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Electronic Supplementary Information

Chemiluminescence Detection of DNA/ MicroRNA Based on Cation-Exchange of CuS Nanoparticles and Rolling Circle Amplification

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EXPERIMENTAL SECTION

Reagents and Materials. DNA oligonucleotides were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The oligonucleotides (0.1 mM) were dissolved in deionised water and diluted to the desired concentrations with PBS (10 mM NaH_2PO_4-Na_2HPO_4, 100 mM KCl, pH 7.4). MiRNAs were synthesised by GenePharma (Shanghai, China), and the buffers used for miRNA detection were autoclaved and treated with diethylpyrocarbonate (DEPC). The sequences of DNA and miRNAs are listed in Table 1 and Table 2. The T4 DNA ligase (5000 U/mL) and phi29 DNA polymerase (10000 U/mL) were ordered from Thermo Scientific. The deoxynucleotide mixture (dNTPs) was purchased from SBS Genetech Co., Ltd. (Beijing, China). Luminol was purchased from ABCR GmbH & Co. Hydrogen peroxide (30%) and silver nitrate ($AgNO_3$) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The carboxyl groups modified magnetic beads (MBs, 1.0 μm) were purchased from Tianjin Baseline ChromTech Research Centre (China). 3-Mercaptopropionic acid (3-MPA), N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma. Imidazole and sodium sulfide (Na_2S) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All solutions were prepared with deionized water.

A 0.01 M luminol stock solution was prepared by dissolving 0.1773 g of luminol in 100 mL of 0.1 M NaOH solution then storing in the dark for 1 week prior to use. Working solutions of H_2O_2 were prepared by freshly diluting 30% (w/w) H_2O_2.

Table S1 DNA sequences used in CXCLAmp strategy

name	sequence
capture DNA	5'-CTA CTA CCT CAA AAAA-NH2-3'
target DNA	5'-TGA GGT AGT AGG TTG TAT AGTT-3'
report DNA	5'-NH2- AAAAAA CTA TAC AAC-3'
two-base mismatched DNA	5'-TGA GGT AGT AGG TTC TAT AGAT-3'
random DNA	5'-CTA TAC AAC CTA CTA CCT CAGG-3'

Table S2 DNA and RNA sequences used in RCA-CXCLAmp strategy

name	sequence
Capture adapter	5'-*TAT TAT TTT A*AA CTA TAC AAC CTA CTA CCT CAA AAAA-NH_2-3'
Circular template	5'-phosphate-CGT AGA TTC CCA AAC CGT AGA GCA CAC ATG AGA CTT ACA ACG TAT TCG ACT TCT TCA GAT GC-3'
Report DNA	5'-CGT AGA GCA CAC ATG AAA AAA- NH_2-3'
Liagate adapter	5'-phosphate-*TAA AAT AAT A*AA AAA AAA AAT TGG GAA TCT ACG GCA TCT GAA GAAG-3'
Let-7a	5'-UGA GGU AGU AGG UUG UAU AGUU-3'
Let-7b	5'-UGA GGU AGU AGG UUG UGU GGUU-3'
Let-7c	5'-UGA GGU AGU AGG UUG UAU GGUU-3'
Let-7d	5'-AGA GGU AGU AGG UUG CAU AGU-3'
Let-7e	5'-UGA GGU AGG AGG UUG UAU AGU-3'
Let-7f	5'-UGA GGU AGU AGA UUG UAU AGUU-3'
Let-7g	5'-UGA GGU AGU AGU UUG UAC AGU-3'
Let-7i	5'-UGA GGU AGU AGU UUG UGC UGUU-3'

Apparatus. The chemiluminescence (CL) intensity was measured using a TriStar LB 942 Multimode Reader (Berthold Technologies, Germany). A 96-well plate (Berthold white plate) was used as a sample reservoir. ICP-MS/MS (Agilent, Model 8800, USA) was used to determine the concentration of cupric ions released to the solution. The concentration of total RNA was determined by a NanoPhotometer P300 (Implen, Germany) at an absorption of 260 nm.

