Supplementary materials:

Hollow AuPt alloy nanoparticles as enhanced immunosensing platform for multiple analytes detection

Na Liu, Hongliang Han, Zhengnan Yuan, and Zhanfang Ma*

Experimental Section

1. Materials and reagents

Mouse anti human monoclonal antibody to carcinoembryonic antigen (anti-CEA) (Source: Mouse Ascites), mouse anti human alpha-fetoprotein monoclonal antibody (anti-AFP) (Source: Mouse Ascites), carcinoembryonic antigen (CEA) (Source: Human colon cancer), alpha-fetoprotein (AFP) (Source: Human fetal cord serum) and Human immunoglobulin G (IgG) (Source: Normal Human Serum) were purchased from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). graphene oxide (GO) and graphene were obtained from JCNANO (Nanjing, China). Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \bullet xH_2O$), vinylferrocene, dihydrogen hexachloroplatinate (IV) hexahydrate ($H_2PtCl_6 \bullet 6H_2O$), potassium tetrachloroplatinate (K_2PtCl_4), thionin acetate (Thi), D-(+)-glucose, ascorbic acid (AA) and uric acid (UA) were achieved from Alfa Aesar (Tianjin, China). Clinical human serum samples were provided by the capital normal university hospital (Beijing, China). Trisodium citrate, cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), NaH_2PO_4, Na_2HPO_4, KCl, KOH, C_2H_5OH, H_2O_2, H_2SO_4 and BSA were obtained from Beijing Chemical Reagents Company (Beijing, China). All the reagents were of analytical grade and used as received.

2. Apparatus

In all the procedures, the water used was purified through an Olst ultrapure K8 apparatus (Olst, Ltd., resistivity=18.2 MΩ cm^{-1}). Transmission electron microscopy (TEM) was performed with a JEOL-100CX electron microscope under 80 kV accelerating voltage. Scanning electron microscopy (SEM) images were obtained from a Hitachi SU8010 SEM. X-ray photoelectron spectroscopy (XPS) was conducted using an Escalab 250 X-ray Photoelectron Spectroscope (Thermofisher, American) employing a monochromatic Al Kα radiation. Electrochemical measurements were carried out on CHI-832 electrochemical workstation (Chenhua Instruments Co., Shanghai, China). High-resolution transmission electron microscope (HRTEM) images were obtained on a jeol-2011 electron microscope. Electrochemical impedance spectroscopy (EIS) was measured by Advanced Electrochemical System of PARSTAT 2273 (Princeton Co. USA). A three-electrode system was used in the experiment with a glassy carbon electrode (4 mm in diameter) as the working electrode, an Ag/AgCl electrode (saturated KCl) and a Pt wire electrode as reference electrode and counter-electrode, respectively. Supersonic cleaner was purchased from Kunshan Ultrasonic Instruments Co., Ltd. High speed centrifuge was purchased from Shanghai Anting Scientific Instrument Factory.

3. Synthesis of Au@Pt nanoparticles (Au@Pt NPs)

Au@Pt nanoparticles were synthesized according to literature reports.[1] In brief, 1.5 mL 1% trisodium citrate dehydrate was quickly added to 25 mL 0.01% boiling $HAuCl_4$ under vigorous stirring. The color changed from pale yellow to deep red. Boiling was continued for 15 min and then stirring until the sample had cooled to room temperature. Then, 25 mL water was added and heated to 100 °C, and 2.5 mL of H_2PtCl_6 was added under stirring. Afterwards, 2 mL of 100 mM AA was dropped into the solution in 2 min, and the reaction mixture was heated for 30 min to yield the Au@Pt nanoparticles.

4. Synthesis of hollow Pt nanoparticles (hPt NPs)

The hollow Pt nanoparticles were synthesized following the previous report with a little modification.[2] Briefly, 20 mg trisodium citrate and 8.5 mg $CoCl_2 \cdot 6H_2O$ were dissolved in 50 mL water with vigorous mechanical stirring at room temperature. 10 mL $NaBH_4$ (1 mg mL^{-1}) was dropwise added into the solution. 2 Mm, 4 mL of K_2PtCl_4 aqueous solution was then mixed with the above solution under rapid agitation. The resulting solution was then stirred for 1 hour. The products were centrifuged for 16000 rpm 15 min and washed with ultrapure water.

