Supporting information for

Concanavalin A Coated Activated Carbon for High Performance Enzymatic Catalysis

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9 pages, 10 figures and 2 tables

Materials and Methods

Materials

Peroxidase from horseradish (product number: V900503), Laccase from Trametes versicolor (product number: 38429), Concanavalin A from Canavalia ensiformis, Lipase from Candida Rugosa, bovine serum albumin, 20-40 mesh AC, 100 mesh AC, 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS), 4-nitrophenyl butyrate (p-NPB), potassium ferricyanide(III), 4-aminoantipyrine, fluorescein isothiocyanate and rhodamine B isothiocyanate were purchased from Sigma-Aldrich. 8~16 mesh AC were purchased from Aladdin. Hydrogen peroxide (H_2O_2) was purchased from Alfa Aesar. Phenol was purchased from Sinopharm Chemical Reagent Co., Ltd. Other chemicals were all of analytical grade. The enzymes were purified before use while other chemicals were applied without pretreatment.

Methods

Laser confocal microscopy was conducted on a Zeiss LSM-710 3-channel system with Plan-Apochromat 40×/0.95 Korr M27 objectives. The excitation wavelengths for ConA/FITC and HRP/RhB (or laccase/RhB) were 488 nm and 590 nm, respectively.

SEM was conducted on a JEOL JSM 7401F field emission SEM with an accelerating voltage of 3.0 kV.

DLS was performed on a Malvin Zetasizer ZS-90.

FTIR were performed on a Nicolet iS10 FT-IR Spectrometer.

UV analysis were performed on a Shimadzu UV-2450 UV-VIS Spectrophotometer.

High Performance Liquid Chromatography (HPLC) of ConA, free enzymes and supernatant after enzyme adsorption on ConA coated activated carbon was performed on TSK-GEL G2000SWXL column (5 μm, 7.8×300 mm) with a SHIMADSU SPD-10AVP UV-Vis detector at volume flowrate of 1ml/min.

Contact angle was measured on a Dataphysics OCAH200 instrument and the samples were prepared by tabletting AC under 10 MPa for 5 min.

The surface area and pore size distribution were measured on a Quantachrome Quadrasorb S1 analyzer.

Enzyme activity assays

For immobilized laccase, ABTS was used as the substrate. The enzymatic activity was determined by adding 10 mg immobilized laccase to 10 mL 2.5 mM ABTS in acetate buffer solution (0.1 M, pH 4). The increased absorbance of supernatant at 415 nm was measured after shaking for 1 min. For free laccase, the enzymatic activity was determined by adding 20 μL 2 mg mL^{-1} laccase to 980 μL ABTS in acetate buffer solution (0.1 M, pH 4). The increased absorbance at 415 nm was measured for 1 min.

For immobilized HRP, ABTS and H_2O_2 were used as the substrates. The enzymatic activity was determined by adding 10 mg immobilized HRP and 0.5 mL 0.3 % H_2O_2 to 9.5 mL 0.5 mM ABTS in PBS solution (50 mM, PH 7). The increased absorbance of supernate at 415 nm was measured after shaking for 1 min. For free HRP, the enzymatic activity was determined by adding 50 μL 5 mg mL^{-1} HRP and 50 μL 0.3 % H_2O_2 to 900 μL ABTS in PBS solution (50 mM, pH 7). The increased absorbance at 415 nm was measured for 1 min.

For immobilized CRL, p-NPB was used as the substrate. The enzymatic activity was determined by adding 10 mg immobilized CRL to 1 ml 0.5 mM p-NPB in PBS solution (50 mM, PH 7) containing 1.25 % (w/v) Triton X-100. The increased absorbance of supernate at 348 nm was measured after shaking for 2 min.

Conversion of 1 μmol substrate per minute in the mentioned condition was defined as one enzyme unit activity ($U \cdot mg^{-1}$).

Synthesis of FITC-labelled ConA and Rhodamine B-labelled enzymes

During a run, 0.4 mL 5 mg mL^{-1} FITC in DMSO solution was added into 4 mL 5mg mL^{-1} HRP in Na_2CO_3-$NaHCO_3$ buffer (50 mM, pH 9), and then stirred for 12 h at 4 ^oC. After stopping the reaction with 50 mM NH4Cl, the mixture was dialyzed in PBS (50 mM, pH 7) for 48 h at 4 ^oC to remove the unreacted dye. HRP and laccase were labelled by RhB with the same method.

