Supplementary Material (ESI) for Chemical Communications

Supporting Information

Ultrasensitive and Selective DNA Detection by Hydroxylamine Assisted Gold Nanoparticle Amplification

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Apparatus. CL measurements were performed with a BPCL chemiluminescence analyzer (Beijing, China) and Fluoroskan Ascent FL (Thermo). Particle size-distribution was measured with a NICOMP^{TM} 380 ZLS (Particle Sizing System, Santa Barbara, USA). Sizes and morphologies of Au NPs were determined at 80 kV using a JEOL JEM-1230 transmission electron microscope.

Reagents. All chemicals were of analytical grade and were used as received. The water was prepared using Milli-XQ equipment. DNA-BIND 96-well plates were obtained from Corning Incorporated. 5-nm, 10-nm, 30-nm and 50-nm naked Au NPs, 40-nm streptavidin-gold was bought from BB International. 10-nm streptavidin-gold and streptavidin-HRP were bought from Sigma-Aldrich. HRP substrate kits were purchased from Millipore Corporation, USA. NH_2OH, $HAuCl_4$, and other chemical reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. Oligonucleotides were acquired from Invitrogen Biotechnology Co., Ltd (Shanghai, China) and had the following sequences (Table S1).

Table S1. DNA sequences used in this work

Name	Sequence
Capture DNA	5'-NH_2-$(A)_{20}$ ACC TTT AAC CTA ATC TCC TC-3'
Target DNA	5'-TGG GAG GAG TTG GGG GAG GAG ATT AGG TTA AAG GT-3'
Reporter DNA	5'-CCC CAA CTC CTC CCA AAA AAA AAA A-biotin-3'
C-C mismatch	5'- TGG GAG CAG TTG GGG GAG GAG ATT AGG TTA AAG GT-3'
C-A mismatch	5'- TGG GAG AAG TTG GGG GAG GAG ATT AGG TTA AAG GT-3'

C-T mismatch	5’-TGG GAG TAG TTG GGG GAG GAG ATT AGG TTA AAG GT-3’
T-T mismatch	5’- TGG GAG GTG TTG GGG GAG GAG ATT AGG TTA AAG GT-3’
A-A mismatch	5’-TGG GAG GAG TTG GGG GAG GAG ATT AGG ATA AAG GT-3’
A-G mismatch	5’-TGG GAG GAG TTG GGG GAG GAG ATT AGG GTA AAG GT-3’
A-C mismatch	5’-TGG GAG GAG TTG GGG GAG GAG ATT AGG CTA AAG GT-3’
Two-base mismatch	5’-TGG GAG GAG TTG GGG GAG GAG ATT AGG AAA AAG GT-3’
Noncomplementary strand	5’-TGA GGT AGT AGG TTG TAT AGT T-3’
Capture DNA 24	5’-NH_2-$(A)_{20}$ CCT AAT CTC CTC-3’
Reporter DNA 24	5’-CCC CAA CTC CTC AAA AAA AAA A-biotin-3’
Target DNA 24	5’-GAG GAG TTG GGG GAG GAG ATT AGG-3’
Capture DNA 30	5’-NH_2-$(A)_{20}$ TAA CCT AAT CTC CTC-3’
Reporter DNA 30	5’-CCC CAA CTC CTC CCA AAA AAA AAA A-biotin-3’
Target DNA 30	5’-TGG GAG GAG TTG GGG GAG GAG ATT AGG TTA-3’
Capture DNA 40	5’-NH_2-$(A)_{20}$ ACC TTT AAC CTA ATC TCC TC-3’
Reporter DNA 40	5’-CCC CAA CTC CTC CCA GTC TTA AAA AAA AAA-biotin-3’
Target DNA 40	5’-AGA CTG GGA GGA GTT GGG GGA GGA GAT TAG GTT AAA GGT-3’
Capture DNA 45	5’-NH_2-$(A)_{20}$ ACC TTT AAC CTA ATC TCC TC-3’
Reporter DNA 45	5’-CCC CAA CTC CTC CCA GTC TTT AAA CAA AAA AAA AA-biotin-3’
Target DNA 45	5’-GTT TAA AGA CTG GGA GGA GTT GGG GGA GGA GAT TAG GTT AAA GGT-3’

