Supporting information

One-pot synthesis of luminol functionalized silver nanoparticles with chemiluminescence activity for ultrasensitive DNA sensing

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1. Experimental section

1.1 Chemicals and Apparatus

$HAuCl_4 \cdot 4H_2O$, $AgNO_3$ and Absolute ethanol (G, R) were purchased from Shanghai Reagent (Shanghai, China). $AgNO_3$ (5 mmol L^{-1}) and $HAuCl_4$ stock aqueous solution (6 mmol L^{-1}) were prepared by dissolving $AgNO_3$ and $HAuCl_4 \cdot 4H_2O$ in purified water and stored at 4℃. A stock solution of luminol (0.01 mol L^{-1}) was prepared by dissolving luminol (Sigma) in 0.1 mmol L^{-1} NaOH aqueous solution. Working solutions of H_2O_2 were prepared fresh daily from 30% (v/v) H_2O_2 (Xin Ke Electrochemical Reagent Factory, Bengbu, China). Streptadvin and bovine serum albumin (BSA) were obtained from Solarbio (Beijing, China). The buffer solutions used for the H_2O_2 detection included 0.02 mol L^{-1} carbonate buffer (CBS, $NaHCO_3$–Na_2CO_3, pH=10.53), pH 7.4 phosphate saline buffer (PBS). pH 8.0 TE buffer (Tris-HCl buffer, 0.05mol L^{-1}) containing 0.05 mol L^{-1} Ethylene Diamine Tetraacetic Acid (EDTA). All other reagents were of analytical grade. Ultrapure water was prepared by a Millipore Milli-Q system and used throughout. All glassware used in the following procedures was cleaned in a bath of freshly prepared HNO_3/HCl (3:1, v/v), rinsed thoroughly in redistilled water, and dried prior to use. Synthetic *mycobacterium tuberculosis* (*M. tuberculosis*) single-stranded DNA and other oligonucleotides were obtained from Sangon Biotech (Shanghai, China) as shown in Table 1.

TEM images were obtained from High Resolution Transmission Electron Microscopy (Jeol Ltd., JEOL-2010, Japan). UV-Vis detection was carried out on a UV-Vis spectrophotometer (Agilent 8453, USA). X-ray diffraction (XRD) spectrum was obtained from a Japan Rigaku D/max X-ray diffractometer. X-ray photoelectron spectroscopy (XPS) was performed on an ESCALABMK IIelectron spectrograph (VG Scientific, UK) with Al KR radiation as the X-ray source. The XPS data were fitted by XPS Peak and further plotted by Origin Pro 7.0. Thermogravimetric analyze (TGA) data were acquired on a Shimadzu TA-50 thermal analyzer (Shimadzu, Japan) at a heating rate of 5 ℃ min^{-1}

Table 1 DNA Sequences and Modification

DNA	Sequence (5'-3')
Capture probe	Biotin-GGCCACGTAGGCGAACC
Biotinylated DNA for signal probe	GGTGAGGTCTGCTACCCAC-Biotin
Target ss-DNA	CCAGCACCTAACCGGCTGTGGGTAGCAGACCTCACC TATGTGTCGACCTGGGCAGGGTTCGCCTACGTGGCC TTTGTCACC

from room temperature to 700 ℃ in a nitrogen atmosphere. ^{1}HNMR spectra were recorded with an AVANCE 300 (300 MHz) NMR spectrometer (Bruker, Switzerland) in dimethyl sulfoxide (DMSO). Tetramethylsilane (TMS) was used as a reference for ^{1}HNMR.

1.2 Synthesis and Characterization of luminol functionalized AgNPs

Luminol functionalized AgNPs (luminol-AgNPs) with different diameters were prepared by the reduction of $AgNO_3$ with luminol in the presence of ethanol. During a typical synthesis, different volumes (2 mL, 3 mL, and 5 mL) of 5 mmol L^{-1} $AgNO_3$ aqueous solution were added to the mixture solution containing 9 mL absolute ethanol and 5 mL ultrapure water under magnetic stirring at room temperature, followed by addition of 0.5 mL 0.01 luminol (0.1 mol L^{-1} NaOH) to the mixture solution as soon as possible. And the solution was maintained at stirring for 2 h, during which time a color change from colorless to primrose yellow to deep yellow, indicating the formation of silver nanoparticles.

The as-prepared luminol-AgNPs were characterized by UV-Vis spectra. As shown in Figure S1, the UV/Vis absorption spectra of luminol-AgNPs with different diameters showed red shift with increasing the diameters. The results demonstrated that the diameter of the AgNPs increased with increasing the ratios of $AgNO_3$ /luminol.