Preparation of CuS NPs. Mercaptopropionic acid-stabilized CuS NPs were prepared according to the literature. [1] Briefly, 15 μL 3-MPA was added to 50 mL of 0.4 mM $Cu(NO_3)_2$, then the pH of the mixture was adjusted to 7.0 with NaOH solution (0.5 M). After bubbled with N_2 for 30 min, Na_2S solution (50 mL, 1.34 mM) was added dropwise to the mixture. The reaction was continued for 24 h under N_2 bubbled until a dark-green solution was obtained. The prepared CuS colloid was dialyzed against distilled water for 48 h using a dialysis membrane with MWCO of 7000, and then stored at 4 °C before use.

Preparation of report DNA-CuS NPs conjugates. 2 mL of 2.0×10^{-6} M of 3'-amino group capped report DNA and 200 μL of 0.1 M imidazole solution (pH 6.8) were added to a small beaker. After shaking at room temperature for 30 min, 100 μL of 0.1 M EDC and 3.0 mL of CuS colloid were added and the mixture was incubated at room temperature for 12 h with stirring. Finally, the mixture was centrifuged at 10 000 rpm for 30 min to remove unbound oligonucleotides. The supernatant was carefully removed and the precipitate was rinsed with deionized water and re-suspended in 3.0 mL deionized water. The resulting solution containing report DNA-CuS NPs conjugates was stored under 4 °C in refrigerator.

Preparation of MB-capture DNA conjugates. First, 100 μL of carboxyl-modified magnetic microbeads were washed three times with 0.1 M imidazole - HCl buffer (pH 6.8). Then actived in 400 μL imidazol-HCl buffer containing 0.2 M EDC at 37 °C for 30 min. After washed thrice with 0.1 M PBS buffer and resuspended in 200 μL imidazole buffer, 100 μL of 1.0×10^{-6} M amino group capped capture DNA was added and incubated at 37 °C for 12 h. Excess DNA was removed by washing and magnetic separation. Finally, the obtained MB-DNA conjugates were suspended in 100 μL of PBS buffer before use.

The assembly of the biosensor without amplification. Different concentrations of target DNA (10 μL) and 30 μL of report DNA-CuS NPs conjugates, obtained as described in the Supporting Information, were added to 10 μL of MB-capture DNA conjugates, and the mixture was incubated

at 37°C for 1 h with gentle shaking. After washing and magnetic separation, the prepared sandwich structure was suspended in 10 μL of PBS.

The assembly of the biosensor amplified by RCA. 10 μL of target miRNA of different concentrations was added to the MB-capture DNA conjugate solution (10 μL) then incubated at 37°C for 1 h with gentle shaking. After washing three times with PBS, 10 μL of conjugate was left. Then, 2 μL of 1.0×10^{-6} M adapter DNA, 1 μL of T4 DNA ligase (5 U/ μL), 2 μL of 10 × T4 DNA ligase buffer and PBS were added to make the final volume of 20 μL. The mixture was shaken at 22 °C for 1 h followed by incubating at 45 °C for 1 h and washing thoroughly to remove the excess DNA, giving 10 μL of solution. Then, 4 μL of 1.0×10^{-6} M padlock DNA, 1 μL of T4 DNA ligase (5 U/ μL), 2 μL of 10 × T4 DNA ligase buffer and PBS were added to make the final volume of 20 μL again. The ligation was carried out by shaking at 22 °C for 1 h followed by washing three times with PBS to give the final volume of 10 μL. The RCA reaction was initiated by adding 3 μL of 10 mM dNTPs, 2 μL of 10 × phi29 DNA polymerase buffer, 1 μL of phi29 DNA polymerase (10 U/ μL) and 4 μL PBS to the 10 μL above mixture, and then shaken at 37 °C for 2 h. After the reaction, the solution was heated at 65 °C for 10 min to inactivate the polymerase. Finally, 100 μL of the report DNA-CuS NPs conjugates obtained above were added and shaken at 37°C for 1 h. Then, the obtained conjugates were washed five times to give a RCA product with substantial CuS NP tags.

