Supporting Information

Ultrasensitive Immunoassay Based on Pseudobienzyme Amplifying System of Choline Oxidase and Luminol-Reduced Pt@Au Hybrid Nanoflowers

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Preparation of luminol-AuNFs

Briefly, a seed solution was prepared by mixing 5 mL of CTAB (0.2 M) and 100 μL of $HAuCl_4$ (1%, wt) with 0.6 mL of freshly prepared 10 mM ice-cold $NaBH_4$ solution. The color of the solution changed from dark yellow to brownish yellow under vigorous stirring, indicating the formation of the seed solution. For the synthesis of AuNFs, 1.0 mL of luminol stock solution was mixed with 100 μL $HAuCl_4$ stock solution and added to 50 mL of 0.2 M CTAB solution. After adding, the mixture was heated to boiling. The solution was maintained at the boiling point for 30 min, during which time a color change from pale yellow to dark brown was observed before a blue color was reached. While this mixture was continuously stirred, 120 μL of the seed solution was added to initiate the growth of the gold nanoflowers. These luminol-AuNFs were aged for 20 h to ensure full growth. The obtained luminol-AuNFs were centrifugally washed extensively with doubly distilled water for

three times, and then it was dispersed in 2 mL 0.1 M PBS.

Preparation of luminol-Pt@Au hybrid nanoflowers

In a typical experiment, the AuNFs solutions (4 mL) were mixed with 2 mL of 0.2 M CTAB aqueous solution containing 1.0 mL of luminol stock solution. Then the solutions were stirred at 30ºC, followed by adding 10 μL volumes of H_2PtCl_6 stock solution. The mixture was then stirred in a 30ºC water bath for 14 h. Thus luminol-Pt@Au hybrid nanoflowers were obtained based on the above method.

Preparation of Ab_2/luminol-Pt@AuNFs/ChOx nanocomposites

The Ab_2/luminol-Pt@AuNFs/ChOx nanocomposites were synthesized according to the following steps (Fig. 1A). Firstly, the anti-cTnI was connected with luminol-Pt@AuNFs by slowly adding 0.2 mL anti-cTnI to 1 mL prepared luminol-Pt@AuNFs suspension under softly stirring and incubated for 6 h at 4ºC, followed by centrifugation at 9000 rpm for 15 min at 4ºC to discard excess reagents. Subsequently, 1 mL of ChOx (14 U mL^{-1}) was implemented to the residual complexes under gently stirring for about 4 h at 4ºC to block the remaining active sites of the luminol-Pt@AuNFs surface. At last, the Ab_2/luminol-Pt@AuNFs/ChOx nanocomposites were collected by centrifugation and redispersed in 1 mL PBS (pH 7.4) and then stored at 4ºC when not use.

Synthesis of MnO_2@MWNTs nanocomposites

As previously reported with slight modifications [1], 0.1 g MWNTs were dispersed into an aqueous solution of $KMnO_4$ (0.01 M). Then the mixture was subjected to

ultrasound at room temperature for 8 h, in the form of 100 kHz ultrasonic waves at 600 W output power. A bath-type ultrasound washer was used to generate ultrasound. Finally the obtained MnO_2@MWNTs were centrifuged three times and dispersed in 4 mL double distilled water and then dried at 120ºC for 10 h.

1 M. F. Wang, G. F. Wang, W. Zhang, F. Bin, Electroanalysis, 2010, 22, 1123 – 1129

Fabrication of the ECL immunosensor

To obtain a mirror like surface, the GCE was first polished, respectively with 0.3, 0.05 mm alumina powder, followed by washing thoroughly with distilled water and successively sonicating in ethanol, distilled water, respectively. Before modification, the GCE was dried with nitrogen at room temperature.

After that, 8 μL of MnO_2@MWNTs suspension soulation was firstly dropped onto the pretreated GCE. After drying at room temperature, 15 μL of anti-cTnI (Ab_1) was dropped onto the surface of the MnO_2@MWNTs with 8 h incubation at 4ºC. The immunosensor was washed with double distilled water to remove the physical adsorbed Ab_1 and then 15 μL of 5% BSA solutions was placed onto the electrode for 30 min at room temperature to block the non-specific binding sites, followed by washing with double distilled water. Ultimately, the obtained immuneosensor was stored at 4ºC when not in use. The process for the fabrication of the immunosensor was shown in Scheme 1.

