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ARTICLE TYPE

# A Novel Electrochemical Immunosensor Based on Dual Signal Amplification of Gold Nanoparticles and Telomerase Extension Reaction

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A novel electrochemical immunosensor has been developed for the detection of human immunoglobulin G (IgG) by using gold nanoparticles (AuNPs) and telomerase extension reaction as dual signal amplification. The immunosensor was implemented based on a heterogeneous sandwich procedure on the gold electrode surface. Goat anti-human IgG (Ab) and telomerase primer P1 co-labelled gold nanoparticles (Ab-DNA-AuNP complexes) was used as secondary antibody for telomerase extension and binding with human IgG. After the telomerase extension reaction, the extension products then hybridized with the biotinylated probe P2, following with binding of streptavidin-labelled alkaline phosphatase (SA-ALP). The ALP converted ascorbic acid 2-phosphate (AA-P) into ascorbic acid, which reduced the silver ions in the solution into metal silver, leading to the deposition of silver onto the electrode surface. Linear sweep voltammetry (LSV) was used to quantify the amount of the deposited silver which was proportional to the concentration of human IgG. The electrochemical immunosensor showed a dynamic range of 0.1-100  $\mu\text{g mL}^{-1}$  with a detection limit of 0.02  $\mu\text{g mL}^{-1}$ , acceptable precision, reproducibility and stability. The real human serum sample assay results demonstrated this approach could be used for clinical diagnosis.

## 1. Introduction

Highly sensitive and selective method for detecting and quantifying proteins is important in proteomics and clinical diagnostics. Enzyme-linked immunosorbent assay (ELISA) is widely used for protein detection due to its high specificity and sensitivity.<sup>1</sup> This method usually involves in antibody-antigen recognition. In general, a secondary antibody is functionalized with different types of labels for detection, such as enzymes including horseradish peroxidase, alkaline phosphatase (ALP), and glucoseoxidase to catalyze specific reactions for photometric or electrochemical detection,<sup>2-6</sup> fluorescence labels<sup>7</sup> and radioisotope labels.<sup>8</sup> Among these approaches, electrochemical detection has gained considerable attention due to its high sensitivity, short analysis time, field portability, low reagent consumption and intrinsic simplicity. Recently, the electrochemical immunoassay based on biocatalytic metal deposition has been developed for bioanalysis.<sup>9-13</sup> This strategy allows accumulation of metallic product onto electrode surface to prevent the diffusion of enzyme-catalyzed product into solution, which represents a promising approach to achieve highly sensitive immunoassay.

Gold nanoparticles (AuNPs) have been attracted great attention in bioanalytical field because of their advantages such as good biocompatibility, easy and rapid synthesis, narrow size distribution and excellent stability.<sup>14</sup> AuNPs have been applied in electrochemical immunoassay for signal amplification to achieve high sensitivity due to their ability to easily conjugate many biomolecules.<sup>15-18</sup>

Telomerase is a ribonucleoprotein that synthesizes the chromosomal telomer ends (telomeric repeats) and is responsible

for the continuous growth of cancer and malignant cells.<sup>19-23</sup> It is a specialized DNA polymerase that adds telomeric sequence (TTAGGG)<sub>n</sub> onto chromosome ends.<sup>24,25</sup> In the presence of primer, the enzyme elongates the primer, generating multiple tandem repeats of the telomeric sequences.<sup>24</sup> Previous documents report that the telomerization reaction can produce hundreds of telomeric repeats.<sup>26-29</sup> Telomerization reaction has been widely used for telomerase activity detection.<sup>30-32</sup> Willner's group report the use of telomerase extension reaction to detect the telomerase activity through the hybridization of the extension products with the biotin-labelled nucleic acid and the DNAzyme-labelled nanoparticles.<sup>33-35</sup> However, to our knowledge, there are few explorations of telomerase as signal amplifiers in the field of electrochemical immunoassay.