CXCLAmp reaction and CL detection. 25 μL of 8×10^{-4} M $AgNO_3$ was added to the MB-CuS conjugates and shaken at 25°C for 5 min. Then, the supernatant was collected as a tested sample. During the CL measurement, 100 μL of 1.0×10^{-6} M luminol and 20 μL of sample were added to a 96-well plate. Then, 50 μL of 7.5 mM H_2O_2 was injected automatically. The CL kinetics were recorded for 90 s in 0.05 s intervals.

Isolation of miRNA. Total RNA from the human renal epithelial cell line 293T was extracted using Trizol reagent (Invitrogen, Beijing, China) following the recommended protocol. The total RNA concentration in 100 μL was determined to be 1.14 μg/μL with the NanoPhotometer P300 spectrophotometer. The total RNA extraction solution was first diluted by a TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) to 50 ng/μL then serially diluted with 0.1% DEPC to 10 pg/μL.

RESULTS AND DISCUSSION

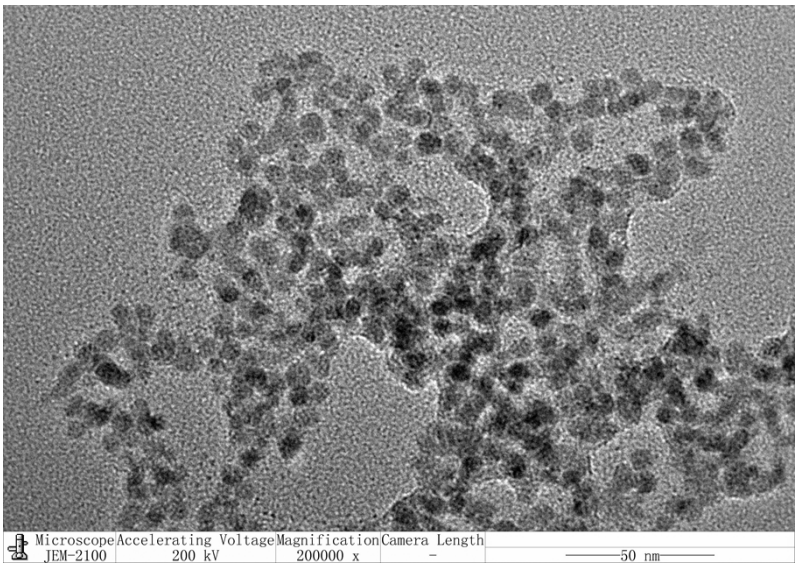

Figure S1 TEM image of prepared CuS NPs.

Conditions for the CL measurement. The conditions for CL reaction were investigated. The CL intensity increased with increasing concentration of luminol (data not shown). To keep the intensity within the measurement range, 5.0×10^{-7} M luminol was used throughout the experiment.

The effect of hydrogen peroxide concentrations on the CL reaction was examined. As shown in Figure S2, CL intensity increased with increasing concentration of H_2O_2 in the range 0.1 mM-1.875 mM and remained constant after 1.875 mM. So the concentration 1.875 mM hydrogen peroxide was chosen for the optimal concentration in this study.

