

## Chemiluminescent Detection of DNA Hybridization Based on Signal DNA Probe Modified with Gold and Cobalt Nanoparticles

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A novel and sensitive chemiluminescence (CL) assay for sequence-specific DNA detection based on signal amplification with gold and cobalt nanoparticles (NPs) was reported. The sandwich-type DNA biosensor was fabricated with the amino-functionalized capture DNA immobilized on the magnetic bead and hybridized with one end of target DNA, the other end of which was recognized with a signal DNA probe labeled on the surface of Au NPs. To amplify the detection signals, a single gold NP was modified with 27 cobalt NPs by a reaction between amino-functionalized DNA on the surface of gold NP and carboxyl-functionalized on the surface of cobalt NPs. The hybridization events were monitored by the CL intensity of luminol-H<sub>2</sub>O<sub>2</sub>-Co<sup>2+</sup> after the cobalt ions were dissolved from the hybrids. This method could detect as low as  $6.0 \times 10^{-17}$  M target DNA and the line range was from  $1.0 \times 10^{-16}$  to  $1.0 \times 10^{-15}$  M.

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**Keywords:** Chemiluminescent DNA detection cobalt nanoparticles luminol-H<sub>2</sub>O<sub>2</sub>-Co<sup>2+</sup>

### 1. INTRODUCTION

Detection of specific DNA sequences is extremely important in clinical diagnosis, gene therapy, and a variety of biomedical studies [1,2,3,4,5]. Many optical [6,7], chemiluminescence (CL) [8,9], surface plasmon resonance [10,11], quartz crystal microbalance [12,13], and electrochemical [14,15] techniques have been used for detecting and quantifying DNA sequence. In these DNA detection processes, CL is a highly sensitive technique because of its excellent sensitivity, wide linear dynamic range, and simple instrumentation [16]. CL DNA biosensors are generally based on measuring the specific luminescence activity of the labels linked to the capture DNA probe for the

detection of target DNA [17,18,19]. These labels include enzymes [20,21], fluorescence dyes [22], lanthanide chelates [23,24], radioisotopes [25,26] and nanoparticles [27] and so on. In recent years, with the development of the nanotechnology, metal or semiconductor nanoparticles (NPs) [28,29,30,31,32] with unique optical and electrical properties have been used as labels for the amplified detection of DNA. These methods can overcome the safety problems, poor sensitivity, and poor stability associated with the radioisotopic, fluorescent, and enzyme labels. At the same time, a large number of metal ions can be released from one metal NPs [33], which significantly enlarged the CL signal and enhanced the detection sensitivity. For example, Wang and co-workers [34] construed a new nanoparticle-based electrical detection of DNA hybridization, based on electrochemical stripping detection of the colloidal gold tag. Its detection limit is 10 fM. Silver NPs were also used as labels for the detection of DNA targets using CL with a detection limit of 5 fM [35]. Recently, Zhang's group developed a new CL scheme for the detection of DNA hybridization based on gold and copper sulfide NPs label with a detection limit of 4.8fM [36]. However, the dissolution of gold NPs need extremely severe conditions (high concentrated  $\text{HNO}_3\text{-HCl}$  or poisonous  $\text{HBr-Br}_2$ ), which result in high CL background and further restrict the detection sensitivity. The labeling procedures of Ag NPs are quite complicated. The step of silver NPs labeled to DNA probe needs 116 h. The coupling CL reaction ( $\text{Ag-Mn-K}_2\text{S}_2\text{O}_8\text{-H}_3\text{PO}_4\text{-luminol}$ ) employed to measure  $\text{Ag}^+$  released from dissolution of silver NP probes was performed at 90 °C for 7 min. Metal sulfide NPs as sulfides have serious pollution on the environment. These disadvantages limit the application of these NPs as labels in DNA detection. As one of the most efficient metal catalyst [37],  $\text{Co}^{2+}$  could be more sensitively measured by luminol- $\text{H}_2\text{O}_2$  CL system compare with  $\text{Cu}^{2+}$  [38],  $\text{Cd}^{2+}$  [39],  $\text{Pb}^{2+}$  [40]. Therefore, the CL sensitivity for the determination of DNA hybridization could be increased 1~2 orders of magnitude. Furthermore, cobalt NPs are very cheap, stable, and safe, and easy to be preserved and dissolved. So far there has been no research reported on cobalt NPs labels in CL bioassays.