Herein, we developed a novel electrochemical immunosensor based on dual signal amplification of AuNPs and telomerase extension reaction coupled with biocatalytic silver deposition for human IgG detection. This approach relies on a sandwich structure formed by goat anti-human IgG (Ab), human IgG, goat anti-human IgG (Ab) and telomerase primer P1 co-labelled AuNPs (Ab-DNA-AuNPs). The detection sensitivity is substantially enhanced by AuNP amplification and telomerase extension reaction. In the presence of ascorbic acid 2-phosphate (AA-P) and silver ions, the ALP converted AA-P to ascorbic acid, a reducing reagent which reduces silver ions into metallic silver on the electrode surface. The amount of deposited silver is correlated with analyte human IgG concentration and can be determined by linear sweep voltammetry (LSV). This design can offer desirable sensitivity and specificity due to signal amplification and specific antibody-antigen recognition. Moreover, the electrochemical method possesses many advantages, such as short analysis time, low reagent consumption

and field portability.

## 2. Experimental section

### 2.1 Reagents and Apparatus

Human immunoglobulin G (IgG) antigen, goat anti-human IgG (Ab), biotinylated goat anti-human IgG (biotinylated Ab), bovine serum albumin (BSA), streptavidin, and streptavidin-alkaline phosphatase (SA-ALP) were purchased from Dingguo biotechnology Co. Ltd. (Beijing, China). ascorbic acid 2-phosphate (AA-P), cysteamine, glutaraldehyde (GA), Hydrogen tetrachloroaurate (II) ( $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ ), and sodium citrate ( $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7$ ) were purchased from Sigma-Aldrich Chemical Co. (USA), 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), Nonidet-P 40 (NP-40) were purchased from Bio Basic. Inc. Sodium deoxycholate, diethyl pyrocarbonate (DEPC), NaCl,  $\text{KH}_2\text{PO}_4$ ,  $\text{Na}_2\text{HPO}_4$ , Tris, EDTA, NaOH, glycine,  $\text{AgNO}_3$ , and  $\text{KNO}_3$  were of analytical purity and obtained from the Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All the other solutions were prepared using ultrapure water ( $>18.2 \text{ M}\Omega$ ) produced by a Millipore Milli-Q water purification system (Billerica, MA, USA).

The synthesized oligonucleotides, all HPLC-purified and lyophilized, were provided by Life Invitrogen Trading Co. Ltd (Shanghai, China), the sequences were listed in Table S1 (in Supplementary Materials). The thermodynamic parameters of all oligonucleotides were calculated using bioinformatics software (<http://www.bioinfo.rpi.edu/applications/>).

### 2.2 Synthesis of gold nanoparticles

Gold nanoparticles (AuNPs) were synthesized by following the method of reduction of tetrachloroauric acid with sodium citrate which has been carried out by Freeman.<sup>36</sup> Briefly, 100 mL of 0.02% (wt)  $\text{HAuCl}_4$  solution was boiled with vigorous stirring, and then 10 mL of 38 mM sodium citrate solution were added to the boiling solution rapidly. Within several minutes, the color of the solution changed from yellow to wine red. The solution was heated under reflux for another 10 minutes to ensure complete reduction. The obtained colloidal solutions were allowed to cool to room temperature and stored at 4 °C for further step. According to a molar extinction coefficient of  $2.7 \times 10^8 \text{ M}^{-1}\text{cm}^{-1}$ , the concentration of AuNPs was calculated to be about 10 nM.

### 2.3 Preparation of Ab-DNA-AuNP complexes

The Ab-DNA-AuNP complexes were prepared according to the literatures with a little modification.<sup>37-39</sup> Firstly, 1 mL of 13 nm gold colloids (10 nM) were adjusted to pH 9.0 by directly using 0.1 M  $\text{Na}_2\text{CO}_3$  aqueous solution. Then, 7  $\mu\text{L}$  of goat anti-human IgG (Ab, 1.0 mg  $\text{mL}^{-1}$ ) was incubated with gold colloids for 60 min at 30 °C. During the process, Ab was covalently bound to gold nanoparticles (AuNPs) by means of the well known gold-sulfur bonds. Afterwards, the antibody modified AuNPs were reacted with alkythiol-modified primer probe P1 (0.5 OD) for 16 hours at 4 °C and then were "aged" in salts (10 mM phosphate, 0.1 M NaCl, pH 7.4) for 8 hours at 4 °C. Following that, the mixture was centrifuged at 12000 rpm for 20 min, and washed

twice with a 10 mM PBS (10 mM PB, 0.1 M NaCl, pH 7.4). The primer probe P1 and Ab-modified gold nanoparticles (Ab-DNA-AuNPs complexes) were then re-dispersed in 1 mL of 10 mM PBS (10 mM PB, 0.1 M NaCl, pH 7.4) containing 1.0% (wt) BSA, and stored at 4 °C until use.

