Supplementary Material (EIS)

A Novel Electrochemiluminescence Strategy for Ultrasensitive DNA Assay Using Luminol Functionalized Gold Nanoparticles Multi-labeling and Amplification of Gold Nanoparticles and Biotin-streptavidin System

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Experimental

Chemicals and solutions. A $HAuCl_4$ stock solution (2‰ $HAuCl_4$, w/w) was prepared by dissolving 1.0 g of $HAuCl_4 \cdot 4H_2O$ (Shanghai Reagent, China) in 412 mL of purified water and stored at 4 ºC. Luminol and 1, 3-propanedithiol were purchased from Sigma-Aldrich (USA). Bovine serum albumin(BSA), streptavidin (SA) were obtained from Solarbio (Beijing, China). All DNA used in this work were synthesized and HPLC-purified by sangon Inc. (Shanghai, China). Their sequences and modification are listed in Table 1. A 0.01 M stock solution of luminol was prepared by dissolving luminol in 0.1 M NaOH solution and was kept at 4 ºC. All other reagents were of analytical grade. Ultra-pure water was prepared by a Millipore Milli-Q system and used throughout.

Table 1 DNA Sequences and Modification

DNA	Sequence(5′-3′)
Capture probe:	5′-biotin-AAAAAAAAAAGACCTAGTCCT TCCAACAGC-3′
Biotinylated DNA for signal probe:	5′- GGGTTTATGAAAAACACTTT-biotin-3′
Target ss-DNA:	5′- AAAGTGTTTTTCATAAACCCGCTGTT GGAAGGACTAGGTC-3′
One-base mismatched sequence:	5′- AAAGTGTTTTTCATAAACCCTCTGTT GGAAGGACTAGGTC-3′
Two-based mismatched sequence:	5′- AAAGTGTTTTTCATAAACCATCTGTT GGAAGGACTAGGTC-3′
Non-complementary sequence:	5′- AAAGTGTTTTTCATAAACCCACTGCT AGAGATTTTCACTA-3′

Preparation of luminol-AuNPs labeled DNA signal probe. The luminol-AuNPs with a diameter of 20 nm were prepared by reducing $AuCl_4^-$ ions with 0.01 M luminol

solution and stored at 4 ºC as described previously. [14] The unreacted reagents and the resulted products (small molecules) were removed via dialysis for 2 days with ultrapure water about six times under stirring by use of a 3500 molecular weight cutoff dialysis membrane to obtain luminol-AuNPs. 1 mL pH-adjusted (pH 8.0) luminol-AuNPs solution was added to 25 μL of SA (1.0 mg/mL), after incubated at room temperature for 0.5 h, the solution was further added with 5 % BSA solution to the final concentration of 1 % BSA, then stirred for 5 min. The conjugate of luminol-AuNPs with SA was centrifuged at 12500 rpm for 20 min (Universal 320, Hettich, Germany), and the red precipitates were dispersed with 0.05 M pH 8.0 Tris-HCl containing 0.3 M NaCl. Biotinylated DNA (4×10^{-7} M, optimized concentration) was added to the red solution and incubated at 37 ºC for 1 h. The as-prepared mixture was centrifuged at 12500 rpm for 10 min, and the red precipitates were suspended with 0.05 M pH 8.0 Tris-HCl containing 0.3 M NaCl to obtain luminol-AuNPs labeled DNA signal probe.

Fabrication of sandwich-type DNA conjugate modified electrode. 60 μL aliquots of the biotinylated DNA capture probe (4×10^{-6} M) was dropped with a pipette on the SA coated AuNPs modified electrode at 37 ºC for 1 h and rinsed by washing buffer (7 mM pH 8.0 Tris-HCl containing 0.17 M NaCl) to remove the absorbed DNA capture probe. Then 60 μL aliquots of the 1%(w/w) BSA in 0.05 M pH 8.0 Tris-HCl was dropped with a pipette on the SA coated AuNPs modified electrode to block both the electrode and AuNPs, the electrode was incubated at 37 ºC for 30 min and rinsed with washing buffer. After that, 60 μL aliquots of target ss-DNA were

dropped with a pipette on the modified electrode at 37 ºC for 1 h and rinsed with washing buffer. Finally, 60 μL aliquots of the luminol-AuNPs labeled DNA signal probe were dropped with a pipette on the modified electrode at 37 ºC for 1 h and rinsed with washing buffer. The gold electrode modified with the "sandwich-type" DNA conjugate was ready for further experiments.

