# "Two-in-One" core-shell nanozyme probes with double signal amplification for high-performing surface plasmon resonance immunosensing

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## Experimental

**Materials and Reagents.** The HIgG, rabbit anti-HIgG and goat anti-HIgG was purchased from Bioss Antibodies Biotechnology Co., Ltd. (Beijing). Staphylococcal protein A (SPA), bovine serum albumin (BSA), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), aniline and 11-Mercaptoundecanoic acid (MUA) were purchased from Sigma-Aldrich. 3,3',5,5'-tetramethylbenzidine (TMB) was purchased from America Acros. Silver nitrate ($AgNO_3$), ascorbic acid (AA), trisodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), tetrachloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$), hydrogen peroxide ($H_2O_2$), hydroxylamine hydrochloride ($NH_2OH \cdot 2HCl$), disodium phosphate ($Na_2HPO_4$), potassium dihydrogen phosphate ($KH_2PO_4$), sodium chloride (NaCl) and potassium chloride (KCl) were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai). All the biological reagents prepared were dissolved or diluted using phosphate buffer solution (PBS) and stored in refrigerator at 4 °C before measurements. The PBS was prepared by dissolving 1.37 mM NaCl, 2.7 mM KCl, 8.72 mM $Na_2HPO_4$ and 1.42 mM $KH_2PO_4$ in ultra-pure water and its pH was 7.4. All other reagents were of analytical

grade and ultra-pure water were used in the experiments.

**Apparatus.** SPR measurements were carried out on a SPR Navi 210A Surface Plasmon Resonance (Bio Navis Co., Finland). All measurements were performed at (25 ± 1) °C under stagnant. Transmission electron micrographs (TEM) was obtained via utilizing a JEM-2100 Emission transmission electron microscope (Japan) at an acceleration voltage of 15 kV. Ultraviolet-visible (UV-vis) experiments were completed with a UV2500 spectrophotometer (Beijing General General Instrument Co., Ltd). D8 Advance polycrystalline X-ray diffractometer (XRD, Bruker AXS, Germany) was utilized to measure X-ray diffractometer spectrum. Scanning electron micrographs (SEM) was observed via using a Zeiss_Supra55 scanning electron microscope (Carl Zeiss AG, Germany) at an acceleration voltage of 15 kV. Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker ARX-400 MHz.

**Synthesis of Ag@Au core-shell nanoparticles (Ag@Au NPs).** The Ag@Au core-shell nanoparticles were synthesized following the procedure according to the literature[S1] with modification. First, Ag nanoparticles were prepared via hydrothermal method. 100 μL of ascorbic acid was added into 46.6 mL of boiling ultra-pure water under stirring. The above mixture was kept boiling for 1.0 min and followed by the addition of the prepared mixture containing 30 μL of NaCl (0.1 M), 2.4 mL of $AgNO_3$ (0.02 M) and 1.0 mL of $C_6H_5Na_3O_7 \cdot 2H_2O$ (34.0 mM). Then, the resulting reaction solution was stirred and heated to boil for 1.0 h. After cooling to room temperature, the obtained product was centrifuged at 11000 rpm and washed several times with water, which was dispersed in ultra-pure water to form Ag nanoparticles hydrosol for use.

Next, 0.1 mL $NH_2OH$ (0.02 M) and 100 μL $HAuCl_4$ (1 wt%) were injected to the Ag nanoparticles hydrosol which was ultrasonicated for 30 min. Thereby the mixture was heated and refluxed for 30 min, and then allowed to cool to room temperature. The obtained Ag@Au core-shell nanoparticles were separated by centrifugation with a rotating speed of 11000 rpm. The eventual product should be dispersed in ultra-pure water. Then 30 μL $H_2O_2$ was dropped slowly into Ag@Au core-shell nanoparticles dispersion under stirring to etch the Au shell, followed by centrifugation and washing with ultra-pure water to obtain porous Ag@Au core-shell nanoparticles.

**Preparation of signal amplification probe (Ag@Au-Ab₂).** In this work, the Goat

anti-HIgG, regarded as secondary antibody ($Ab_2$), was immobilized on Ag@Au core-shell nanoparticles to form signal amplification probe via the following steps. The Ag@Au NPs was dissolved in 2 mL of PBS (0.01 M) with ultrasonic treatment for 30 min to prepare uniform dispersion. Then, 200 μL MUA was injected into the mixture accompanied with stirring for 2 h to modify carboxyl on the surface of Ag@Au NPs. The principle about the modification of carboxyl group can be explained mainly by covalent attachment. 11-Mercaptoundecanoic acid (MUA), which contains one thiol and one carboxyl located at each end of carbon chain. Benefiting from interaction between Au nanomaterials and thiol, Ag@Au can interact with thiol of MUA to form Au-S bond, accompany with effective modification of carboxyl. Taking advantage of 100 μL EDC (10 mg/mL) and 70 μL NHS (10 mg/mL) to activate carboxyl to bind amino site of $Ab_2$, the Ag@Au-$Ab_2$ conjugates were obtained by adding 100 μL Goat anti-HIgG (1.0 mg/mL) into the above mixture under slow stirring at 4 ℃ for 6 h and centrifuged at high speed of 10000 rpm at 4 ℃. The resultant precipitate was redispersed in PBS and stored in refrigerator at 4 ℃ for next measurement.