5. Preparation of Thi-rGO and Fc-rGO

Thionine reduced graphene oxide (Thi-rGO) was synthesized according to our previous method with a little modification.[3] Briefly, 0.2 g thionine was added into 50 mL of GO homogeneous dispersion in water (0.5 mg mL^{-1}). Then, 50 mg KOH was added into the above turbid mixture and then the mixture was subjected to ultrasonication for 30 min. After the ultrasonication, the turbid mixture was transformed into a homogeneous solution. Finally, the homogeneous solution was vigorously stirred at 80 °C for 24 h. The resulting Thi-rGO was subsequently centrifuged, washed with ethanol and ultrapure water, and dispersed in ultrapure water (1 mg mL^{-1}). The thiol functionalized ferrocene reduced GO (Fc-rGO) was prepared by the same method under the absence of KOH.

6. Preparation of nanocomposite

hAuPt NPs decorated Thi-rGO nanocomposite (hAuPt-Thi-rGO) was synthesized by simply blending in an appropriate ratio of Thi-rGO and hAuPt NPs for 2 h. Then, the resulting solution was centrifuged at 4000 rpm, and then washed and dispersed in ultrapure water (1 mg mL^{-1}). The hAuPt NPs decorated Fc-rGO nanocomposite (hAuPt-Fc-rGO) was synthesized using the same method. The hPt NPs (or Au@Pt NPs) decorated Thi-rGO (or Fc-rGO) nanocomposites (abbreviation as hPt-Thi-rGO, Au@Pt-Thi-rGO, hPt-Fc-rGO, and Au@Pt-Fc-rGO, respectively) were prepared by the same method.

7. Preparation of immunosensing probes

The immunosensing probes were fabricated by immobilizing labeled anti-CEA onto hAuPt-Thi-rGO, and labeled anti-AFP onto hAuPt-Fc-rGO, respectively. In brief, the obtained hAuPt-Thi-rGO was dispersed in 1 mL 0.01 M phosphate buffer (pH 7.3). Subsequently, labeled anti-CEA (100 μL, 1 mg mL^{-1}) was added into the dispersion and gently mixed for 12 h at room temperature. After centrifugation, the obtained anti-CEA-hAuPt-Thi-rGO was blocked with 1% BSA solution for 2 h to avoid any nonspecific absorption. After centrifuged and washed several times, the obtained anti-CEA-hAuPt-Thi-rGO was re-dispersed in 1 mL 0.01 M phosphate buffers (pH 7.3) and stored at 4 °C until use. The hAuPt-Fc-rGO nanocomposite was labeled anti-AFP with the same procedure as preparing anti-CEA-hAuPt-Thi-rGO mentioned above. The obtained anti-AFP-hAuPt-Fc-rGO was dispersed in 1 mL 0.01 M phosphate buffers (pH 7.3) and stored at 4 °C until use. The immunosensing probes of anti-CEA-hPt-Thi-rGO, anti-AFP-hPt-Fc-rGO, anti-CEA-Au@Pt-Thi-rGO, anti-AFP-Au@Pt -Fc-rGO were fabricated by the same method.

8. Preparation of immunosensing substrate

The Co_3O_4/graphene nanocomposite was synthesized following the previous report with a little modification.[4] 0.5 mM $CoCl_2 \cdot 6H_2O$ and 0.5 mM urea were added to 10 mL of water and stirred for 10 min. The mixture was then transferred to a Teflon-lined stainless steel autoclave of 30 mL capacity. Subsequently, 2 mL 1 mg mL^{-1} graphene was added into the solution. This was followed by autoclaving at 120 °C for 16 h. After cooling to room temperature, the Co_3O_4/graphene nanocomposite was washed with deionized water and dried at 50 °C. Then, the obtained Co_3O_4/graphene was dispersed in chitosan solution (2 mg mL^{-1}) by 30 min ultrasonication and 2 h vigorous stirring. The chitosan coated Co_3O_4/graphene was stored at 4 °C until use.