Preparation of gold probes. 60-pmol biotinylated reporter sequences were added to 150 μL of buffer A (20 mM Tri-HCl, pH 8.0, 0.5 M NaCl) solution that contained 60 μL of 40-nm streptavidin-gold. The mixture was incubated at 37 ^{o}C for 1 h. The conjugates were washed several times with 500 μL wash buffer (7 mM Tris-HCl, pH 8.0, 0.17 M NaCl, 0.05% Tween 20) by centrifuging at 12000 rpm for 3 min. The soft sediment of gold probes was then resuspended in 120 μL buffer A containing 1% BSA at 4 ^{o}C before use. The concentration of gold probes was estimated by UV-Vis spectroscopy to be about 0.545 nM, based on an extinction coefficient of 9.264 x 10^{9} M^{-1} cm^{-1} at ë=520 nm for 40 nm Au NPs.

Assay procedures on polystyrene microwells. In a typical experiment, amine modified capture probes were diluted to 0.1 pmol per 100 μL of coupling buffer (0.5 M Na_2HPO_4-NaH_2PO_4, pH 8.5) and divided into the wells of a DNA-BIND 96-well plate (100-μL per well). The wells were washed three times with wash buffer after incubating with gentle mixing for 40 min at 37 ^oC. Different amounts of target DNA or mismatched DNA was then added into each well (100-μL per well, in buffer A). Following a 40-min incubation with gentle mixing at 37 ^oC, the wells were washed three times with wash buffer. Four microliters of gold probes were added and incubated in 100-μL buffer A containing 5% BSA at 37 ^oC for 40 min. The wells were then washed three times with wash buffer before 50 μL of 2.5 mM luminol (0.1 M NaOH) was pipetted into the microwells. Finally, 50 μL of 0.1 mM $AgNO_3$ solution was injected and the CL signal was displayed in the Fluoroskan Ascent FL. For the amplification assay procedure, Au NPs that assembled on the surface of the 96-well plate were catalytically enlarged in the presence of 1 mM NH_2OH and 0.1 mM $HAuCl_4$ at room temperature for 20 min. The wells were washed three times with wash buffer, and then detected as described above. For comparison, streptavidin-HRP was also used instead of gold probes, and CL signals on the surface of the 96-well plate were detected directly with 100 μL of CL HRP substrate.

Optimization of Reaction Parameters. Several parameters were investigated systematically to establish optimal conditions for the ultrasensitive DNA detection, including the amounts of capture DNA, streptavidin gold, reporter DNA, luminol , $AgNO_3$, $HAuCl_4$ and NH_2OH, etc.

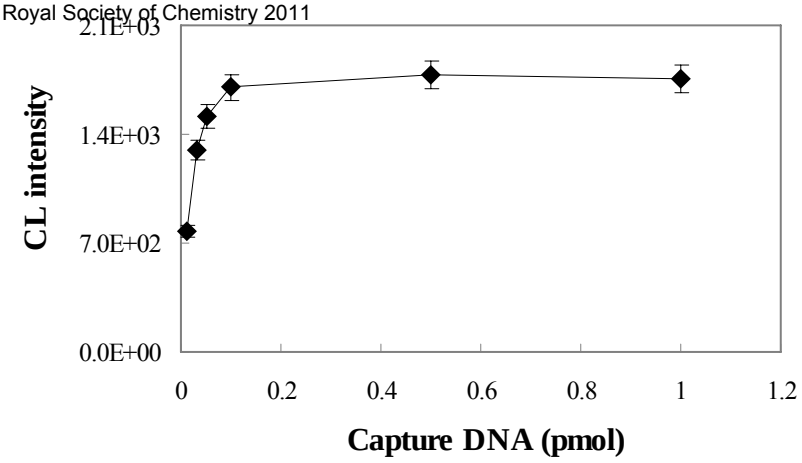

Figure S1. CL intensity vs the amount of capture probes. Experimental conditions: target DNA and biotinylated reporter sequence were 0.006 and 5 pmol, respectively; 40-nm streptavidin-gold was 56 pM; luminol and $AgNO_3$ were 2.5 and 0.1 mM, respectively.