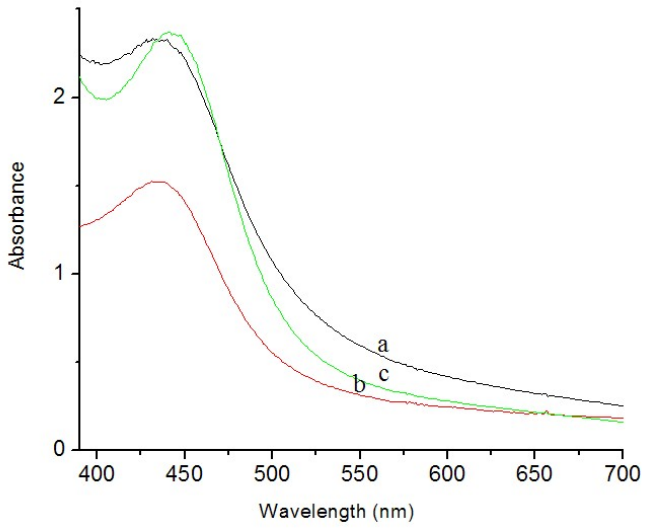

Figure S1 UV/Vis absorption spectra of luminol-AgNPs synthesized with varying amounts of $AgNO_3$ solution: a) 2, b) 3, c) 5 mL

1.3 CL activity of luminol-AgNPs and luminol functionalized AuNPs

The CL activity of luminol-AgNPs and luminol functionalized AuNPs (luminol-AuNPs) by reported method[1] using the same amount of $HAuCl_4$ and $AgNO_3$ were investigated by static injection.

In order to avoid the interference of free luminol in colloids, the luminol-AgNPs and luminol-AuNPs were centrifuged twice and redispersed with 0.1 mol L^{-1} NaOH solution. The CL detection was conducted on a BPCL Luminescence Analyze (Beijing, China) with a fixed voltage of -900V.

1.4 Preparation of streptavidin modified gold nanoparticles and luminol-AgNPs

The gold nanoparticles (AuNPs) was prepared by the classical citrate reduction method[2]. The average diameter of used luminol-AgNPs and AuNPs were 25 nm and 16 nm, respectively. The Streptavidin (SA) modified AuNPs or luminol-AgNPs was synthesized according to the following procedure. Typically, 25 uL of 1 mg mL^{-1} streptavidin dissolved in 0.1 mol L^{-1} PBS buffer was added to 1 mL AuNPs or luminol-AgNPs (pH adjusted to 6.5 using 0.1 M NaOH), followed by incubation at 37 ℃ for 30 min. After that, 250 uL of 5 % BSA solution was added with incubation for 5 min. The mixture was centrifuged at 12500 rpm for 45 min (Universal 320, Hettich, Germany), and the soft sediment was resuspended in 250 uL 0.1 mol L^{-1} PBS (pH 7.4).

1.5 Preparation of luminol-AgNPs labeled DNA probe

luminol-AgNPs labeled DNA probe was prepared as follows. 250 uL of SA modified luminol-AgNPs was added to 250 uL 2 umol L^{-1} signal probe solution (Biotinylated DNA) in TE buffer solution, and incubated for 60 min at 37 ℃. The unreacted reagents were centrifuged at 12500 rpm for 45 min and redispersed with 250 uL TE buffer solution.

1.6 Preparation of gold nanoparticles modified electrode

The Indium tin oxide (ITO) electrode was rinsed with abstergent, acetone, ethanol and ultrapure water, and then dried in air. This freshly pretreated bare ITO electrode was immersed in a 3 % (v/v) 3-mercaptopropyltrimethoxysilane (3-MPTS) ethanol solution, and was incubated at room temperature for 12 hours. The 3-mercaptopropyltrimethoxysilane self-assembled monolayer on the surface of the ITO electrode was rinsed with ethanol and treated 10 min at 100 ℃ to remove the weakly adsorbent 3-MPTS molecules. The ITO electrode partially covered the electrodes with a thin polydimethylsiloxane (PDMS) membrane containing holes (5 mm in diameter). After that, 50 uL of streptavidin modified AuNPs was added to the 3-MPTS modified ITO electrode, followed by a 4 hours-incubation at 4 ℃. After rinsing with 0.02 mol L^{-1} PBS (pH 7.4) buffer solution, the AuNPs modified electrode was ready for further experiments.

1.7 Fabrication of sandwich-type DNA sensor

AuNPs modified electrode was further treated with 50 uL of 0.1 mol L^{-1} Tris-HCl (pH 8.0) buffer solution containing 4 umol L^{-1} capture DNA and incubated 1 h at 37 ℃. The modified electrode was subsequently rinsed thoroughly with 0.02 mol L^{-1} PBS (pH 7.4) buffer solution to remove the weakly absorbed capture DNA. A 50 uL portion of 1 % (w/w) BSA was used to saturate the possible bare AuNPs for 40 min at 37 ℃ and rinsed by 0.02 mol L^{-1} PBS (pH 7.4) buffer solution. Then 50 uL of TE buffer solution containing the target DNA with different concentrations were dropped onto the modified electrode, incubated 1 h at 37 ℃, and subsequently rinsed with 0.02 mol L^{-1} PBS (pH 7.4) buffer solution to remove unbound target DNA. Finally, 50 uL of luminol-AgNPs labeled DNA probe was dropped onto the modified electrode at 37 ℃ for 1 h, and rinsed with 0.02 mol L^{-1} PBS (pH 7.4) buffer solution.