9. Preparation of the immunosensor

Prior to the experiment, the GCE was carefully polished with 1.0, 0.3, and 0.05 μm alumina powder separately, followed by successive sonication in ethanol and distilled water for 5 min and dried in nitrogen. After that, 20 μL of the prepared chitosan coated Co_3O_4/graphene was dropped

onto GCE and then dried in air. Next, 10 μL 1% glutaraldehyde solution and 20 μL antibody mixture solution contained 400 ng mL^{-1} anti-CEA and anti-AFP were dropped onto the electrode, then was kept at 4 °C overnight to immobilize antibodies. Subsequently, excess glutaraldehyde solution and antibodies were washed away with 0.01 M phosphate buffer (pH 7.3), and 1% BSA solution was applied to the electrode and incubated for 1 h at room temperature to block possible remaining active sites against nonspecific adsorption. After another washing with 0.01 M phosphate buffer (pH 7.3), the resulting immunosensor was obtained and stored at 4 °C until use.

10. Simultaneous measurement procedure

Based on the typical procedure of sandwich-type immunoreactions, the prepared immunosensor was first incubated with a sequence of standard solutions of CEA and AFP with different concentrations at 37 °C for 40 min, and then it was incubated with the mixture of 1:1 diluted anti-CEA-hAuPt-Thi-rGO and anti-AFP-hAuPt-Fc-rGO. After every step, the electrodes were washed with 0.01 M phosphate buffer (pH 7.3). Subsequently, SWV was performed to record the electrochemical responses for simultaneous measurement of CEA and AFP in 0.1 M pH 7.0 phosphate buffer solution (PBS), containing 5 mM H_2O_2 and 5 mM glucose. The SWV measurement was taken from -0.6 V to 1 V (vs Ag/AgCl) with a frequency of 15 Hz and a pulse amplitude of 25 mV.

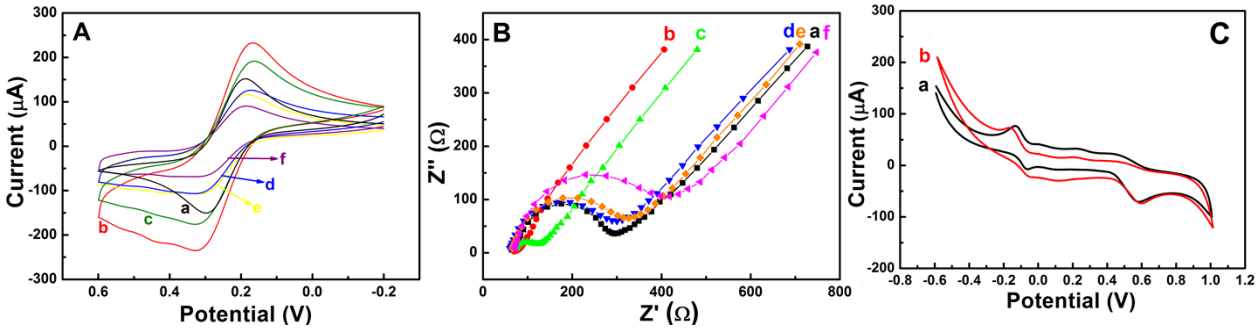

Fig. S1. CV (A) and EIS (B) characterization of the modified procedure of electrodes in PBS-$[Fe(CN)_6]^{4-/3-}$ (pH 7.0): (a) bare GCE, (b) chitosan coated Co_3O_4/graphene modified GCE, (c) 1% glutaraldehyde activated chitosan coated Co_3O_4/graphene modified GCE, (d) anti-CEA and anti-AFP modified electrode, (e) blocked with 1% BSA, and (f) modified GCE electrode after incubation with 3 ng mL^{-1} CEA and AFP. (C) CV measurements using hAuPt-Thi/Fc-rGO as signal probes in 0.1 M PBS without (a) and with (b) 5 mM H_2O_2 and 5 mM glucose.