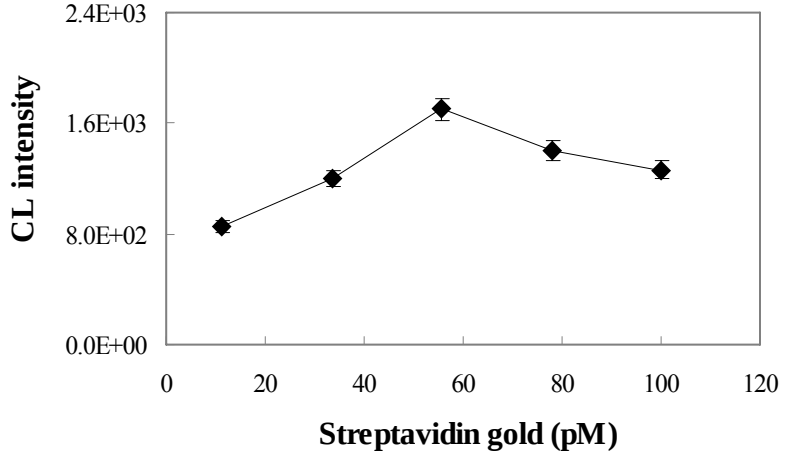

Figure S2. CL intensity vs. the concentration of 40-nm streptavidin-gold. Experimental conditions: capture probe, target DNA and biotinylated reporter sequence were 0.1, 0.006 and 5 pmol, respectively; luminol and $AgNO_3$ were 2.5 and 0.1 mM, respectively.

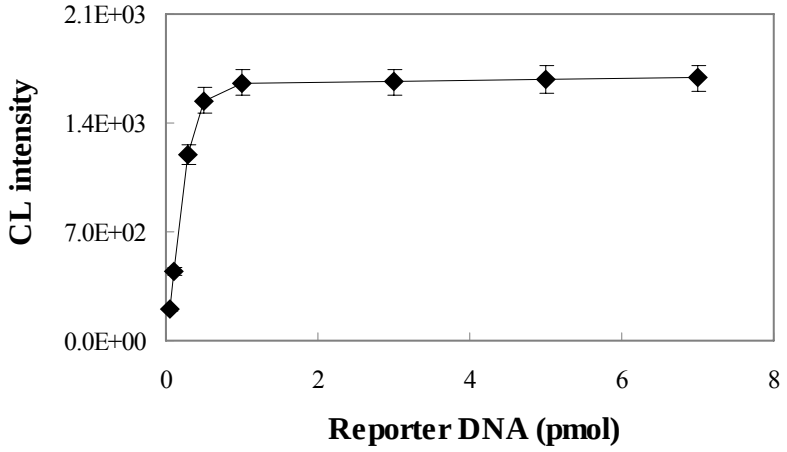

Figure S3. CL intensity vs. the amounts of biotinylated reporter sequence. Experimental conditions: capture probe and target DNA were 0.1 and 0.006 pmol, respectively; 40-nm streptavidin-gold was 56 pM; luminol and $AgNO_3$ were 2.5 and 0.1 mM, respectively.