ECL measurement. ECL measurements were carried out with a homemade ECL system consisted of a model CHI760B workstation (Chenhua, Shanghai, China), a model RFL-1 luminometer (Remax, Xi'an, China), an H-type electrochemical cell (self-designed) and a computer. A three electrode system was used, being composed of a disk gold electrode (Φ=5 mm) modified with the "sandwich-type" DNA conjugate as working electrode, a platinum wire as counter electrode, and a silver wire as the quasi-reference electrode (AgQRE). A 1.0 mM H_2O_2 solution containing 0.02 M carbonate buffer solution (CBS, pH 9.95) was used as working solution for the detection of target ss-DNA. During measurements, a 3.0 mL portion of the working solution and blank solution without H_2O_2 were added to the working compartment and the auxiliary compartment of the ECL cell, respectively. When a double-step potential (30 s pulse period, 0.1 s pulse time, 0.8 V pulse potential and 0 V initial pulse potential) was applied to the working electrode, an ECL signal was generated and recorded.

Scanning Electron Micrograph (SEM) analysis. For the SEM anlysis, the modified gold electrodes were dried under an infrared lamp and measured by SEM (JEOL JSM-6700F, Japan).

Supporting figures

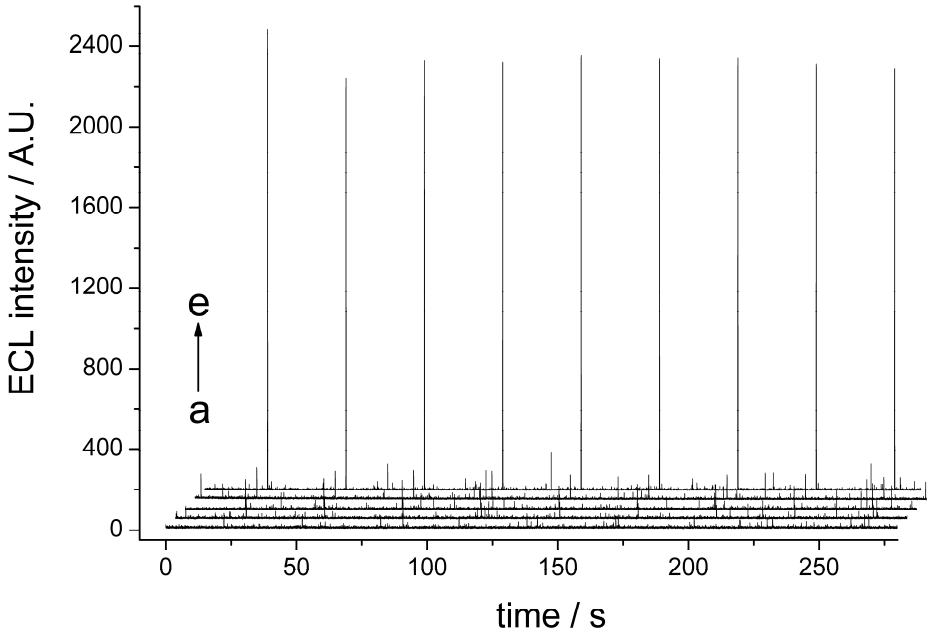

Figure S1. The control study for non-specific absorption of luminol-AuNPs labeled DNA signal probes in each step. ECL signals under pulse potential. Initial potential, 0 V; pulse period, 30 s; pulse time, 0.1 s; pulse potential, 0.8 V. ECL signals with non-specific absorption of luminol-AuNPs labeled DNA signal probes were obtained a) on a bare gold electrode, b) on a SA-coated AuNPs / 1,3-propanedithiol / gold electrode, c) on a DNA capture probe / SA-coated AuNPs / 1,3-propanedithiol / gold electrode, d) on a blank / DNA capture probe / SA-coated AuNPs / 1,3-propanedithiol / gold electrode, e) on a luminol-AuNPs labeled DNA signal probe / target ss-DNA (1×10^{-12} M) / DNA capture probe / SA-coated AuNPs / 1,3-propanedithiol / gold electrode. All ECL signals were measured in 0.02 M CBS (pH 9.95) solution containing 1.0 mM H_2O_2.