In the present work, a highly sensitive CL strategy for the determination of DNA hybridization was developed gold NPs and cobalt NPs as label. Cobalt ions were dissolved from hybrid tagged with cobalt NPs and the concentration of dissolved cobalt ions by the CL intensity of luminol- $\text{H}_2\text{O}_2\text{-Co}^{2+}$ . The linear range was from  $1.0 \times 10^{-16}$  to  $1.0 \times 10^{-15}$  M, and the detection limit of the target DNA was estimated to be as low as  $6.0 \times 10^{-17}$  M.

## 2. EXPERIMENTAL

### 2.1. Reagents

All the DNA sequences were synthesized and purified by SBS Genetech Co. Ltd. (China), and the sequences of this work are as follows:

Capture DNA (1): 5'-NH<sub>2</sub>-TTG GCG CGA ACC GTA TA-3'

Target DNA (2): 5'-GCT TGG CAT ATA TAT AGA TAG TAC GGT TCG CGA-3'

Probe DNA (3): 5'-TCT ATC ATG CCT AGC GGT ACA-SH-3'

Singal DNA (4): 5'-NH<sub>2</sub>-ATC CTG TTC GAA TAT TTG-SH-3'

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was purchased from Sigma. H<sub>2</sub>O<sub>2</sub> with analytical grade was from Shanghai Chemical Reagent Company (Shanghai, China). 6-Mercapto-1-hexanol (MCH) was obtained from Fluka. Luminol was purchased from ABCR GmbH & Co. Imidazole was obtained from Guoyao Chemical Co. A luminol stock solution of  $2.5 \times 10^{-2}$  M was prepared by dissolving 0.4429 g of luminol in 100 mL of 0.1 M NaOH solution and further stored in dark; the luminol solution used for CL determination was diluted by 0.1 M Na<sub>2</sub>CO<sub>3</sub>-NaHCO<sub>3</sub> buffer (pH 11.5). Ferriammonium sulfate was ordered from Tianjin Yaohua Chemical Reagent Co., Ltd. (China). The 0.1M PBS buffer (pH 7.4) and 0.1 M HAc-NaAc buffer (pH 3.5) was prepared according to the standard methods. Deionized and doubly distilled water was used throughout the experiments. All the chemicals employed were of analytical reagent grade and were used without further purification.

## 2.2. Apparatus

The CL measurements were performed with a CL instrument (RFL-1, Remex Analytical Instrument Co. Ltd., Xi'an, China), Transmission electron microscopy (TEM) images were acquired on a JEM-2000EX/ASID2 (HITACHI, Japan). A CARY 500 Scan UV/Vis-NIR spectrophotometer (Varian, USA) was used to record the UV-vis absorption spectra. Carboxyl groups modified magnetic beads (MBs) (10 mg mL<sup>-1</sup> Fig. S1) and magnetic racks were obtained from BaseLine ChromTech Research Centre (Tianjin, China).

## 2.3. Preparation of gold NPs

Gold NPs were prepared by trisodium citrate reduction of HAuCl<sub>4</sub> in aqueous solution [41,42]. HAuCl<sub>4</sub> solution and trisodium citrate solution were filtered through a 0.22 μm microporous membrane filter prior to use, and then 2.0 mL of 1% trisodium citrate was added to 100 mL of boiling 0.01% HAuCl<sub>4</sub> solution and stirred for 10 min at the boiling point. The final Au NPs prepared by this method have an average diameter of ~20 nm as measured by TEM (Fig. 2 A).

## 2.4 Preparation of cobalt NPs

Cobalt NPs were prepared according to the method reported previously with a slight modification [43]. 0.2 M brown colored solution of cobalt acetate tetrahydrate (0.166 g) in diphenyl ether (3.3 mL) was first prepared in the presence of 0.32 mL of oleyl amine and 0.08 g of polyvinyl pyrrolidone. This solution was injected into 20 mL of a hot solution of a mixture of 0.687 g of 1, 2-hexadecanediol and 0.9 mL of trioctylphosphine in diphenyl ether (20 mL) at 100 °C in a 100 mL three necked round bottomed flask under an inert atmosphere of nitrogen. The temperature of the reaction solution was slowly raised to 180 °C, where 0.16 mL of oleic acid (0.02 M) was added as a surfactant.

Upon heating at 240 °C, the color of the reaction solution changed to black within few seconds. Then, the temperature of the reaction solution was raised to 260 °C and the solution was refluxed for 60 min to grow the cobalt NPs. The black colored solution was cooled to room temperature under nitrogen atmosphere and the produced cobalt NPs were precipitated by adding ethanol (~20mL) as a flocculating agent. The precipitate of the cobalt NPs was separated by centrifugation at 6000 rpm for 15 min.