Measurement procedure

The measurement was based on a sandwich immunoassay method. Before

measurement, the immunosensor was incubated with different cTnI standard solutions at 37ºC for 30 min. Next, the modified electrode was incubated with Ab_2 bioconjugates for 30 min at 37ºC. Finally, the resultant immunosensor was investigated with a MPI-A electrochemiluminescence analyzer in 3 mL PBS (pH 7.4) containing 2 mM choline at room temperature. The voltage of the photo-multiplier tube (PMT) was set at 800 V and the applied potential was 0.2 - 0.8 V (vs. Ag/AgCl) with a scan rate of 100 mV/s in the process of detection. With the increasing cTnI antigen concentration, the amount of Ab_2/luminol-Pt@AuNFs/ChOX increased, which implied that the ECL signals of luminol enhanced. Therefore, the changes of ECL intensity directly reflected the concentration changes of cTnI.

The UV absorption spectroscopy of luminol-Pt@AuNFs

The UV absorption spectroscopy was used to examine the successful synthesis of luminol-Pt@AuNFs. Compared the UV-visible absorption spectra of luminol-Pt@AuNFs with luminol (Fig. S1), The absorption spectrum of luminol-Pt@AuNFs at 225, 368 and 385 nm slightly red-shifted compared to luminol at 220, 307 and 348 nm, which could be attributed to the quantum size effect of Pt@AuNFs, [2] thus indicating luminol was capped on Pt@AuNFs. Those changes proved that the luminol-Pt@AuNFs was successfully prepared.

2 Liu, Y.; Tolentino, J.; Gibbs, M.; Ihly, R.; Perkins, C. L., Nano Lett., 2013, 13, 1578

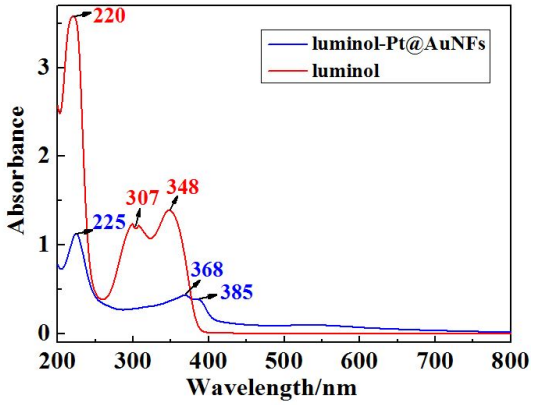

Fig. S1. UV-visible absorption spectrum of luminol-Pt@AuNFs (blue line) and luminol (red line).

X-ray photoelectron spectroscopy

In order to further prove the successful synthesis of luminol-AuNFs (Fig. S2) and luminol-Pt@AuNFs (Fig. S3), X-ray photoelectron spectroscopy (XPS) was used for elemental analysis. As expected, characteristic peaks for O1s, N1s, C1s and Au4f core-level regions could clearly be observed in the obtained luminol-AuNFs spectrum (Fig. S2A). The peaks at 537.68 eV, 405.98 eV, 292.73 eV, and 93.58 eV could be respectively assigned to O1s, N1s, C1s, and Au4f (Fig. S2B, C, D, and E, respectively). Then, characteristic peaks for O1s, N1s, C1s, Au4f and Pt4f core-level regions could clearly be observed in the obtained luminol-Pt@AuNFs spectrum (Fig. S3A). The peaks at 536.23 eV, 405.48 eV, 291.33 eV, 92.63 and 92.28 eV could be respectively assigned to O1s, N1s, C1s, and Au4f (Fig. S3B, C, D, E, and F, respectively). Based on the elemental analysis above, we could confirm that the luminol-AuNFs and luminol-Pt@AuNFs were successfully prepared.

