

# **The fabrication of CuBTC@PW<sub>12</sub> with Prominent Peroxidase-Mimicking Activity for Colorimetric detection of H<sub>2</sub>O<sub>2</sub> and ascorbic acid via “On–Off” Switch**

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## **Experimental section**

### **1.1 Effects of pH, catalyst concentration, response time and reaction temperature on the peroxidase activity of CuBTC@PW<sub>12</sub> Chemicals**

The effect of fixed H<sub>2</sub>O<sub>2</sub> (1.0 mM), H<sub>2</sub>O<sub>2</sub> (1.0 mM), in 0.2 M acetate buffer (pH 4.0) on pH was investigated. The concentration of CuBTC@pPW<sub>12</sub> was fixed at 1.0 mg/mL at 25 °C and varying pH (3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4 and 5.6) and a reaction time of 3 minutes. Other conditions were consistent with those described above and the temperature was kept at 30°C. Optimisation of the catalyst concentration was carried out. The concentration range of the CuBTC@pPW<sub>12</sub> solution (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.6, 0.7, 0.8, 0.9 and 1.0 mg/mL). To explore the optimum time, other conditions were kept constantly and the times were varied: 1, 2, 3, 4, 5 and 10 min. The effect of temperature on the catalytic oxidation of TMB was carried out over a temperature range of 20 to 50 °C. The above conditions were kept constantly and the mixed solutions were incubated for 3 min at a range of temperatures (25, 30, 40 and 50 °C).

### **1.2 Detection of H<sub>2</sub>O<sub>2</sub>**

100 µL of TMB solution (1.0 mM) was dissolved in 150 µL of acetate (pH 4.0) buffer solution, 100 µL of 0.06 mg/mL CuBTC@pPW<sub>12</sub> was added, followed by 100 µL of different concentrations of 150 µL of different concentrations of H<sub>2</sub>O<sub>2</sub> (0.01 to 1.0 mM), in that order. The resulting solutions were used to perform absorption spectroscopy measurements by incubation at 652 nm for 3 min at 40 °C with a UV-Vis spectrophotometer.

### 1.3 Steady-state kinetic analysis.

Kinetic experiments were investigated by measuring the variance of absorbance at 652 nm over 3 min. The TMB concentration (1.0 mM) was fixed with the variety of H<sub>2</sub>O<sub>2</sub> concentration (0.0-2.0 mM), the H<sub>2</sub>O<sub>2</sub> concentration (1.2 mM) was fixed with the variety of TMB concentration (0.0-2.0 mM) was evaluated respectively. The kinetic parameters were determined by Lineweaver – Burk diagram of the double inverse of the Michaelis – Menten equation:

$$v = V_{\max}[S]/(K_m + [S])$$

where  $v$ ,  $V_{\max}$ ,  $K_m$  and  $[S]$  are the initial velocity, maximal reaction velocity, Michaelis constant and the concentration of the substrate, respectively.

### 1.4 Detection method of hydroxyl radicals ( $\cdot\text{OH}$ ).

The generation of  $\cdot\text{OH}$  during catalysis was proved by EPR technique measuring with a EPR 100 M spectrometer using DMPO as spin-trapping agents.