**Fabrication of SPR immunosensor and immunoassay procedure.** $H_2O_2$ (30%), $NH_3 \cdot H_2O$, and ultrapure water were mixed in a volume ratio of 1:1:5 and boiled, and then the gold chips were placed in the boiling mixture for 10 min. Then, the treated gold chips were rinsed with the ultrapure water and ethanol followed by drying with nitrogen for use. The pre-treated gold chips were loaded into the surface plasmon resonance detection cell to start assembling the immunosensor. First of all, the channels were filled with 0.01 M PBS to balance the SPR response. In order to amplify the SPR response to mass change of the substance, a sandwich immunoassay was constructed in this work. As it is depicted in Scheme 1, SPA, Rabbit anti-HIgG, BSA, HIgG, Ag@Au-$Ab_2$, mixture of aniline and $H_2O_2$, all the above mentioned were consecutively added to construct the sandwich immunoassay and signal amplification, which was detected by SPR. SPA (100 μg/mL) was injected to functionalize the chips via the interact of Au-S bond for immobilizing Rabbit anti-HIgG ($Ab_1$, 100 μg/mL). The BSA (200 μg/mL) was syringed after the injection of $Ab_1$ to prevent nonspecific binding. Then, HIgG was injected to bind specifically with $Ab_1$, which is beneficial to incubated Ag@Au-$Ab_2$ to form signal magnification. In the light of the POD-like activity of the Ag@Au for

catalyzing the $H_2O_2$ to generate reactive oxygen species to induce the polymerization of aniline forming polyaniline on the surface of gold chips, the injection of mixture of aniline (30 mM) and $H_2O_2$ (5 mM) was performed to fabricate signal amplification again. It is noted that PBS was utilized to rinsed the unreacted substance after every injection. Additionally, the above-mentioned samples in this research used a flow rate and reaction time of 30 μL/min and 30 min, respectively.

**The characterizations of Ag@Au core-shell nanozyme.**



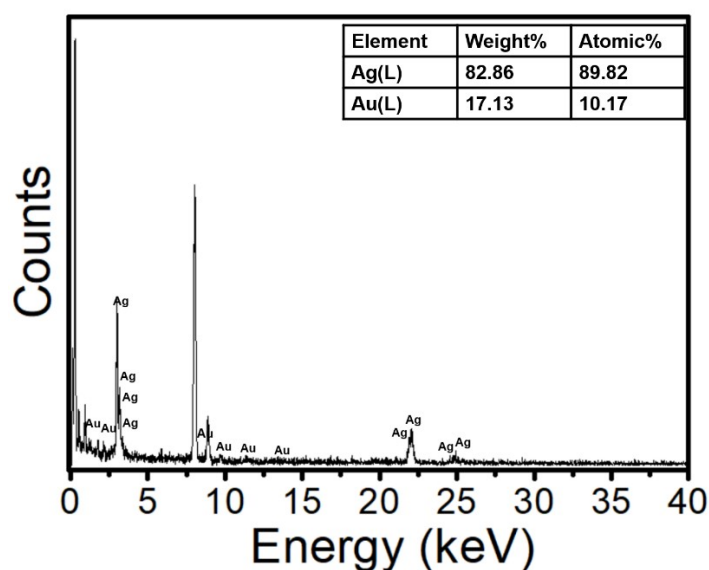| Element | Weight% | Atomic% |
| --- | --- | --- |
| Ag(L) | 82.86 | 89.82 |
| Au(L) | 17.13 | 10.17 |

**Fig. S1.** EDX spectra and quantification table of etched Ag@Au core-shell nanoparticles.

**Verifying formation of polyaniline catalyzed by Ag@Au nanozyme**

In order to verify the formation of polyaniline, SEM images of bare Au chip and polyaniline formed on the Au chip surface were studied. Additionally, NMR-spectra of aniline and polymerization of aniline on the Au chip surface were recorded on Bruker ARX-400 MHz to further confirm the generation of polyaniline.

