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Electronic Supplementary Information

Monitoring the Electrochemical Reactions on Gold Nanoelectrode Array via Fluorescence-Enabled Electrochemical Imaging

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Experimental

Materials and Reagents. Indole-5-carboxaldehyde (5FIn, 98%), cresyl violet acetate, 1-hexyl-3-methylimidazolium chloride ([HMIM]Cl, 98 %), acetonitrile (ACN, 99.5%), chloroplatinic acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$), sodium citrate, citric acid, cysteamine, sodium borohydride ($NaBH_4$), sodium sulfate anhydrous (Na_2SO_3), lithium perchlorate, and ascorbic acid were purchased from Sigma-Aldrich (Shanghai, China). Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) was obtained from Alfa Aesar (Shanghai, China). Anodic aluminum oxide (AAO) membranes (Pore: ~140 nm, space: ~450 nm, thickness: 40~60 μm) were received from TopMembranes Technology Co., Ltd (Shenzhen, China). Potassium ferricyanide ($K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$), potassium chloride (KCl), ethylene diamine tetraacetic acid (EDTA), disodium hydrogen phosphate (Na_2HPO_4), ammonium citrate trihydrate and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). ECL detection kit was received from KeyGEN Biotech (Nanjing, China). Diamond grinding discs with a diameter of 3 or 0.5 μm and diamond suspension with a diameter of 0.05 μm both were received from Buehler (USA). All reagents were analytical pure grade and used without further purification. Second distilled water (18.2 MΩ cm) from the Millipore system (Direct-Q 3UV, Millipore, USA) was used in all experiments.

Instruments and Measurements. The morphology was characterized by scanning electron microscopy (SEM, FEI Inspect F50, USA). All the electrochemical experiments were carried out using a CHI 740D electrochemical workstation (Chenhua, Shanghai). The E-beam vacuum evaporation system (Kurt J. Lesker, USA) was applied to evaporate silver film at one side of the AAO membrane. Fluorescence imaging of

individual gold nanoelectrodes array was performed using inverted confocal microscopy (Leica TCS SP8) coupled with a 561 nm laser, an oil immersion objective (100x or 60x), and a photomultiplier tube (PMT) detector. During the scanning, the laser power was 10 %, and the gain value of PMT was set at 600 V. The fluorescence images were obtained under 128×128 pixels at zoom factor 20, or 256×256 pixels at zoom factor 10. And the obtained fluorescence images were dealt with by the ImageJ software.

Preparation of Gold Nanoelectrode Array.

The gold nanoelectrode array was prepared according to Qin's method with a repeating chronopotentiometry at 8.512 mA/cm^2 in an electrolyte containing 25 g/L gold, 80 g/L ammonium citrate trihydrate, 150 g/L sodium sulfate anhydrous (Na_2SO_3), and 60 g/L ethylene diamine tetraacetic acid (EDTA)[S1,2]. As shown in **Scheme S1**, the electrodeposition experiment was carried out in a traditional three-electrode system with a saturated calomel electrode and platinum electrode as a reference and a counter electrode, respectively. The available anodic aluminum oxide (AAO) membrane was used as a template, which was mounted on the specific holder to serve as the working electrode. After the electrodeposition, the obtained sample was immersed in a nitrite acid solution to remove the silver layer for 5 s at room temperature. Then the sample was rinsed with ethanol, deionized water in turn, and polished to obtain a flat gold nanoelectrodes array.

Electro-polymerization of P5FIn Film. Gold nanoelectrode array-based closed bipolar electrodes (c-BPEs) system was constructed according to our previous report[S2]. The electrochemical workstation was connected to a pair of Ag/AgCl driving electrodes equipped with a c-BPEs system to apply the external voltage. And the fluorescence

signals of the reduced or oxidized states of cresyl violet were studied with the combined setup of cyclic voltammetry and fluorescence microscopy (**Scheme S2**). The excitation laser from the fluorescence microscope was focused on the reporting pole above the cover glass surface to record the fluorescence imaging.