### 2.4 Cell culture and telomerase extraction

Hela cells were prepared as described in our recent paper.<sup>30</sup> Briefly, Hela cells were cultured in DMEM medium supplemented with 10% fetal calf serum (FBS) and 100 IU/mL of penicillin-streptomycin, and the cells were maintained at 37 °C in a humidified atmosphere (95% air and 5%  $\text{CO}_2$ ) and were kept in logarithmic growth phase by routine passage every 2~3 days. Then, cells were collected in EP tube, washed twice with 10 mM PBS (10 mM PB, 0.1 M NaCl, pH 7.4) by centrifuging at 2000 rpm for 3 min at 4 °C. The cells were suspended in 200  $\mu\text{L}$  of ice cold NP-40 lysis buffer (10 mM Tris-HCl, 1% NP-40, 0.25 mM sodium deoxycholate, 10% glycerol, 150 mM NaCl, 0.1 mM AEBSF, pH 8.0) by swaying at least three times at a concentration of  $1.0 \times 10^7$  cells/mL, kept on ice for 30 min and then centrifuged at 12000 rpm for 20 min at 4 °C. Last, the supernatant was carefully moved to the sterilized tube, and was frozen at -80 °C for further experiment.

### 2.5 Preparation of Ab modified gold electrode

The gold electrodes (2 mm diameter, 99.99% polycrystalline, CH Instrument Inc.) were treated with piranha solution ( $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2 = 3:1$  in volume) for 3 h, and were then polished with 0.05  $\mu\text{m}$  alumina slurry for 5 min followed by sonicating in ethanol and double distilled water for 3 min twice. Subsequently, the gold electrodes were doused with ultrapure water and dried under nitrogen gas. 7  $\mu\text{L}$  of 10 mM cysteamine solution was added on the pretreated gold electrode overnight at 4 °C to produce a self-assemble monolayer (SAM) of cysteamine. The electrode surface was then rinsed in double distilled water for 10 min and dried under nitrogen gas. After immersing in 2.5% glutaraldehyde (GA) aqueous solution at 37 °C for 1 h, the electrode was extensively rinsed with double distilled water for 10 min and dried in a nitrogen gas. Subsequently, 7  $\mu\text{L}$  of 1 mg  $\text{mL}^{-1}$  Ab in 10 mM PBS (10 mM PB, 0.1 M NaCl, pH 7.4) was dripping onto the electrode at 37 °C for 1 h. The unreacted glutaraldehyde was blocked with 5 mg  $\text{mL}^{-1}$  BSA solution at 37 °C for 30 min. Then, the electrode was rinsed with 10 mM PBS (10 mM PB, 0.1 M NaCl, pH 7.4) for 10 min and dried with nitrogen gas for further experiment.

### 2.6 Electrochemical immunoassay and telomerase extension reaction

A series of 80  $\mu\text{L}$  samples containing either purified human IgG antigen or serum at various concentrations in 10 mM PBS (10 mM PB, 0.1 M NaCl, pH 7.4) were incubated with the Ab-modified electrode at 37 °C for 1 h. After washing with 10 mM PBS for 10 min and dried under nitrogen gas, 7  $\mu\text{L}$  of Ab-DNA-AuNP complexes were placed on the electrode and incubated at 37 °C for 1 h for completing the immunoassay. Afterwards, the

electrode was rinsed by 10 mM PBS for 10 min and dried under nitrogen gas.