## 2. Result and discussion

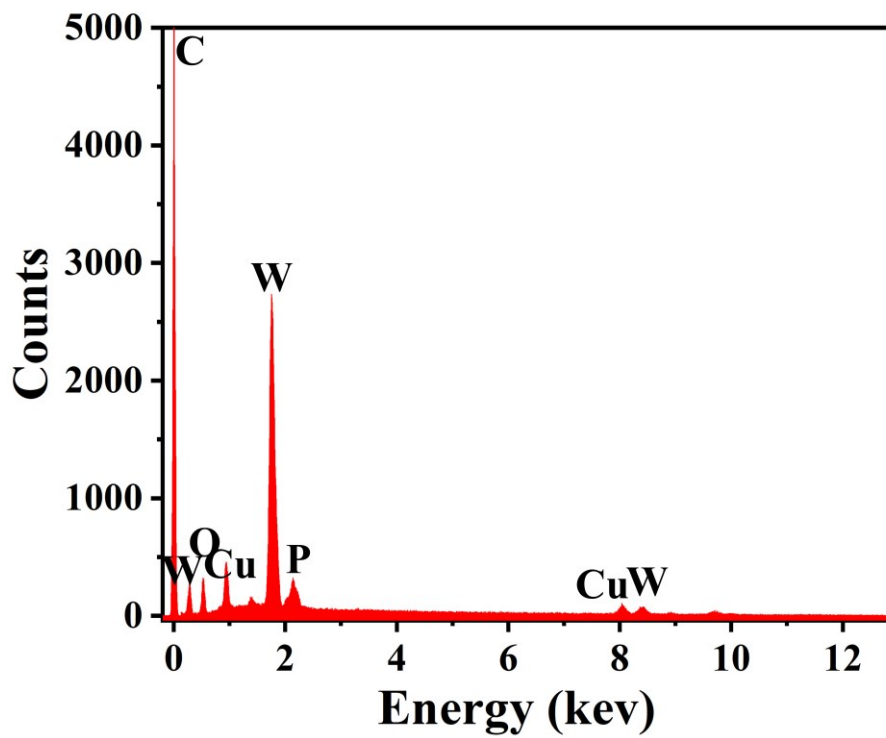
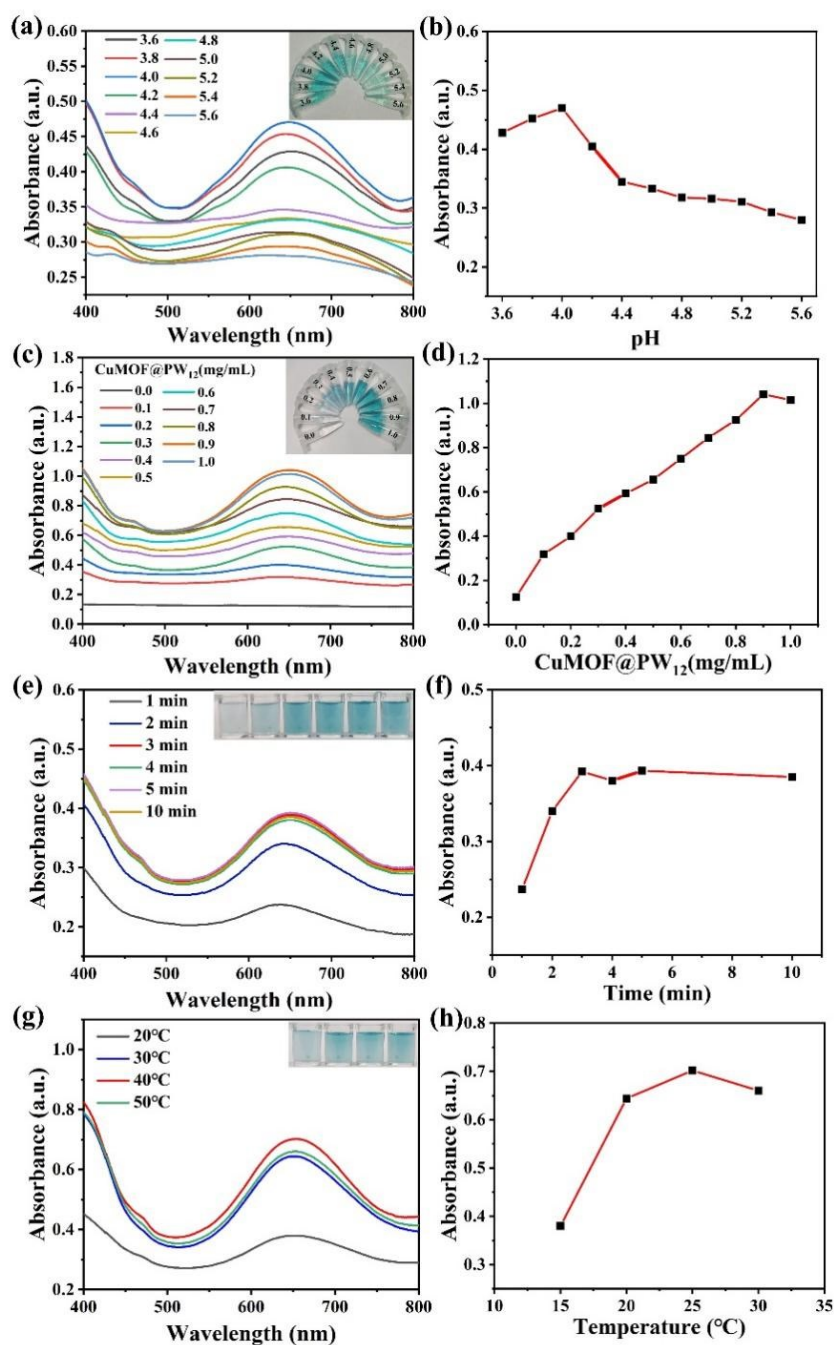
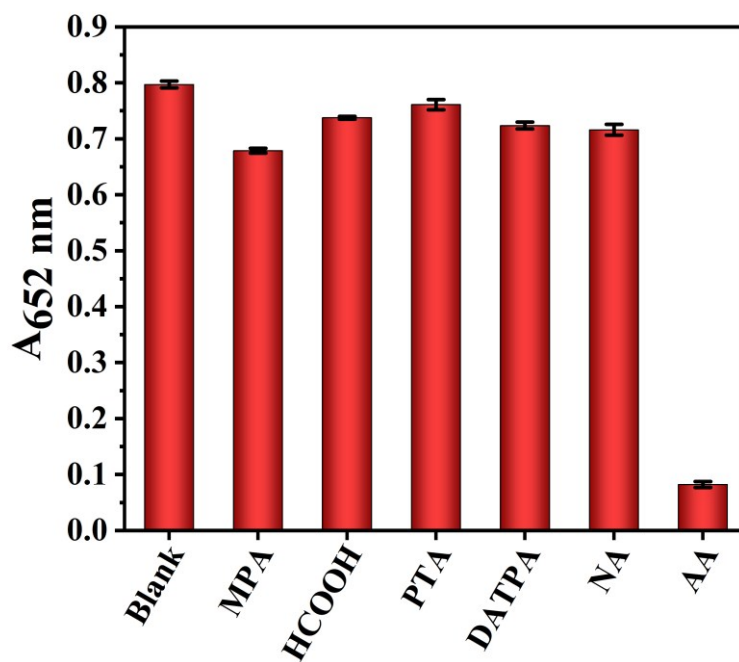


