Electronic Supplementary Information

Ultrasensitive DNA detection based on Au nanoparticles and isothermal circular double-assisted electrochemiluminescence signal amplification

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EXPERIMENTAL SECTION

Reagents.

Labelled DNA oligonucleotides were ordered from Shenggong Bioengineering Ltd (Shanghai, China), and their sequences are listed in Table 1.

Table 1. DNA Sequence Used in This Work

Name	Sequences[a] (5' to 3')
Hairpin DNA Probe	NH_2-TTT *TCT TGG ACG* AGT CAA TCG ATG GAC CTC AGC *GTC CAA GA* -SH
Target	TGAGGTCCATCGATTGACTCGTCCA
One-base mismatch	TGAGGTCCATCGATTCACTCGTCCA
noncomplementary	CATTTAGTTGACGAGTCTAATCAAT
bio-bar-code DNA	SH-GCGCGAACCGTATA
Primer	TCTTGGAC

[a] The italicized region of the hairpin DNA probe identifies the stem sequence and the underlined region identifies the complementary sequence to the primer. The NEase recognition site of the target is red. The mismatched positions are highlightened in blue.

Bovine serum albumin (BSA), imidazole, 3-mercaptopropionic acid (MPA), N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and tri(2-carboxyethyl) phosphine hydrochloride

(TCEP) were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. NEB buffer 2 solution and Nb.BbvCI nicking endonuclease were from New England Biolabs, Inc. The deoxynucleotide solution mixture (dNTPs) and polymerase Klenow fragment exo_ were purchased from TaKaRa Bio Inc. (Dalian, China). 0.1 M phosphate buffer solution (KH_2PO_4-K_2HPO_4-NaCl; PBS) containing 0.05 M $K_2S_2O_8$ (pH 8.3) as a coreactant was used for ECL detection, and 0.1 M Tris-HCl buffer containing 0.1 M NaCl (pH 7.4) for hybridization and preparation of DNA stock solutions. All other reagents were of analytical grade and used as received. Millipore ultrapure water (resistivity $\geqslant$ 18.2 M Ω cm) was used throughout the experiment.

Apparatus.

The electrochemical and ECL emission measurements were conducted on a MPI-A multifunctional electrochemical and chemiluminescent analytical system (Xi'An Remax Electronic Science &Technology Co. Ltd., Xi'An, China) at room temperature. The spectral width of the photomultiplier tube (PMT) was 350-650 nm, and the voltage of the PMT was set at 500 V in the process of detection. The experiments were carried out with a conventional three-electrode system. The working electrode was a 3 mm diameter glassy carbon electrode (GCE) modified with NCs composite film, meanwhile, a Pt wire and SCE electrode served as the counter and reference electrodes, respectively. Transmission electron microscopy was performed with a JEOL model 2000 instrument operating at 200 kV accelerating voltage. The UV-vis absorption spectra were obtained on a Shimadzu UV-3600 UV-vis-NIR photospectrometer (Shimadzu Co.).

Preparation of Au nanoparticles (Au NPs).

Au NPs were prepared by sodium borohydride reduction of $HAuCl_4$ according to the methods reported previously with a slight modification.[1,2] All glassware was cleaned with chromate washings (cleaning solution), rinsed with water, and oven-dried prior to use. Briefly, 600 μL of ice cold 0.1 M $NaBH_4$ was added to 20 mL aqueous solution containing 2.5×10^{-4} M $HAuCl_4$ under stirring. The mixture immediately turned to orange-red color, indicating the formation of gold

nanoparticles. Keep on stirring in ice bath for 10 min. Then, the solution reacted at room temperature with continuous stirring for another 3 h till the color changed from orange-red to wine red. The average diameter of the prepared gold nanoparticles was about 5 ± 1 nm as characterized by transmission electron microscope (Fig. S1), and their UV-vis spectrum which exhibited an absorption maximum at ca. 519 nm (Fig. 1B, curve d) was characterized. The prepared colloid Au NPs were stored in brown glass bottles at 4 ºC for further use and used to label oligonucleotides.

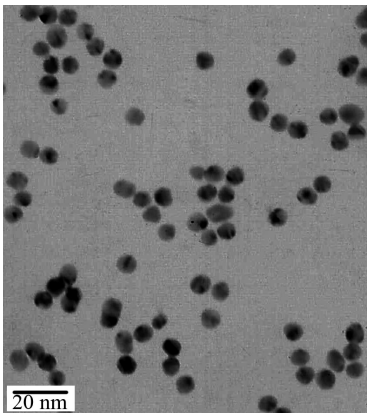

Fig. S1. TEM picture of Au NPs.

Synthesis of CdS NCs.