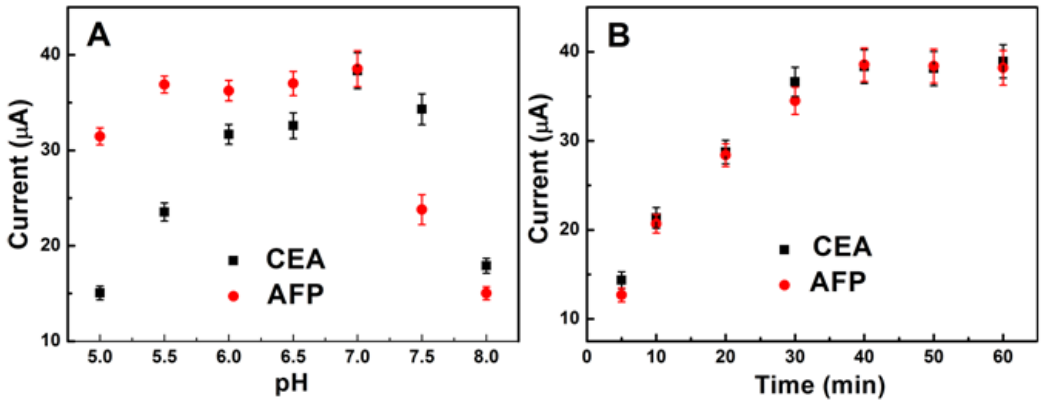

Fig. S2. Effect of pH of detection solution (A) and incubation time (B) on SWV responses to 3 ng mL^{-1} CEA and AFP at the immunosensor array.

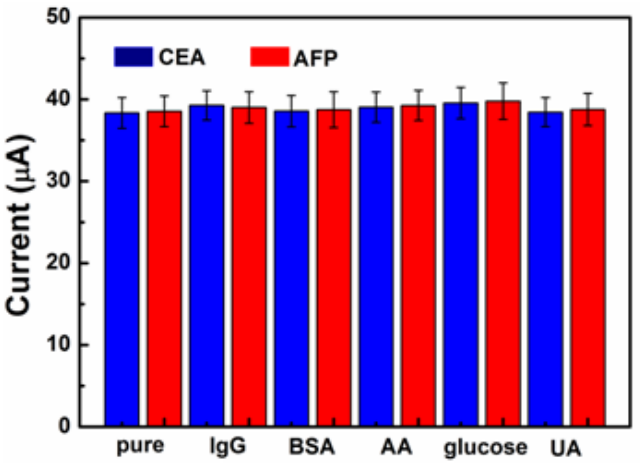

Fig. S3. Current response of the immunosensor to 3 ng mL^{-1} CEA and AFP, 3 ng mL^{-1} CEA and AFP+100 ng mL^{-1} IgG, 3 ng mL^{-1} CEA and AFP+100 ng mL^{-1} BSA, 3 ng mL^{-1} CEA and AFP+100 ng mL^{-1} AA, 3 ng mL^{-1} CEA and AFP+100 ng mL^{-1} glucose, 3 ng mL^{-1} CEA and AFP+100 ng mL^{-1} UA.

Table S1. Interference degree or cross-talk level.

sample type	concentration (ng mL^{-1})	current shift at CEA position (μA)[a, b]	current shift at AFP position (μA)[a, c]
CEA	0.3	23.53	0.10
	30	56.17	0.13
AFP	0.3	0.09	21.12
	30	0.12	56.03
CEA +AFP	0.3+0.3	23.51	21.06
	30+30	55.89	55.79

[a] The average value of three measurements in PBS, pH 7.0, containing 5 mM H_2O_2 and glucose.

[b] The SWV peak current was ~ 0.617 μA for zero CEA analyte.

[c] The SWV peak current was ~ 0.623 μA for zero AFP analyte.