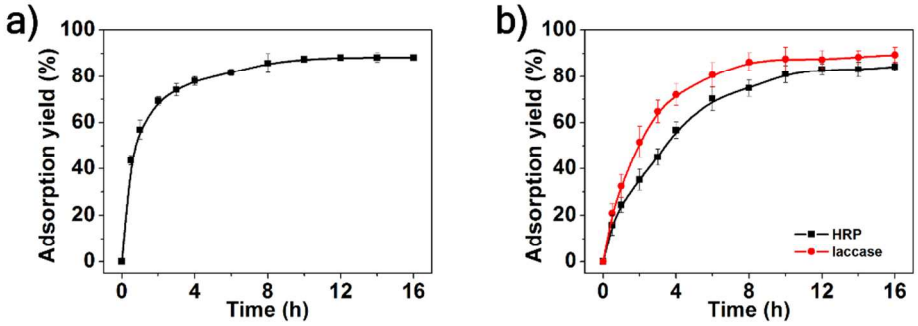


Figure S1. Adsorption kinetic curves of (a) ConA and (b) laccase and HRP to AC

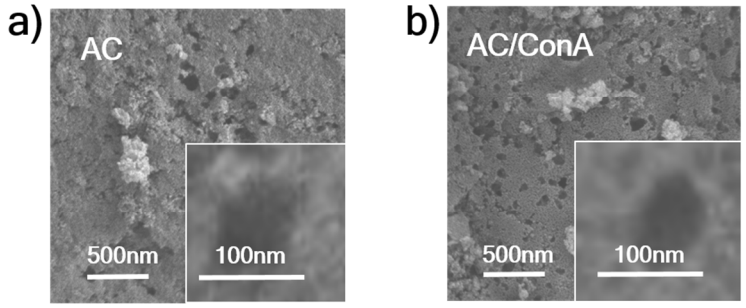


Figure S2. SEM images of (a) AC and (b) AC/ConA (inset: high-resolution SEM)

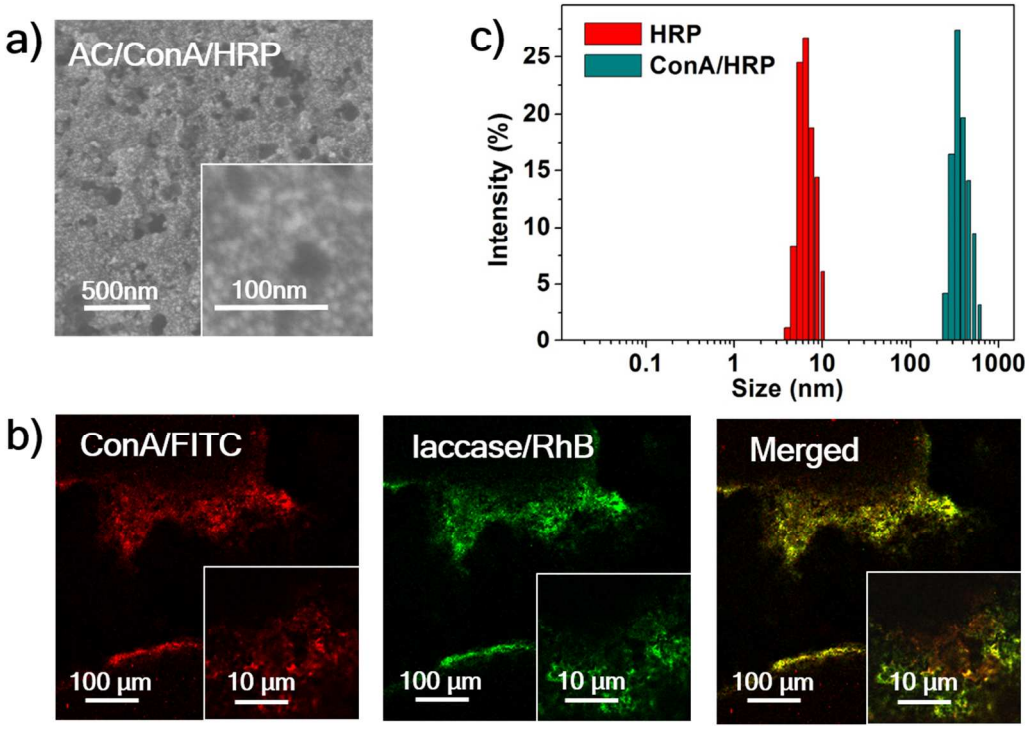


Figure S3. Structure characterizations of HRP immobilized on AC coated with ConA. (a) SEM (inset: high-resolution SEM); (b) laser confocal microscopy, in which HRP were labelled with RhB (red) and ConA was labelled with FITC (green), respectively; (c) DLS of HRP and ConA/HRP.