CdS NCs were prepared according to the literature.[1] Briefly, $Cd(NO_3)_2 \cdot 4H_2O$ (0.1683 g) was dissolved in 30 mL ultra-pure water, and heated to 70ºC under stirring, then a freshly prepared solution of Na_2S (0.5960 g) in 30 mL ultra-pure water was slowly injected and instantly orange-yellow solution was obtained. The reaction was held at 70ºC for 3 h with continuous refluxing. The final reaction precipitates were centrifugated and washed thoroughly with absolute ethanol two times and ultrapure water two times. Then the obtained precipitate was redispersed into water for centrifugation to collect the upper yellow solution of CdS NCs. The average size of synthesized CdS NCs was about 5 nm, as indicated by transmission electron microscopy (Fig. S2, right) and UV-vis spectrum (Fig. S2, left). The final solution was stored at 4℃ when not in use.

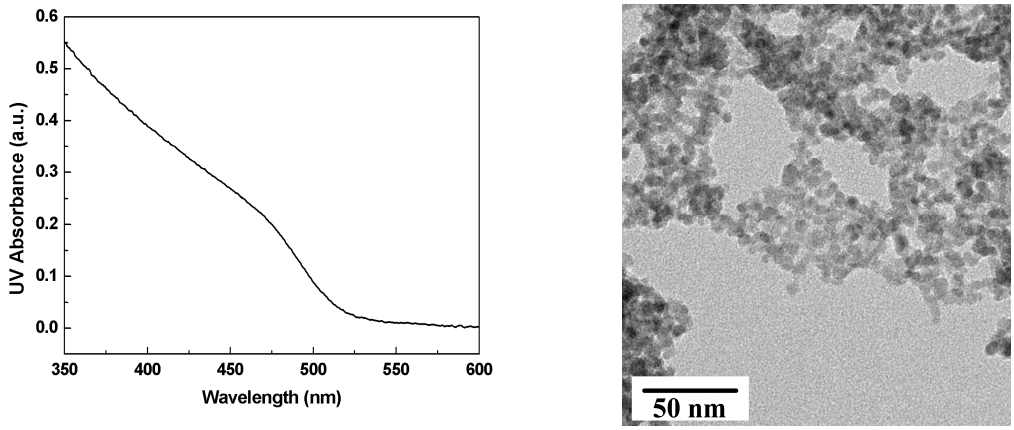

Fig. S2. UV- visible absorption spectrum (left) and TEM picture (right) of synthesized CdS NCs

Preparation of bio-bar-code DNA/hairpin DNA probe/Au NPs composite (bbc-h-DNA-Au NPs).

The bbc-h-DNA-Au NPs were prepared according to the reference with a slight modification.[3] Briefly, 50 μL of 1 μM hDNA and 4 μM bbcDNA (noncomplementary to tDNA) in 0.1 M NaCl + 0.1 M Tris-HCl buffer (pH 7.4)were activated with 1.5 μL 10 mM TCEP before use, in order to reduce disulfide bonds, then added into 500 μL Au colloidal solution containing 0.1M NaCl and 0.5 mM $MgCl_2$. hDNA was complementary to tDNA and bbcDNA was noncomplementary, the low density of hDNA on Au NPs would be favourable to the one-to-one combination of tDNA and the bbcDNA was used to avoid physical absorption or cross-reaction. The resulting colloidal solution was kept in refrigerator at 4 ºC for 16 h. Finally, the resulting bbc-h-DNA-Au NPs were washed three times with 0.1 M Tris-HCl buffer, and resuspended in solutions containing up to 0.5 M NaCl and stored at 4 ºC for further use.

Preparation of CdS NCs film.

The GCE was polished in sequential order with 1.0, 0.3 and 0.05 μm alumina. Then the GCE was thoroughly rinsed with water, sonicated in ethanol and ultrapure water in turn and finally dried in air. The CdS NCs film was achieved by dropping 10 μL of

CdS NCs solution onto the pretreated surface of GCE and evaporated in air at room temperature. At last, the CdS NCs modified GCE was stored in 0.1 M NaCl + 0.1 M Tris-HCl buffer (pH 7.4) for characterization and further modification.

Assembling of bbc-h-DNA-Au NPs composites to CdS NCs film on GCE.

The CdS NCs modified GCE was immersed in 1.0 mL of 0.1 M NaCl + 0.1 M Tris-HCl buffer (pH 7.4) containing 3 mM MPA for 6 h at 4 ºC for assembly of MPA. After rinsed thoroughly with water and Tris-HCl buffer, the terminal carboxylic acid groups of the MPA/CdS/GCE were activated by immersion in 1.0 mL of 0.1 M imidazol-HCl buffer (pH 7.0) containing 20 mg EDC and 10 mg NHS for 1 h at room temperature. Then the electrode was rinsed with 0.1 M Tris-HCl buffer (pH 7.4) to wash off the excess EDC and NHS. Finally, the resulting linker/MPA/CdS/GCE was soaked in the stable colloidal solution of bbc-h-DNA-Au NPs composites (100 μL) for 24 h at 4 ºC. Finally, 2 wt% BSA solution was used at 4 ºC for 1 h for the purpose of blocking the non-specific active binding sites of the CdS NCs. The electrode surface was rinsed with 0.1 M NaCl-Tris-HCl buffer (pH 7.4) after each step to remove nonspecifically adsorbed species.

