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Supporting information

Hierarchically Porous and Hydrophilic Metal-Organic Frameworks with Enhanced Enzyme Activity

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Experimental section

1. Materials

2-Methylimidazole (HmIM), lithocholic acid (LCA), protease and catalase were purchased from Aladdin (Shanghai, China). Phospholipase B (PLB) was prepared by our laboratory and stored in the pH7.0 Tris-HCl buffer at 4 ℃. $ZnSO_4$ was purchased from Xilong chemical company (Shantou, China). *Sn*-Glycero-3-phosphocholine (α-GPC) and phosphatidylcholine (PC) were purchased from Sigma−Aldrich (Saint Louis, MO), Egg yolk lecithin, isooctane, n-hexane, n-heptane, dichloromethane, and butyl alcohol were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). All chemicals used in this work were of analytical grade and used without further purification.

2. Preparation of Phospholipase B (PLB)

A gene of PLB from in *Pseudomonas fluorescens* was cloned into the *Nco*I/*Xho*I restriction sites of the pET28a vector to generate the expression plasmid pET28a-*PLB*. The sequence of the resulting expression plasmid was verified by DNA sequencing (Springen Biotech, Nanjing, China). Chemically competent *E. coli* BL 21 (DE3) cells were transformed with pET28a-*PLB* and protein expression was performed by standard protocols. Cells were grown in lysogeny broth (LB) supplemented with kanamycin (10 μg/mL) at 37 °C to an OD_{600} of 0.6, and protein expression was induced by adding isopropyl-β-d-thiogalactopyranoside (IPTG) with a final concentration of 0.5 mM. The cultures were incubated at 28 °C and 200 rpm for an additional 10 h, and cells were harvested by centrifugation. Cell pellets were washed

with a NaCl solution (0.9%, w/v) and frozen at -20 °C for later purification.

The cell pellets were thawed on ice and resuspended in lysis buffer (20 mM Tris-HCl buffer containing 50 mM imidazole, pH 7.0). Then the cells were disrupted by sonication (SCIENTZ-IID Ultrasonic cell crusher) with cooling on ice. Cell debris was removed by centrifugation at 10000 g for 30 min at 4 °C. The supernatant was filtered through a 0.25 μm PVDF filter and was loaded onto a Ni-NTA column (HitrapQFF) equilibrated with lysis buffer. After loading of the filtered lysate, the column was washed with six column volumes of wash buffer (20 mM Tris-HCl buffer containing 50 mM imidazole, pH 7.0). Finally, The eluent was ultrafiltrated by a Millipore ultrafiltration membrane to remove imidazole from the wash buffer, and fractions containing target enzyme were pooled at 4 °C in Tris-HCl buffer (pH 7.0).

3. Synthesis of ZIF-8

In a typical experiment, 20 mL of HmIM aqueous solution (1.25 M) and 2 mL of zinc sulfate aqueous solution (0.31 M) were mixed and stirred for 30 min at room temperature, followed by 3 cycles of centrifugation at 8000 rpm for 5 min, washing, and then vacuum drying.

4. Synthesis of PLB/ZIF-8

In a typical experiment, 20 mL of HmIM aqueous solution (1.25 M), 1 mL of PLB aqueous solution (0.8 mg mL^{-1}) and 2 mL of zinc sulfate aqueous solution (0.31 M) were mixed and stirred for 30 min at room temperature, followed by 3 cycles of centrifugation at 8000 rpm for 5 min, washing, and then vacuum drying.

5. Synthesis of LCA@ZIF-8

In a typical experiment, 20 mL of HmIM aqueous solution (1.25 M), 2 mL of zinc sulfate aqueous solution (0.31 M) and l mL of LCA aqueous solution (1 mM) were mixed and stirred for 30 min at room temperature, followed by 3 cycles of centrifugation at 8000 rpm for 5 min, washing, and then vacuum drying.

6. Synthesis of LCA@PLB/ZIF-8

In a typical experiment, 20 mL of HmIM aqueous solution (1.25 M), l mL of PLB aqueous solution (0.8 mg mL^{-1}), 2 mL of zinc sulfate aqueous solution (0.31 M) and l mL of LCA aqueous solution (1 mM) were mixed and stirred for 30 min at room temperature, followed by 3 cycles of centrifugation at 8000 rpm for 5 min, washing, and then vacuum drying.

7. Assay of PLB activity

PLB activity was determined by measuring the amount of free fatty acids released by the 0.05 M NaOH titration method during the hydrolysis of 10 g L^{-1} (w/v) egg yolk lecithin in 0.2 M phosphate buffer solution (PBS, pH 5.0) at 30 ℃ for 30 min at 1000 rpm for maintaining the emulsion of the reaction substrate. One unit of PLB activity was defined as the amount of enzyme that released 1 μmol of free fatty acid per min under the assay conditions.