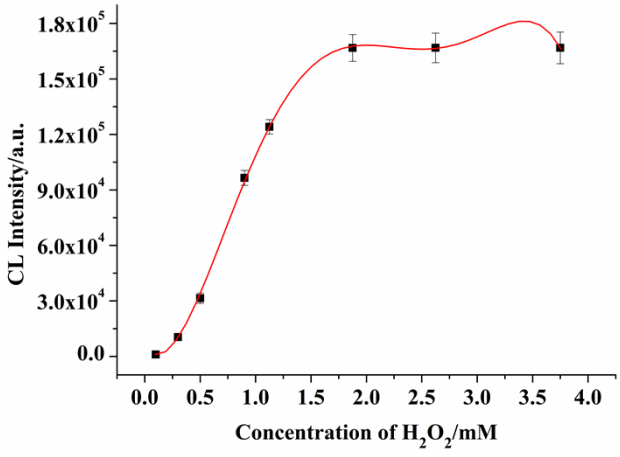

Figure S2 The effect of H_2O_2 concentration to the CL intensity. Concentration of luminol is 5.0×10^{-7} M; pH during CL measurement is 10.5; Concentration of target DNA is 1.0×10^{-12} M.

The effect of the pH on CL intensity during CL measurement was studied in the pH range 8.5-12.0. As shown in Figure S3, CL intensity increased from pH 8.5 to 10.5, and the maximum CL

intensity was obtained at pH 10.5. Therefore, 0.1 M NaOH-$NaHCO_3$ buffer with pH 10.5 was selected for the best CL measurement response in following experiments.

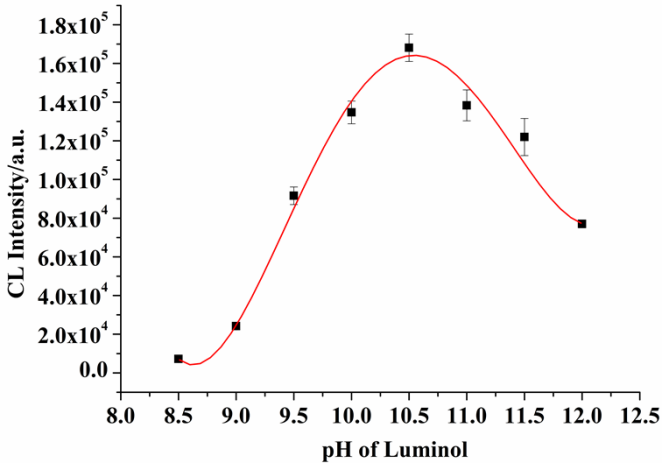

Figure S3 The effect pH to the CL intensity. Condition: luminol, 5.0×10^{-7} M; H_2O_2, 1.875 mM; target DNA, 1.0×10^{-12} M.

The influence of Ag^+ ions on the CL reaction

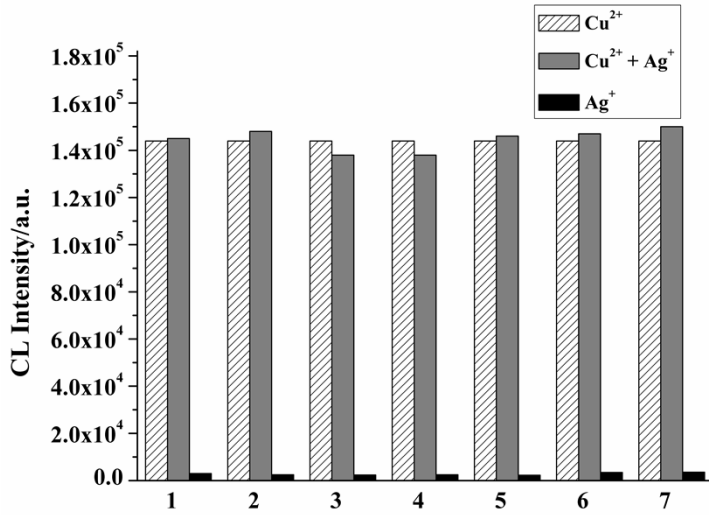

Figure S4 The influence of Ag^+ on the CL of the luminol-H_2O_2 system. The concentration of Ag^+ was (1) 4.0×10^{-4} M; (2) 4.0×10^{-6} M; (3) 4.0×10^{-8} M; (4) 4.0×10^{-10} M; (5) 4.0×10^{-12} M; (6) 4.0×10^{-14} M; (7) 4.0×10^{-16} M. The concentration of Cu^{2+} was 1.0×10^{-8} M.

