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Supporting Information

Hierarchical self-assembly of double structured Prussian blue film for highly sensitive biosensors

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Supplement 1 (S1). Experimental section

Reagents:

Analytical grade $K_4[Fe(CN)_6]{\bullet}3H_2O$ (Sigma-Aldrich), $FeCl_3{\bullet}6H_2O$ (Sigma-Aldrich) and 30 wt% H_2O_2 (Sinopharm Chemical Reagent Co., Ltd, China) were used. No further purification processes were applied and all solutions were prepared with deionized water.

Characterization:

The electrochemical properties were studied using an electrochemical workstation (CHI 660C, Shanghai Chenhua, China). Cyclic voltammetry (CV) analysis was conducted in 0.05 M phosphate buffer solution (PBS, pH= 6.5) with 0.1 M KCl at 25 °C. Pt wire and Ag/AgCl were used as the counter and reference electrodes, respectively. The scan rate was 50 mV/s. H_2O_2 and the response was observed under a potential of -0.05 V, using a chronoamperometric method.

Microscale observations of H_2O_2 detection were conducted by scanning electrochemical microscopy (SECM) (M370, USA). The electrochemical cell was a classical three-electrode system placed in PBS as the CV setting. A Pt ultramicroelectrode (UME) with a diameter of 100 μm was used as the probe for receiving signals. To switch the probe into a generation-collector, the probe was biased at 0.25 V, while the PB modified electrode was operated at -0.05 V during the redox cycle with a 1 μm/s scan rate.

All field emission scanning electron microscopy images (FESEM) (Hitachi-4800, Japan) were acquired at magnifications of 600×, 20000× and 40000×. The prepared electrodes were used directly with samples and observed at 5 kV.

Methods:

Pretreatment on the platinum foil surface ($7{\times}2$ mm^2) was required, in order to keep the electrode clean. The surface of the Pt electrode was first polished like a mirror using metallographical sand paper, then dipped into a piranha solution (7:3 mixture of H_2SO_4/H_2O_2, v/v) for 30 min, and finally rinsed with water. The Pt electrode was ready for use after a 30 min ultrasonic wash in water. Two solutions were prepared for the deposition of PB films. Solution 1 (S1): 0.01 M $K_4[Fe(CN)_6]$ + 0.1 M KCl + 0.1 M HCl. Solution 2 (S2): 0.01 M $FeCl_3$ + 0.1 M KCl + 0.1 M HCl. The preparation process is shown in Fig. 1. The two Pt sheets served as working and counter electrodes and were maintained in synchronous movement during preparation. It should be noted that the two Pt sheets were kept parallel to maintain the vertical electric field between the two electrodes. The distance between working and counter electrodes was kept constant at 8 mm. The two electrodes were first dipped into S1 for 60 s. The electrodes were then cleaned in deionized water for 15 s, for a total of three washes. The electrodes were next placed in S2 and the same procedure repeated, as for S1. Finally, the electrodes were again washed in deionized water. This process was conducted once, for self-assembly, and one layer of PB was deposited on the Pt surface. The self-assembly time and operation were controlled accurately using a Dip-Robot (DR-3, Riegler & Kirstein GmbH, Germany) for the whole process. The number of self-assembled layers and the electric field intensity were modified as required. The prepared electrodes were cleaned again with deionized water and dried at 100˚C for one hour.

Supplement 2 (S2). The equation for calculation of PB surface concentration

Because the redox reaction is reversibility for PB, its surface concentration can be calculated as follow equation:

$$\Gamma_T = \frac{Q}{nFA} \qquad \text{(Eq. S2)}$$

Where Q is the electric quantity of one single peak in CV diagram; n is average electrons transfer, which should amount to 57 milivolts divided by the different between oxidation and reduction potential; F is the Faraday constant and A is the area of electrode surface.

Γ_T can describe the amount of PB per unit area. From the comparison of different samples, one can observe the surface coverage change with the preparation parameters.

Supplement 3 (S3). The morphology of electrode prepared at 0.1 V

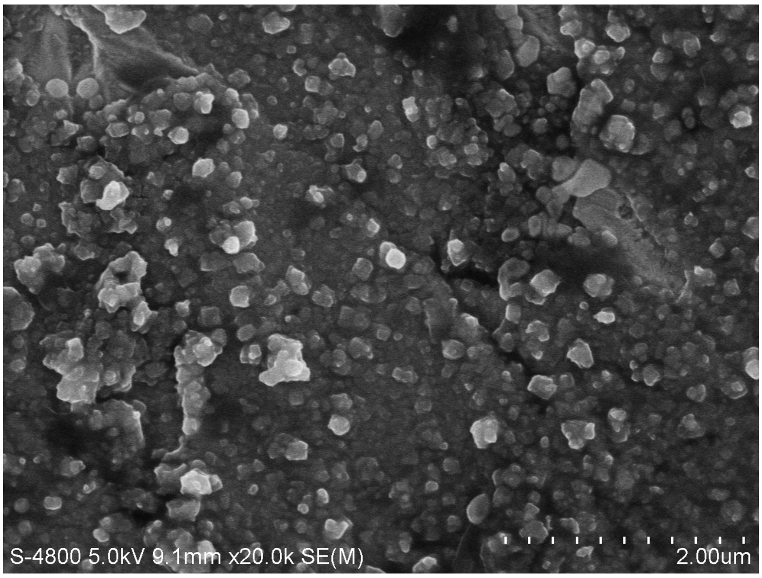

Fig. S3. The FESEM image of electrode prepared in 0.1 V.