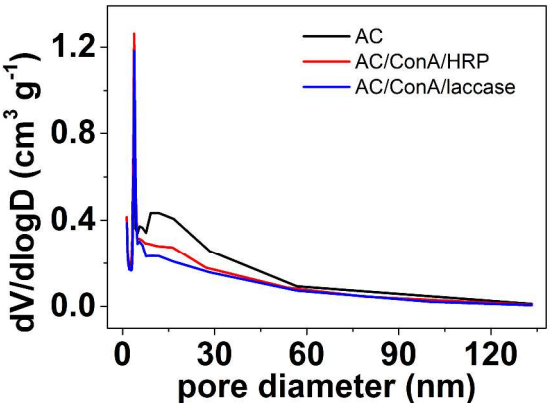


Figure S4. Pore size distribution of 20-40 mesh AC before and after protein adsorption.

Table S1. The surface area of 20-40 mesh AC before and after protein adsorption

	surface area (m^2 g^{-1})
AC	582.8
AC/ConA/laccase	491.3
AC/ConA/HRP	525.5

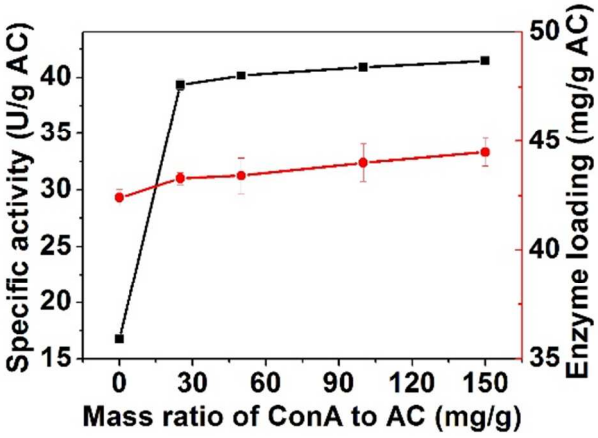


Figure S5. Effect of ConA on HRP immobilized on AC

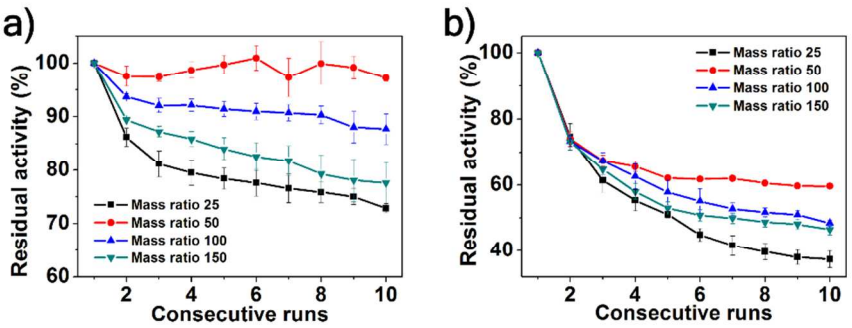


Figure S6. Effect of ConA on the reusability of immobilized enzyme on AC, and mass ratio means mass ratio of ConA to AC (mg g^{-1}).