### 2.5 Preparation of carboxyl-functionalized cobalt NPs

The carboxyl-functionalized Co NPs were achieved with solution polymerization. The surface modification reaction was conducted in a 150 mL three necked round bottomed flask under an inert atmosphere of nitrogen. First, 100 mL 5% acrylic acid aqueous solution, 1mL 1% ammonium persulfate solution and 0.1~0.2 g cobalt NPs were added into three necked round bottomed flask. Then the mixture was stirred for 15 min. Finally the flask was placed in an oven at 60 °C for 2 h. The carboxyl-functionalized cobalt NPs have an average diameter of ~20 nm as measured by TEM (Fig. 2 B).

### 2.6 Modification of signal DNA probe with gold NPs and cobalt NPs

The oligonucleotide-modified gold NPs were prepared according to the reference with a slight modification [44]. Briefly, 20  $\mu\text{L}$  of  $1.0 \times 10^{-7}$  M probe DNA solution and 200  $\mu\text{L}$  of  $1.0 \times 10^{-7}$  M single DNA solution were added to 1 mL of the prepared gold NPs solution. After shaking gently, the solution was allowed to stand, followed by centrifugation. Following the red oily precipitate was washed with phosphate buffer containing 0.1 M NaCl (pH 7.0), recentrifuged and then 100  $\mu\text{L}$  of 0.1M EDC solution and different amounts of prepared cobalt NPs solution modified with carboxyl were added to the above solution. Finally, the signal DNA and probe DNA tagged with gold NPs and cobalt NPs was collected by centrifugation at 8000 rpm. The precipitate was washed and then resuspended in water. The solution of signal DNA and probe DNA modified with gold and cobalt NPs was stored at 4 °C for the hybridizations.

### 2.7 Fabrication of the CL biosensor

The process for fabrication and CL detection of the DNA biosensor is schematically shown in Scheme1. 20  $\mu\text{L}$  of the MBs modified with carboxyl was washed with 500  $\mu\text{L}$  0.1 M imidazole solution (pH 6.8) then 100  $\mu\text{L}$  capture DNA was added into MBs and incubated, then washed it with 0.1 M phosphate buffer containing 0.1 M NaCl (pH 7.0). In order to avoid consequent nonspecific adsorption in the following hybridization steps, 200  $\mu\text{L}$  0.1 M MCH was added into the MBs for 2 h to block the uncovered MBs surface. The sandwich-type format assay used consists of two steps. First, the modified MBs was added into 0.1M PBS buffer containing target DNA with the different concentrations at 37 °C. The modified MBs was separated from the solution on a magnetic rack after 2

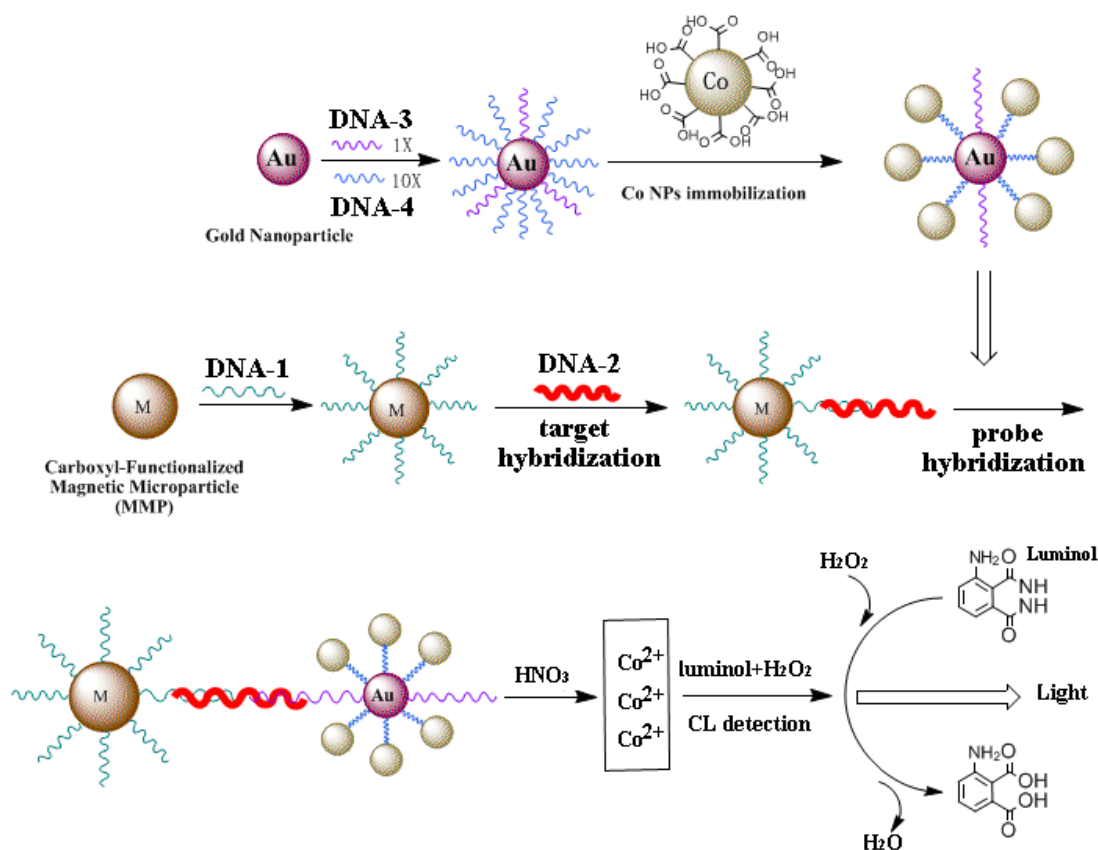
h and added into 0.1M PBS buffer containing signal DNA and probe DNA functionalized with gold and cobalt NPs for 12 h. The MBs were rinsed with 0.1M phosphate buffer.