8. Protease treatment of PLB/ZIF-8 and LCA@PLB/ZIF-8

20 mg of samples was incubated in 0.5 mL of 50 mM Tris buffer (pH 6.0) for 30 min and subsequently added to 100 μL of 50 mM Tris buffer (pH 6.0) with 2 mg protease for 1 h, followed by 3 cycles of centrifugation at 8000 rpm for 5 min, washing, and subsequent re-testing of the catalytic activity.

9. Characterization

Scanning electron microscopy (SEM) was performed using a Hitachi s-3400 at 3.0 kV accelerating voltage (Japan). Transmission electron microscopy (TEM) was carried out using a JEM-200CX analytical transmission electron microscope (Japan). Elemental mapping was carried out using an energy dispersive X-ray spectroscopy (EDS) with TEM. Surface area, pore volume and pore diameter and adsorption-desorption isotherm were performed using a Micromeritics 3Flex surface analyzer (USA). The chemical functional groups were analyzed by a Thermo Corporation Nexus FTIR spectrophotometer (USA). Thermal gravity analysis (TGA) was performed on a Q500 thermogravimetric analyzer (TA, USA). The sample was heated from 25 °C to 800 °C at a rate of 10 °C/min under air atmosphere. X-ray diffraction (XRD) was performed on a Rigaku Ultima IV diffractometer with Cu Kα X-rays. X-ray photoelectron spectroscopy (XPS) was performed using a Kratos AXIS Ultra DLD XPS system equipped with a hemispherical energy analyzer and a monochromatic Al Kα source. The source was operated at 15 keV and 150 W; pass energy was fixed at 40 eV for the high-resolution scans. All samples were prepared as pressed powders supported on a metal bar for the XPS measurements. Water contact angle was carried out using a LSA100 optical contact angle tension measuring instrument (Germany). Size distribution of LCA@PLB/ZIF-8 was performed on a Zetasizer Nano ZS90 laser particle size analyzer (UK).

10. Protein adsorption experiment with different size

20 mg of samples was incubated in 0.5 mL of 50 mM Tris buffer (pH 6.0) for 30

min and subsequently added to 100 μL of 50 mM Tris buffer (pH 6.0) with 4 mg protease (size, around 3 nm) or catalase (size, around 8 nm) for 1 h, followed by 3 cycles of centrifugation at 8000 rpm for 5 min, vigorous washing, and subsequent testing of the adsorption capacity by Bradford method.

11. Measurement of the content of LCA, PLB, Zn and HmIM in the composites

20 mg of samples was dissolved in disodium ethylenediamine tetraacetate (EDTA) solution (0.1 mol L^{-1}, pH 7) for 1 h. Then, the content of LCA in samples was analyzed by HPLC-ELSD. Briefly, HPLC was performed on an Agilent HC-C18 column (5 μm×4.6 mm×250 mm, Agilent). Mobile phase contained 75% methanol and 25% acetic acid solution (1%). The flow rate was 1.0 mL min^{-1}. The column temperature, nebulizing temperature, and evaporating temperature were controlled at 25 ℃, 70 ℃, and 70 ℃, respectively, and nitrogen was used as the nebulizing gas. The nitrogen gas flow rate was 2.0 SLM. The LCA was determined from the retention time using calibration solutions of LCA, and the concentrations of the LCA in the samples were calculated from the peak areas by integration. The content of PLB in samples was analyzed by Bradford method. Zn content of samples was analyzed by using the inductively coupled plasma mass spectrometry (ICP-MS) from Aglient 7500a (USA). The content of HmIM of samples was calculated by mass subtraction. $M_{HmIM}= M_{Total}-M_{LCA}-M_{PLB}-M_{Zn}$. M_{HmIM} is the content of HmIM in samples, M_{Total} is the total content of samples, M_{LCA} is the content of LCA in samples, M_{PLB} is the content of PLB in samples, M_{Zn} is the content of Zn in samples.

12. Effect of temperature and pH on enzyme activity

6 mg of free PLB, PLB/ZIF-8 or LCA@PLB/ZIF-8 (equivalent quality of enzyme) were incubated in 10 mL of 0.2 M PBS buffer solution at a range of temperature (20—60°C) or pH (5.0—8.0) with 10 g L^{-1} (w/v) egg yolk lecithin for 30 min. Then, 5 mL of anhydrous alcohol was added to end the reaction. Then, the amount of free fatty acids released was determined by the 0.05 M NaOH titration method. Relative activity of free PLB, PLB/ZIF-8, or LCA@PLB/ZIF-8 was determined.