Electrochemical impedance spectroscopy (EIS) was used to monitor the fabrication procedures. EIS experiment was carried out on a CHI760B electrochemical workstation (Chenhua, China). PBS (0.1 mol L^{-1}, pH 7.0) containing 1 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ was used as working solution. The EIS results were recorded in the frequency range from 0.1 to 10 KHz at the formal potential of the corresponding redox couple and with a 5 mV amplitude of the alternative voltage.

1.8 ECL Measurements

ECL measurements were performed with a home made ECL/electrochemical cell system, including a model CHI760B electrochemical workstation (Chenhua, China), an H-type electrochemical cell (self-designed), a model CR-105 photomultiplier tube (PMT) (Beijing, China), a model RFL-1 luminometer (Xi′an, China), and a computer, as described previously[3]. A three-electrode system composed of an ITO electrode attached by a sandwich-type DNA complex as the working electrode, a platinum wire as the counter electrode, and a silver wire as the quasi-reference electrode (AgQRE). Although the potential of the AgQRE was essentially stable during an experiment, the measurements of $\triangle E=E_{Ag/Ag+} - E_{SCE}$ in different solutions were taken for potential calibrations. A H_2O_2 solution containing CBS buffer (pH =10.53) was used as working solution for the detection of target DNA. During measurements, a 3.0 mL portion of the working solution and the 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. High voltage applied to the PMT was maintained at -900 V.

2. Analysis of surface composition of luminol-AgNPs

It was found that luminol could be oxidized by $AgNO_3$ to the excited aminophthalate ion (AP^{2-}) in aqueous solution, giving rise to CL[4]. In the reaction, the final oxidation product of luminol was ground state AP^{2-}. Herein, it deduced that $AgNO_3$ was reduced by luminol to form luminol-AgNPs and luminol was oxidized to AP^{2-}. Considering that both luminol and AP^{2-} including the aromatic amine group in their molecular structure, it was reasonable to presume that luminol and AP^{2-} were coexisted on the surface of luminol-AgNPs through the weak covalent interaction between silver and nitrogen atoms, according to the earlier reports on the interaction between amine compounds and AgNPs [5]. UV-Vis, ^{1}HNMR spectroscopy, XPS and TGA were used to characterize chemical compositions of the surface of Ag nanoparticles. The luminol-AgNPs of diameter 25 nm were selected for following characterizations.

2. 1 UV/Vis spectroscopy

A 24 hour dialysis operation was carried out to remove free luminol and AP^{2-} molecules from luminol-AgNPs. After that, it can be seen that the three absorption peaks (Figure S2, curve a, 219, 300, and 350 nm) of luminol approximately disappeared in the UV-Vis spectrum of the dialyzed luminol-AgNPs (curve c) compared with that of initial luminol-AgNPs (curve b), and the maximal absorption wavelength remained at 427 nm except the intensity of the peak decreased, which was ascribed to the dilution effect during the dialysis procedure. However, the three peaks of luminol could be observed in the dialyzed-out solution during the first dialysis operation (curve d) and it disappeared in the dialyzed-out solution during last dialysis (curve e).