### 2.8 CL detection

After hybridization, the MBs containing gold and cobalt NPs labeled DNA probe was immersed into a cell containing 200  $\mu\text{L}$  of  $1.0 \times 10^{-3}$  M nitric acid solution (pH 2.8) for 4 h. The cobalt NPs anchored onto the hybrids were dissolved. The solution was pipette out by magnetic separation into an analytical cell. The 50  $\mu\text{L}$  above solution and 200  $\mu\text{L}$   $1.0 \times 10^{-4}$  M luminol solutions were added into the sample cell. Then 200  $\mu\text{L}$   $1.0 \times 10^{-3}$  M  $\text{H}_2\text{O}_2$  solutions were injected, and cobalt ions were reacted with the mixture of luminol and  $\text{H}_2\text{O}_2$  to produce CL signal. The concentration of target DNA was quantified based upon the concentration of dissolved cobalt ions, which was quantified by the CL intensity.

## 3. RESULTS AND DISCUSSION

### 3.1. Fabrication of the biosensor and the detection process



**Scheme 1.** Schematic representation of chemiluminescence detection of DNA hybridization biosensor

The CL detection strategy of DNA hybridization based on two steps of NPs-amplification through sandwich-type is shown in Scheme 1. Amino-functionalized capture DNA was assembled on the magnetic beads (MBs) with carboxyl groups, cobalt NPs labeled signal DNA and probe DNA were conjugated with gold NPs, both of which flank the target DNA resulting in the fabrication of a sandwich-type detection protocol. Since a single gold NP could be loaded with a plenty of signal DNA strands labeled with cobalt NPs, the first significant amplification for the detection of target DNA was obtained. At the same time, once the cobalt NPs conjugated on the signal DNA were dissolved, a large amount of cobalt ions were released as the secondary amplification and could be sensitively determined by the luminol- $\text{H}_2\text{O}_2$ - $\text{Co}^{2+}$  CL reaction system which generated a strong CL signal. The CL intensities were proportional to the concentration of target DNA based upon the concentration of dissolved cobalt ions.

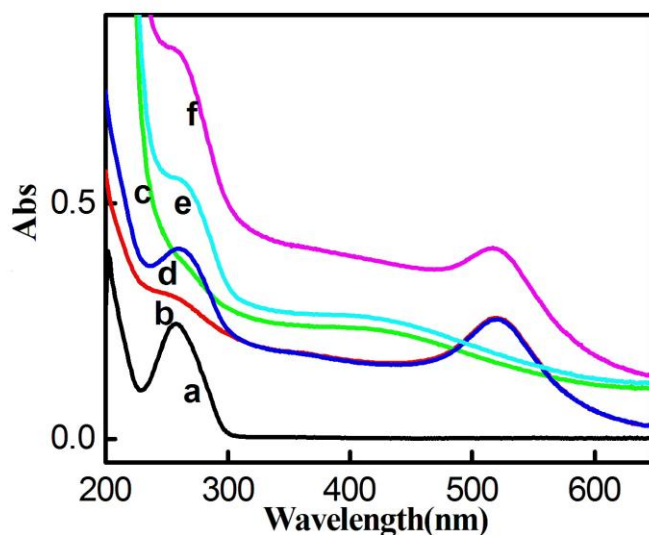
Capture DNA sequence (1): 5'-NH<sub>2</sub>-TTG GCG CGA ACC GTA TA-3'

