injection volume was 20 μL. All experiments were triplicated and data reported were mean values of three independent measurements.

Supplementary Figures

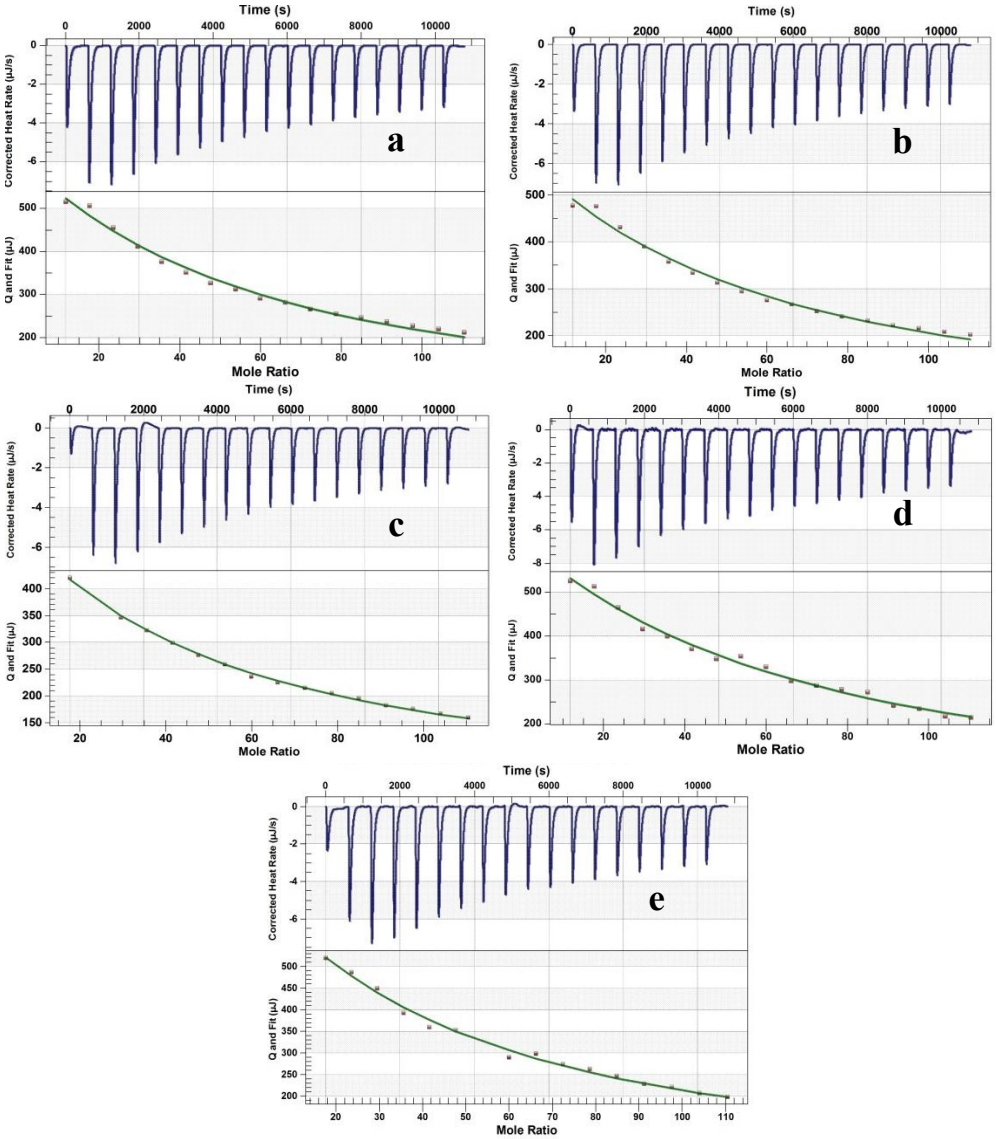


Fig. S1 Thermogram of raw heat and enthalpy with independent model fit for the isothermal titration of 10 mmol·L^{-1} ABTS into 0.01 mmol·L^{-1} enzyme: (a) HRP@PDA@MHCo_3O_4-(500 ℃); (b) HRP@PDA@MHCo_3O_4-(600 ℃); (c) HRP@PDA@Co/C-(500 ℃); (d) HRP@PDA@Co/C-(600 ℃); (e) HRP@PDA@Co/C-(800 ℃).