Amplifield ECL detection of tDNA.

The bbc-h-DNA-Au NPs/CdS/GCE was immerged in 100 μL solution consisting of 50 mM NaCl, 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 50 mM Tris-HCl (pH 7.9), 1.25 mM dNTPs, 5.0×10^{-8}M primer, 15 U polymerase Klenow fragment exo_ and 30 U Nb.BbvCI nicking endonuclease in 0.1% Triton X100. A series of tDNA at different concentrations were then added to the mixture solution and incubated the reaction at 37 ºC for 40 min. Subsequently, the electrode was washed thoroughly with the 0.1 M NaCl-Tris-HCl buffer (pH 7.4) to remove unhybridized oligonucleotide followed by the measurement of ECL. ECL detection was accomplished with the electrodes in each step were in contact with 0.1 M PBS (pH 8.3) containing 0.05 M $K_2S_2O_8$ and scanned from 0 to -1.4 V, the voltage of the PMT was set at 500 V in the process of detection. ECL signals related to the tDNA concentrations could be measured. The data of three independent measurements are presented with an error margin of one standard deviation.

Nondenaturing Polyacrylamide Gel Electrophoresis (PAGE).

The hairpin DNA probe (molecular beacon), and the products by the DNA machine's isothermal strand-displacement polymerization reaction were characterized by 20% native polyacrylamide gel electrophoresis (Acr = acrylamide, Bis = N,N'-methylenebisacrylamide; Acr/Bis = 19/1). Tris-acetate-EDTA (TAE) (pH= 8.3) was used as the separation buffer. Electrophoresis was carried out at 120 V for 2 h at 25°C. The visualization and photography were performed using a digital camera under UV illumination.

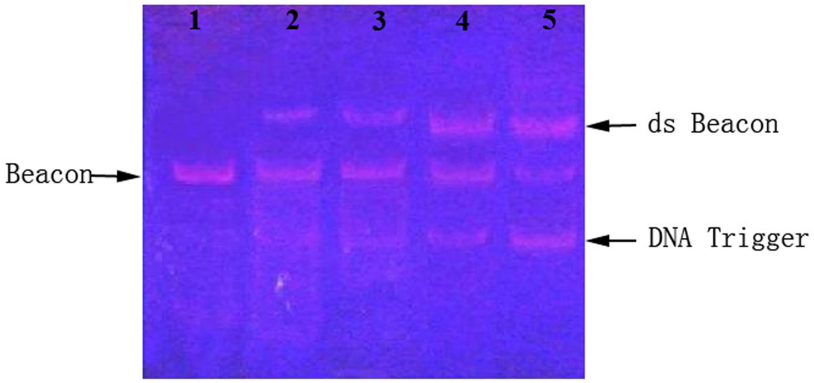

Fig. S3. Nondenaturing PAGE analysis the DNA trigger as machine's product by addition of tDNA in the presence of molecular beacon, polymerase and NEase in the mixture at 37 ºC for different time (lane1: 0 min, lane 2: 10 min, lane 3: 20 min, lane 4: 30 min, lane 5: 40 min). Experimental conditions: tDNA,1×10^{-10} M; molecular beacon, 1×10^{-7} M; polymerase, 15 U; and NEase, 30 U.

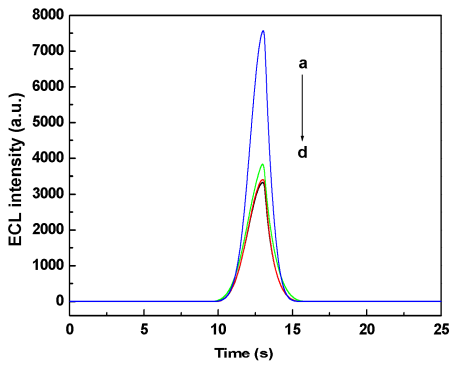

Fig. S4. ECL signals of sensor combined with isothermal circular system initiated with different tDNA (1.0×10^{-16} M) containing polymerase (15U) and NEase (30U) in the mixture at 37 ºC for 40 min. (a) complementary sequences; (b) one-base

mismatched sequences; (c) noncomplementary sequences; (d) without tDNA (blank).