Target DNA sequence (2): 5'-GCT TGG CAT ATA TAT AGA TAG TAC GGT TCG CGA-3'

Signal DNA probe sequence (3): TCT ATC ATG CCT AGC GGT ACA-SH-3'

DNA sequence (4): 5'-NH<sub>2</sub>-ATC CTG TTC GAA TAT TTG-SH-3'

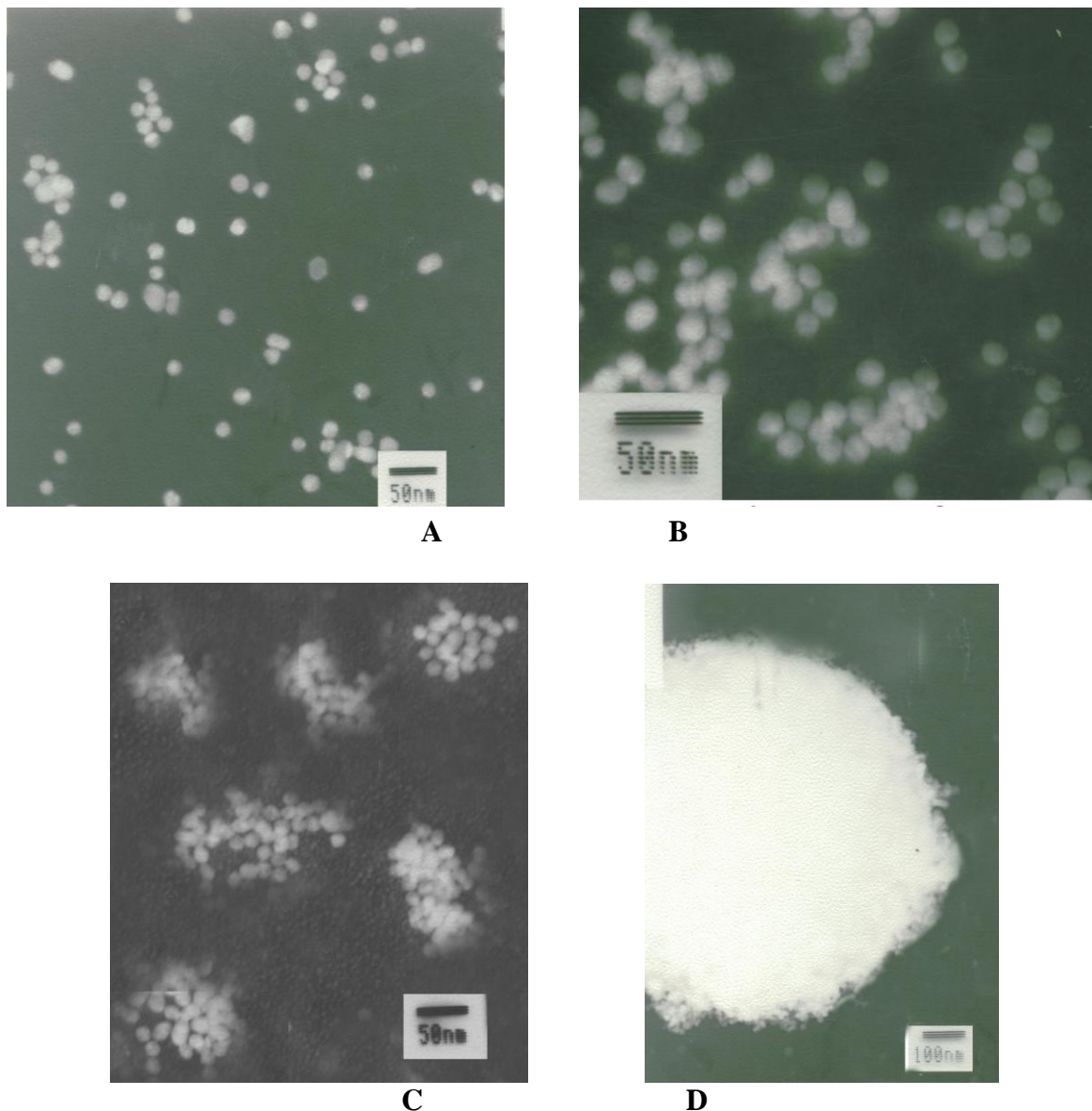
### 3.2. UV-Visible spectra of the DNA-NP conjugates