The electro-polymerization of indole-5-carboxaldehyde (5FIn) on one side of the gold nanoelectrode array was carried out with a two-electrode system. In this case, 5 mM $K_3[Fe(CN)_6]$ solution was added to one pole of c-BPEs (named sensing pole), while the acetonitrile solution containing 0.1 M lithium perchlorate and 0.05 M 5FIn monomer was introduced into another pole (named reporting pole). The thickness of conductive P5FIn film was controlled by scanning cycles and the scanning rate was obtained by cyclic voltammetry.

Preparation of platinum nanoparticles. According to the reported literature, platinum nanoparticles (PtNPs) were synthesized by the seed method with some modifications.[S3,4] Firstly, 1.1 mL of the mixture solution including 0.05% citric acid and 1% sodium citrate was quickly injected into 48.9 mL of 0.4 M $H_2PtCl_6 \cdot 6H_2O$ boiling solution. Then, the freshly prepared mixture solution including 0.08% $NaBH_4$ was added to the above solution. After kept stirring for about 15 min and then cooling to room temperature, the Pt seed solution was obtained.

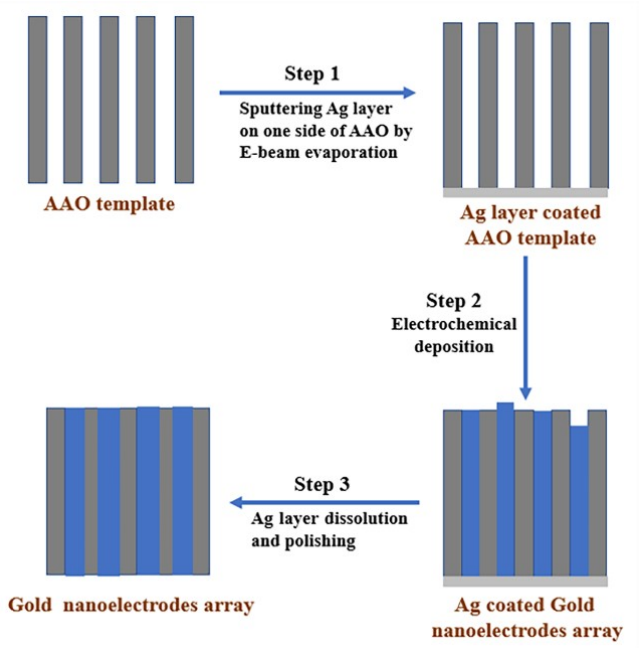
Secondly, the precursor solution containing 1 mL of resultant Pt seed solution and 0.045 mL of $H_2PtCl_6 \cdot 6H_2O$ (0.4 M) was added to 29 mL of the ultrapure water, followed by the introduction of a 0.5 mL mixture solution including 1% sodium citrate acid and 1.25% ascorbic acid (AA). Then slowly raise the temperature to boiling point by stirring, and keeping for 30 min and PtNPs with a diameter of around 75 nm were harvested.

Thirdly, 0.25 mL of PtNPs (*d*: ~ 75 nm) and 0.045 mL of $H_2PtCl_6 \cdot 6H_2O$ (0.4 M)

solution were introduced to 29 mL of ultrapure water. Then 0.5 mL mixture solution including 1% sodium citrate acid and 1.25% ascorbic acid was injected into the above solution. After that, the temperature of the system was slowly raised to boiling point and the whole reaction was kept for 30 min to obtain PtNPs with a diameter of around 100 nm.