It was observed from Figure S1a, S1b, S1c, S1d that almost no ECL signals were gained on bare gold electrode, SA-coated AuNPs / 1, 3-propanedithiol / gold electrode and DNA capture probe / SA-coated AuNPs / 1, 3-propanedithiol / gold electrode and these signals were the same as that gained from blank control experiment (no target ss-DNA). These results reveal that there is no nonspecific absorption of luminol-AuNPs labeled DNA signal probes in each step.

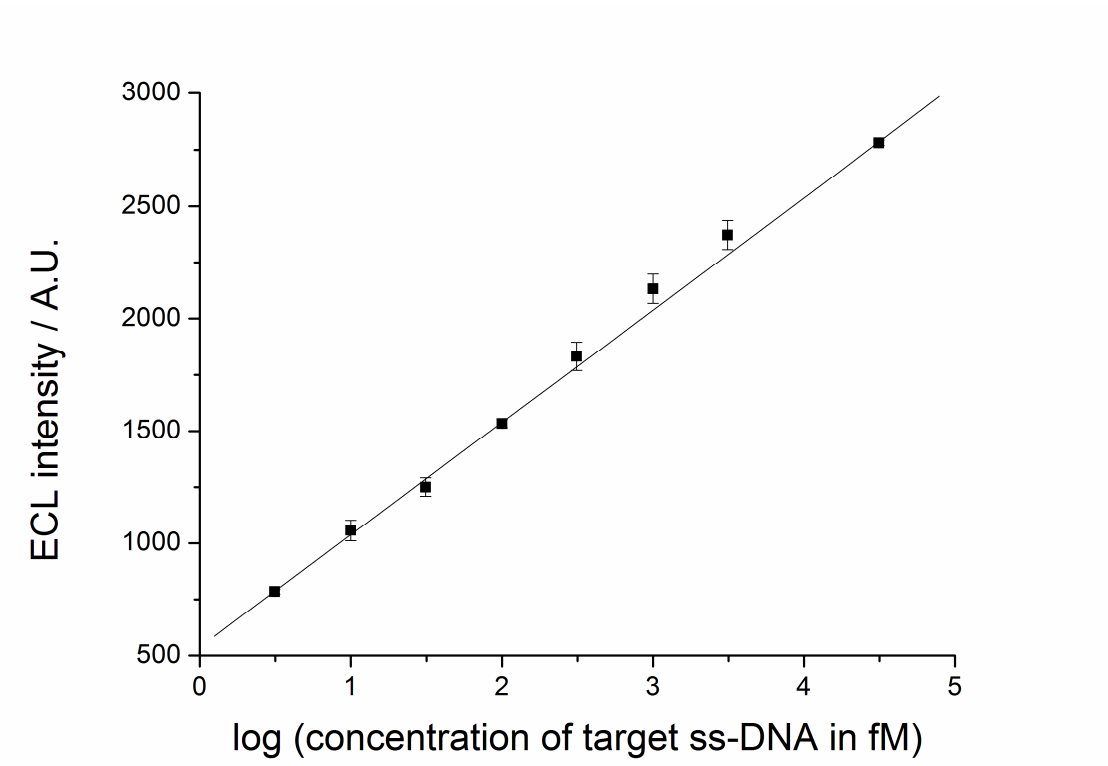

Figure S2. Calibration curve of target ss-DNA. Initial potential, 0 V; pulse period, 30 s; pulse time, 0.1 s; pulse potential, 0.8 V; H_2O_2, 1.0 mM; CBS, 0.02 M, pH 9.95; DNA capture probe, 4×10^{-6} M.