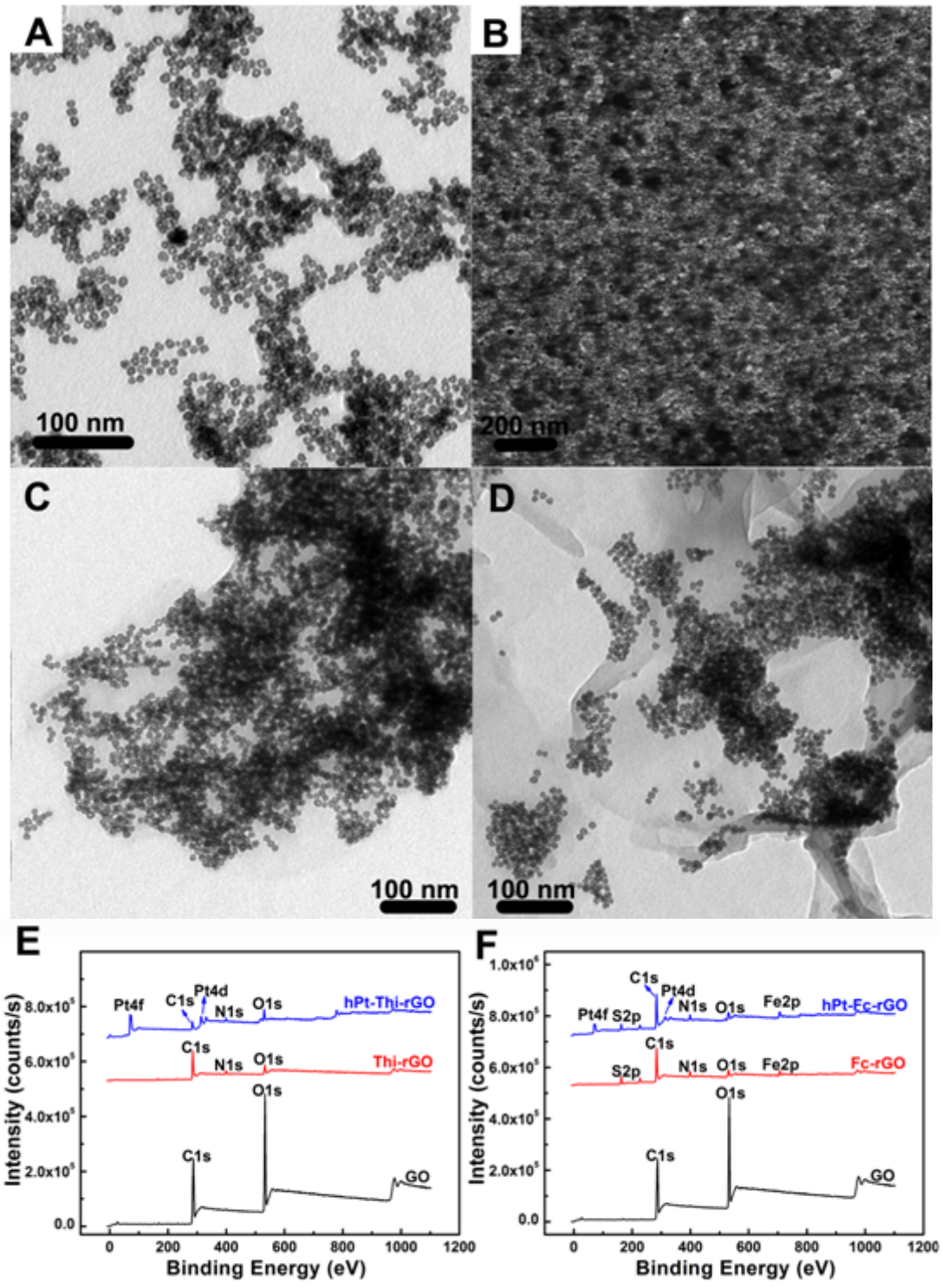

Fig. S4. Typical TEM (A) and SEM (B) images of hPt NPs, TEM images of (C) hPt-Thi-rGO and (D) hPt-Fc-rGO, survey XPS spectra of (E) GO, Thi-rGO, and hPt-Thi-rGO and (F) GO, Fc-rGO, and hPt-Fc-rGO.

The hollow Pt nanoparticles (hPt NPs) were fabricated by a one-step galvanic displacement between Co nanoparticles and K_2PtCl_4. From the TEM image (Fig. S4A), the shallow cores can