13. Thermo and pH stability

6 mg of free PLB, PLB/ZIF-8 or LCA@PLB/ZIF-8 (equivalent quality of enzyme) were incubated in 5 mL of 0.2 M PBS buffer solution at a range of temperature (30, 40, and 50 °C) or pH (6.0, 7.0, and 8.0), and then 5 mL of 10 g L^{-1} (w/v) egg yolk lecithin PBS buffer solution were added to the tube and incubated at 40°C for 30 min. Then, 5 mL of anhydrous alcohol was added to end the reaction. Then, the amount of free fatty acids released was determined by the 0.05 M NaOH titration method. Relative activity of free PLB, PLB/ZIF-8, or LCA@PLB/ZIF-8 was determined.

14. Organic solvent tolerance

6 mg of free PLB, PLB/ZIF-8 or LCA@PLB/ZIF-8 (equivalent quality of enzyme) were incubated in 5 mL of 0.2 M PBS buffer solution with 50% organic solvent (isooctane, n-hexane, n-heptane, dichloromethane, and butyl alcohol) in the described assay of PLB activity. Relative activity of free PLB, PLB/ZIF-8, or LCA@PLB/ZIF-8 was determined.

15. Kinetic and thermodynamics

The hydrolysis of egg yolk lecithin to releasing free fatty acids was used as a model reaction. The maximum reaction rate (V_{max}) and the Michaelis–Menten constant (K_m) of free PLB, PLB/ZIF-8, or LCA@PLB/ZIF-8 were determined according to intercept of the fitting line of 1/V ($\mu mol \cdot L^{-1}$ min^{-1}) versus 1/[S] ($mmol \cdot L^{-1}$). The activation energy (E_a) could be measured according to the method in the Arrhenius function which was plotted as lg (V_0) versus 1/T (K) and the E_a was counted from the slope of the fitting line.

16. Storage stability

Free PLB, PLB/ZIF-8 and LCA@PLB/ZIF-8 were stored at 4 ℃ for 30 consecutive days. Every 3 days, samples of the free PLB, PLB/ZIF-8 and LCA@PLB/ZIF-8 were incubated in the described assay of PLB activity. Relative activity of free PLB, PLB/ZIF-8, or LCA@PLB/ZIF-8 was determined.

17. Reusability

PLB/ZIF-8 and LCA@PLB/ZIF-8 was incubated in the described assay of PLB activity. PLB/ZIF-8 and LCA@PLB/ZIF-8 were recovered through centrifugation and washed and dried. The recovered PLB/ZIF-8 and LCA@PLB/ZIF-8 were used for the next batch. This procedure was repeated ten times to determine the reusability of PLB/ZIF-8 and LCA@PLB/ZIF-8.

18. PLB-catalyzed synthesis of α-GPC

6 mg of free PLB, PLB/ZIF-8 or LCA@PLB/ZIF-8 (equivalent quality of enzyme) were incubated in 5 mL of 0.2 M PBS buffer solution at 40°C and pH 6.0

with PC (40, 60 and 80 g L^{-1}) for 8 h at 1000 rpm for maintaining the emulsion of the reaction substrate. Samples were taken at regular intervals and detected by HPLC. Samples were analyzed using an Agilent 1260 HPLC (USA) equipped with a Chromachem evaporative light scattering detector (ELSD). The samples from PLB-catalyzed synthesis of α-GPC were diluted by methanol. HPLC was performed on a ZORBAX Rx-SIL silica gel column (5 μm×4.6 mm×250 mm, Agilent). Mobile phase contained 90% methanol and 10% water. The flow rate was 1.0 mL min^{-1}. The column temperature, nebulizing temperature, and evaporating temperature were controlled at 25 ℃, 65 ℃, and 65 ℃, respectively, and nitrogen was used as the nebulizing gas. The nitrogen gas flow rate was 1.5 SLM. The α-GPC was determined from the retention time using calibration solutions of α-GPC, and the concentrations of the α-GPC in the samples were calculated from the peak areas by integration.