Regeneration of the biosensor

Although oxidation by nitric acid can release cupric ions from CuS NPs, it could destroy the structure of DNA completely at the same time. However, the cation-exchange reaction needs no strong acids or corrosive oxidants, which can maintain the integrity of the oligonucleotide. During the regeneration process, 20 μL of 0.1 M NaOH was added to break the hydrogen bonds between the bases of the capture DNA and the target DNA. The results shown in Figure S5 demonstrated

that after three cycles of regeneration, the CL intensity retained 97.0% of the initial signal, and 89.8% of the initial value was retained after four regeneration cycles.

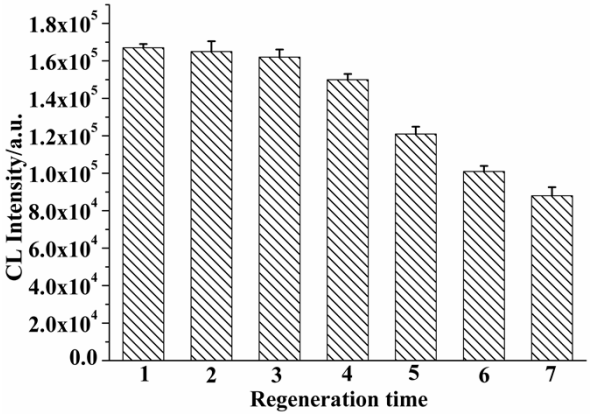

Figure S5 The CL intensity as a function of the number of denaturation–regeneration cycles using 0.1 M NaOH. The concentration of the target DNA was 1.0×10^{-12} M.

Sensitivity of CXCLAmp strategy. To illustrate the high amplification efficiency of CuS based CXCLAmp strategy, the linear calibration curve of this assay was measured. As shown in Figure S6 and Figure S7, the CL intensity increased with the increasing target DNA concentration in the range from 5.0×10^{-14} M to 1.0×10^{-11} M. The linear regression equation was $I = 21708.7 + 1.28 \times 10^{17} C$ (I represents CL intensity; C is the concentration of target DNA, n=9) with a coefficient of 0.9991. A detection limit of 1.0×10^{-14} M was estimated using 3σ. A series of eleven repetitive measurements of 5.0×10^{-13} M target DNA were used for estimating the precision, and the relative standard deviation was 4.6%.

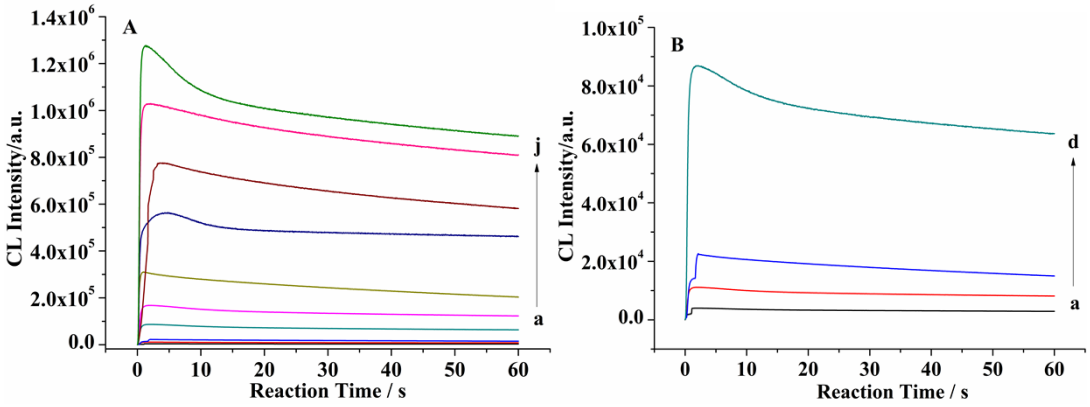

Figure S6 (A) Real-time CL detection curves in response to different concentrations of target DNA. [DNA] $/10^{-12}$ M: (a) 0; (b) 0.050; (c) 0.10; (d) 0.50; (e) 1.0; (f) 2.0; (g) 4.0; (h) 6.0; (i) 8.0; and (j) 10. (B) Partial enlarged drawing of (A).