be obviously observed, demonstrating the formation of the hollow interior structures. The monodispersed hPt NPs with a diameter of approximately 10 nm were shown in Fig. S4B. The hPt-Thi-rGO and hPt-Fc-rGO nanocomposites were fabricated by self-assembling the hPt NPs and Thi-rGO or Fc-rGO via electrostatic interaction. Morphologies of the synthesized hPt-Thi-rGO and hPt-Fc-rGO nanocomposites were characterized by TEM (Fig. S4C and S4D). It was clearly observed that the hPt NPs were absorbed on the surface of graphene. XPS measurements were employed to further analyze the chemical composition of the hPt-Thi-rGO and hPt-Fc-rGO. The fully scanned spectra demonstrated the existence of C and O elements in GO and N elements in the Thi-rGO. The characteristic peaks for Pt 4f, C 1s, N 1s, and O 1s core level regions could be obviously observed at the hPt-Thi-rGO nanocomposite in Fig. S4E. The N1s core level was mainly derived from the thionine and the Pt 4f (71.2eV and 74.5eV) doublet indicated the presence of Pt^0. Fig. S4F showed the Pt 4f, C 1s, N 1s, S 2p, O 1s and Fe 2p core level regions of the hPt-Fc-rGO nanocomposite. It could be found that the Fe 2p core level region (710 eV and 727 eV) originated from ferrocene.