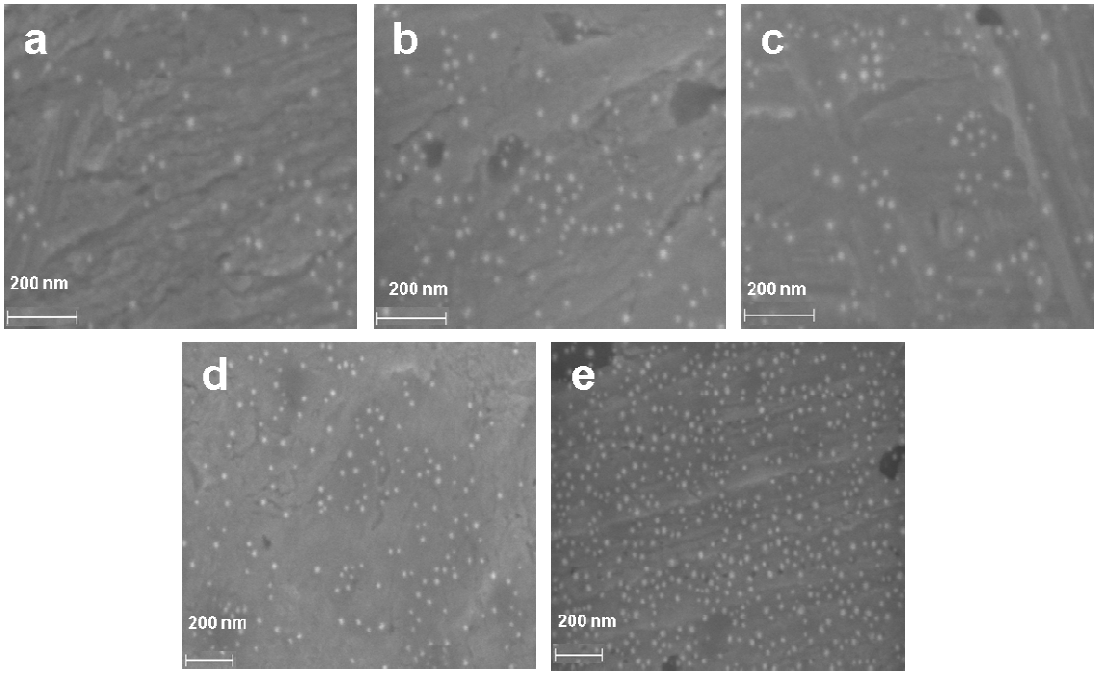

Figure S3. SEM images of gold electrodes modified with different targets in Tris-HCl buffer. a) blank; b) non-complementary sequence (1.0×10^{-12} M); c) two-bases mismatched sequence (1.0×10^{-12} M); d) one-base mismatched sequence (1.0×10^{-12} M); e) target ss-DNA (1.0×10^{-12} M); Other conditions are the same as Fig. 2.

