

Supporting Information

Reliable Method for Detection of Horseradish Peroxidase Activity and Enzyme kinetics

EXPERIMENTAL SECTION

Reagents. All inorganic salts were obtained at highest purity. Horseradish peroxidase was bought from Sigma-Aldrich as the lyophilized enzyme powder, whose value of specific activity is equal or greater than 300 U/mg. The hydrogen peroxide (30%), N,N-dimethylformamide (DMF), guaiacol, sodium dihydrogen phosphate and disodium hydrogen phosphate were obtained from Sinopharm Chemical Reagent Co., Ltd. The potassium chloride and molybdenum disulfide were obtained from Shanghai Ling Feng Chemical Reagent Co., Ltd., and J&K Scientific Ltd., respectively. The ultrapure water, whose electric conductivity was less than $0.065 \mu\text{S cm}^{-1}$, was used in every experimental procedure.

Preparation of MoS₂ Nanoparticles. The MoS₂ nanoparticles were prepared according to the previous report.¹ The MoS₂ powder was mixed with DMF to obtain a black mixture with the concentration of 1 mg mL⁻¹. This mixture was ultrasonicated 4 h by a KQ-200KDE sonifier (Kunshan Ultrasound Equipment Co., Ltd., China) at room temperature ($25 \pm 2 \text{ }^{\circ}\text{C}$) to form black slurry. Then the slurry was successively processed for centrifugalization at 3000 rpm for 15 min and 6000 rpm for 15 min. The precipitates were collected respectively. After that, the supernatant was centrifuged at 12 000 rpm for 30 min and the precipitation was gathered. A TG16MW centrifugal machine (Hunan Herexi Instrument& equipment Co., Ltd., China) was used in these procedures.

Fabrication of H₂O₂ Biosensors. MoS₂ nanoparticles precipitation was dispersed into DMF with the concentration of approximate 2 mg mL⁻¹. Then, 5 μL of the mixed solutions were dropped on the GC electrode (diameter = 3 mm) and dried in the air. The upper part of this modified electrode was then put into ethanol for 30 min with a slow stir to remove the residual DMF, which was left on the electrode surface. The MoS₂ nanoparticles would form very stable films even without the protection of Nafion.

Preparation of Solutions. Ascorbic acid solution and hydrogen peroxide solution with the concentrations of 1.0 M and 0.5 M respectively should be prepared firstly. Put the 250 μL GA liquid into 4750 μL deionized water to obtain 5 mL mixture. Then, an injector (10 mL)'s needle was put into mixture solution. Pulled and pushed repeatedly. The preparation of GA emulsion wasn't finished until the mixture

turn into an oyster white emulsion. The HRP solution had been prepared with the pH of 7.0, the concentration of 0.1 M and containing 0.1 M KCl.

Detection of HRP Activity. 5.0 mL 0.1 M pH 7.0 PBS (containing 0.1 M KCl) of actual sample leached solution was added into a 10 mL electrolytic cell. The oxygen was deaerated by pure nitrogen. After 20 min, 3.0 μ L 1.0 M AA was added. Three-electrode system was employed, using a saturated calomel electrode as a reference electrode, a platinum wire electrode as a counter electrode, and the Nano-MoS₂ modified GCE ($\varnothing$ = 3 mm) as the working electrode. The cyclic voltammetry (CV) was used to scan number of turns for getting a steady baseline. The scanning potential range is from -0.8 V to 0 V. Chronoamperometry testing was carried out at the applied potential of -0.3 V with magnetic stirring. The first 60s scanning was used to make the current become stable. Then 10 μ L of 0.5 M H₂O₂ solution was injected into the electrolytic cell for five times with the same time interval. After that, spend 60s in collecting the blank signal, then injected 50 μ L of 0.45 M GA emulsion. The detection was finished while current signal substantially returning to baseline position. The above operations were using the 50 μ L-liquid micro-injector for manual injection, and the CHI 660C electrochemical workstation (Chenhua, China) was used in this process. All electrochemical measurement was carried out at room temperature ($25 \pm 1^\circ\text{C}$).

Reference

- (1) Wang, T.; Zhu, H.; Zhuo, J.; Zhu, Z. *Anal. Chem.*, **2013**, *85*, 10289–10295.