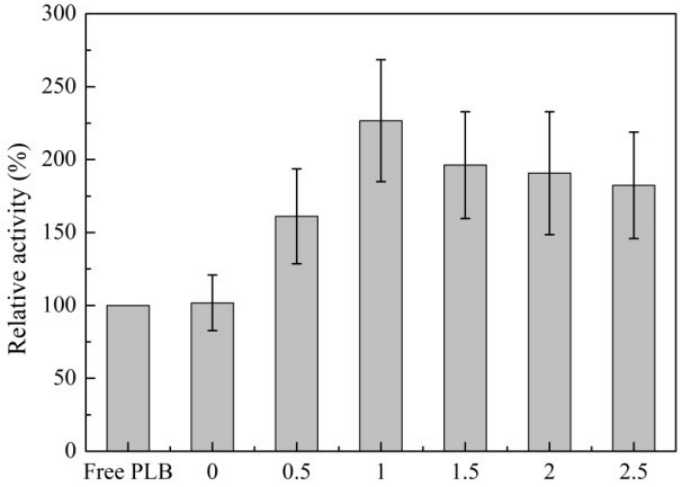


Fig. S1. Relative activity of LCA@PLB/ZIF-8 at different concentration of LCA

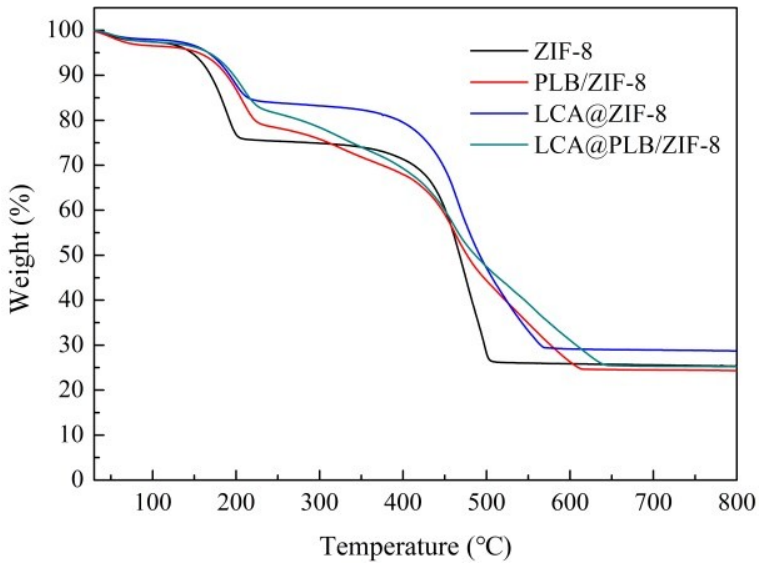


Fig. S2. Thermal gravity analysis (TGA) curves of ZIF-8, PLB/ZIF-8, LCA@ZIF-8, and LCA@PLB/ZIF-8 in an air atmosphere