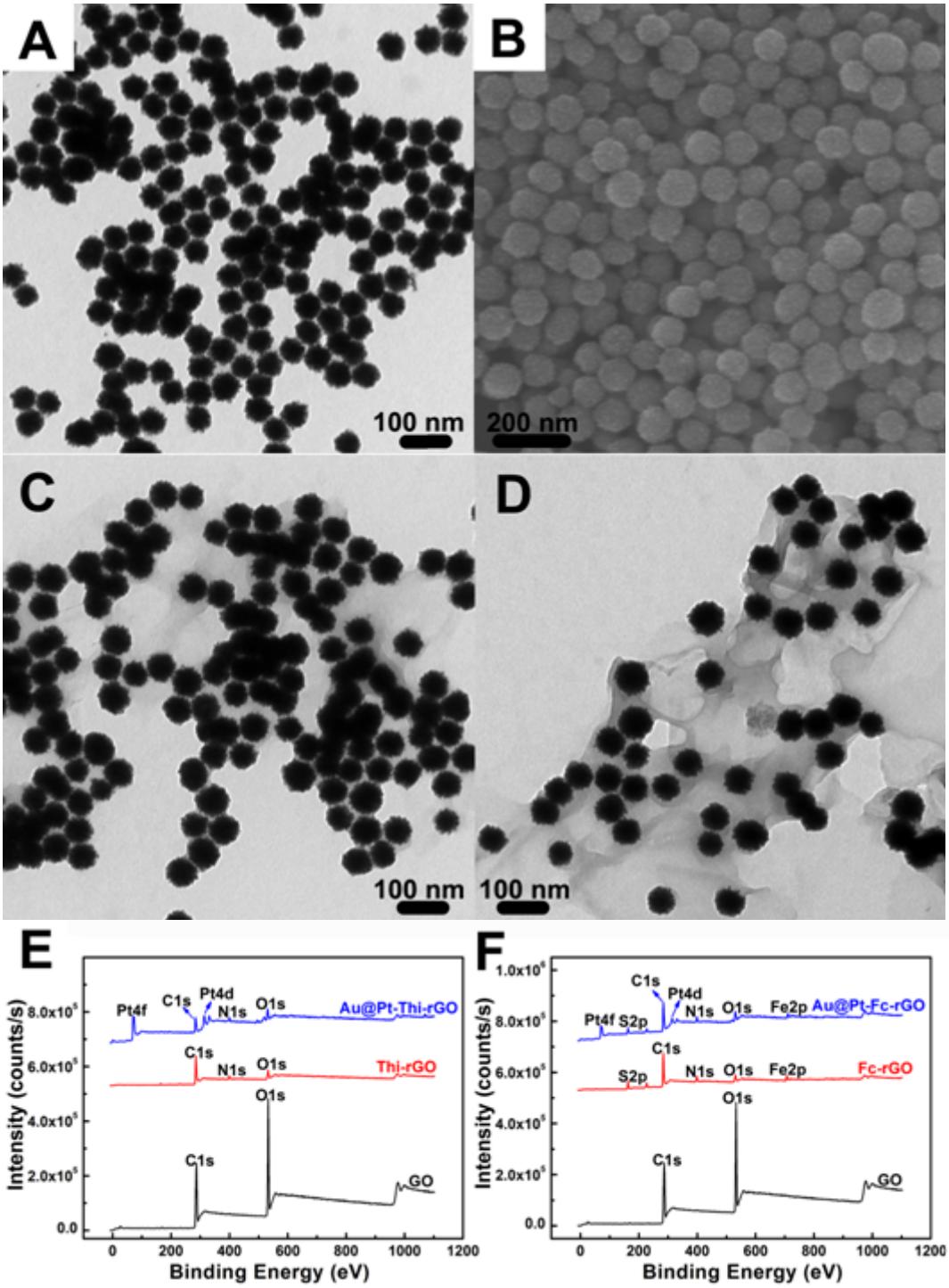

Fig. S5. Typical TEM (A) and SEM (B) images of Au@Pt NPs, TEM images of (C) Au@Pt-Thi-rGO and (D) Au@Pt-Fc-rGO, survey XPS spectra of (E) GO, Thi-rGO, and Au@Pt-Thi-rGO and (F) GO, Fc-rGO, and Au@Pt-Fc-rGO.

The Au@Pt nanoparticles (Au@Pt NPs) were synthesized according to the literature.[5] The prepared Au@Pt NPs were about 50 nm and urchin-like morphology (Fig. S5A and S5B).

Morphologies of the Au@Pt-Thi-rGO and Au@Pt-Fc-rGO nanocomposites were characterized by TEM (Fig. S5C and S5D). It can be observed that the Au@Pt NPs were absorbed on the surface of graphene. XPS data of the Au@Pt NPs-Thi-rGO or Au@Pt NPs -Fc-rGO composites was shown in Fig. S5E and S5F.

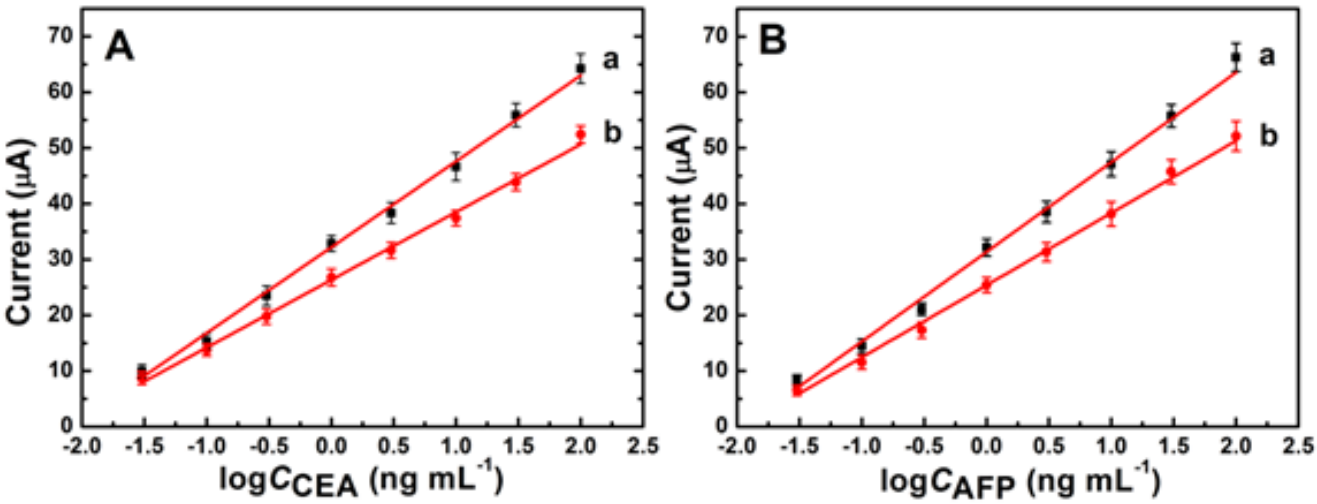

Fig. S6. Electrochemical responses of the multiplexed immunoassay with two types of immunosensing substrates, (a) chitosan coated Co_3O_4/graphene and (b) chitosan coated graphene toward various concentrations of (A) CEA and (B) AFP standards, using hAuPt-Thi-rGO and hAuPt-Fc-rGO as immunosensing probes.

References

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