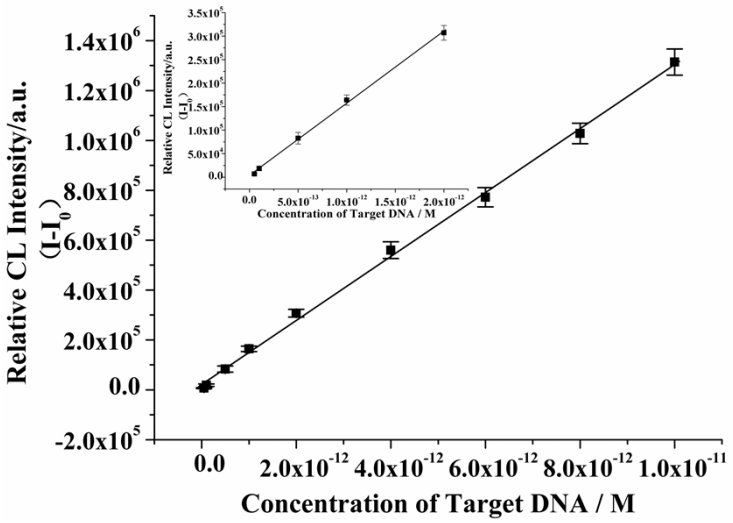

Figure S7 The relative CL intensity is linear correlation with the amount of DNA in the range from 5.0 × 10 $^{-14}$ M to 1.0 × 10 $^{-11}$ M.

Selectivity of the CXCLAmp strategy. The selectivity of the prepared DNA biosensor was evaluated through hybridization with the same concentration of non-complementary sequences, two-base mismatched DNA sequences as well as complete complementary target DNA sequences. From the results shown in Figure S8, we found that only the perfectly matched DNA produced prominent signals, which could be well distinguished from two-base mismatched sequences, let alone non-complementary sequences. These results indicated that this novel DNA assay showed good selectivity.

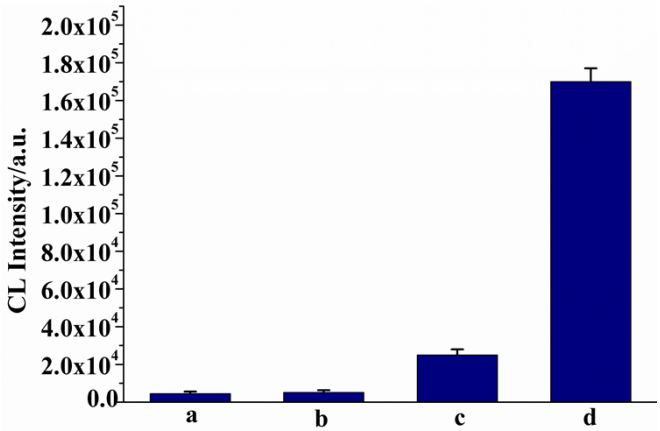

Figure S8 Selectivity of CXCLAmp biosensor for (a) blank solution, (b) 1.0 × 10^{-12} M noncomplementary DNA, (c) 1.0 × 10^{-12} M two-base mismatched DNA, (d) 1.0 × 10^{-12} M complementary DNA.

Condition for the RCA reaction. The amount of enzyme plays crucial roles in DNA cyclic

amplification. From the results shown in Figure S9, 0.5U μL^{-1} of phi29 DNA polymerase was chosen as the optimum amount.