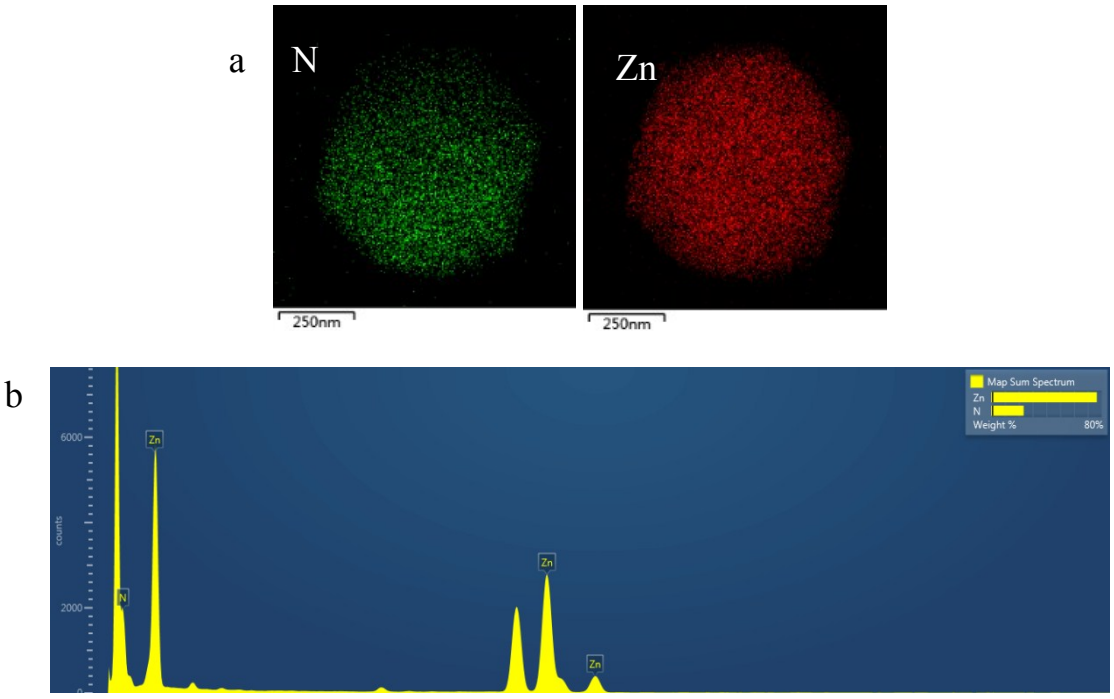


Fig. S3. a) The EDS mapping images of N and Zn and b) EDS mapping of ZIF-8

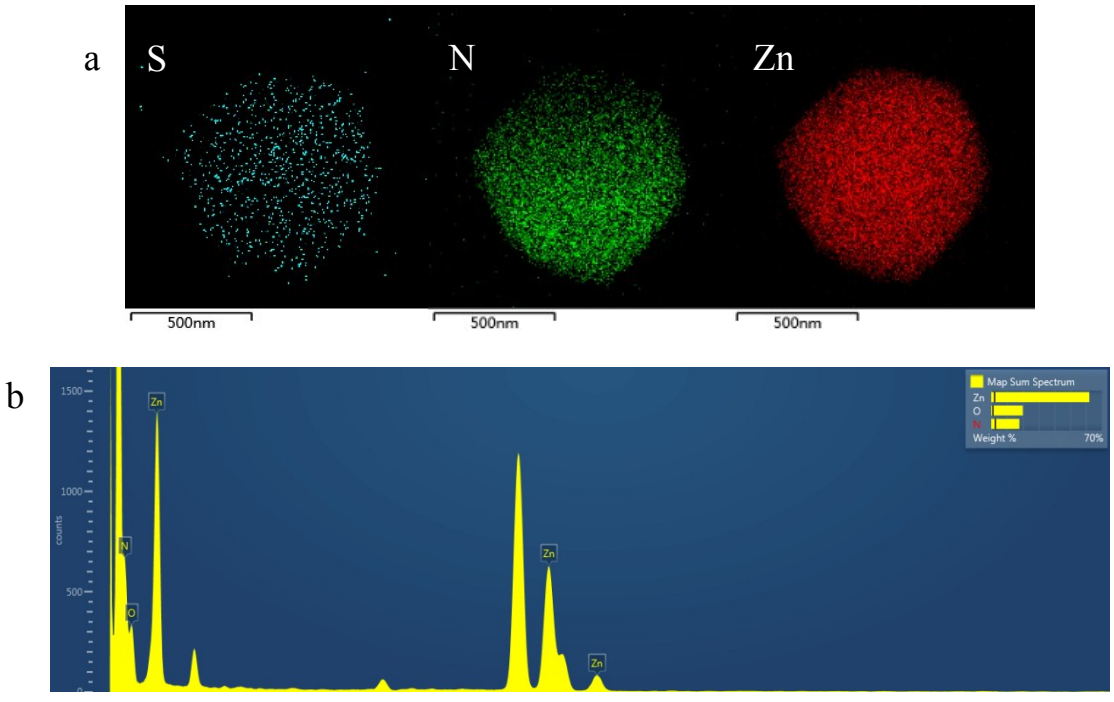


Fig. S4. a) The EDS mapping images of S、N and Zn and b) EDS mapping of PLB/ZIF-8

