

**Controlled synthesis of enzyme-inorganic nanocrystal composite assembled into
3D structure with ultrahigh enzymatic activity**

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(A) Controlled synthesis of laccase microflowers

Laccase from *Trametes versicolor*, copper (II) sulfate pentahydrate and 2,6-dimethoxyphenol (DMP), were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS, 1X, pH 7.4) was purchased from Invitrogen. In a typical experiment of the preparation of laccase-copper phosphate hybrid microflowers, 0.8 mM CuSO₄ water solution was added to PBS containing 0.1 mg/mL laccase at pH 7.4 and 25 °C. After three-day incubation, the laccase hybrid microflowers were obtained as precipitates. For the preparation of laccase-copper phosphate hybrid microflowers in the presence of EDTA, 10 mM, 20 mM, 30 mM, 40 mM and 50 mM of EDTA were directly added in PBS solution respectively, followed by the same procedure to synthesize the laccase microflowers.

(B) SEM and XRD analysis

Scanning electron microscope (SEM) images of samples were taken on a Sirion 200 SEM at an accelerating voltage of 10.0 kV. Powder X-ray diffraction (XRD) patterns were recorded using a Bruker D8 Advance X-Ray diffractometer with a Cu K α anode ($\lambda = 0.15406$ nm) at 40 kV and 40 mA.

(C) Activity assay of the laccase microflowers

The laccase activity was measured using DMP (1 mM) as the enzymatic substrate at 25 °C in pH 5.0, 0.05 M phosphate buffer. The molar extinction coefficient of the

oxidation product of DMP at 470 nm is 49.6 mM/cm. One unit of activity is defined as the amount of laccase required to oxidize 1 mmol of DMP in 1 min.

(D) Detection of phenol in water solution by the laccase microflowers immobilized on membrane

The suspension of the microflowers (2 mg/mL microflowers, 0.3 mL) in PBS was injected into a syringe filter (Whatman Puradisc 30, ϕ 30 mm, pore size 0.2 μ m), followed by drying at room temperature. In a typical detection experiment, 2 mL of phenol solution in 50 mM pH 6.0 phosphate buffer was mixed with 2 mL of 4-aminoantipyrine water solution (1 mg/mL). Then 400 μ l of the solution was injected into the syringe filter and kept for 5 minutes. After that the solution was pushed out and subjected to the UV-Vis analysis at 495 nm using Shimadzu UV2550 instrument. For the repeated use of the syringe filter, the membrane was rinsed by filtering 2 mL of deionized water and dried at room temperature before the next run.

(E) The effect of pH on the enzyme activity

pH	4	5	6	7
laccase	90%	100%	75%	60%
laccase microflowers	900%	1100%	700%	540%

The influence of pH on the enzyme activity was investigated. From the results, keeping other parameters as constant, the optimal pH range for free laccase and laccase microflowers to oxidize anthracene was about 5.0.