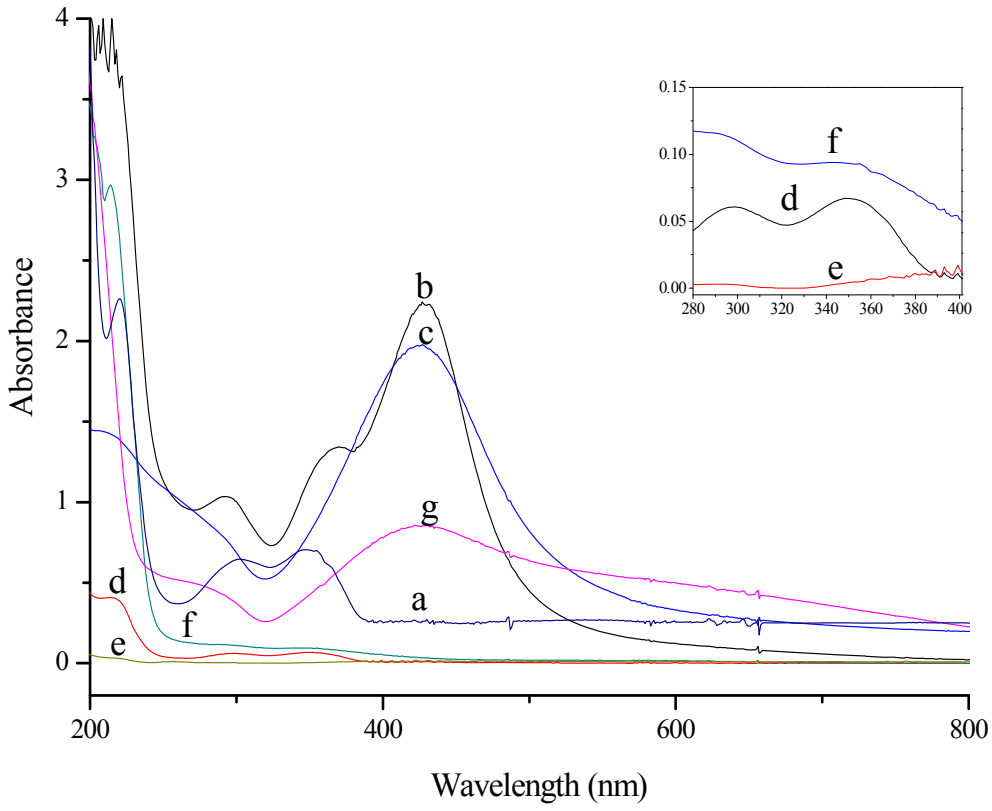

Figure S2 UV/Vis absorption spectra of 25 nm luminol-AgNPs before and after dialysis and salt-out procedures. a) luminol solution ,b) original 25 nm luminol-AgNPs, c) dialyzed luminol-AgNPs, d, e) dialyzed-out solution for the first and last dialysis procedures, respectively, f) supernatant after salt-out of dialyzed luminol-AgNPs, and g) redispersed luminol-AgNPs precipitated during the salt-out process. Inset: magnification of curves e-f in the range of 280-400 nm

These results indicated that the dialysis procedure was feasible and effective in removing all free luminol and its oxidation product (AP^{2-}) from the luminol-AgNPs, while keeping the diversity and stability of the luminol-AgNPs. Subsequently, a further salt-out operation was executed on dialyzed luminol-AgNPs and observable coagulation appeared after the addition of salts. The three peaks of luminol appeared again in the supernatant after centrifugation (curve f). The sediments were redispersed in 0.1 mol L^{-1} Na_2CO_3 solution. Compared with initial luminol-AgNPs (curve b), The surface plasmon absorption wavelength of luminol-AgNPs increased (curve g) and the peak became wide, which might be due to the diameter of nanoparticles increased and the monodispersity became worse during the salt-out procsess. The information attained from UV/Visible spectra confirmed that the protective molecules were partially dissociated from the surface of the luminol-AgNPs and repulsive interaction became weak during the salt-out process, which was the reason why the luminol

molecules could be detected again in the supernatant. In summary, these results provided evidence for the existence of luminol on the surface of luminol-AgNPs.

2. 2 XPS spectroscopy

Figure S3 showed the Ag 3d, C 1s and N 1s X-ray photoelectron spectra of pure luminol and the as-prepared luminol-AgNPs after further treatments. All binding energies were calibrated with respect to the C 1s at 284.7 e V. The spin-orbit splitting of doublet components for Ag $3d_{5/2}$ and Ag $3d_{3/2}$ (Figure S3A) were measured to be 6 e V, which was in good agreement with previous reports[6]. This result was supporting evidence for the presence of Ag^0 in as-prepared luminol-AgNPs. As can be seen from Figure S3B, the C 1s spectrum of pure luminol was curve-fitted into three components at 284.7, 285.6 and 287.7 e V with the same full width at half-maximum (fwhm, 1.03 e V), which might correspond to carbon atoms from 1 to 3 in the molecular structure of luminol. The maximal peak centered at 284.7 (C1 in the molecular structure of luminol) was attributed to the carbon atom in the -CH- group. The component at 285.6 e V (C2) was associated with the carbon atom in the -C-NH- group. The component at 287.7 e V (C3) was due to the carbon atom in the -CO-NH- group. The C 1s spectrum of luminol-AgNPs is shown in Figure S3D. Compared with the C 1s of pure luminol, a new component centered at 288.7 eV (C4) appeared, as well as the three components similar to those in the pure luminol C 1s spectrum which was attributed to the carbon atom in the carboxylic group (-COO-) of the oxidation product of AP^{2-}. The results supported that luminol and its oxidation product coexisted on the surface of the luminol-AgNPs.

The analysis of the N 1s spectra was consistent with the above discussion. The N 1s spectra of pure luminol (Figure S3C) was curve-fitted into two components at 399.6 and 400.3 eV, attributed to the nitrogen atoms in the -N-C- group and -N-CO- group, respectively. The N 1s spectrum of luminol-AgNPs was shown in Figure S3E, peak of the nitrogen atoms in the -N-C- group had a negative shift (-0.3 e V). This was due to that the -N-CO- group of luminol was oxidized to the -COO- group of AP^{2-}, Notably, the N 1s spectrum of luminol AgNPs also confirmed the absence of the protonated state ($-NH_3^+$) reported to be at approximately 402.3 e V. Consequently, the electrostatic interaction between the protonated amino group and negatively charged AgNPs did not exist, which was reported to occur in the other fatty-amine-protected AgNPs in aqueous solution. In addition, the Ag-N covalent interaction was the only force between the silver core and the luminol/its oxidation product capping molecules.