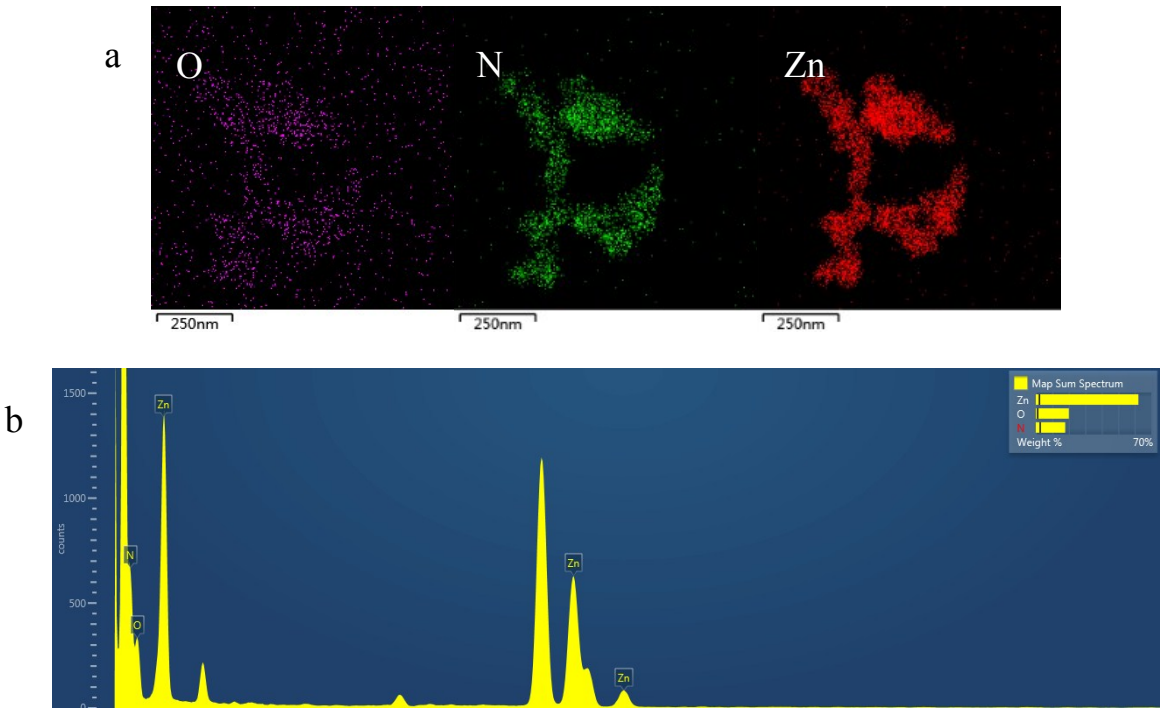


Fig. S5. a) The EDS mapping images of O、N and Zn and b) EDS mapping of LCA@ZIF-8

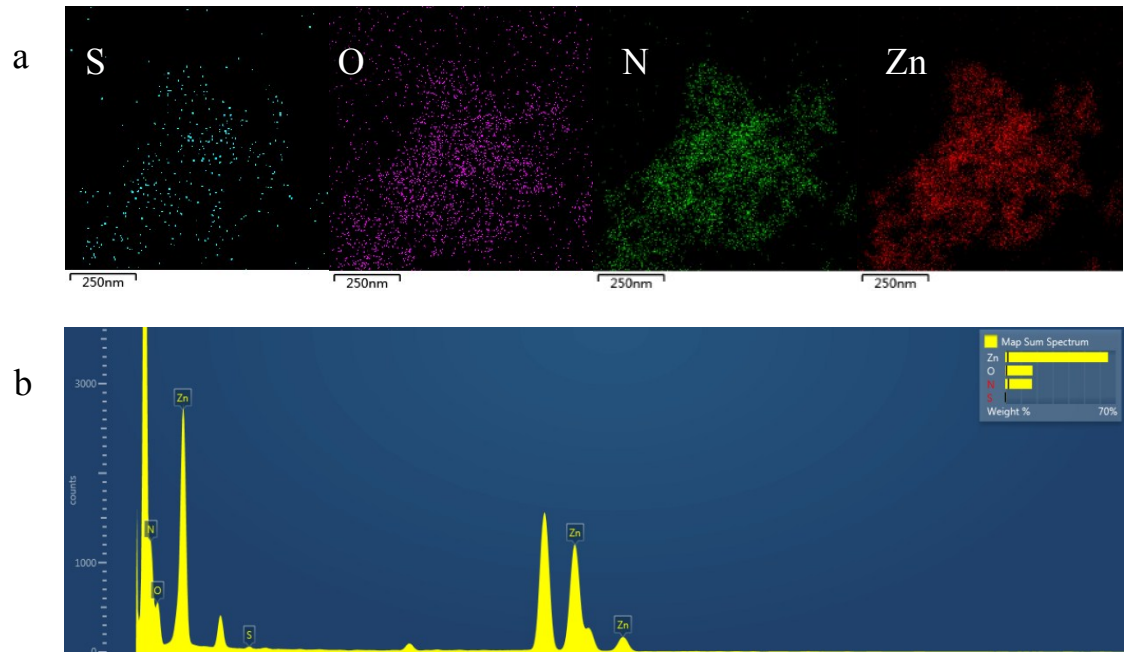


Fig. S6. a) The EDS mapping images of S、O、N and Zn and b) EDS mapping of LCA@PLB/ZIF-8