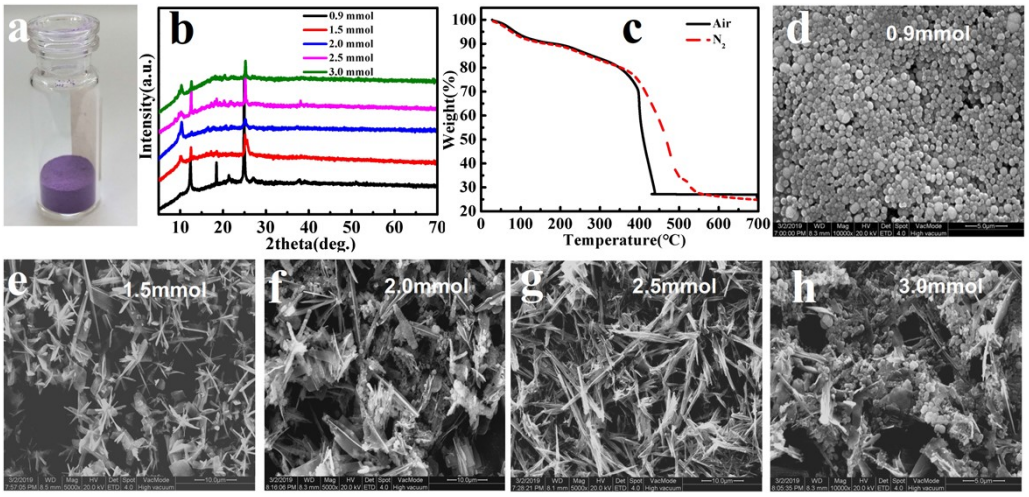
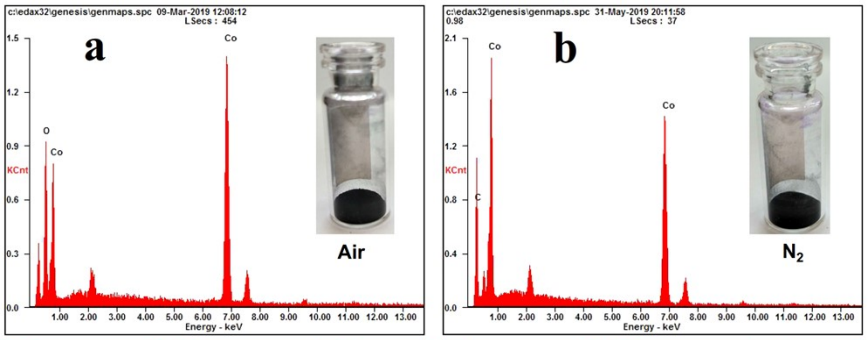


Fig. S2 Digital photo (a), XRD patterns (b), TGA curves (c), SEM images (d, e, f, g, h) of Co-MOF.



Element	*Wt%*	*At%*
OK	18.77	45.99
CoK	81.23	54.01
Matrix	Correction	ZAF

Element	*Wt%*	*At%*
CK	46.33	80.90
CoK	53.67	19.10
Matrix	Correction	ZAF

Fig. S3 Digital photoes and EDX spectra of $MHCo_3O_4$ (a) and Co/C (b).