Finally, the prepared PtNPs were coupled to the sensing pole surface of the *c*-BPEs array based on a gold nanoelectrodes array by cysteine as the linker. *I*) the prepared and cleaned gold nanoelectrode array was activated in 5% sulfuric acid solution, and the sulfuric acid solution was rinsed off with ultrapure water; *II*) 200 μL of 5 mg/mL cysteine was dropped onto one side of the gold nanoelectrodes array and incubated at room temperature. After 1.5 h, the excess cysteine was removed by rotating; *III*) the 50 μL PtNPs solution prepared above was dropped onto the surface of the cysteine modified-gold nanoelectrodes array; *IV*) After 0.5 h, the gold nanoelectrodes array was placed on the spinning instrument and coated at 2000 rpm for 30 s to remove the excess uncoupled PtNPs. Finally, PtNP was successfully coupled to the surface of a single independent gold nanoelectrode.

Scheme S1. Schematic images of template-assisted preparation procedure of gold nanoelectrode array. **Step 1**. A thin silver layer was evaporated onto one side of the AAO film by an e-beam evaporation system (Kurt J. Lesker); **Step 2**. Gold nanoelectrode were electrodeposited into the AAO pores; **Step 3**. Selective removal of the silver layer and polishing to obtain the regular gold nanoelectrode array.



Scheme S2. Schematic diagram of the used experimental setup. The reporting pole of the closed bipolar electrode (c-BPEs) array based on the gold nanoelectrode array with a bottom made out of a transparent thin cover glass was placed above the microscope objective of an inverted fluorescence microscope. The driving electrodes were a pair of Ag/AgCl wires to connect the electrochemical analytical system. Both excitation laser beam and fluorescence collection occur through the objective.

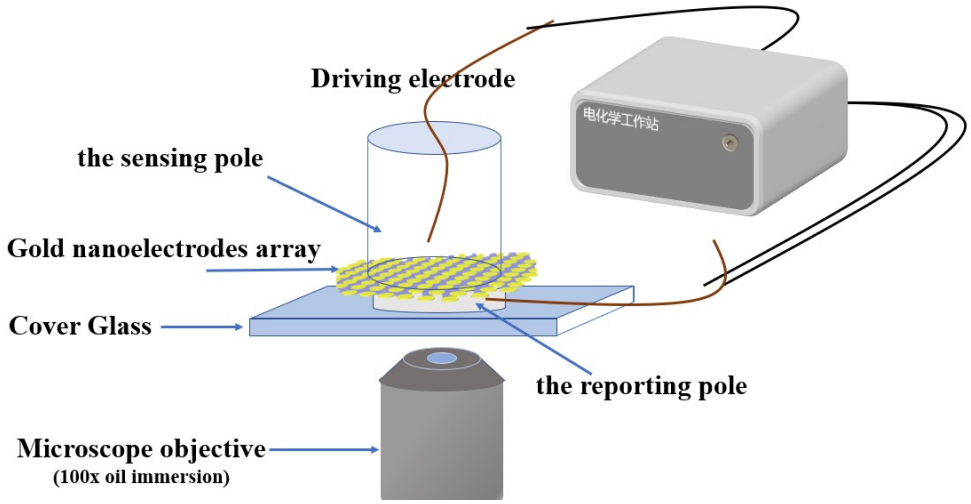


Fig. S1. Top-view SEM images of AAO template (a), a sliver film evaporated on the AAO template (b), and gold nanoelectrode array (c).

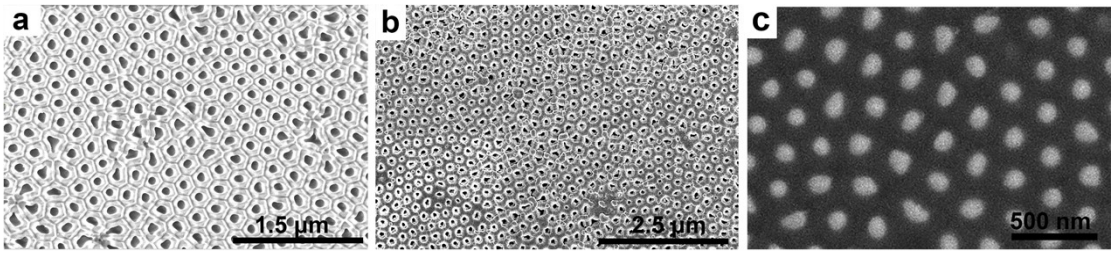


Fig. S2. SEM images of gold nanoelectrode array coated with conductive film by electro-polymerization under different conditions of cyclic voltammetry. (a) Scan rate: 0.1 V s^{-1}, number of segments: 20, (b) 0.1 V s^{-1}, number of segments: 14, (c) scan rate: 0.1 V s^{-1}, number of segments: 10, (d) scan rate: 0.1 V s^{-1}, number of segments: 6. Potential: 0-1.2 V. Scale bars: 500 nm.