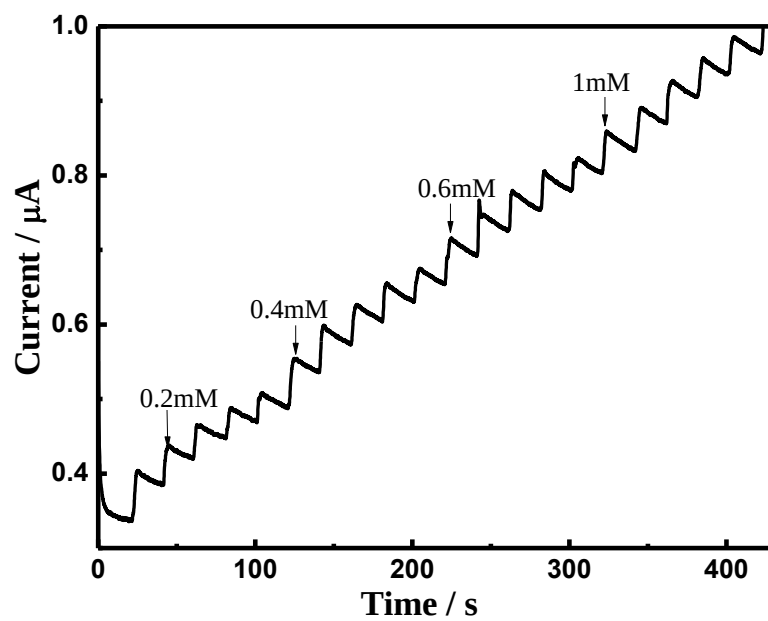


Figure S1. Amperometric responses of the modified electrode to the successive addition of H_2O_2 in a N_2 saturated 0.1 M PBS containing 0.1 M KCl at -0.3 V.