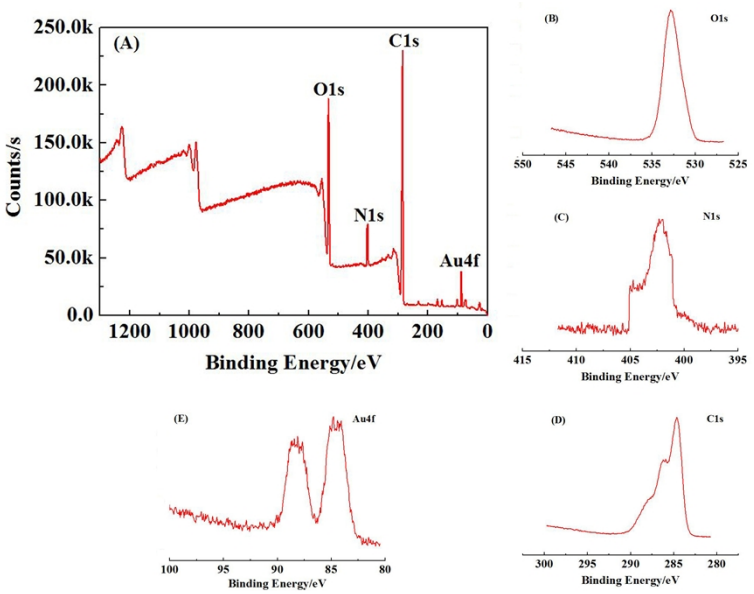

Fig. S2. XPS analysis for A) the full region of XPS for luminol-AuNFs, B) O1s region, C) N1s region, D) C1s region, E) Au4f region

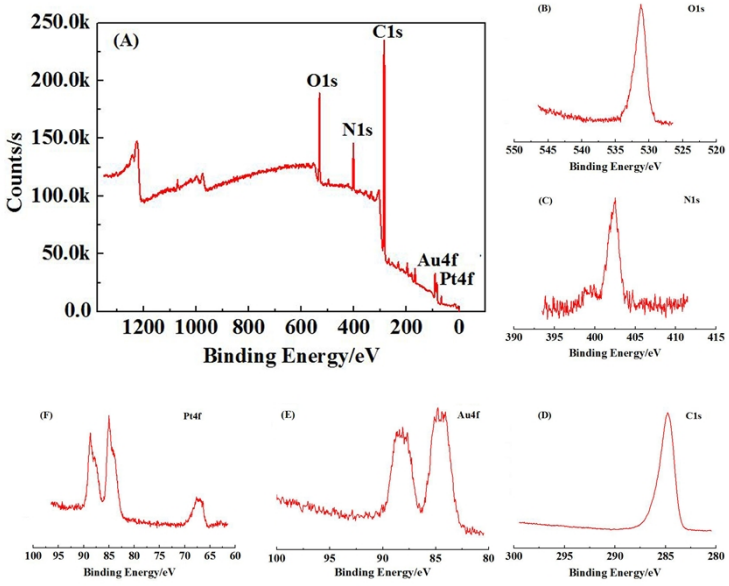

Fig. S3. XPS analysis for A) the full region of XPS for luminol-Pt@AuNFs, B) O1s region, C) N1s region, D) C1s region, E) Au4f region, F) Pt4f region

Transmission electron microscopy

As shown in Fig. S4, the morphological changes in the synthetic process of nanomaterials were monitored using transmission electron microscopy (TEM). The AuNFs were seen to be flower-like nanoparticles with a diameter of 45 ± 5 nm (Fig. S4A). Because Pt nanoparticles grew on the AuNFs surface, Pt@AuNFs have a larger

diameter of 110 ± 10 nm (Fig. S4B).