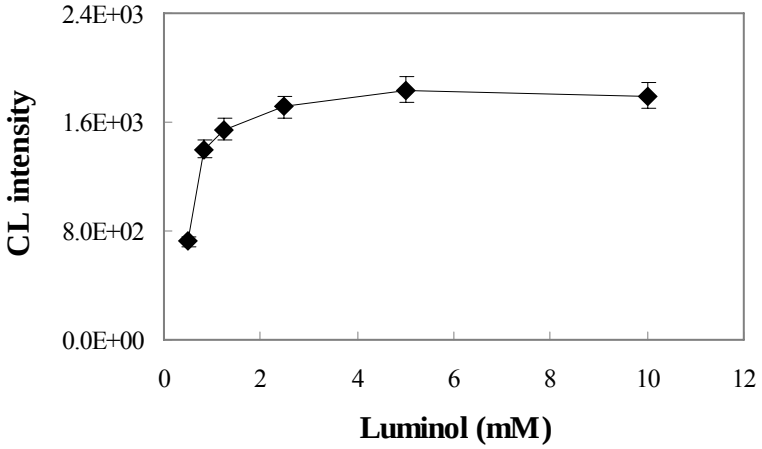

Figure S4. CL intensity vs. the concentration of luminol. Experimental conditions: capture probe, target DNA and biotinylated reporter sequence were 0.1, 0.006 and 5 pmol, respectively; 40-nm streptavidin-gold was 56 pM and $AgNO_3$ was 0.1 mM.

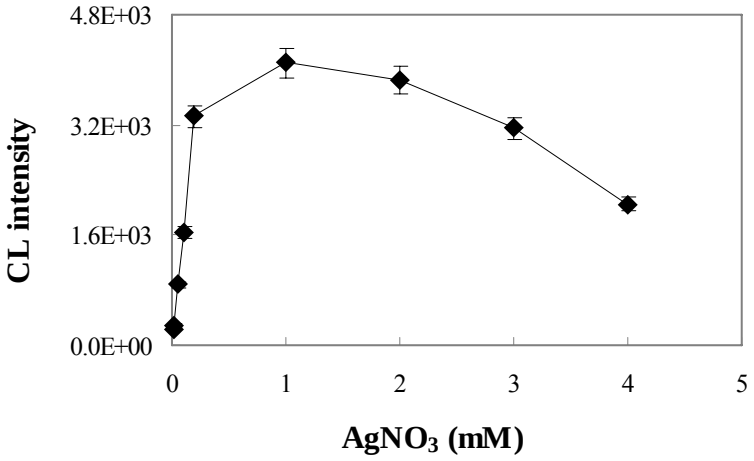

Figure S5. CL intensity vs. the concentration of $AgNO_3$. Experimental conditions: capture probe, target DNA and biotinylated reporter sequence were 0.1, 0.006 and 5 pmol, respectively; 40-nm streptavidin-gold was 56 pM and luminol was 2.5 mM.

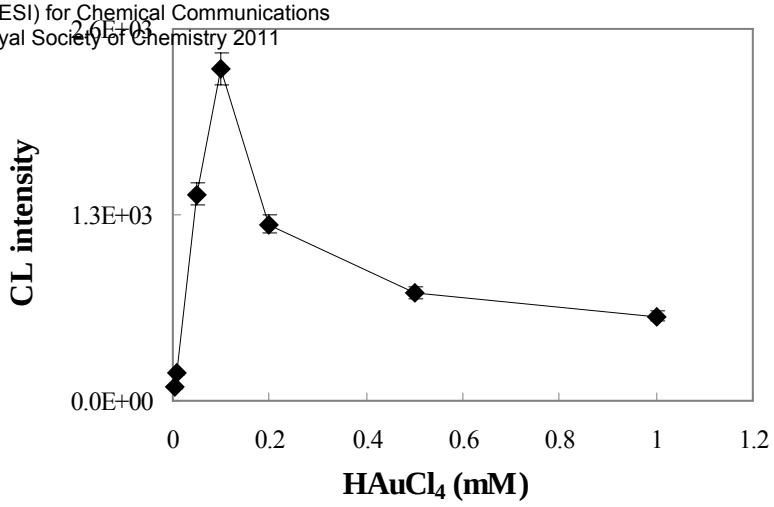

Figure S6. CL intensity vs. the concentration of $HAuCl_4$. Experimental conditions: capture probe, target DNA and biotinylated reporter sequence were 0.1, 0.0001 and 5 pmol, respectively; 40-nm streptavidin-gold was 56 pM; luminol, $AgNO_3$ and NH_2OH were 2.5, 1 and 1 mM, respectively.