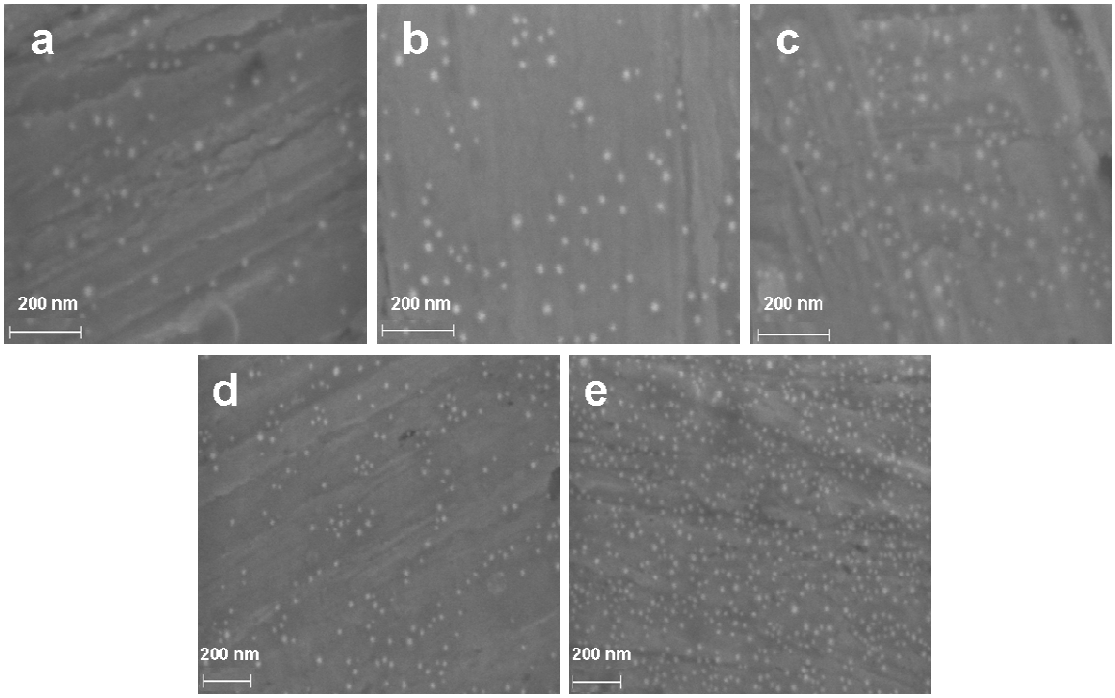

Figure S4. SEM images of gold electrodes modified with different targets in 50% serum sample 1. a) blank; b) non-complementary sequence (1.0×10^{-12} M); c) two-bases mismatched sequence (1.0×10^{-12} M); d) one-base mismatched sequence (1.0×10^{-12} M); e) target ss-DNA (1.0×10^{-12} M); Other conditions are the same as Fig. 2.

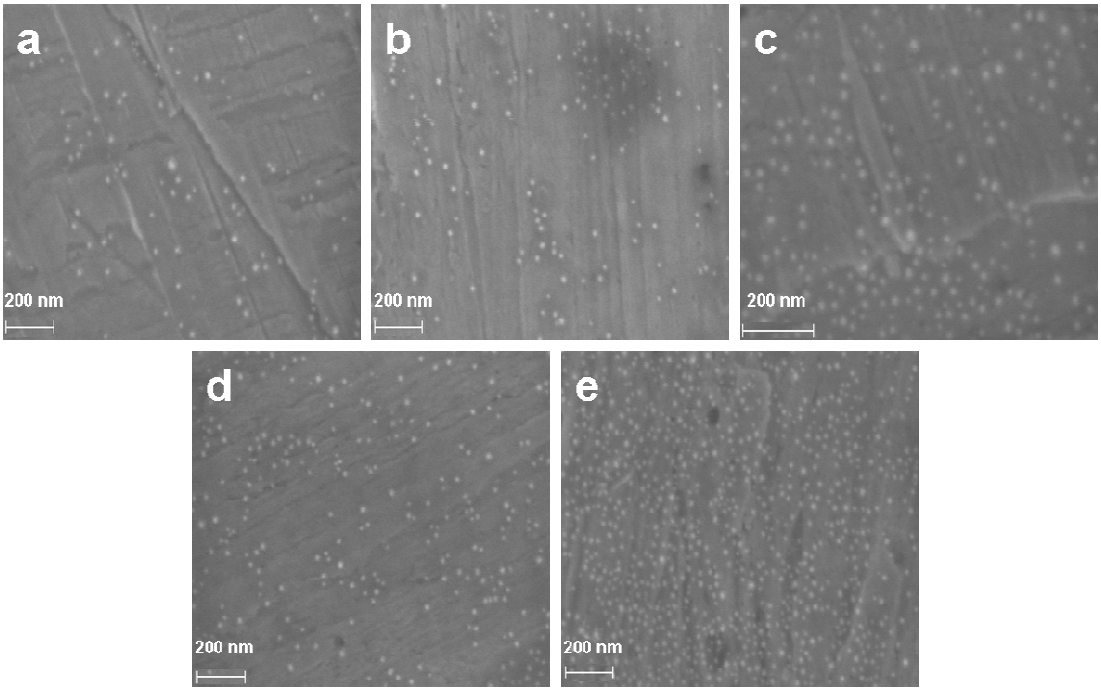

Figure S5. SEM images of gold electrodes modified with different targets in 50% serum sample 2. a) blank; b) non-complementary sequence (1.0×10^{-12} M); c) two-bases mismatched sequence (1.0×10^{-12} M); d) one-base mismatched sequence (1.0×10^{-12} M); e) target ss-DNA (1.0×10^{-12} M); Other conditions are the same as Fig. 2.