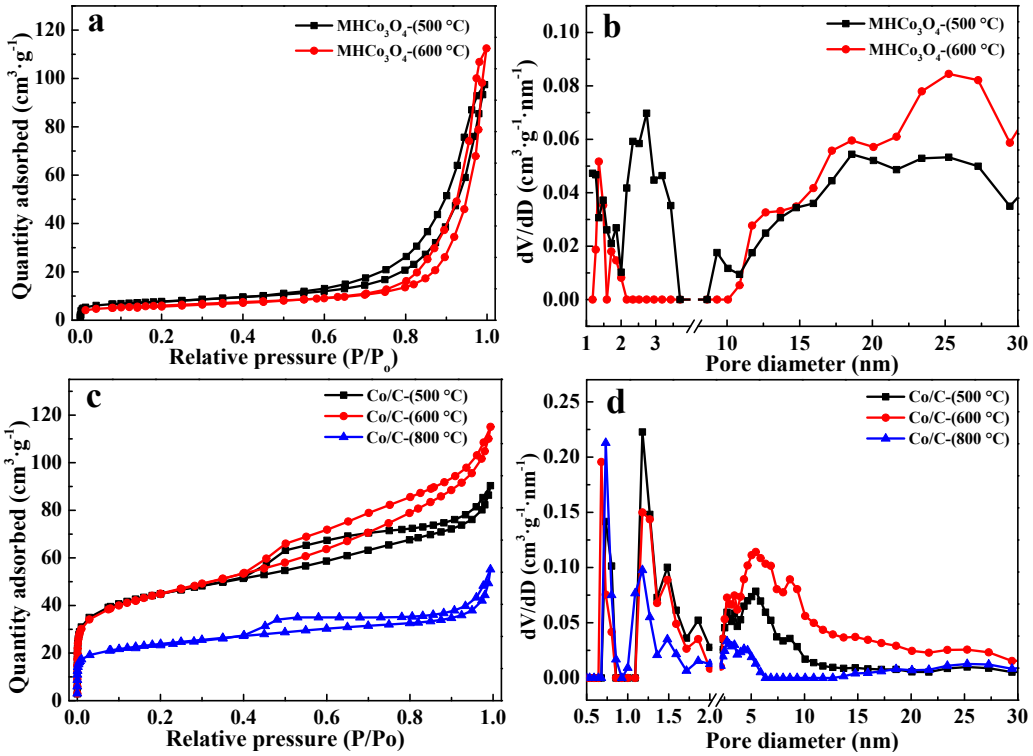


Fig. S4 Nitrogen adsorption-desorption isotherms (77K) (a) and pore size distribution curves (b) of $MHCo_3O_4$ calcinated at 500 ℃ and 600 ℃; Nitrogen adsorption-desorption isotherms (77K) (c) and pore size distribution curves (d) of Co/C calcinated at 500 ℃, 600 ℃ and 800 ℃.

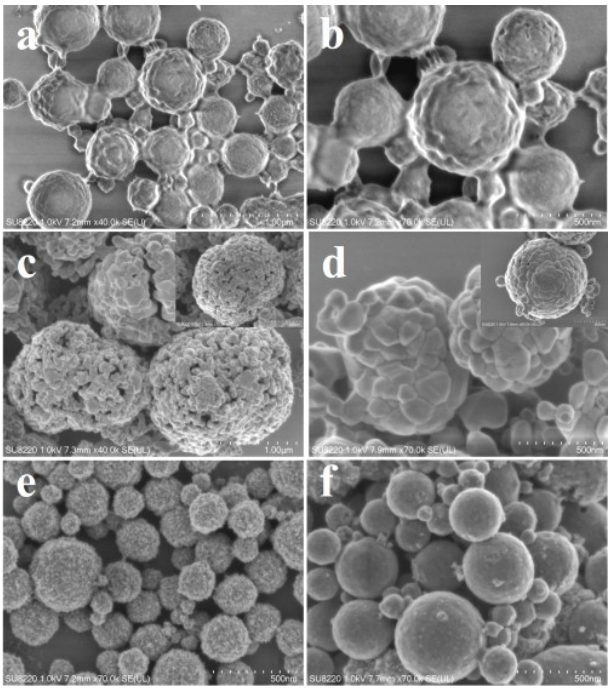


Fig. S5 FESEM images of Co-MOF (a,b), $MHCo_3O_4$ (c), HRP@PDA@$MHCo_3O_4$ (d), Co/C (e) and HRP@PDA@Co/C (f).