Subsequently, the electrode was immersed in 80  $\mu\text{L}$  of telomerase extract solution with 10  $\mu\text{L}$  of telomerase extracts (1.0 $\times 10^7$  cells/mL), 8  $\mu\text{L}$  of 10 $\times$ TRAP reaction buffer (200 mM Tris-HCl, 15 mM MgCl<sub>2</sub>, 630 mM KCl, pH 8.3), 8  $\mu\text{L}$  of 10 mM dNTP mixture (dATP, dCTP, dGTP, dTTP) and 54  $\mu\text{L}$  of DEPC water at 30  $^\circ\text{C}$  for 2 h. After telomerase extension reaction, the electrode was then rinsed with 10 mL mixture solution of 1 mL 10 $\times$ TRAP reaction buffer, 1 mL NP-40 lysis buffer and 8 mL DEPC water at room temperature ( $\sim 25$   $^\circ\text{C}$ ) for 10 min and dried under nitrogen gas for the next experiments.

## 2. 7 Enzymatic silver deposition and electrochemical detection

An aliquot of 80  $\mu\text{L}$  of 10  $\mu\text{M}$  biotinylated probe P2 in 10 mM PBS (10 mM PB, 0.3 M NaCl, pH 7.4) was incubated with the prepared electrode at 33  $^\circ\text{C}$  for 2 h followed by rinsing the electrode with 10 mM PBS for 10 min and dried under nitrogen gas. Then, 7  $\mu\text{L}$  of 100  $\mu\text{g mL}^{-1}$  SA-ALP solution was dispensed to the gold electrode. After incubation at 37  $^\circ\text{C}$  for 1 h, the electrode was thoroughly rinsed with 10 mM PBS (pH 7.4) for 10 min. Then, the gold electrode was immersed into the freshly prepared 50 mM glycine-sodium hydroxide buffer (pH 9.08) containing 1 mM AgNO<sub>3</sub> and 1 mM ascorbic acid 2-phosphate (AA-P), and incubated at 37  $^\circ\text{C}$  for 30 min. The resulting electrode was rinsed with ultrapure water for the followed electrochemical detection.

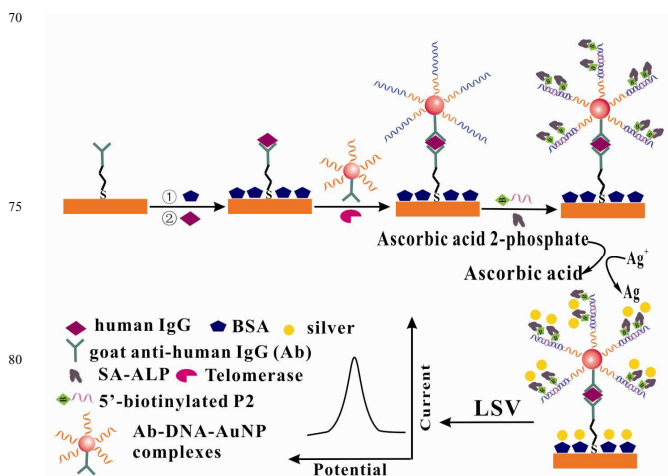
Electrochemical measurements including linear sweep voltammetry (LSV) and electrochemical impedance spectroscopy (EIS) were performed on an electrochemical analyzer CHI-660C (CH Instruments, Shanghai, China) at room temperature ( $\sim 25$   $^\circ\text{C}$ ). A three-electrode system consisting of a KCl saturated calomel reference electrode (SCE), a platinum counter electrode and the working electrode (gold electrode) were used. LSV was performed at a potential range from 0.2 to 1.0 V (vs SCE) with a 100 mV/s scanning rate using the 0.6 M KNO<sub>3</sub> solution containing 0.1 M HNO<sub>3</sub> as the supporting electrolyte. EIS was performed in 1/15 M PB (pH 7.4) containing 5 mM Fe(CN)<sub>6</sub><sup>3-</sup>/Fe(CN)<sub>6</sub><sup>4-</sup> (1:1 mixture) and 0.1 M KCl in the frequency range from 0.1 Hz to 100 KHz at a bias potential of -0.19 V (vs SCE) with frequency modulation of 5 mV.