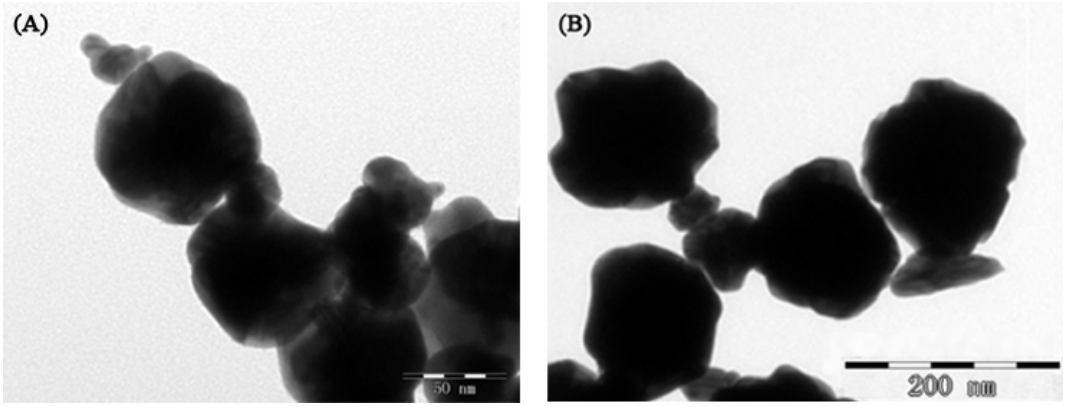

Fig. S4. TEM images of A) luminol-AuNFs, B) luminol-Pt@AuNFs.

The electrochemical characterization of the proposed immunosensor

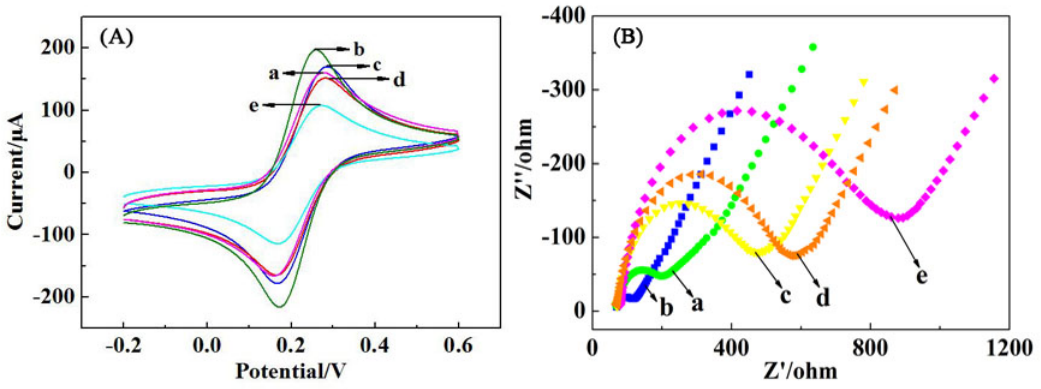

Fig. S5. CVs **(A)** and EIS **(B)** of different modified electrodes at pH 7.4 PBS containing 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) as redox probe: (a) bare GCE, (b) MnO_2@MWNTs/GCE, (c) anti-cTnI/MnO_2@MWNTs/GCE, (d) BSA/anti-cTnI/MnO_2@MWNTs/GCE, (e) cTnI/BSA/anti-cTnI/MnO_2@MWNTs/GCE. Scanning of CVs was from - 0.2 to 0.6 V with a rate of 100 mV/s and all potentials are given against SCE.

Optimization of the concentration of choline

The concentration of choline as the most important factor that influenced the performance of the immunosensor had been discussed. As shown in Fig. S6, the ECL intensity enhanced with the increasing of the concentration of choline. However,

when the concentration of choline reached 2 mM, the ECL intensity increased slowly and then reached a constant value. Thus, 2 mM was chosen as the optimal concentration of choline in this experiment.

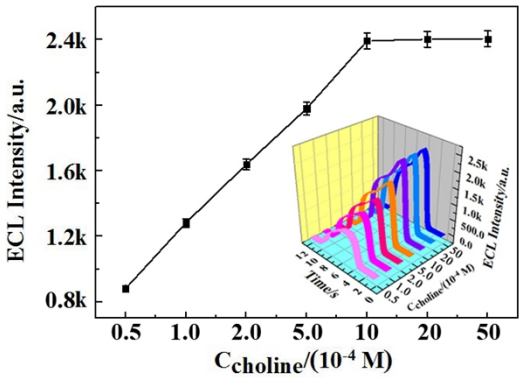

Fig. S6. Optimization of the concentration of choline on the immunosensor.

