

## Supporting Information for

### Enzyme-copper Nanoparticle Hybrid Catalyst Prepared from Disassembly of Enzyme-inorganic Nanocrystal Three-dimensional Nanostructure

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## Experimental

### 1. Materials

*Candida antarctica* lipase B (CALB), phosphate buffer saline (1×), polyvinylpyrrolidone (PVP), p-nitrophenyl butyrate (p-NPB), p-nitrophenol (p-NP) and copper powder were purchased from Sigma-Aldrich. Cupric sulfate pentahydrate was purchased from Alfa Aesar. Sodium borohydride, potassium phosphate monobasic and disodium phosphate were purchased from Sinopharm Chemical Reagent Co., Ltd.

### 2. Synthesis of CALB@Cu and CALB&PVP@Cu

In a typical experiment, 1.5 mL of cupric sulfate (200 mM) in water solution was first added into 50 mL of phosphate buffer saline (10 mM, pH 7.4) solution containing 0.25 mg/mL of CALB. After stirring vigorously, the mixture was then incubated at 4 °C for 24 h. By this co-precipitation method, the enzyme-copper phosphate composites (CALB@CP) were obtained after centrifugation. Then, the as-prepared CALB@CP composites were re-suspended into 50 mL of deionized water, followed by adding 1.5 mL of sodium borohydride (NaBH<sub>4</sub>) (1M). After 24 hour reducing reaction at 4 °C, the enzyme-copper nanoparticle hybrid catalysts (CALB@Cu) were collected by centrifugation at 12000 rpm and washing with water for three times. By the same procedure, CALB&PVP@Cu were prepared in which 250 mg of polyvinylpyrrolidone (PVP) was added into the suspension prior to the addition of NaBH<sub>4</sub>.

### **3. Determination of enzyme loading amount**

After the CALB@CP composites were obtained by centrifugation, the supernatant was collected. The amount of protein in the supernatant was determined by the standard BCA method. Then the amount of enzyme encapsulated in the CALB@CP composites can be calculated. By the same procedure, the loading amount of enzyme in CALB@Cu and CALB&PVP@Cu can be calculated.

### **4. Enzymatic activity assay**

In the enzymatic activity assay, 35  $\mu\text{L}$  of p-NPB was first dissolved in 1 mL of acetonitrile. Then the substrate stock solution was diluted by adding 99 mL of phosphate buffer (25 mM, pH 7.0). The hybrid catalysts CALB@CP, CALB@Cu and CALB&PVP@Cu were re-suspended into phosphate buffer (25 mM, pH 7.0) solution with the same enzyme concentration of 0.25 mg/mL. In the enzymatic activity assay, 100  $\mu\text{L}$  of hybrid catalyst suspension and free enzyme with same enzyme concentration was added into 900  $\mu\text{L}$  substrate stock solution respectively, followed by monitoring the increase of absorbance at 400 nm for 5 min at room temperature.

### **5. Catalytic activity of copper**

1 mmol of solid  $\text{NaBH}_4$  was first added into 2 mL of p-NP (100mM) solution. Upon the addition of  $\text{NaBH}_4$ , the initial maximum absorbance of p-NP solution immediately shifted from 340 nm to 400 nm due to the formation of deprotonation of p-AP (Fig. S1a). Immediately after that, different hybrid catalysts as well as copper powder aqueous suspensions were added to the above solution under magnetic stirring at room temperature. The reaction progress was monitored by taking out aliquots of the solution (0.02 mL) at different times, diluting it to 2 mL with deionized water, followed by measuring the absorbance at 400 nm on a Shimadzu UV-2450 UV-VIS spectrophotometer.

### **6. Cascade reaction**

0.25 mL of p-NPB (100 mM) acetonitrile solution was diluted in 2.25 mL of phosphate buffer solution (25 mM, pH 7.0). Enzyme-copper nanoparticle hybrid catalysts were then added into the solution. After stirring for about 1 hour, the p-NPB was decomposed to p-NP completely. Then, 1 mmol of sodium borohydride was added into the solution. The reaction progress was monitored by taking out aliquots of the solution (0.02 mL) at different times,

diluting it to 2 mL with deionized water, followed by measuring the absorbance between 250 nm and 500 nm.