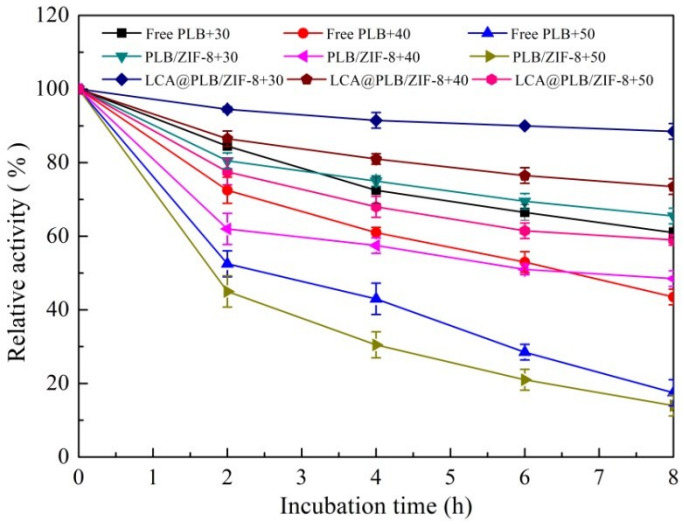


Fig. S7. Thermal stability for free PLB, PLB/ZIF-8, and LCA@PLB/ZIF-8 at 30, 40, and 50℃.

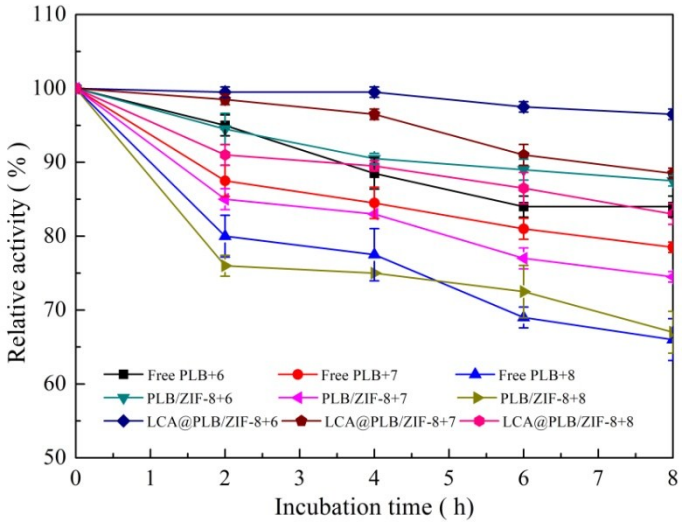


Fig. S8. pH stability for free PLB, PLB/ZIF-8, and LCA@PLB/ZIF-8 at pH 6.0, 7.0 and 8.0.

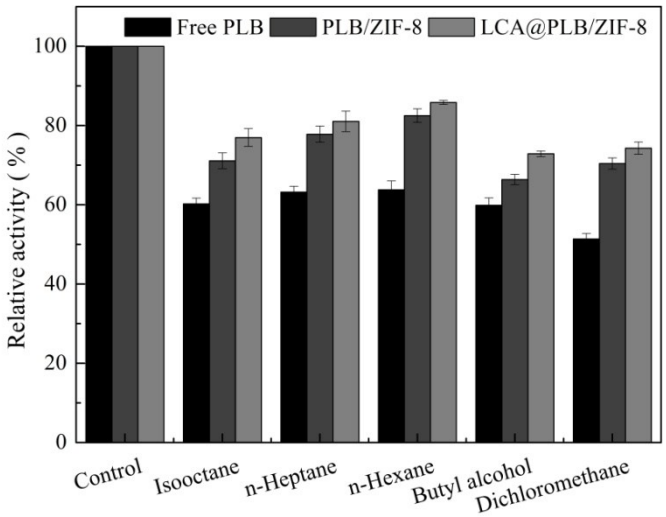
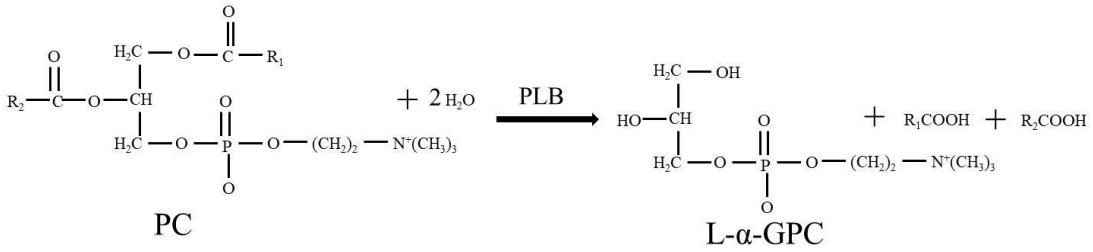


Fig. S9. Organic solvent tolerance for free PLB, PLB/ZIF-8, and LCA@PLB/ZIF-8.