## 3. Results and discussion

### 3. 1 Analytical principle of the electrochemical immunosensor

The analytical principle of the electrochemical immunosensor is illustrated in Scheme 1. Firstly, goat anti-human IgG (Ab) is immobilized on gold electrode through cysteamine and glutaraldehyde reaction and the analyte human IgG antigen binds with Ab specifically. Then goat anti-human IgG (Ab) and telomerase primer co-labelled AuNPs (Ab-DNA-AuNP complexes) are used as a secondary antibody to combine with human IgG antigen to form a sandwich structure. In the presence of telomerase, telomerase extension reaction is initiated to add TTAGGG tandem repeat unites to the 3'-end of the primer. Probe P2 is designed for hybridization with telomerase extension product. Then streptavidin labelled alkaline phosphatase (SA-

ALP) is employed to connect ALP onto electrode surface via specific binding between biotin and streptavidin. After washing steps, the modified gold electrode is incubated with ascorbic acid 2-phosphate (AA-P) and silver ions. The ALP converts AA-P to ascorbic acid, and the latter reduces silver ions to form a metallic silver layer on the electrode surface. The amount of deposited silver is correlated with analyte human IgG concentration and can be determined by linear sweep voltammetry (LSV). This approach offers high sensitivity through dual signal amplification of AuNPs and telomerase extension reaction. Moreover, silver deposition can enhance sensitivity because the silver layer can prevent the diffusion of enzyme-catalyzed product into solution.

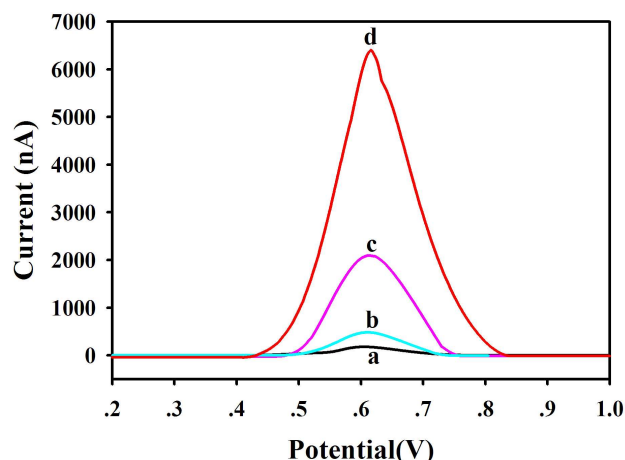


**Scheme. 1** Illustration of the electrochemical immunosensor based on dual signal amplification of AuNPs and telomerase extension reaction for human IgG detection.

### 3. 2 Evaluation of dual signal amplification by AuNPs and telomerase extension reaction

To investigate the dual signal amplification of the AuNPs and telomerase extension reaction, we compared the performance of different detection approach using gold electrode for the determination of 100  $\mu\text{g mL}^{-1}$  human IgG by linear sweep voltammetry (LSV). As can be seen from Fig. 1, in the absence of analyte human IgG, a negligible anodic stripping peak current was obtained even if the Ab-DNA-AuNP complexes were introduced (curve a). On the contrary, a substantial anodic peak current was observed in the presence of 100  $\mu\text{g mL}^{-1}$  human IgG (curve d), and a signal-to-background ratio as high as 35-fold was achieved when using the dual signal amplification of the AuNPs and telomerase extension reaction. On the other hand, when using biotinylated Ab and streptavidin-ALP instead of Ab-DNA-AuNP complexes to perform the enzymatic metallic deposition, the assay for 100  $\mu\text{g mL}^{-1}$  human IgG gave a small anodic peak current (curve b). The peak current of the Ab-DNA-AuNP complex amplification immunoassay was about 30 times as much as that of the method based on direct biotinylated Ab and SA-ALP assay, implying that the dual signal amplification of gold nanoparticles and the telomerization extension reaction could offer a  $\sim 30$ -fold amplification. Moreover, when the biotinylated telomerase primer (Probe P3) was used to initiate the telomerase extension reaction through the biotinylated Ab and streptavidin

bridges rather than through the Ab-DNA-AuNP complex, the peak current of the assay for  $100 \mu\text{g mL}^{-1}$  human IgG was obviously lower than that of method based on the Ab-DNA-AuNP complex amplification strategy (curve c), indicating that the gold nanoparticles decorated with many telomerase extension primers indeed enhanced the assay sensitivity.