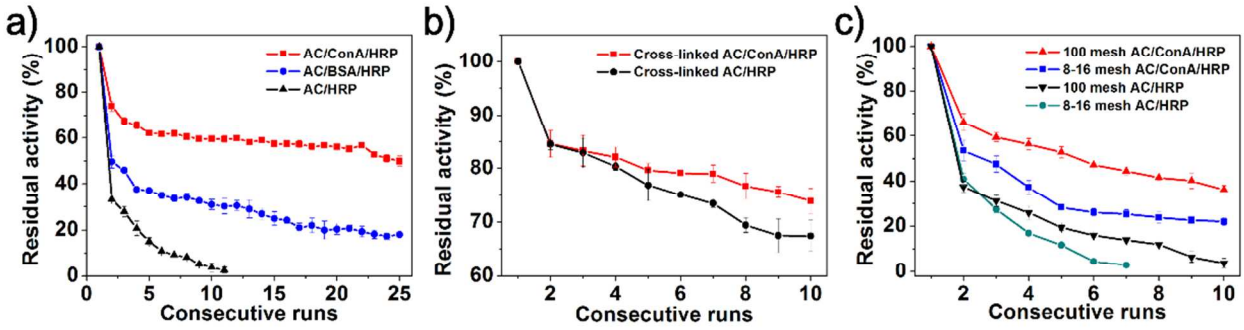
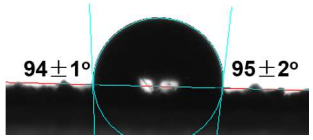
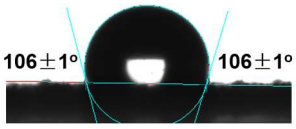
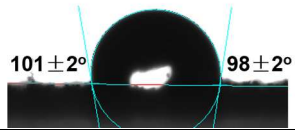
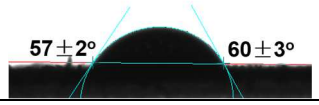
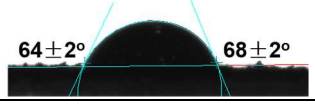
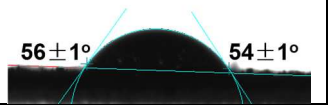
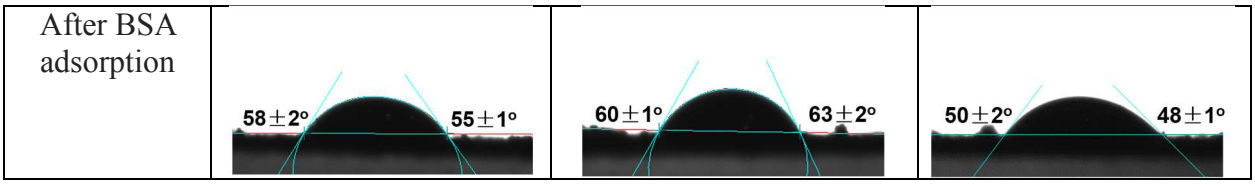


Figure S7. Reusability of (a) HRP immobilized on ConA coated AC, BSA coated AC, AC (20-40 mesh), (b) cross-linked HRP immobilized on ConA coated AC and AC and (c) HRP immobilized on ConA coated AC and AC in 8-16 mesh and 100 mesh.

Table S2. The contact angle of AC before and after protein adsorption

	8-16 mesh	20-40 mesh	100 mesh
Before			
After ConA adsorption			



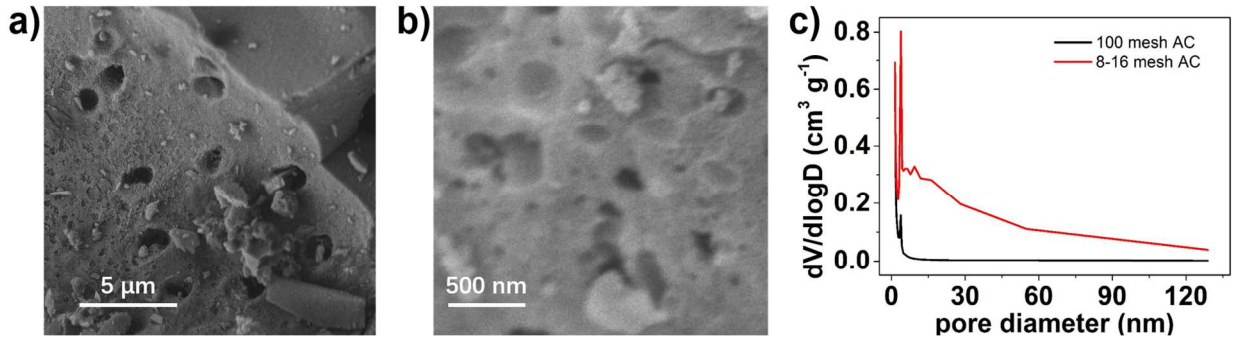


Figure S8. SEM images of (a) 8-16 mesh AC and (b) 100 mesh AC; (c) pore size distribution of 8-16 mesh AC and 100 mesh AC.