The shifts of binding energies in N 1s implied an interaction between luminol/its oxidation product and Ag atoms at the interface. The electron clouds inclined to transfer from the Ag surface to luminol/its oxidation product.

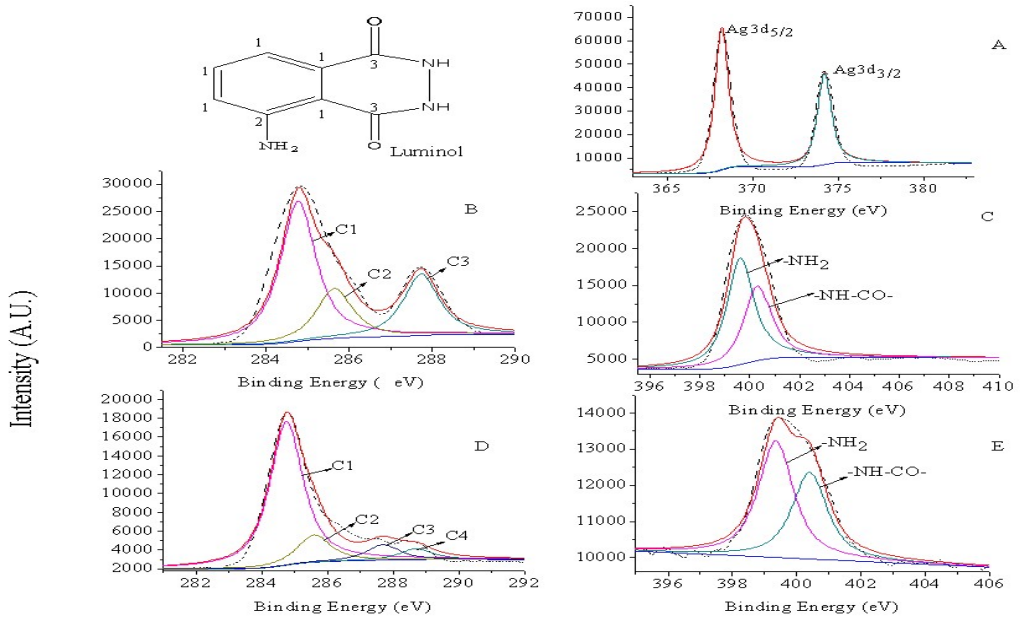

Figure S3. X-ray photoelectron spectra of A) Ag 3d and B) C 1s and C) N 1s of pure luminol and D) C 1s and E) N 1s of luminol-AgNPs. The inset showed the molecular structure of luminol.

2. 3 ^{1}HNMR spectroscopy

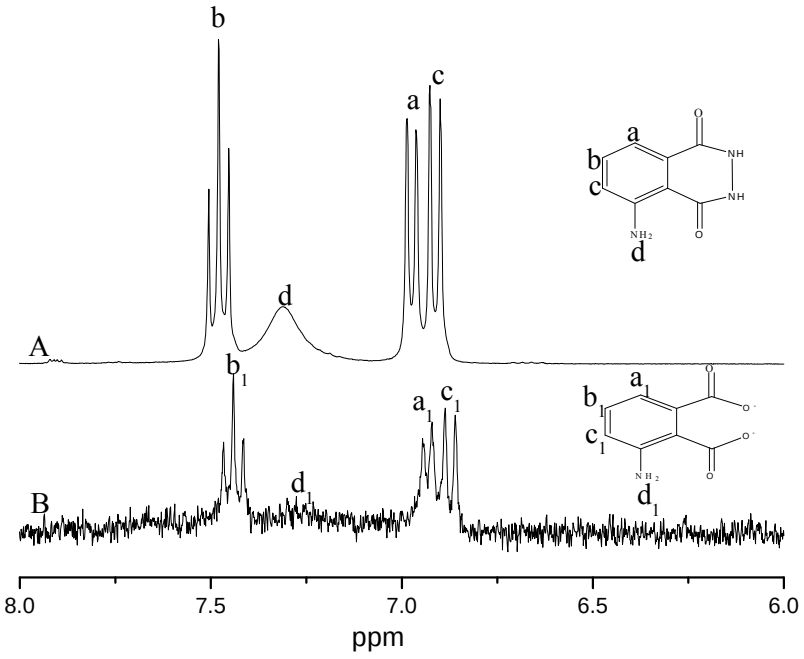

Figure S4. ^{1}H NMR spectra of luminol (A) and luminol-AgNPs (B).