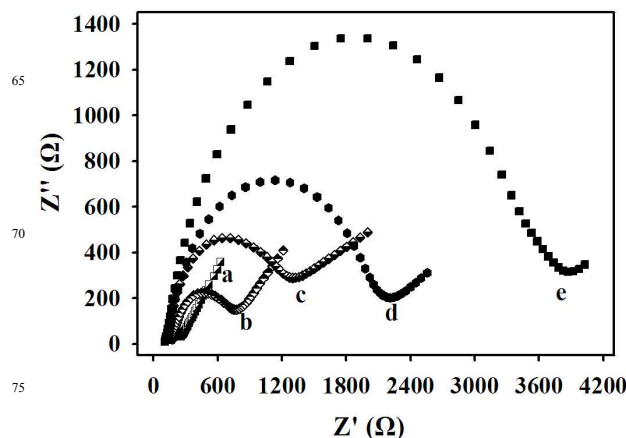
**Fig. 1** LSV measurements for  $100 \mu\text{g mL}^{-1}$  human IgG using different detection approaches in  $0.6 \text{ M KNO}_3/0.1 \text{ M HNO}_3$  solutions. Dual signal amplification strategy using the Ab-DNA-AuNP complexes in the absence (a) and presence (d) of human IgG; Direct biotinylated Ab and streptavidin-ALP detection strategy (b); Telomerase extension amplification triggered by biotinylated telomerase primer through the biotinylated Ab and streptavidin bridges (c). Scan rate:  $100 \text{ mV/s}$ .

### 3. 3 EIS characterization of the electrochemical immunosensor

In order to investigate the modification processes of the developed approach, electrochemical impedance spectroscopy (EIS) with different interfacial processes was performed. As shown in Fig. 2, the bare gold electrode behaved as an ideal conductor and the impedance spectra gave a linear plot (curve a). The Ab-modified electrode showed an increased Ret (resistance of electron-transfer) compared with bare gold electrode (curve b) since the formation of a barrier for the electron transfer at the electrode interface. After blocked with BSA, the Ab/BSA modified electrode gave a larger Ret (curve c), indicating that the treatment of BSA could block the unreacted glutaraldehyde to prevent the nonspecific adsorption. When Ab/BSA modified electrode was incubated with analyte human IgG antigen, a bigger Ret was observed (curve d) due to the formation of another barrier on the electrode interface for the electron transfer. After Ab/BSA/human IgG modified electrode interacting with Ab-DNA-AuNP complexes, the Ret (curve e) greatly increased. This phenomenon was due to the fact that the DNA backbone of the telomerase primer P1 coated on the AuNPs had strong electrostatic repulsion with  $\text{Fe}(\text{CN})_6^{3-/4-}$ . These results gave immediate evidences for the surface binding events.

### 3. 4 Optimization of experimental conditions

In the electrochemical immunoassay, biotinylated detection probe P2 was used to hybridize with the telomerase extension product and combine with the SA-ALP. Hence, the concentration of the



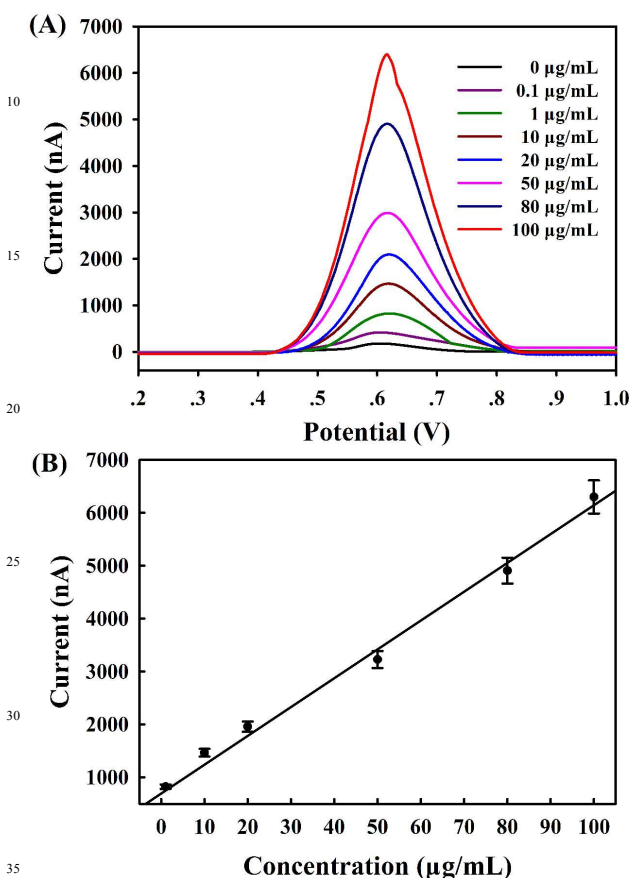
**Fig. 2** Nyquist plot of a bare gold electrode (a), a Ab modified electrode (b), a Ab/BSA modified electrode (c), a Ab/BSA/target modified electrode (d), a Ab/BSA/target/Ab-DNA-AuNP nanocomplexes modified electrode (e). Data was obtained in PBS buffer ( $10 \text{ mM pH } 7.4$ ) containing  $5 \text{ mM Fe}(\text{CN})_6^{3-/4-}$  (1:1 mixture) as the redox probe at  $0.24 \text{ V}$ , the frequency range is  $0.1\text{--}10^5 \text{ Hz}$  and the amplitude was  $5.0 \text{ mV}$ .