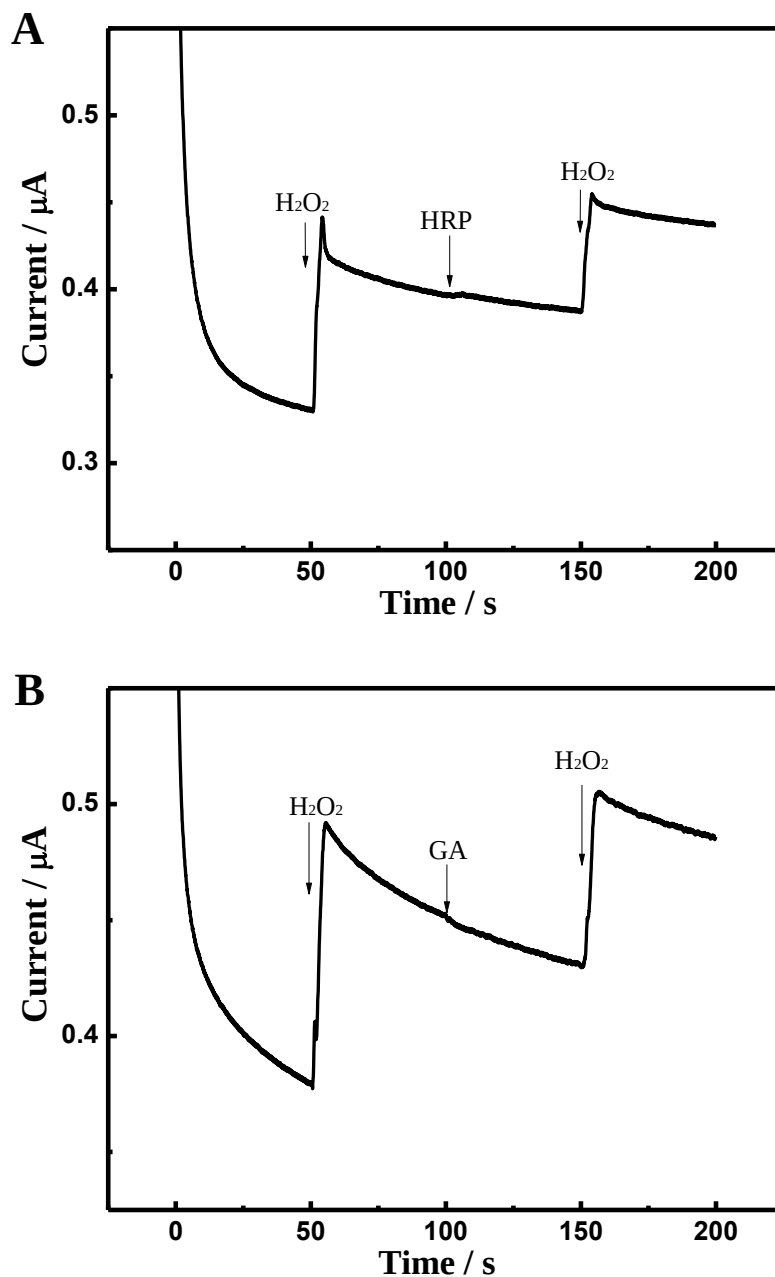


Figure S2. Amperometric responses of the modified electrode to the successive addition of H_2O_2 , (A) HRP and (B) GA in a N_2 saturated 0.1 M PBS containing 0.1 M KCl at -0.3 V. Every H_2O_2 -injection made the concentration of H_2O_2 increased 1 mM. The injection of HRP is 12 μg and the injection of GA is 22.5 μmol . The volume of PBS containing KCl is 5.0 mL.

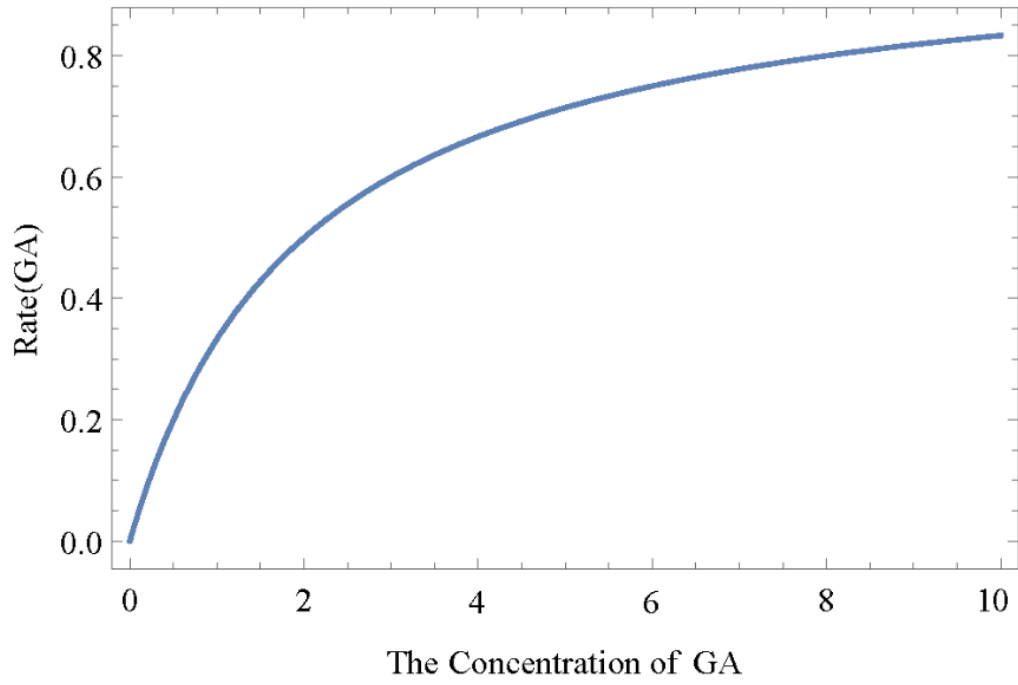


Figure S3. $r(R)$ - R curve plotted by Wolfram Mathematica 10, according to the equation (10)

$$r = \frac{k_1 \times k_3 \times k_5 \times S \times R \times E_0}{(k_1 \times S + k_2)k_5 + (k_1 \times S + k_5 \times R)k_3}$$

Because the other constants were set as 1, the above equation can be simplified as

$$r = \frac{R}{3 + R}$$