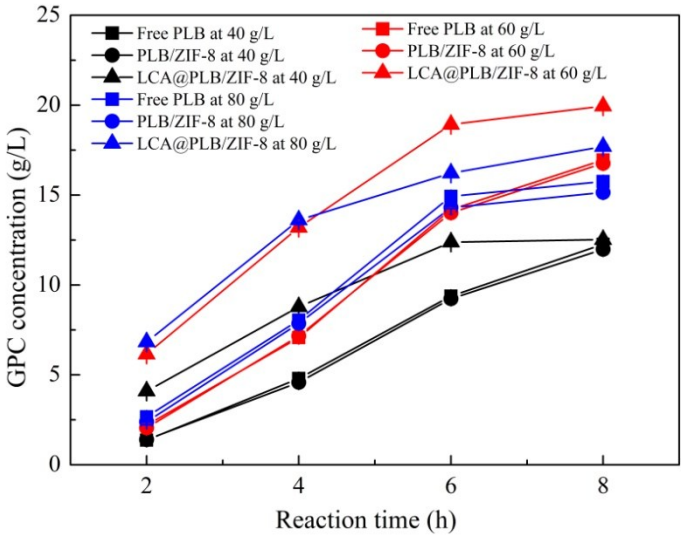


Fig. S10. L-α-GPC yield with different PC concentrations at free PLB, PLB/ZIF-8, and LCA@PLB/ZIF-8.

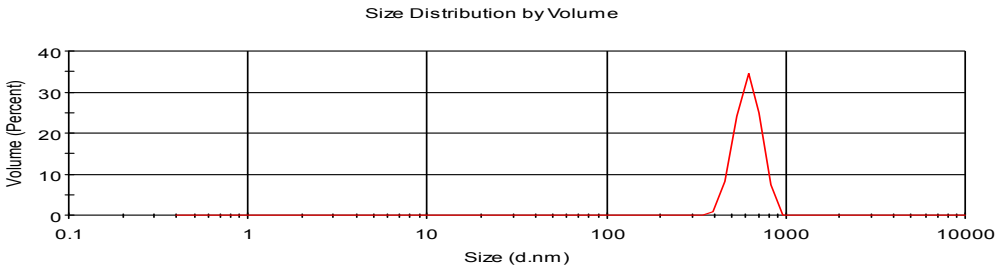


Fig. S11. Size distribution of LCA@PLB/ZIF-8 in the reaction medium (0.2 M PBS buffer solution, pH 6.0).

Table S1. Specific surface area, pore volume, and average pore diameter of ZIF-8, PLB/ZIF-8, LCA@ZIF-8, and LCA@PLB/ZIF-8.

Sample	S_{micro}[a] [m^2/g]	S_{BET}[b] [m^2/g]	V_{micro}[c] [cm^3/g]	V_{total}[d] [cm^3/g]	D_{micro}[e] [nm]	D_{total}[f] [nm]
ZIF-8	1559.8	1722.4	0.750	0.817	1.432	1.898
PLB/ZIF-8	1277.1	1397.1	0.916	1.123	1.432	3.214
LCA@ZIF-8	1800.9	1892.5	1.246	1.753	1.220	3.706
LCA@PLB/ZIF-8	1250.1	1289.4	1.418	1.637	1.432	5.078

[a] S_{micro}: micropore surface area calculated with density functional theory (DFT).

[b] S_{BET}: Brunauer–Emmett–Teller (BET) surface area.

[c] V_{micro}: micropore volume calculated with density functional theory (DFT).

[d] V_{total}: total pore volume determined by using the adsorption branch of the N_2 isotherm at P/P_o=0.99.

[e] D_{micro}: micropore volume calculated with density functional theory (DFT).

[f] D_{total}: average pore diameter calculated from the adsorption branch of the N_2 isotherm.

Table S2. Protein adsorption capacity with different size of ZIF-8, PLB/ZIF-8, LCA@ZIF-8, and LCA@PLB/ZIF-8.

Sample	Protein adsorption capacity [mg/g]	
	Protease (size, around 3 nm)	Catalase (size, around 8 nm)
ZIF-8	---	---
PLB/ZIF-8	0.01	---
LCA@ZIF-8	3.6	2.8
LCA@PLB/ZIF-8	2.1	1.7

Table S3. Kinetic parameters, and apparent activation energies for free PLB, PLB/ZIF-8 and LCA@PLB/ZIF-8.

Sample	V_{max} [μM/min]	K_{cat} [1/s]	K_m [μM]	E_a [kJ/mol]
Free PLB	181.16	32.71	111.91	5.709
PLB/ZIF-8	176.68	31.90	124.43	5.246
LCA@PLB/ZIF-8	268.10	48.41	74.89	4.915