^{1}HNMR spectra were an important tool for studying the surface composition of metal nanoparticles. The NMR of luminol and luminol-AgNPs was showed in Figure S4A and S4B, respectively. The results implied that the NMR of luminol-AgNPs was very similar to that of luminol. It was reasonably deduced that the surface of luminol-AgNPs had a similar chemical composition with luminol. As it well known, luminol and its oxidation product AP^{2-} presented analogous structures. Compared with luminol (Figure S4A), the NMR of luminol-AgNPs had a slight high-field shift of aromatic protons and amine protons (Figure S4B), which was ascribed to AP^{2-} with a dual carboxylate ion that decreased the electron-attractive ability of carbonyl group, resulting in low NMR chemical shift. Nevertheless, The NMR of luminol did not emerge, which was potentially due to its low concentration on the surface of luminol-AgNPs. The results supported that AP^{2-} existed on the surface of luminol-AgNPs.

2. 4 Thermogravimetric analysis

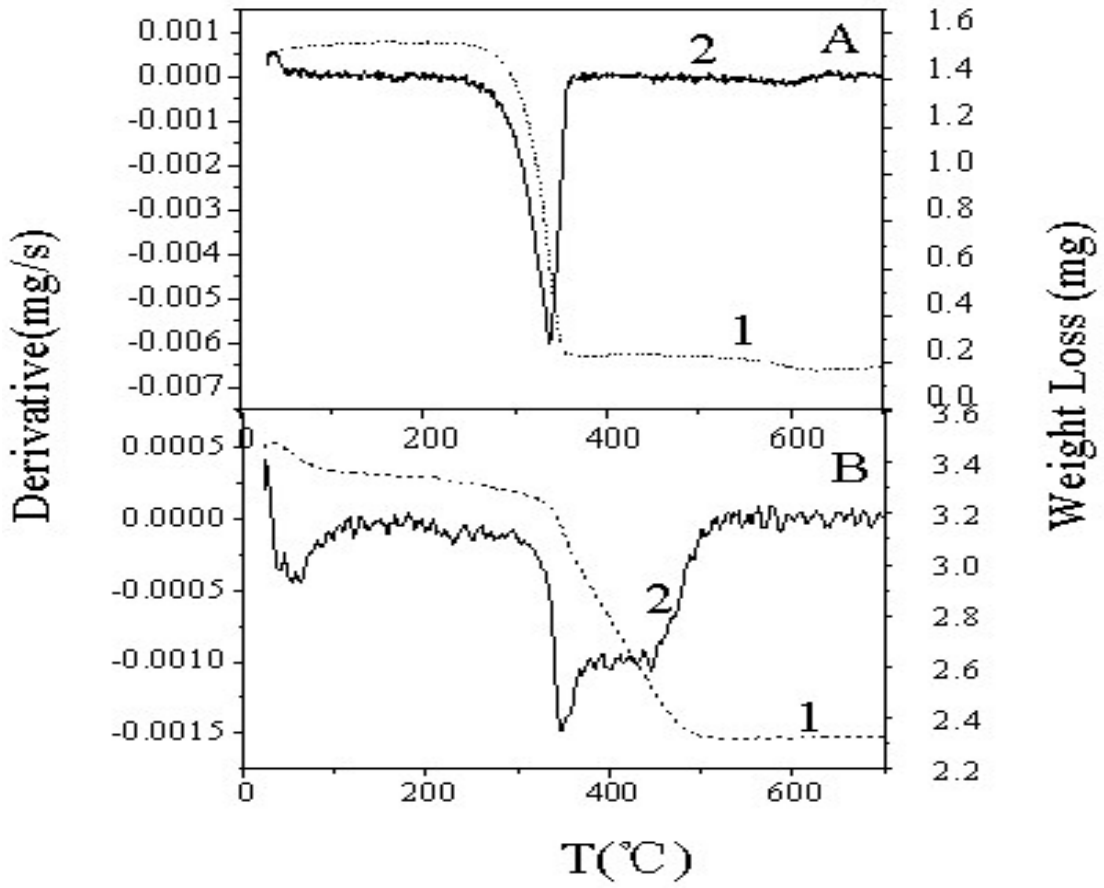

Figure S5 TGA (1) and DTG (2) curves of A) pure luminol and B) luminol-AgNPs.

Figure S5 showed the TGA and correspond differential thermogravimetry (DTG) plots of pure luminol and luminol-AgNPs (Figure S5A and S5B, respectively). Curve 1 was the TGA curve (right

axis) and curve 2 was the corresponding DTG curve (left axis) in both plots. For pure luminol, a nearly total weight loss appeared increasing the temperature to 700 ℃, which started at 280 ℃ and ended at 360 ℃, with the maximal rate of weight loss at approximately 340 ℃. For luminol-AgNPs, a similar weight loss was observed at approximately 340 ℃ (Figure S5B). The results demonstrated that luminol exited on the surface of luminol-AgNPs. The weight loss around 65 ℃ in Figure S5B was assigned to the moisture content due to the preparation method. The weight loss of luminol-AgNPs occurred at 400 ℃, which was consistent with the previously reported TGA result of amine-capped AgNPs[7]. In that work, two prominent weight loss peaks were observed at 210 and 380 ℃ for the Ag-glycocholate complex. The lower characteristic temperature was ascribed to the loss of the desorption of bile salt presented in the AgNPs, and the higher was due to the loss of the covalent interaction between them. The absence of weight loss at approximately 210 ℃ and the presence of weight loss at approximately 400 ℃ in the TGA data of luminol-AgNPs further confirmed the results from UV/Vis and ^{1}NMR spectra, which indicated that there was only covalent interaction and almost no adsorption in these luminol-AgNPs. It was concluded that luminol and AP^{2-} coexisted on the surface of luminol-AgNPs, which was also agreement with the results of UV/Vis，XPS and ^{1}NMR spectra.