Fig. S1. EDX analysis of CuBTC@PW<sub>12</sub>.

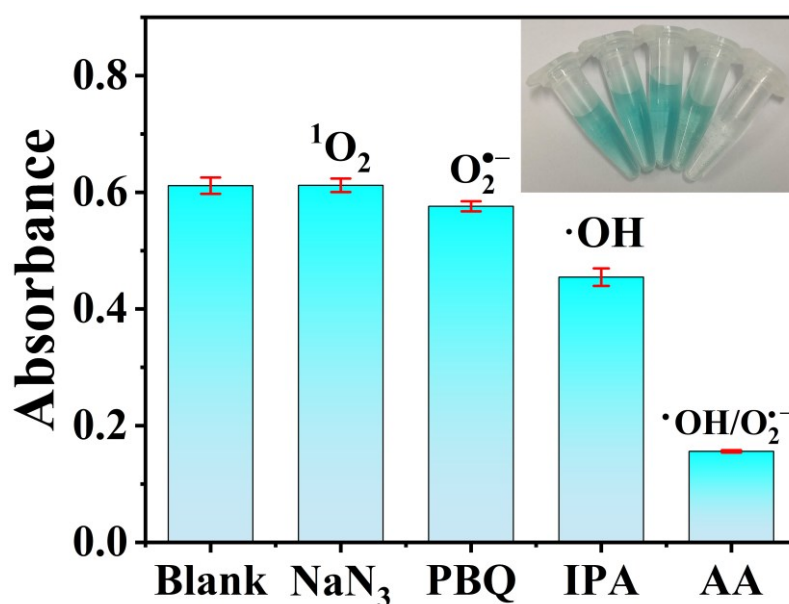


**Fig. S2.** Optimization of TMB oxidation conditions with: (a) the absorbance with various pH and (b) corresponding statistical data, (c) the absorbance with various concentration of CuBTC@PW<sub>12</sub> and (d) corresponding statistical curve, (e), (f) the evaluation of reaction time and (g), (h) temperature, the corresponding colorimetric images were inserted.



**Fig. S3.** Selectivity analysis of the system for AA based on the A<sub>652 nm</sub> of the TMB/H<sub>2</sub>O<sub>2</sub> /CuBTC@PW<sub>12</sub> system within 200 μM of MPA, HCOOH, PTA, DATPA, NA, AA (The error bars are one standard deviation of three independent tests.)

Considering the favorable peroxidase-like catalytic ability, a series of experiments were performed to identify the mechanism of detection. The relative types of ROS (such as singlet oxygen ( $^1\text{O}_2$ ), superoxide radical ( $\text{O}_2^{\cdot-}$ ), and hydroxyl radical ( $\cdot\text{OH}$ )) can be scavenged by sodium azide ( $\text{NaN}_3$ ), p-benzoquinone (PBQ) and isopropanol (IPA), respectively<sup>10</sup>. After co-incubation of CuBTC@PW<sub>12</sub>, H<sub>2</sub>O<sub>2</sub>, and TMB with  $\text{NaN}_3$ , PBQ, IPA, and AA, respectively, the absorbance at 652 nm with AA emerged dramatically decreased, the IPA sample also declined almost 20%, while other samples occurred slight change, implying that  $\cdot\text{OH}$  and  $\text{O}_2^{\cdot-}$  caused the oxidation of TMB (Figure S4). These findings confirm that the peroxidase-mimicking activity of CuBTC@PW<sub>12</sub> derives from the formation of  $\cdot\text{OH}$  and  $\text{O}_2^{\cdot-}$  species.



**Fig. S4.** The intensity of the UV-vis absorption of CuBTC@PW<sub>12</sub> with various ROS scavenger.