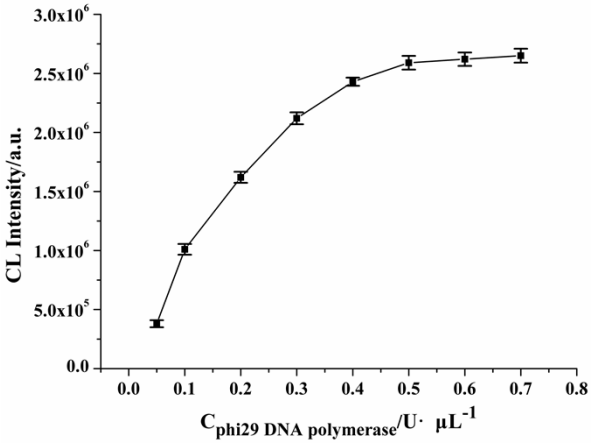

Figure S9 Influence of the amount of phi29 DNA polymerase on the CL signal responding to 5.0 × 10^{-12} M miRNA.

In order to obtain higher sensitivity, the RCA duration time was investigated. As shown in Figure S10, CL intensity increased rapidly at first and then increased slowly. Considering the time consuming, 120 min was chosen as the optimal reaction time for RCA reaction.

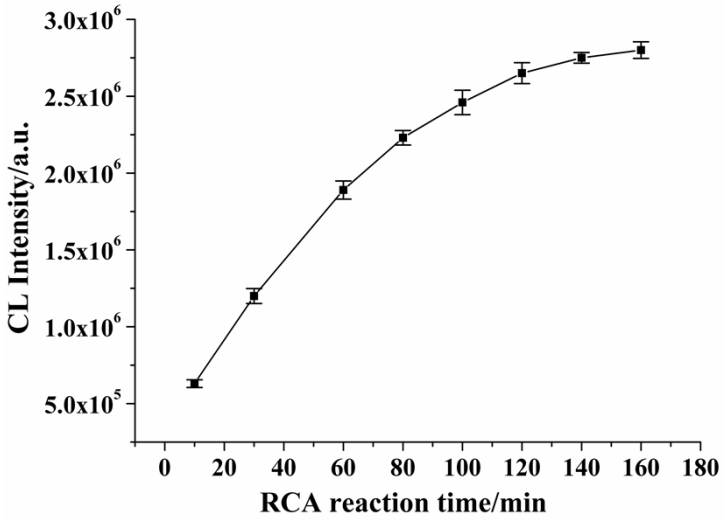

Figure S10. Influence of RCA reaction time on the CL signal responding to 5.0 × 10^{-12} M

Sensitivity of the RCA-CXCLAmp strategy.