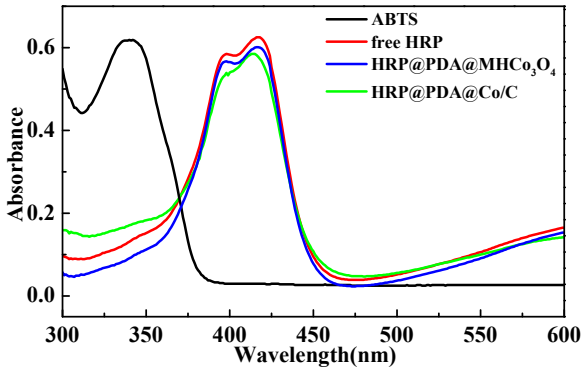


Fig. S6 UV-Vis absorbance spectra of peroxidation of ABTS catalyzed by free HRP and HRP@PDA@$MHCo_3O_4$ & Co/C.

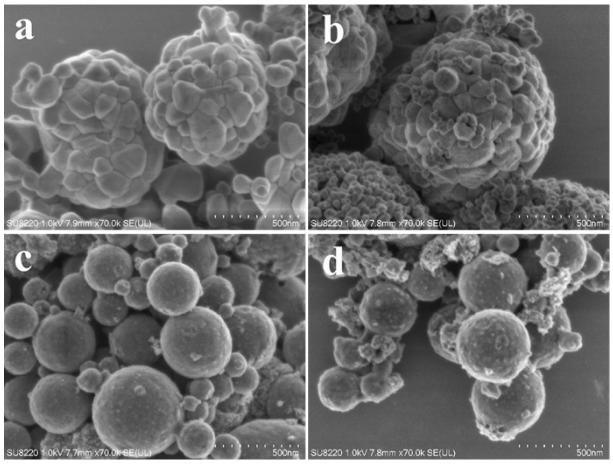


Fig. S7 FETEM images of enzymatic reactors: (a) newly prepared HRP@PDA@ $MHCo_3O_4$; (b) HRP@PDA@$MHCo_3O_4$ after repeated use for 30 times; (c) newly prepared HRP@PDA@Co/C; (d) HRP@PDA@Co/C after repeated use for 30 times.

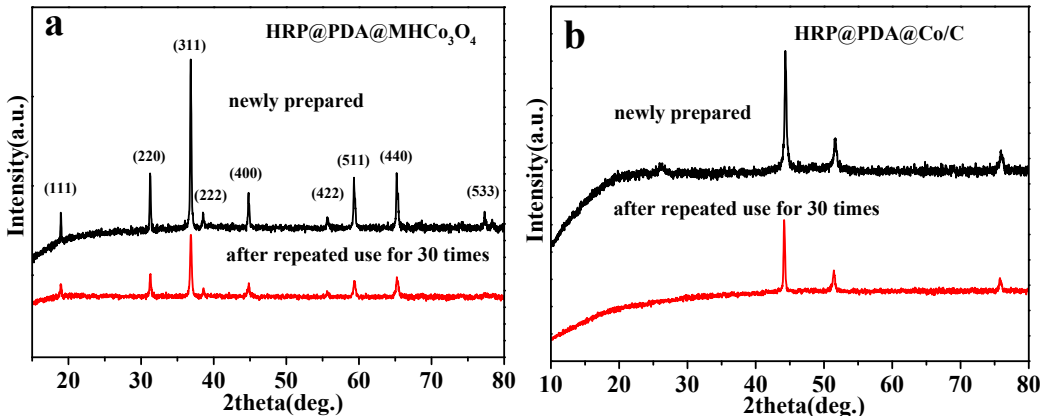


Fig. S8 XRD patterns of enzymatic reactors before and after repeated use: (a) HRP@PDA@$MHCo_3O_4$; (b) HRP@PDA@Co/C.