The ECL characterization of the amplified strategy

By comparing detection limit and liner range, we also prove the superiority of the proposed immunosensor (Ab_2/luminol-Pt@AuNFs/ChOx), 4 sets of Ab_2 labeled probes were prepared, including (1) Ab_2/luminol-AuNFs/ChOx, (2) Ab_2/luminol-PtNFs/ChOx, (3) Ab_2/Pt@luminol-AuNFs/ChOx (in which ascorbic acid displace luminol as reducer to product Pt nanoparticles) and (4) Ab_2/luminol-Pt@AuNFs.

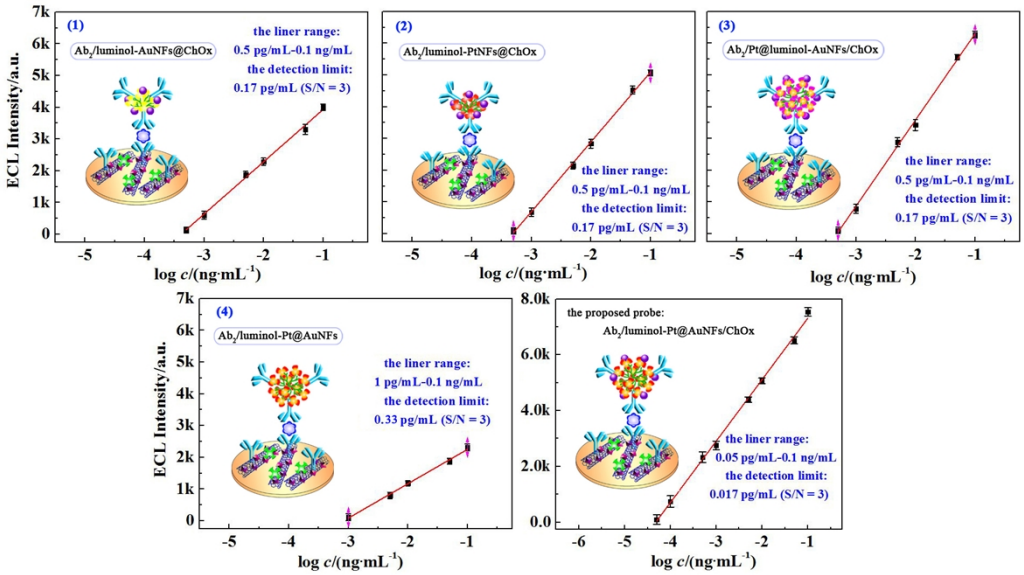

Fig. S7. ECL-concentration profiles of the different sandwich format immunosensor: (1) Ab_2/luminol-AuNFs/ChOx labeled immunosensor, (2) Ab_2/luminol-PtNFs/ChOx labeled immunosensor, (3) Ab_2/Pt@luminol-AuNFs/ChOx labeled immunosensor, (4) Ab_2/luminol-Pt@AuNFs labeled immunosensor and the proposed immunosensor: Ab_2/luminol-Pt@AuNFs/ChOx labeled immunosensor. The concentration of cTnI was 0.1 ng/mL. Working solution, PBS (pH 7.4) containing 2 mM ChOx. Scan rate was 100 mV/s.

It could be found that the proposed immunosensor exhibited low detection limit and wide liner range by comparing the calibration curves. In summary, the results adequately indicated that the proposed probe of Ab_2/luminol-Pt@AuNFs/ChOx could be effectively utilized for ultrasensitive detection of cTnI with the amplification of the ECL signal.

Table S1. Comparison of our research with other methods for cTnI detection.

Measurement protocol	Linear range / (pg·mL^{-1})	Detection limit / (pg·mL^{-1})	References
Electrochemical immunoassay	800-5000	500	Guo et al.,2005
Optomagnetic biosensor	30-6500	30	Dittmer et al.,2010
ELISA[a]	100-10000	27	Cho et al.,2009
Electrochemiluminescence	2.5-10000	2	Shen et al.,2011
Electrochemiluminescence	0.25-100	0.083	Present work

[a] enzyme-linked immunosorbent assay.

Table S2. Preliminary analysis of real samples.

Sample number	Added / (ng·mL^{-1})	Found / (ng·mL^{-1})	Recovery / %
1	0.0500	0.0520	104
2	0.0400	0.0390	97.5
3	0.0300	0.0315	105
4	0.0200	0.0218	90.0
5	0.0100	0.0107	107
6	0.00500	0.00456	91.2