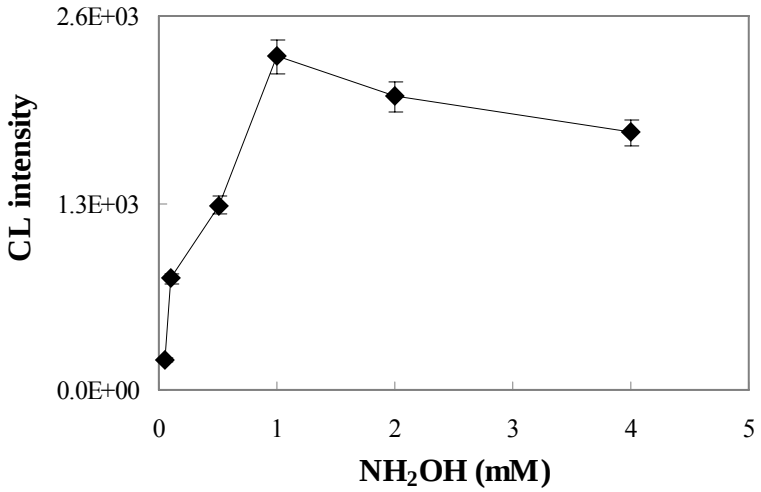

Figure S7. CL intensity vs. the concentration of NH_2OH. Experimental conditions: capture probe, target DNA and biotinylated reporter sequence were 0.1, 0.0001 and 5 pmol, respectively; 40-nm streptavidin-gold was 56 pM; luminol, $AgNO_3$ and $HAuCl_4$ were 2.5, 1 and 0.1 mM, respectively.

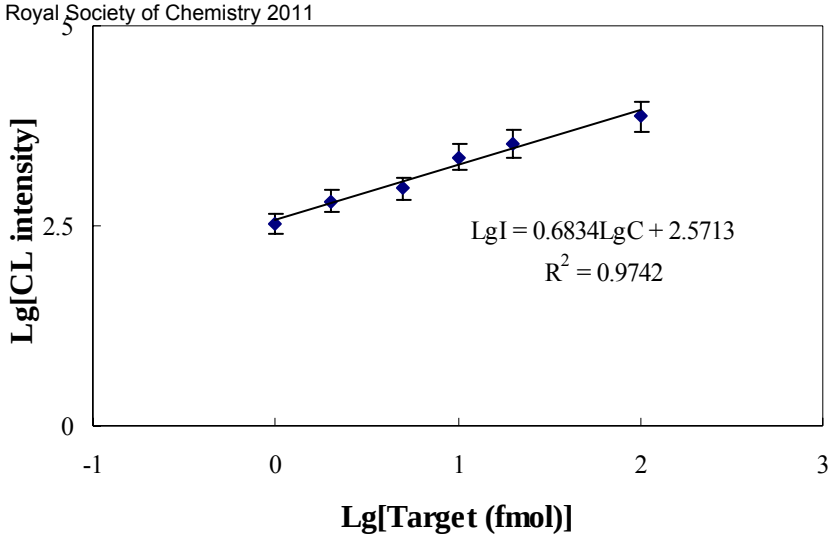

Figure S8. Log-Log calibration plot for the HBV target with 10-nm streptavidin-gold. Experimental conditions: capture probe and biotinylated reporter sequence were 0.1 and 5 pmol, respectively; 10-nm streptavidin-gold was 56 pM; luminol and $AgNO_3$ were 2.5 mM and 1mM, respectively.