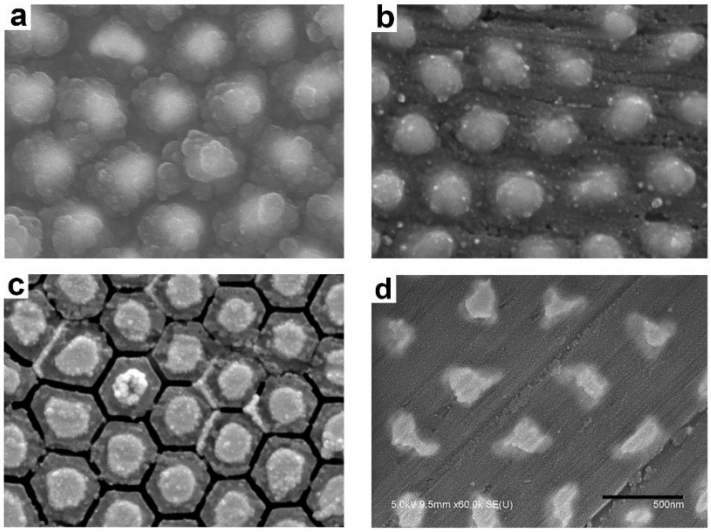
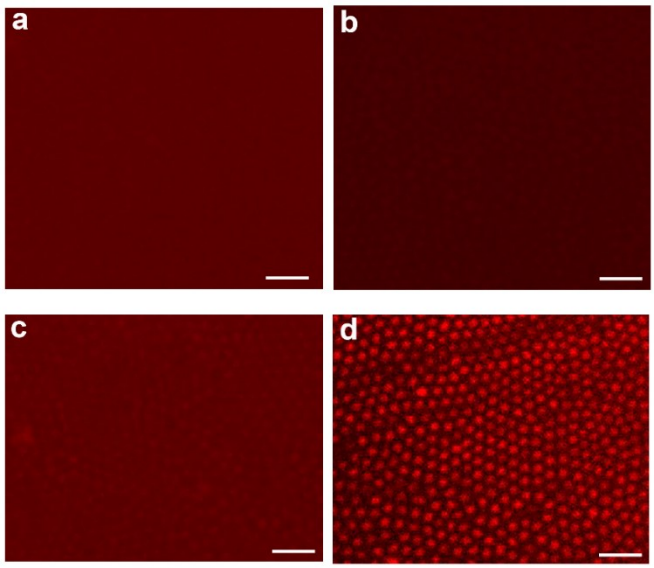


Fig. S3. Fluorescence images of the c-BPEs array at the reporting pole coated with conductive film by electro-polymerization under the assistance of cyclic voltammetry. Number of segments: (a) 20, (b) 14, (c) 10, and (d) 6. Scan rate: 0.1 V s^{-1}, potential: 0~1.2 V, and the condition of fluorescence imaging: after electro-polymerization, the cresyl violet was covalentalized with *via* aldehyde-amine condensation to produce a fluorescence signal. The sensing pole of the *c*-BPEs array: 0.01 M PBS (pH 7.0), and the reporting pole: [HMIM]Cl. Driving potential: 1.5 V. Scale bars: 1 μm.