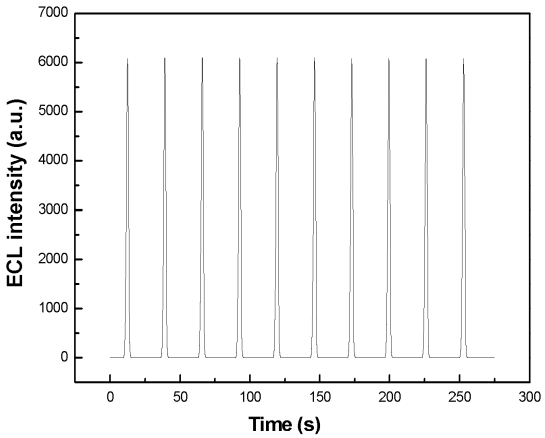

Fig. S5. ECL emission from CdS NCs film on GCE in 0.05M $K_2S_2O_8$ + 0.1M PBS (pH 8.3) under continuous cyclic potential scan for 10 cycles. Scan rate, 100 mV s^{-1}.

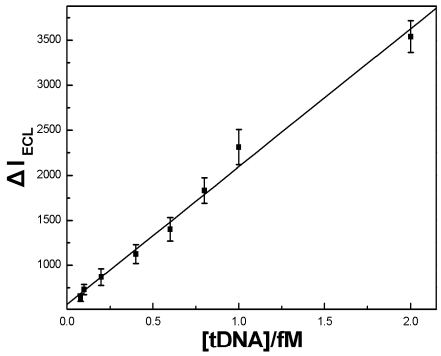

Fig. S6. The relationship between relative ECL intensity (ΔI) and concentration of tDNA after incubation at 37 ℃ for 40 min without polymerase and NEase in the mixture, three measurements for each point. The concentration of tDNA was 8.0×10^{-17} M, 1.0×10^{-16} M, 2.0×10^{-16} M, 4.0×10^{-16} M, 6.0×10^{-16} M, 8.0×10^{-16} M, 1.0×10^{-15} M, 2.0×10^{-15} M, respectively. And the regression equation was expressed as y = 563.9169 + 1531.7886 x (x was the concentration of tDNA, fM, y was ΔI, R = 0.995).

Estimation of the cycles of the amplification reaction

We can estimate the cycles of the amplification reaction within 40 min, and the time of an amplification cycle roughly according to the changes of relative ECL intensity (ΔI) in different experimental conditions.

The relative ECL intensity (ΔI = I - I0) was 640 when the circular amplification system initiated by 8.0×10^{-17} M tDNA in the absence of polymerase and NEase in the mixture at 37 ºC for 40 min; The ΔI was 1832 when the circular amplification system initiated by 8.0×10^{-17} M tDNA in the presence of only polymerase in the mixture at 37 ºC for 40 min; And the ΔI was 3540 when the circular amplification system initiated by 8.0×10^{-17} M tDNA in the presence of polymerase and NEase in the mixture at 37 ºC for 40 min.

From the results of the additional experiment without polymerase and NEase (Fig. S6, ESI), the calibration curve of tDNA could be expressed by the equation: y = 563.9169 + 1531.7886 x (x was the concentration of tDNA, fM; y was ΔI, R = 0.995). From the equation, the concentrations of tDNA were calculated to be 8.3×10^{-16} M and 1.9×10^{-15} M when the ΔI was 1832 and 3540, respectively. Then, based on the results, the multiple of the amplification with only polymerase was: $(8.3\times10^{-16}-8.0\times10^{-17})$ / 8.0×10^{-17} = 9.4 fold.

Because above amplification is obtained entirely due to the isothermal circular system (Scheme 1, (I)) with effect of polymerase, we could hypothesize that 9 cycles of the isothermal circular system (Scheme 1, (I)) were accomplished in the presence of only polymerase within 40 min. Similarly, the multiple of the amplification with the "DNA machine" (Scheme 1, (II)) within 40 min were calculated as below:

$(1.9\times10^{-15}-8.3\times10^{-16})$ / 8.0×10-17 = 13.4

So, 13 cycles of the reaction of "DNA machine" (Scheme 1, (II)) were accomplished in the presence of polymerase and NEase within 40 min.

Therefore, the cycles of the amplification reaction within 40 min were 22 (9+13), and an amplification cycle averagely takes 1.82 min (40 min / 22).

References:

1 Y. Shan, J.-J. Xu and H.-Y.Chen, *Chem. Commun.*, 2009, 905-907.

2 A. Gole and C. J. Murphy, *Chem. Mater.*, 2004, **16,** 3633-3640.

3 K. C. Hu, D. X. Lan, X. M. Li and S. S.Zhang, *Anal. Chem.*, 2008, **80,** 9124-9130