**Figure 1.** UV spectra of probe DNA (a), Au NPs (b), Co NPs (c), probe DNA difunctionalized with Au NPs (d), probe DNA difunctionalized with Co NPs (e), probe DNA difunctionalized with Co NPs and Au NPs (f)

The UV-visible absorption spectra of the signal DNA (a), gold NPs (b), cobalt NPs (c), gold NPs functionalized probe DNA (d), cobalt NPs functionalized probe DNA (e), both cobalt and gold NPs difunctionalized probe DNA (f) were shown in Fig. 1. Curve f exhibited all the characteristic absorbance of DNA (curve a), gold NPs (curve b) at ~520nm and cobalt NPs (curve c). The results indicated that both the cobalt NPs and gold NPs had been successfully labeled on the DNA probe. At

the same time, from Fig. 2 (C), the cobalt NPs covers the Au NPs around. From Fig. 2 (D), the gold and cobalt NPs cover the whole surface of the MB and retain their original sizes, while in the absence of signal DNA, only individual NPs were observed (in Fig. 2 (A), (B)). So far the TEM indicated that the MB was modified with gold NPs and cobalt NPs.

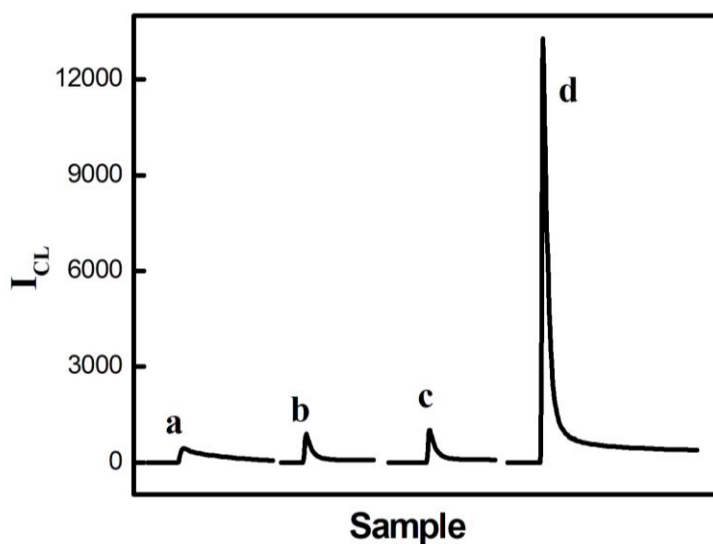


**Figure 2.** The TEM images of Au NPs (A), Co NPs (B), Au NPs modified with Co NPs (C), MB modified with Au NPs and Co NPs.

### 3.3 CL behavior of the luminol- $H_2O_2$ catalyzed by cobalt ions

The CL curves of luminol- $H_2O_2$  before and after catalyzing by cobalt ions were shown in Fig. 3. It was found that the CL intensities of target DNA probe (curve a), gold NPs functionalized signal DNA probe (curve b), cobalt and gold NPs (curve c) were almost the same with the baseline of

luminol- $\text{H}_2\text{O}_2$  system. However, the CL intensity obviously increased when the signal DNA probe was labeled by cobalt and gold NPs (curve d). This could be attributed to the fact that the cobalt ions recognized by signal DNA could catalyze the luminol- $\text{H}_2\text{O}_2$  system. Moreover, more cobalt NPs could be loaded on the hybrids through gold NPs-amplification technique, and thus the CL intensity was greatly enhanced. The amplification could be confirmed by the ratio (1:27) of gold NPs and cobalt NPs (the calculation results see supporting information).



**Figure 3.** CL behavior of the luminol- $\text{H}_2\text{O}_2$ . CL intensities of DNA probe (a), DNA probe labeled with Au NPs (b), Co and Au NPs (c), DNA probe labeled with Au and Co NPs (d). All the concentration of the different target DNA is  $1.0 \times 10^{-10}$  M. Samples are measured after injection of 10s.