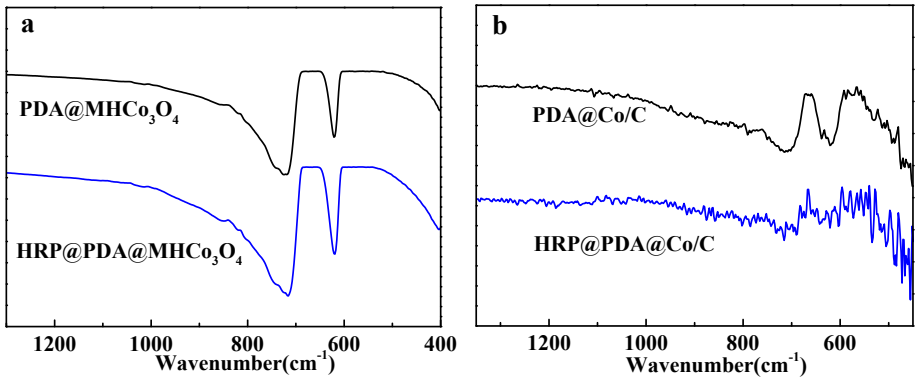


Fig. S9 (a) IR spectra of PDA@$MHCo_3O_4$ and HRP@PDA@$MHCo_3O_4$; (b) IR spectra of PDA@Co/C and HRP@PDA@Co/C

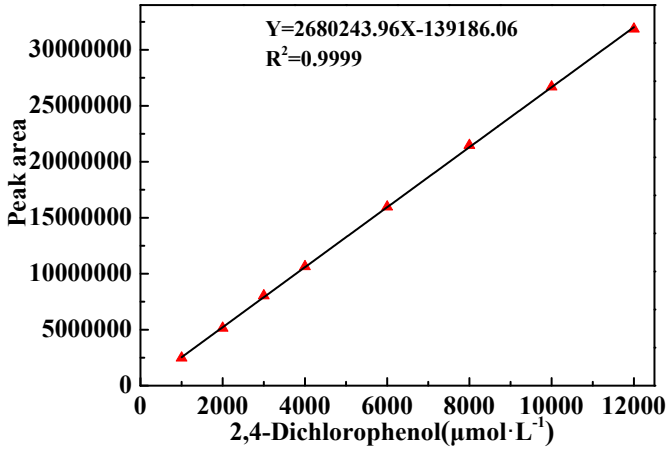


Fig. S10 The standard curve in determination of 2,4-dichlorophenol.