Figure S2A shows SEM image of Au chip, and a smooth surface was observed. After catalysis polymerization of aniline resulted by nanozyme probe, an obvious fiber-shaped polyaniline was observed from SEM image of polyaniline formed on gold chip. As depicted in Figure S3, the NMR-spectra can be analyzed as following: (A) 1H NMR (400 MHz, Chloroform-d) δ 7.23-6.40 (m, 5H), 3.56 (s, 2H), (B) 1H NMR (400 MHz, Chloroform-d) δ 7.23-6.56 (m, 6H), 4.85-4.62 (m, 1H), 3.63 (s, 2H). As shown in

Figure S3A, 1H NMR of aniline, 5H presents within the chemical shift (δ) range of 7.23-6.40, which can be attributed to hydrogen on benzene ring. Besides, 2H exists at chemical shift of 3.56 ascribed to hydrogen of amino group. Comparison with 1H NMR of aniline, 6H appears in the chemical shift range of 7.23-6.56 accompanying with 1H in the range of 4.85-4.62 and 2H at δ 3.63 (Figure S3B). The excess hydrogen can be attributed to the polymerization of aniline. All the experiment results can confirm the formation of polyaniline on the surface of gold chip.
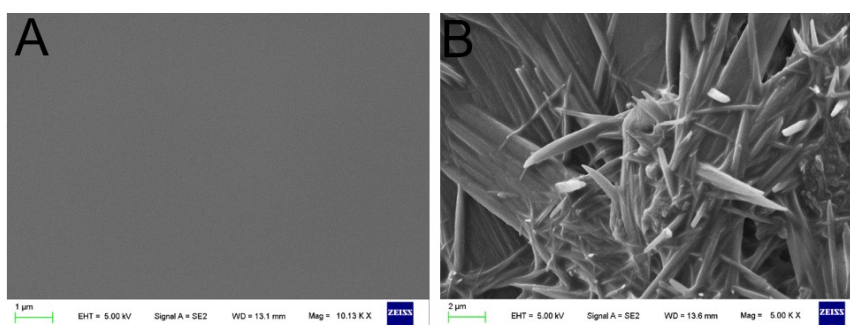


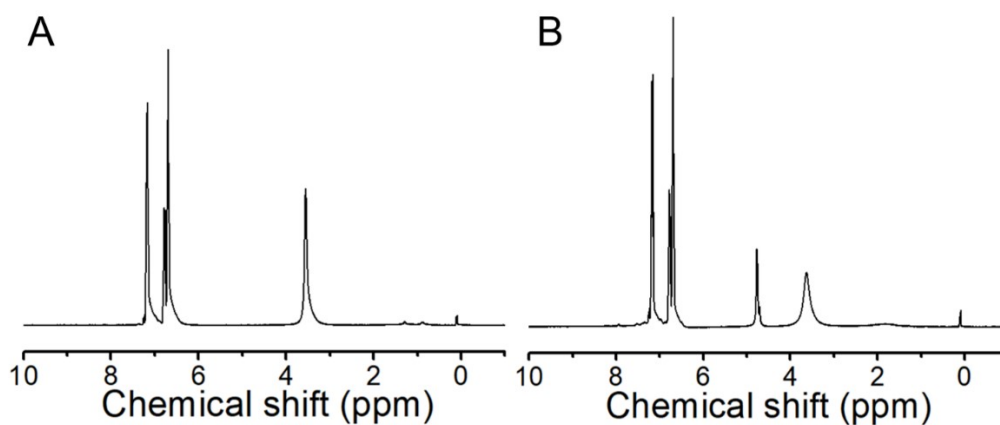**Fig. S2.** SEM images of (A) bare Au chip, (B) polyaniline formed on gold chip



**Fig. S3.** 1H NMR of aniline (A) and formation of polyaniline (B).

**Table S1 Recoveries for HIgG by the proposed SPR immunosensor (n = 5)**

| Sample | Added (µg/mL) | Detected (µg/mL) | Recovery (%) |
|--------|---------------|------------------|--------------|
| 1 | 0.10 | 0.09 | 94.6 |
| 2 | 0.30 | 0.28 | 93.3 |
| 3 | 0.60 | 0.63 | 105.5 |
| 4 | 0.80 | 0.77 | 96.3 |
| 5 | 1.00 | 1.03 | 103.2 |

**Table S2** Comparison of performance of the proposed method and other material-based SPR immunoassay methods

| Materials | methods | Detection limit (µg/mL) | Linear range (µg/mL) | Reference |
|-----------|---------|-------------------------|----------------------|-----------|
| Ag@Au nanozyme | SPR | $1.4\times10^{-3}$ | 0.01-1 | This work |
| Polydopamine | SPR | 0.9 | 2-100 | S2 |
| Graphene oxide/silver | SPR | 0.04 | 5-30 | S3 |
| Reduced graphene oxide | SPR | 0.0625 | 0.625-25 | S4 |
| Graphene oxide with gold bipyramids | SPR | 0.15 | 0.15-40 | S5 |
| ZnO@Au based GSAB-Ab$_2$ sandwich assay | SPR | 0.0375 | 0.0375-40 | S6 |

GSAB: Gold nanorods streptavidin biotin

**References**

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