According to the CV results, we knew that if the PB film grew at 0.1 V, the amount was much less than that at 0.2 or 0.25 V. This may lead to the imperfect growth of crystals. From the FESEM characterization, we can find that the film have not covered the whole electrode surface. Some places exposure the substrate of Pt surface. In addition, the assembly of crystals is not obvious and most of PB particles exist solely or are of little congregation. This is because the potential imposed between two electrodes was so weak that the effects of electric field could not strongly affect the growing direction of PB. Besides that, the fewer amount of PB cannot provide enough crystals to recover the blank of electrode surface. Consequently, we can expect that the electrode prepared at this potential will show the worse performance due to the deficiency.

Supplement 4 (S4). The roughness characterization of the bare Pt electrode

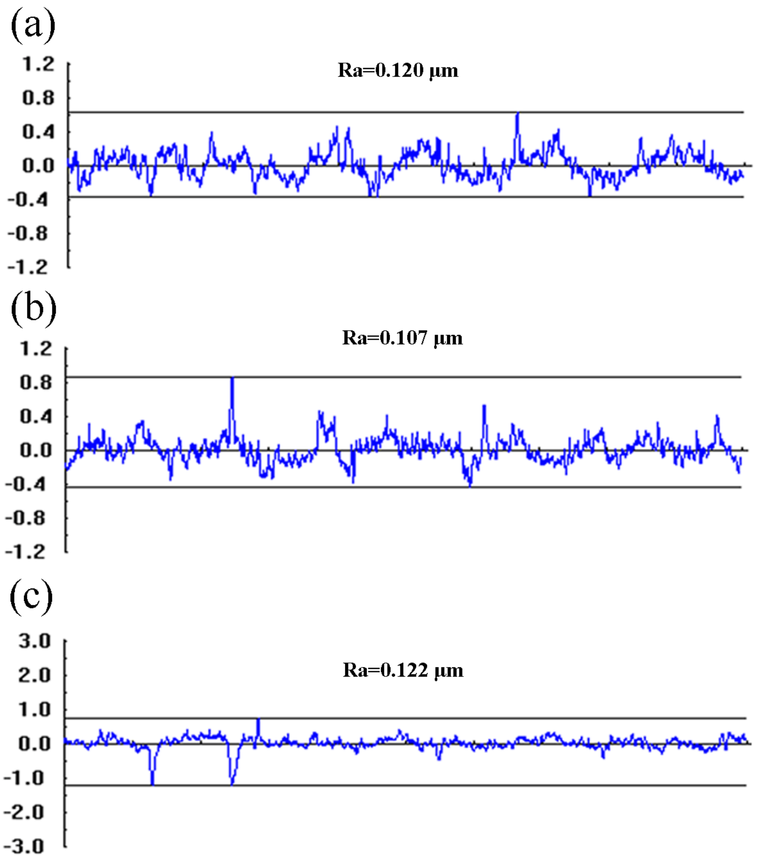

Fig. S4. (a) to (c) are the roughness of bare Pt electrode for different areas. The scanning range is 0.5 mm. Ra is the average roughness.

The surface roughness of bare Pt electrode was characterized by surface roughness measuring instrument (JB-4C, Shanghai Taiming Optical Instrument Co., Ltd., Shanghai). Detections of different areas were operated three times for the repeated experiments. The results show that the surface of Pt is not absolutely even. The average roughness of three areas is 0.116 μm. There are a lot of peaks and valleys on the surface. In this aspect, the formation of crystal nucleus will not uniform.

Supplement 5 (S5). Reduction ability of the prepared electrode in microscopic area.

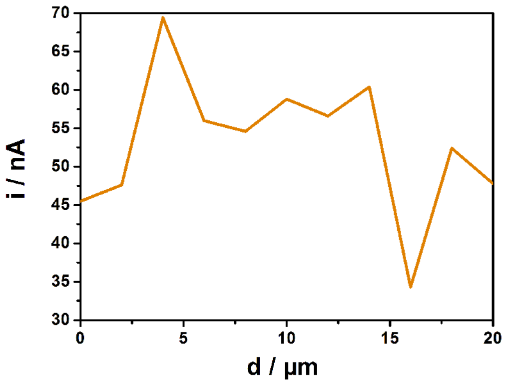

Fig. S5. The SECM image of prepared electrode in $K_3[Fe(CN)_6]$ solution. The scanning area is 20 μm. The imposed potential of electrode is -0.05 V and the tip potential is 0.25 V. The sample electrode was prepared at 0.25 V for 40 layers by weak electric induced self-assembly.

To investigate the reduction ability of the prepared electrode, the electrode was characterized in 10 mM $K_4[Fe(CN)_6]$ solution by linear scanning mode of SECM. $[Fe(CN)_6]^{3-}$ is the common electron mediator of redox in SECM method. During the detection, the $[Fe(CN)_6]^{3-}$ will be reduced to $[Fe(CN)_6]^{4-}$ by PB film and then be oxidized back to $[Fe(CN)_6]^{3-}$ at the scanning tip. The result can be applied to represent the reduction ability of the whole electrode surface. As shown in Fig. S6, the current curve exhibits the tendency of fluctuation, which is just consistent with the H_2O_2 detection (the top diagram of Fig. 6 (b) in main text). Accordingly, the peak currents were also caused by the micro-cubic structure. We can find that the peak current is much higher than the valley current, about two times than the lowest value. This illustrates that, on the whole surface, the micro-cubes own the much stronger reduction ability than the ground film. Consequently, the area around “antenna” can rapidly produce electrocatalytic reaction and accumulate more amounts of electrons to cause the high signals for the improvement of sensitivity.