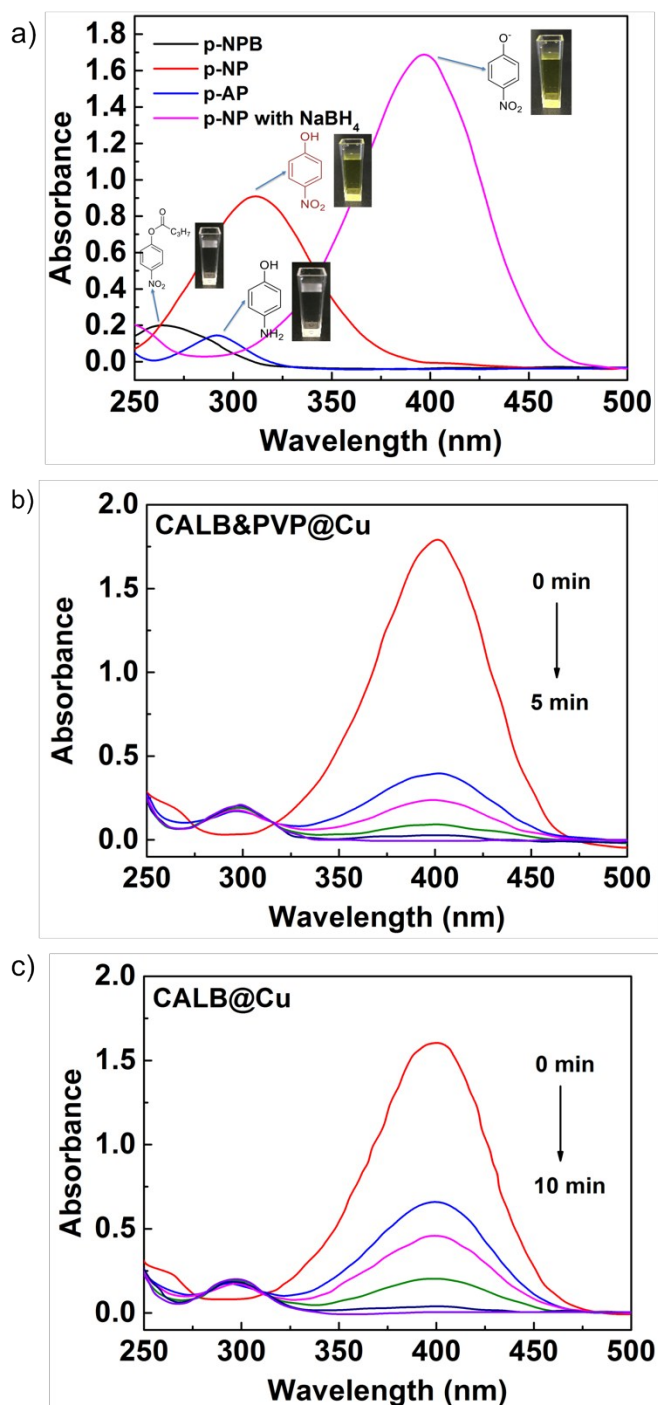


Figure S1. (a) UV-Vis absorbance of substrates and products of the cascade reaction; (b) Change in absorbance at 400 nm during the reaction when CALB&PVP@Cu acted as catalyst; (c) Change in absorbance at 400 nm during the reaction when CALB @Cu acted as catalyst;

## 7. Electron microscope analysis

Scanning electron microscope (SEM) images were taken on a Sirion 200 SEM at an accelerating voltage of 10.0 kV. Samples for SEM measurements were prepared by first suspending the composites in methanol and then 1-10 microliters of the sample solution was dropped onto a silica wafer. After all methanol was evaporated, the silica wafer was attached to a carbon paste and then sputter-coated with a thin layer of conductive gold to improve the electrical conductivity. For transmission electron microscope (TEM) analysis, a drop of methanol suspension containing the composites was added on a carbon grid and dried at room temperature. TEM images were taken on a JEOL JEM-2010 high-resolution TEM with an accelerating voltage of 120 kV.

### 8. X-ray diffraction (XRD) analysis

Powder X-ray diffraction (XRD) patterns were recorded using a Bruker D8 Advance X-Ray diffractometer with a Cu K $\alpha$  anode ( $\lambda = 0.15406$  nm) at 40 kV and 40 mA. 2 Theta ranged from 0 to 80 degree, with a scanning rate at 5 degrees per minute.

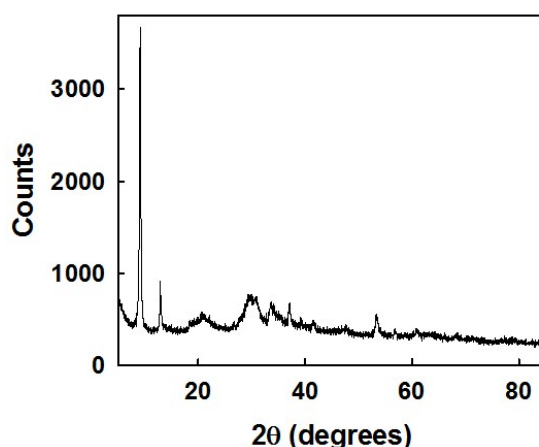


Figure S2. XRD of CALB-copper phosphate composites.

### 9. Dynamic light scattering (DLS) analysis

The sample was re-suspended in deionized water. DLS analysis was carried out on a Malvern Zetasizer ZS-90 instrument.