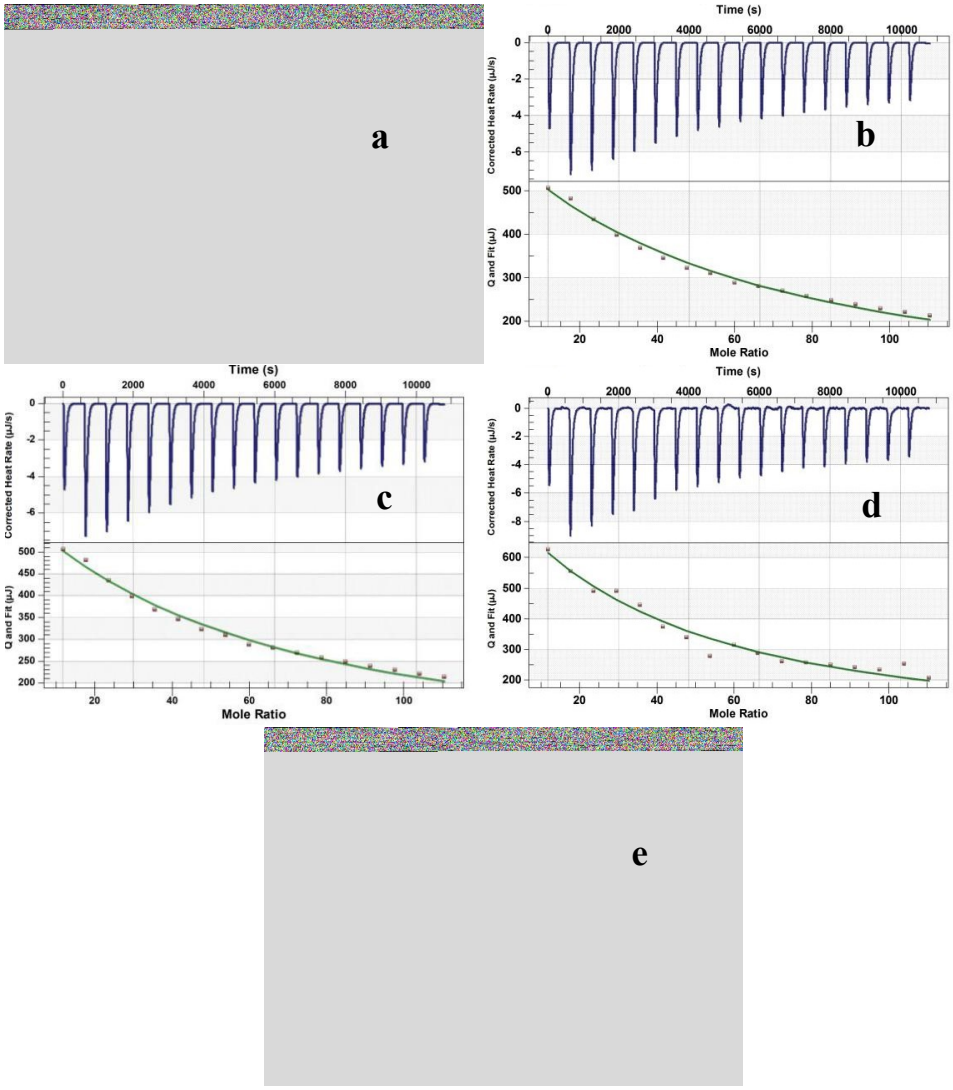


Fig. S11 Thermogram of raw heat and enthalpy with independent model fit for the isothermal titration of 10 mmol·L^{-1} ABTS into 0.01 mmol·L^{-1} enzyme: (a) HRP; (b) HRP@$MHCo_3O_4$; (c) HRP@PDA@$MHCo_3O_4$; (d) HRP@Co/C; (e) HRP@PDA@Co/C.

Supplementary Table

Table S1 Thermodynamic binding parameters for enzymatic reactors.

Enzyme	$K_a \times 10^{-3}$ (M^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	n	ΔG (kJ mol^{-1})
HRP@PDA@$MHCo_3O_4$-(500 ℃)	1.24±0.01	97.8±0.01	387.3±0.1	6.23±0.1	-17.66±0.06
HRP@PDA@$MHCo_3O_4$-(600 ℃)	1.00±0.01	54.91±0.01	241.6±0.1	2.43±0.1	-17.13±0.06
HRP@PDA@Co/C-(500 ℃)	0.96±0.02	231.7±0.02	834.3±0.2	4.08±0.1	-17.05±0.05
HRP@PDA@Co/C-(600 ℃)	1.01±0.02	37.3±0.02	182.5±0.2	5.79±0.1	-17.14±0.05
HRP@PDA@Co/C-(800 ℃)	0.88±0.02	893.5±0.02	3054.7±0.2	5.43±0.1	-16.79±0.06