detection probe P2 was important for the assay. In order to obtain a maximal anodic stripping peak current, the concentration of the detection probe P2 was optimized ranging from  $2 \mu\text{M}$  to  $10 \mu\text{M}$ . As shown in Fig. S1 (in Supplementary Materials), the anodic stripping peak current first increased with the increasing concentration of detection probe P2 and then reached a maximal value when the P2 concentration was above  $10 \mu\text{M}$ . So, a detection probe P2 concentration of  $10 \mu\text{M}$  was selected. The effect of the hybridization temperature on the immunosensor was also investigated. As shown in Fig. S2 (in Supplementary Materials), the anodic stripping peak current reached its maximum when the hybridization reaction temperature was set at  $33 \text{ }^\circ\text{C}$ . Therefore, the hybridization reaction temperature of  $33 \text{ }^\circ\text{C}$  was used throughout the subsequent experiments. Also, hybridization reaction time of probe P2 with telomerase extension products was investigated. Fig. S3 (in Supplementary Materials) showed the effect of hybridization time on the electrochemical readout of the immunoassay. The anodic stripping peak current increased rapidly with the increasing reaction time up to 2 h, and no further increase in the signal was observed when the hybridization time was more than 2 h. Thus, 2 h was selected as the optimum time for the hybridization reaction.

### 3. 5 Analytical performance of the electrochemical immunosensor

The ability of our electrochemical immunosensor for quantitative analysis of human IgG was investigated by performing a series of IgG solution with different concentrations. Fig. 3A showed the linear sweep voltammogram responses of the enzymatic amplification immunoassay based on dual signal amplification. We can observe that the anodic stripping peak currents increased with the increasing human IgG concentration in the range from  $0.1$  to  $100 \mu\text{g mL}^{-1}$ . The calibration curve of the immunosensor was shown in Fig. 3B. One can observe that the peak current was proportional to the concentration of human IgG in the range

from 0.1 to 100  $\mu\text{g mL}^{-1}$ . The linear regression equation was  $I_p = 697.4 + 54.45C$  (where  $I_p$  represented the peak current and  $C$  represented the human IgG concentration) with a correlation coefficient of 0.9913. The detection limit was estimated to be 0.02  $\mu\text{g mL}^{-1}$  in terms of the  $3\sigma$  rule. This detection limit was comparable to those detection limits reported previously.<sup>40,41</sup>



**Fig. 3** (A) LSV of the electrochemical immunosensor in 0.6 M  $\text{KNO}_3/0.1$  M  $\text{HNO}_3$  solution with different human IgG concentrations: 100, 80, 50, 20, 10, 1, 0.1 and 0  $\mu\text{g mL}^{-1}$  (from upper to lower). Scan rate, 100 mV/s. (B) Calibration curve of peak current as a function of human IgG concentration. The error bars represented the standard deviation of three repetitive experiments.