### 3.4. Optimization of luminol- $\text{H}_2\text{O}_2$ - $\text{Co}^{2+}$ CL system

The concentrations, and pH of buffer, luminol and  $\text{H}_2\text{O}_2$  concentrations and pH of  $\text{Co}^{2+}$  standard solution have a dramatic effect upon the CL assay. Furthermore, these parameters of the luminol- $\text{H}_2\text{O}_2$ - $\text{Co}^{2+}$  CL system were investigated systematically to obtain the optimal conditions for the CL reactions. From Fig. S2, the optimal pH values of the luminol were 11.5 in 0.1M  $\text{Na}_2\text{CO}_3$ - $\text{NaHCO}_3$  buffer solutions (Fig. S2 A). The concentrations of luminol and  $\text{H}_2\text{O}_2$  were found to be the optimum for CL reaction in  $1.0 \times 10^{-4}$  M and  $1.0 \times 10^{-3}$  M, respectively ((Fig. S2 B, S2 C). The pH of  $\text{Co}^{2+}$  standard solution is another critical factor for luminol- $\text{H}_2\text{O}_2$ - $\text{Co}^{2+}$  CL reaction system. Through adjusting the pH of  $\text{Co}^{2+}$  standard solution in the range from 1.5 to 4.5, the maximum CL signal was obtained at pH 3.5 for  $\text{Co}^{2+}$  standard solution (Fig. S2 D).

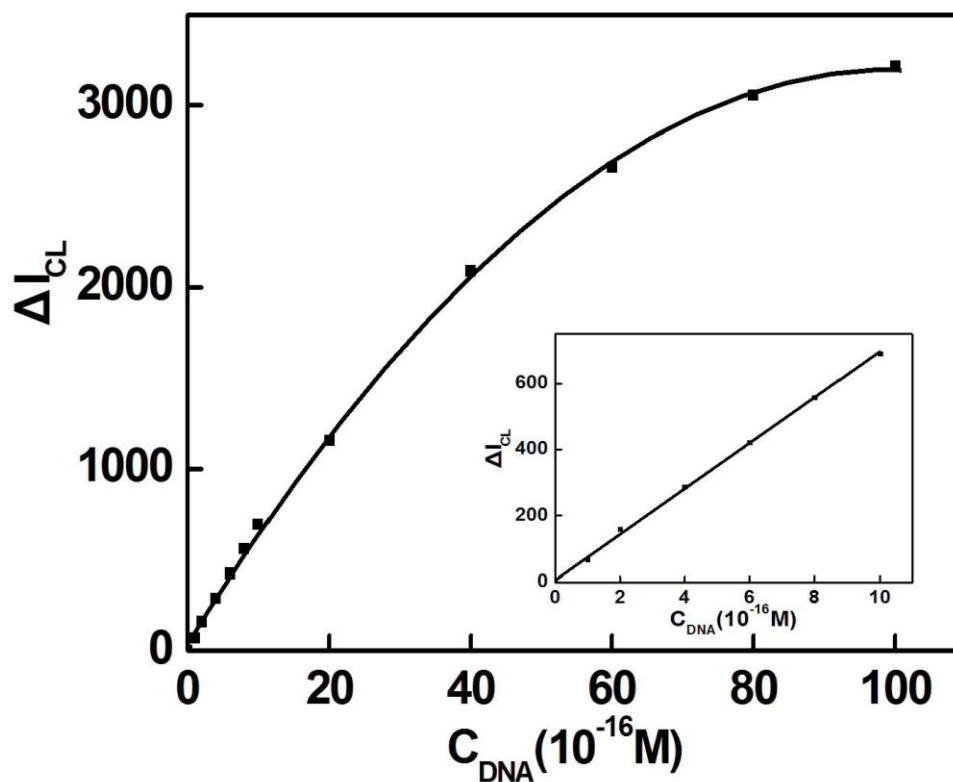
### 3.5. Sensitivity of the DNA biosensor

Based on the combination of the remarkable sensitivity of the CL method with the NPs-amplification technique, the CL response with DNA concentration was shown in Fig. 5. The CL



intensities of luminol-H<sub>2</sub>O<sub>2</sub>-Co<sup>2+</sup> increased with the increase of concentration of the target DNA range from  $1.0 \times 10^{-16}$  to  $1.0 \times 10^{-14}$  M. The nonlinear function for target DNA was  $I_{CL}=38.75 + 63.24C - 0.3165C^2$  ( $I_{CL}$  is the CL intensity;  $C$  is the concentration of target DNA,  $10^{-16}$  M;  $n = 12$ ,  $R^2 = 0.9794$ ). The linear range was achieved from  $1.0 \times 10^{-16}$  to  $1.0 \times 10^{-15}$  M with the equation of  $I_{CL} = 68.88C + 7.529$  ( $I$  is the CL intensity;  $C$  is the concentration of DNA,  $10^{-16}$  M;  $n = 7$ ,  $R^2 = 0.9965$ ) and with the detection limit as low as  $6.0 \times 10^{-17}$  M estimated using  $3\sigma$ . The improved detection limit is attributed to 27 cobalt NPs (see supporting information for detail) conjugated with a gold NPs and more cobalt ions released from cobalt NPs after oxidizing by nitric acid and then quantitatively determined by a simple and sensitive Co<sup>2+</sup>-catalyzed luminol-H<sub>2</sub>O<sub>2</sub> CL reaction.