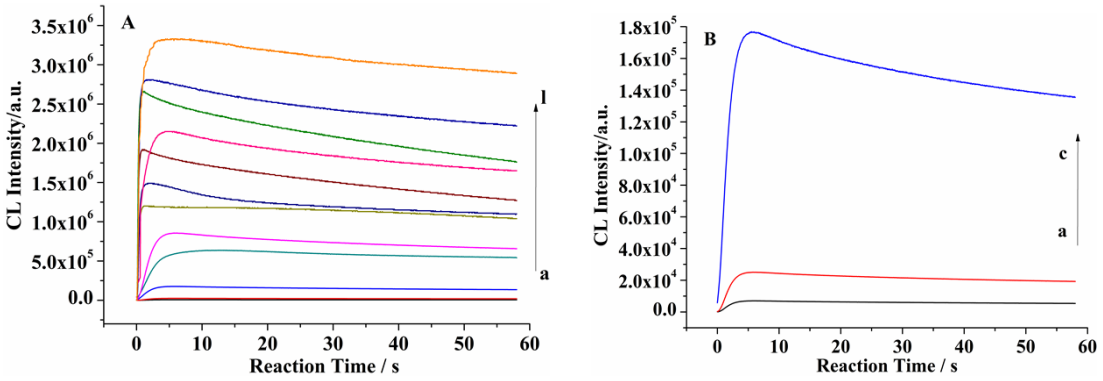

Figure S11. (A) Real-time CL detection curves in response to different concentrations of let-7a: (a) 0; （b）6×10^{-16} M; (c) 1×10^{-15} M; (d) 5×10^{-15} M; (e) 1×10^{-14} M; (f) 5×10^{-14} M; (g) 1×10^{-13} M; (h) 5×10^{-13} M; (i) 1×10^{-12} M; (j) 5×10^{-12} M; (k) 1×10^{-11} M; (l) 5×10^{-11} M. (B) Partial enlarged drawing of (A).

Table S3. Comparison of different strategy for miRNA detection

Methods	Transducer	LOD	Methods	Transducer	LOD
Toehold-initiated RCA assay	fluorescence	0.72 fm[2a]	Electrocatalytic nanoparticle tags	electrochemistry	80 fM[2l]
Exponential strand-displacement amplification	fluorescence	16 zmo[2b]	DNAzyme-based RCA DNA machine	color change	2 aM[2m]
Target-primed and branched RCA detection	fluorescence	10 fM[2c]	Graphene oxide fluorescence quenching and endonuclease.	fluorescence	3.0 fM[2n]
Nanocrystal clusters combined RCA amplification	fluorescence	91 amo[2d]	Duplex-specific nuclease based EIS test	electrochemistry	1.0 fM[2o]
Hairpin probe-based circular exponential amplification assay	fluorescence	0.38 pM[2e]	Padlock probe-based exponential RCA	fluorescence	0.24 zmol[2p]
Deposition of an insulating film and electrochemical impedance spectroscopic (EIS) detection	electrochemistry	2.0 fM[2f]	Graphene fluorescence switch-based cooperative amplification	fluorescence	10.8 fM[2q]
Size-coded ligation chain reaction	fluorescence	0.2 fM[2g]	Bifunctional strand displacement mediated hyperbranched RCA	fluorescence	0.18 pM[2r]
Target-guided formation of nanowires in nanogaps	electrochemistry	5.0 fM[2h]	Cyclic enzymatic repairing and strand-displacement	fluorescence	0.1 fM[2s]
Isothermal exponential amplification coupled with fluorescent DNA-scaffolded AgNCs	fluorescence	2 aM[2i]	A competitive hybridization assay using the bioluminescent enzyme as a label	bioluminescence	1.0 fmol[2t]
Cross-catalyst based chemiluminescence resonance energy transfer	chemiluminescence	0.68 fM[2j]	This method	chemiluminescence	0.17 fM (1.7 zmol)
Using duplex-specific nuclease	fluorescence	100 fM[2k]			

Determination of miRNAs in real samples. To determine the ability of the proposed method for quantifying miRNAs in real samples, the total RNA sample extracted from the 293T cell line was investigated. 5 μL of diluted cell extract (containing 50 pg of total RNA) was further diluted with 5 μL of ultrapure water and then subjected to the RCA-CXCLAmp assay. The calculated content of let-7a in the 293T cell was 0.102 amol (2.0 amol/ng), which was similar to a previously

reported value (1.3 amol/ng).[2g] In addition, the reliability of the assay was confirmed by a standard addition method through adding 5 μL of let-7a of three different concentrations to 5 μL of diluted cell extract (Table 3). The recovery for the spiked let-7a was between 96.0 and 101.7%, and the RSD was lower than 6.5% (n=3), suggesting that the proposed method can be used to quantify miRNAs in real-world samples with great accuracy and reliability.

Table 3. Recovery ratio of let-7a miRNA in the 293T cell samples

Nos.	Average let-7a content in 50 pg samples (amol)	Added let-7a (amol)	Detected let-7a (amol)	Recovery (%)	RSD (n=6) (%)
1	0.102	0.080	0.179	96.2	5.3
2	0.102	0.100	0.198	96.0	6.5
3	0.102	0.120	0.224	101.7	4.3

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