Supplementary Information

Disposable Electrochemical Immunosensor for Simultaneous Assay the Panel of Breast Cancer Tumor Markers

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1. Synthesis of Glutathione-Protected Au clusters

Glutathione protected Au clusters (AuCs) with diameter 6.0 nm were prepared according to the literature [1,2] with a slight modification by the reduction of $HAuCl_4 \cdot 3H_2O$ using sodium borohydride in the presence of glutathione. In short, 5.0 mL 10 mmol/L of $HAuCl_4 \cdot 3H_2O$ and 2.5 mL 10 mmol/L of glutathione were added to a mixture of solvents, methanol (4.5 mL) and acetic acid (1.0 mL) dissolved by stirring for 5 min, resulting in a light yellow solution. The $NaBH_4$ solution (15 mg/mL) was added drop by drop into above solution with vigorous stirring for 10 h. During this period, the color of the colloid gradually changed, from yellow to brown, and the red fluorescence emission indicated the nucleation of AuCs. The as-synthesized glutathione-protected gold nanoparticles (GSH- AuCs) were soluble in water. The particle solution was filtered through a 5000 MW cutoff, centrifuged at 4000 rpm and washed with Milli-Q water for four times. The solution was then dissolved in 20 mmol/L PBS (pH 7.4) buffer.

2. Synthesis of PVP-Protected Graphene

Graphene oxide (GO) was synthesized from graphitic power according to Hummer's method with some modification [3,4]. In brief, graphite powder (3 g, 325 mesh) was added into a mixture of 12 mL of concentrated H_2SO_4, 2.5 g of $K_2S_2O_8$, and 2.5 g of P_2O_5, and reacted for 5 h at 80 ℃. Next, the mixture was cooled to room

temperature and diluted with 500 mL Milli-Q water. After residual acid was removed by filtrating and washing through 0.2 μm nylon film with water, the product was dried naturally overnight and added to concentrated H_2SO_4 (120 mL). Successively, $KMnO_4$ (15 g) was added gradually under stirring, while keeping the temperature around 5 ℃. This mixture was stirred at 40 ℃ for 50 min. Then, the mixture was diluted with water (250 mL) and kept at 55 ℃ for 30 min. After the resulting mixture was stirred for 2 h, 600 mL of water was then injected into the mixture followed by adding 30 mL of 30 % H_2O_2 dropwise. The mixture was filtered and washed with 1 mol/L HCl aqueous solution and water many times to remove residues. Finally, the product was further purified by dialysis for 1 week to remove the remaining metal species. By sonicating dispersion under ambient conditions for 30 min, the homogeneous GO suspension (0.1 mg/mL) was obtained, which was stable for several months. The PVP/GNs was prepared by a modified method according to the literature [5,6]. In a typical experiment for chemical conversion of graphene oxide to PVP/GNs, 400 mg of PVP was put into 100 mL of homogeneous GO dispersion (0.25 mg/mL), followed by stirring for 12 h. Then, to the resulting dispersion were added 30 mL of hydrazine solution (80 %). The mixture was stirred for 12 h at 90 ℃. Finally, the stable black dispersion was centrifuged two times and redispersed in 25 mL of water (1 mg/mL).

3. Optimization of pH and Incubation time

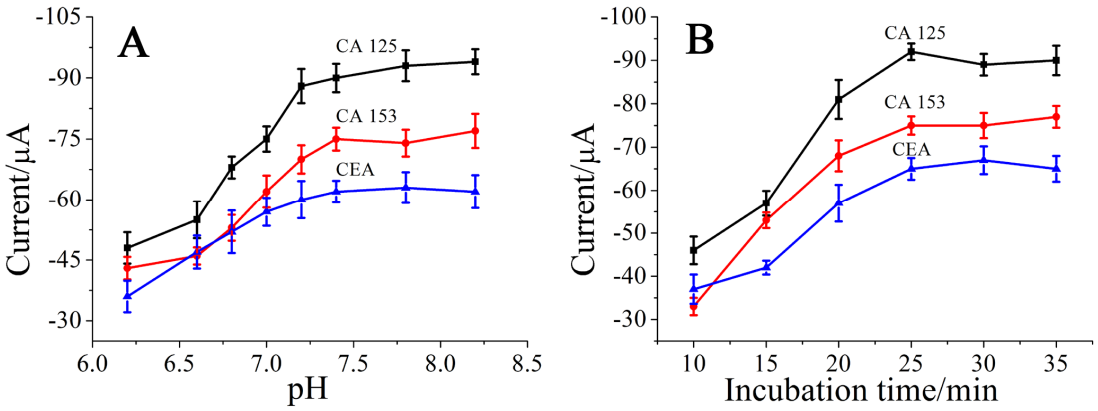

Figure S1 Effect of pH(A) and incubation time(B) on stripping current of Ag NPs in 1.0 M KCl for 10.0 U/mL CA 153, CA 125 and 10.0 ng/mL CEA.

Reference

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