This method and some other amplified techniques, which can greatly improve the sensitivity of the DNA assay, are listed in Table 1. In Table 1, the reported lowest detection limit of CL reaction is  $4.8 \times 10^{-15}$  M based on gold NPs and copper sulfide NPs. Compared with their work, there are three main advantages of this assay. First, to the best of our knowledge, it is the first time that both gold and cobalt NPs labels amplification were employed simultaneously in the most sensitive CL method for DNA detection with the merits of being simple and fast. Second, the CL reaction of luminol-H<sub>2</sub>O<sub>2</sub>-Co<sup>2+</sup> was quite efficient with a detection limits of  $1.25 \times 10^{-8}$  M Co<sup>2+</sup>, which is much more sensitive than Cu<sup>2+</sup> detection in luminol-H<sub>2</sub>O<sub>2</sub> CL system [45]. Third, the amount of cobalt ions and cupric ions were approximately  $3.49 \times 10^{17}$ :  $2.34 \times 10^{17}$  in the luminol-H<sub>2</sub>O<sub>2</sub> CL reaction systems. So the detection limit of this present work was found to be increased 2 orders of magnitude.



**Figure 4.** CL signal of cobalt ions dissolved from different hybrids. Inset is the amplification of the dots 1-7.

**Table 1.** Comparison between the Proposed CL Assay and Other Reported Techniques for the Detection of DNA Hybridization

format	label	techniques	detection limit of ssDNA
nanoparticle and nanostructure-based methods for DNA hybridization	Au NPs (cross-linked) [46]	colorimetric	~10 nM
	Au NPs (cross-linked) [47]	electrochemical	100 fM
	Au NPs (non-cross-linked) [48]	colorimetric	60 nM
	Au NPs [31]	laser diffraction	~50 fM
	Au NPs [49]	SPR	10 pM
	Au NPs [48]	PSA	15 nM
	Au NPs[34]	electrochemical stripping	10 fM
	Au NPs with amplification[50,51]	Ag Scanometric	50 fM
	Au NPs with amplification[52]	Ag Raman spectroscopy	~20 fM
	Au NPs with amplification[53]	Ag electrical	500 fM
	Au NPs with amplification[31]	Ag PSA	32 pM
	silver NPs[35]	CL	5 fM
	silver NPs[32]	ASV	0.5 pM
	CuS NPs[54]	CL	550 fM
	ZnS, CdS, PbS NPs[46]	stripping voltammetry	270 pM
	ZnS and CdSe quantum dots[28]	fluorescence	2 nM
	Liposome[53]	liposome-amplified electrochemical	50 fM
	Au NPs with amplification[30]	Ag bio-bar-code amplified scanometric	0.5 fM
Au and CuS NPs[36]	CL	4.8 fM	
Au and Co NPs(this method)	CL	60.0aM	

#### 4. CONCLUSIONS

In conclusion, a simple and sensitive biosensor has been developed for the detection of DNA hybridization based on CL and signal amplification by gold and cobalt NPs was reported. The constructed CL DNA biosensor platform exhibits the following advantages. First, after dissolution by nitric acid, a large amount of cobalt ions are released and are oxidized into  $\text{Co}^{2+}$  which exhibited a high sensitivity for the luminal- $\text{H}_2\text{O}_2$  CL system. Compared with the severe dissolution conditions for gold

NPs, cobalt NPs can be easily dissolved by a nitric acid solution of low concentration. Second, owing to the inherent high sensitivity of the CL method for metal ions, an ultrasensitive detection of DNA hybridization is achieved by the luminal-H<sub>2</sub>O<sub>2</sub>-Co<sup>2+</sup> CL system with the detection limit as low as 6.0 × 10<sup>-17</sup> M without any preconcentration process. Third, no complicated instrument and no expensive reagents are required during the analytical procedure. Considering the easy operation and simple labeling procedure, it is promising that the NP modified NP will open up a new signal amplification possibility for biological assays and clinical diagnoses. In view of these intrinsic advantages, this method has widely potential applications in immunoassay and pathogenic detection.

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