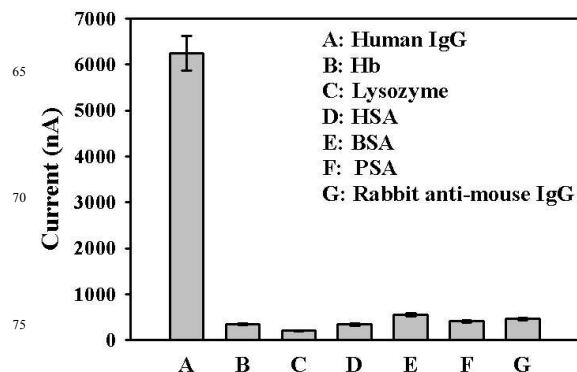
### 3. 6 Specificity evaluation of the electrochemical immunosensor

To evaluate the specificity of the developed electrochemical immunosensor, the influences of different proteins, including haemoglobin (Hb), human serum albumin (HSA), prostatic specific antigen (PSA), bovine serum albumin (BSA), lysozyme and rabbit anti-mouse IgG were investigated. The concentration of these proteins were used about 10-fold higher than human IgG concentration in the assay. As shown in Fig. 4, the peak currents of these proteins were very small compared with that of human IgG. This result demonstrated that our electrochemical immunosensor afforded great selectivity for human IgG analysis due to the specific antibody-antigen recognition.

#### Detection of the real human serum samples

In order to investigate the feasibility of the proposed

electrochemical immunosensor for clinical analysis, five real



**Fig. 4** Specificity of the electrochemical immunosensor. The concentration of human IgG was 100  $\mu\text{g mL}^{-1}$ , the concentrations of all the other proteins were 1 mg  $\text{mL}^{-1}$ . The results were the average of three repetitive experiments, with error bars indicating the standard deviation.

human serum specimens were obtained from XiangYa School of Medicine, Central South University (Changsha, China) and analyzed by the developed electrochemical immunosensor. A commercial CLIA kit was used as a reference method. The results were shown in Table 1. The human IgG concentrations determined by our electrochemical immunosensor were consistent with those determined by commercial CLIA kit with relative deviations smaller than 6.95%, indicating that it was feasible to apply the developed electrochemical immunoassay for quantitative detection of human IgG in human serum samples.

Table 1. The comparison of the developed electrochemical immunosensor<sup>a</sup> (EI) with the chemiluminescent immunoassay (CLIA)

Serum samples	EI ( $\mu\text{g mL}^{-1}$ )	CLIA ( $\mu\text{g mL}^{-1}$ )	RSD (%)
1	43.52±0.53	44.67	-2.57
2	51.34±0.78	50.03	2.62
3	58.64±0.64	54.83	6.95
4	67.62±0.39	71.32	-5.19
5	85.43±0.91	82.76	3.23

<sup>a</sup> The data are given as the average  $\pm$  S.D. obtained from five independent experiments ( $n=5$ ). As the IgG concentration in human serum is very high, the human serum specimens have to be diluted to the detection range of electrochemical immunoassay by using phosphate buffer saline solution.

### 95 Conclusions

We have developed a novel electrochemical immunosensor based on dual signal amplification of AuNPs and telomerase extension reaction coupled with biocatalytic silver deposition for human IgG detection. This strategy offered desirable sensitivity and high specificity. The analysis results for real human serum samples demonstrated that the developed electrochemical immunosensor could be used for quantitative analysis of practical and clinical samples. This approach held the potential to be extended to a

common electrochemical immunoassay platform for different protein assay.

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## Notes and references

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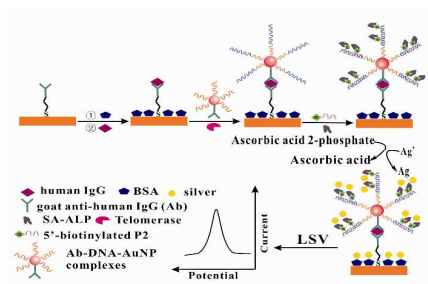
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## A Novel Electrochemical Immunosensor Based on Dual Signal Amplification of Gold Nanoparticles and Telomerase Extension Reaction

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### Graphical and text abstract



Highly sensitive electrochemical immune analysis was achieved based on dual signal amplification of AuNPs and telomerase extension reaction.