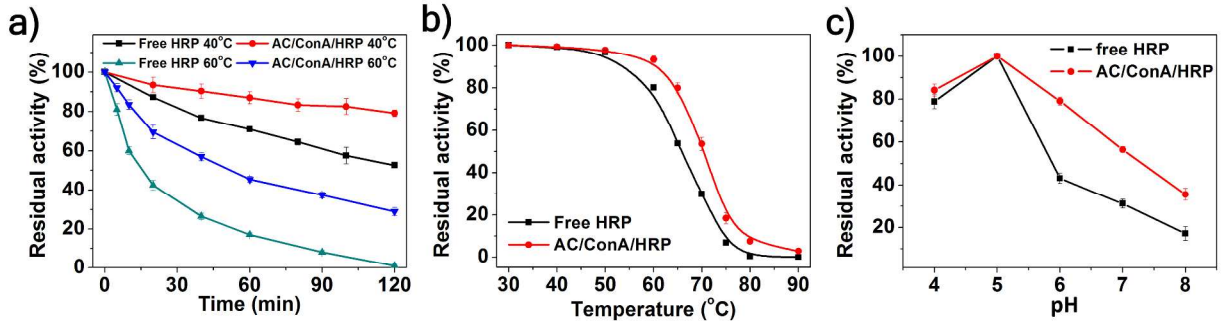


Figure S9. Stability characterization of AC/ConA/HRP and free HRP. (a) thermostability in 60 ^{o}C and 65 ^{o}C; (b) effect of temperature on the apparent activity; (c) effect of pH on the apparent activity.

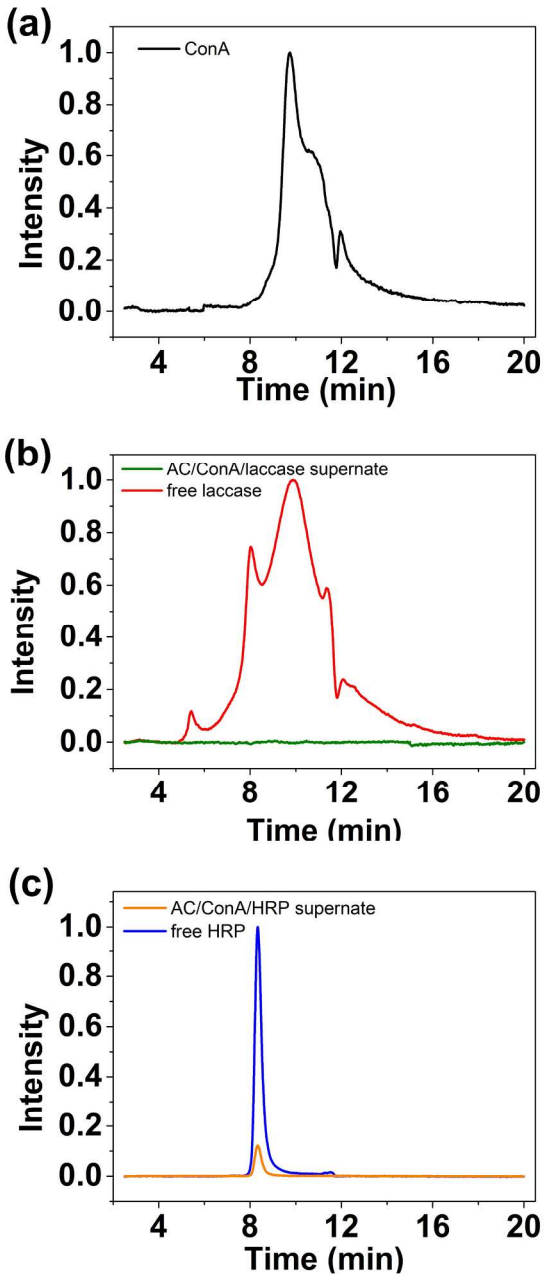


Figure S10. Size exclusion chromatography of proteins. (a) free ConA, (1mg mL^{-1}); (b) free laccase (1 mg mL^{-1}) and the supernatant of AC/ConA/laccase (1 mg mL^{-1} ConA, 4 mg mL^{-1} laccase); (c) free HRP (1 mg mL^{-1}) and the supernatant of AC/ConA/HRP (1 mg mL^{-1} ConA, 1 mg mL^{-1} HRP)