3. EIS of DNA sensor

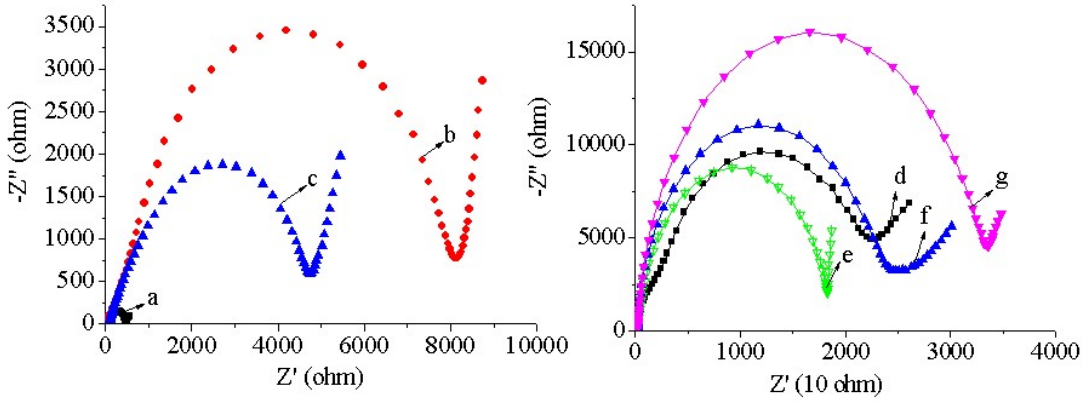

Figure S6 a bare ITO electrode, b 3-mercaptopropyltrimethoxysilane /ITO electrode, c streptavidin-AuNPs/3-mercaptopropyltrimethoxysilane /ITO electrode, d capture DNA-biotin/streptavidin-AuNPs/3-mercaptopropyltrimethoxysilane /ITO electrode, e BSA/capture DNA-biotin/streptavidin-AuNPs/3-mercaptopropyltrimethoxysilane /ITO electrode, f target DNA /BSA/ capture DNA-biotin/streptavidin-AuNPs/3-mercaptopropyltrimethoxysilane /ITO electrode, g luminol-AgNPs labeled probe DNA /target DNA /BSA/ capture DNA-biotin/streptavidin-AuNPs/3-mercaptopropyltrimethoxysilane /ITO electrode in 0.1 mol L^{-1} PBS

(pH =7.0) with 1 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$. Scan rate is 100 mV L^{-1}. The frequency range is between 0.1 and 10^4 Hz, alternate voltage 5 mV.

Figure S6 showed the EIS curves of the modified ITO electrode during the step-by-step buildup process using $[Fe(CN)_6]^{3-/4-}$ as electro-active probe couple. In the Nyquist plot, the diameter of the semicircle at higher frequency range was equal to the electron transfer resistance, which represented the electron transfer kinetics of the electroactive probes at the electrode interface. It was clear that the diameter of semicircle after the assembly of 3-mercaptopropyltrimethoxysilane (curve b) was larger than that of bare ITO electrode (curve a), indicating an increase of the electron transfer resistance after the assembly of 3-mercaptopropyltrimethoxysilane on the ITO electrode. The electron transfer resistance decreased after the assembly of AuNPs (curve c), which was ascribed to AuNPs accelerate electron transfer between a bare ITO electrode and the redox species[8]. When capture biotin-DNA was assembled successively on the modified electrode above, the electron transfer resistance increased gradually (curve d). Interesting when BSA as a block regent further assembled on the modified electrode above, the electron transfer resistance decreased (curve e). This fact indicated that BSA might replace some non-specific absorbed capture DNA on the modified electrode above. The electron transfer resistance increased after the assembly of target DNA and luminol-AgNPs labeled DNA probe owing to their bad electronic conductivity. These results confirmed that the electrode was modified as expected.