To obtain a high spatial resolution fluorescence imaging at a single gold nanoelectrode, we performed a series of optimizations on the experimental conditions of the electro-polymerization substrate carrier. Herein, the conducting polymer film was obtained from the anodic oxidation of indole-5-carboxaldehyde (5FIn) monomer by cyclic voltammetry, and the thickness and spatial ductility of the conducting polymer film was controlled by the scan rate and the number of segments. As shown in **Fig. S3**, when the scanning rate was selected as 0.1 V s^{-1}, with the change of the scanning segment, from twenty to six segments, the fluorescence signal changed from almost a piece of fluorescence on the entire array surface, which could not distinguish the

fluorescence signal on the surface of a single gold nanoelectrode (**Fig. S3a-c**). When the segment number was selected as six, the fluorescence signal between nanoelectrodes can be distinguished (**Fig. S3d**). The results indicate that the independence of the substrate carrier can be effectively improved by optimizing the conditions of electro-polymerization, which lays an important foundation for the subsequent realization of fluorescence high-resolution imaging. Based on the above results, the scan rate and the number of segments were selected as 0.1 V s^{-1} and 6 for the electro-polymerization of carrier monomers and further fixation of fluorescent molecules.

Fig. S4 (a) TEM images of platinum nanoparticles. (b) Dynamic light scattering distribution of the prepared platinum nanoparticles.

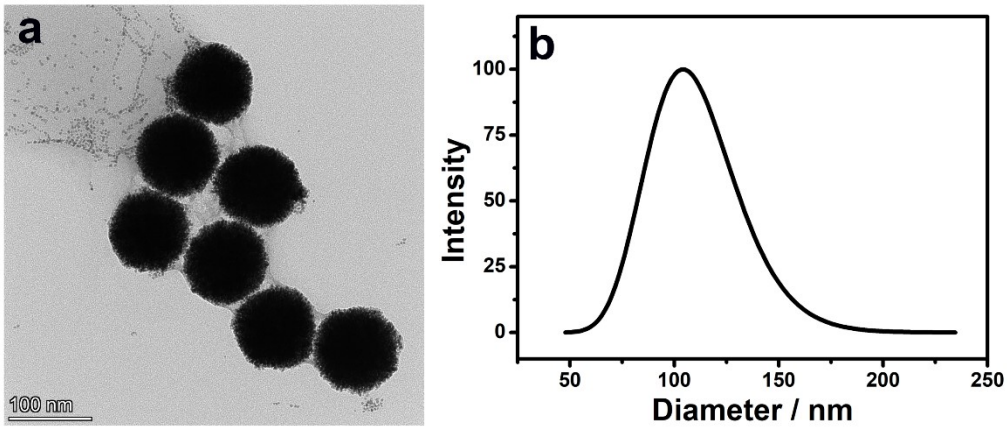
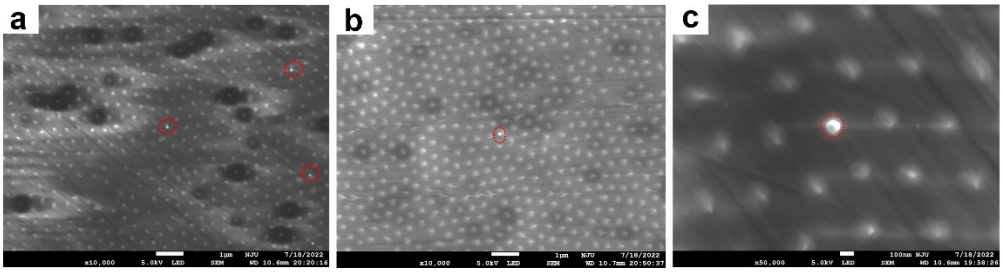


Fig. S4a shows that the platinum nanoparticles (Pt NPs) prepared by the seed methods possess a relatively homogeneous particle size distribution and a diameter of about 100 nm. **Fig. S4b** further demonstrates the above results, indicating that Pt NPs with uniform size have been successfully prepared.

Fig. S5 Several SEM images of gold nanoelectrodes modified with a single platinum

nanoparticle.



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