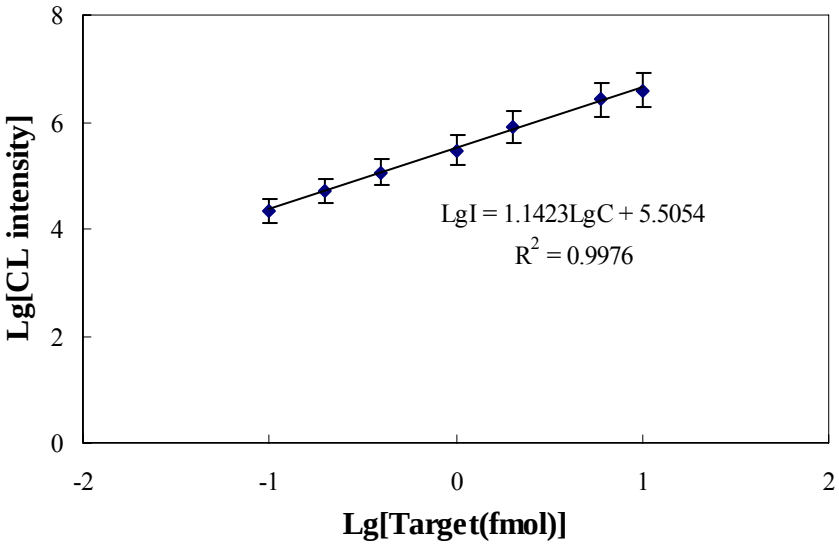

Figure S9. Log-Log calibration data for the HBV target with HRP. Experimental conditions: capture probe and biotinylated reporter sequence were 0.1 and 5 pmol, respectively; streptavidin-HRP was 20 ng; CL HRP substrate was 100 μL.

Table S2. Comparison of sensitivity for different DNA assay methods

Analytical method	Label	No. of target bases	Detection limit
Electrochemical detection	Ferrocene	21	1 fM [1]
Electrochemical detection	Label-free	33	1 fM [2]
Electrochemical detection	Liposome	27	1.2 pM [3]
Electrochemical detection	HRP	22	17 pM [4]
Fluorescence imaging	Cy5	30	1 pM [5]
Fluorescence imaging	Silica NPs	27	0.8 fM [6]
CL detection	Label-free	60	5 nM [7]
CL detection	DNAzyme	36	1 nM [8]
CL detection	Pt NPs	27	10 pM [9]
CL detection	CuS NPs	18	0.55 pM [10]
ECL detection	$Ru(bpy)_3^{2+}$	12	5 pM [11]
Colorimetric detection	Ag/SiO_2	24	100 pM [12]
Surface plasmon resonance	Label-free	16	10-100 pM [13]
Circular dichroism	Label-free	8 to 32	5 μM [14]
CL detection (this work)	Au NPs	35	8 fM
CL detection (this work)	Au NPs	35	300 aM

Table S3. Comparison of sensitivity for different DNA assay methods based on Au NPs

Analytical method	Label	No. of target bases	Detection limit
Electrochemical detection	Au NPs	19	15 nM [15]
Electrochemical detection	Au NPs	19	6 pM [16]
Electrochemical detection	Au NPs	27	100 fM [17]
Electrochemical detection	Au NPs	35	0.6 fM [18]
Colorimetric detection	Au NPs	15	60 nM [19]
Colorimetric detection	Au NPs	30	10 nM [20]

Colorimetric detection	Au NPs and nicking endonuclease	24-80	10 pM [20]
SPR	Au NPs	30	1 pM [21]
ICPMS	Au NPs	40	0.2 pM [22]
Flatbed scanner	Au NPs	27	50 fM [23]
SERS spectroscopy	Au NPs and dyes	30	20 fM [24]
Scanometric	Au NPs and Ag	27	500 aM [25]
CL detection	Au NPs and CuS	42	4.8 fM [26]
CL detection (this work)	Au NPs	35	8 fM
CL detection (this work)	Au NPs	35	300 aM

Table S4. Comparison of linear ranges and detection limits for different target lengths

Target DNA bases	Magnetic beads-based detection		96-well plate-based detection	
	Linear range (fmol)	Detection limit (fmol)	Linear range (fmol)	Detection limit (fmol)
24	0.1~100	0.1	0.0025~1	0.0025
30	0.1~100	0.1	0.0025~1	0.0025
35	0.1~100	0.1	0.0025~1	0.0025
40	0.1~100	0.1	0.0025~1	0.0025
45	0.1~100	0.1	0.0025~1	0.0025

(Experimental conditions: streptavidin-gold was 56 pM; luminol and $AgNO_3$ was 2.5 and 1 mM, respectively. For magnetic beads-based detection: magnetic beads were 20 μg; capture probe and biotinylated reporter sequence were 1 and 5 pmol, respectively; for 96-well plate-based detection: capture probe and biotinylated reporter sequence were 0.1 and 5 pmol; respectively. Note also that the linear range and detection limit can be further improved for some of the target lengths, such as 40 and 45 bases)

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