4. **Optimization of Detection Conditions**

When a double-step potential was applied to the electrode, a pulse ECL signal was obtained. The average intensity of stable pulse ECL signal was used for the detection of target DNA. Some important experimental parameters influenced the ECL reaction, including the pH of solution, the concentrations of H_2O_2, and some electrochemical parameters, were optimized. The effect of pH in the range of 9.51 ~ 10.83 (CBS, 0.02 mol L^{-1}) and 11.5 (NaOH solution) was examined as shown in Figure S7. The ECL intensity increased with the increase of pH value from 9.51 to 10.53 and reached a maximum at pH 10.53. Thus, the optimal pH value was 10.53. The ECL intensity of this system was also dependent on the concentration of H_2O_2 concentration (Figure S7B). The ECL intensity increased with the increase of the concentration of H_2O_2 (0.2 mmol L^{-1}~1.1 mmol L^{-1}). When the concentration of H_2O_2 was over 1.0 mmol L^{-1}, the background increased and did not get stable ECL signal. Therefore, 1.0 mol L^{-1} H_2O_2 was selected in the following experiments. Figure S7C and S7D showed the effects of potential value and pulse time. The effect of the pulse potential over the range 0.6 ~ 1.1 V (versus SCE) was studied. The ECL intensity increased with increasing pulse potential from 0.6 to 1.1 V. Nevertheless , if the

potential larger than 0.8 V was used, the stability and reproducibility of the modified electrode declined due to the electro-oxidation of AuNPs on the surface of electrode and the break of Au-S bond between the 3-mercaptopropyltrimethoxysilane and the AuNPs. Therefore, a pulse potential of 0.8 V was chosen as the optimum value. The ECL intensity increased with the pulse time in the range of 0.05 ~ 0.1 s. When the pulse time was longer than 0.1 s, The ECL signal was unstable (Figure S7D). Thus, the pulse time of 0.1 s was chosen as the optimum time.

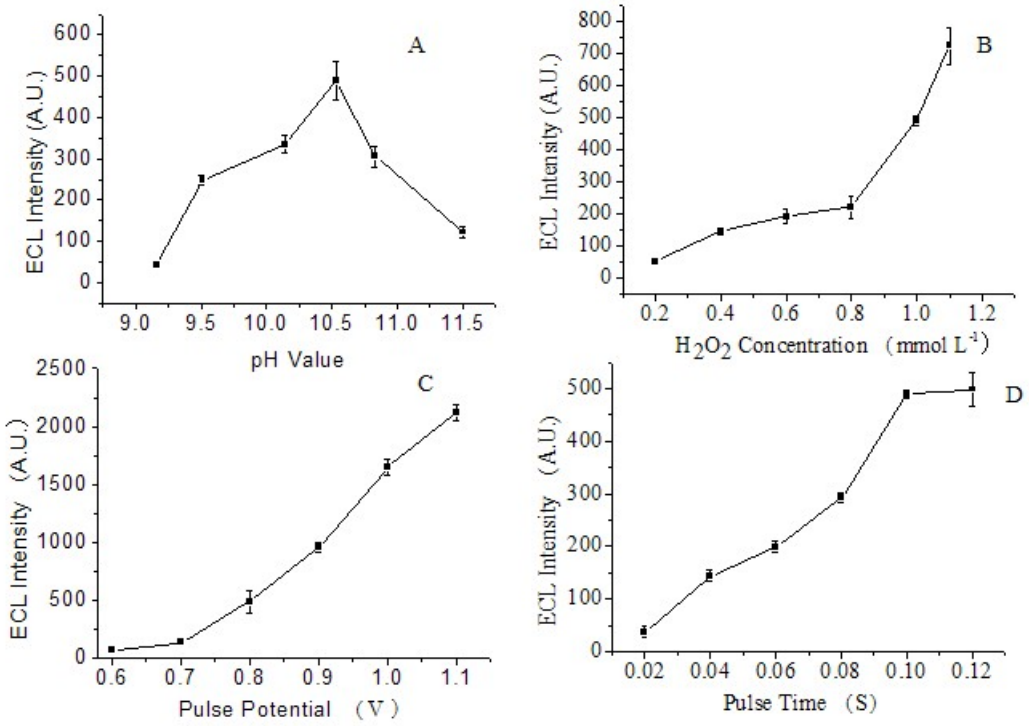

Figure S7 Effect of pH value (A), H_2O_2 concentration (B), pulse potential (C) and pulse time (D).

References

[1] H. Cui, W. Wang, C. F. Duan, Y. P. Dong and J. Z. Guo, *Chem. Eur.J.* 2007, **13**, 6975.

[2] G. Frens, Nature Phys. Sci. 1973, **241**, 20.

[3] H. Cui, G. Z. Zou and X. Q. Lin, *Anal. Chem.* 2003, **75**, 324.

[4] D. L. Giokas, A. G. Vlessidis, G. Z. Tsogas and N. P. Evmiridis, *Trends Anal. Chem.* 2010, **29**, 1113.

[5] M. Chen, Y. G. Feng, X. Wang, T. C. Li, J. Y. Zhang and D. J. Qian, *Langmuir* 2007, **23**, 5296.

[6] P. Jiang, S. Y. Li, S. S. Xie, Y. Gao and L. Song, *Chem. Eur.J.* 2004, **10**, 4817.

[7] J. Kasthuri and N. Rajendiran, *Colloid. Surface.B.* 2009, **73**, 387.

[8] S. Zhang, N. Wang, H. Yu, Y. Niu and C. Sun, *Bioelectrochemistry* 2005, **67,** 15.