Electronic Supplementary Information

Ultrasensitive and Selective DNA Detection Based on Nicking Endonuclease Assisted Signal Amplification and Its Application in Cancer Cells Detection

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EXPERIMENTAL SECTION

Chemicals All of oligonucleotides were custom-ordered from SBS Genetech Co. Ltd. (China), and their sequences are listed in Table S1. NEase (Nt.AlwI, 10 units) was obtained from New England Biolabs. Tri(2-carboxyethyl)phosphine hydrochloride (TCEP, 98%) was purchased from Alfa Aesar (Massachusetts). H_2O_2 with analytical grade was purchased from Shanghai Chemical Reagent Company (Shanghai,China). Luminol standard powder and hemin was ordered from Sigma-Aldrich. A luminol stock solution (1.0×10^{-2} M) was prepared by dissolving it in 0.1 M NaOH solution and storing it in dark. A hemin stock solution (5.0×10^{-3} M) was prepared in DMSO and stored in dark at -20 ^{o}C. Double-distilled, deionized water was used throughout the experiments. All the chemicals employed were of analytical reagent grade and were used without further purification.

Table S1. DNA Sequence Used in This Work

oligonucleotides name	sequences[a]
1	5'-CCT CCC GGT GTT CGA TCC-3'
1'	5'-CCT CCC GGT GTT CG*C* TCC-3'
1"	5'-*GGA GGG CCA CAA GCG AGG*-3'
2	5'-CCC TTA AGT GTC CC-3'
3	5'-CAC AGG GTT GGG CGG GAT GGG TGG ATC GAA CAC CGG GAG GTT GGG TAG GGC GGG TTG GGA ATT-3'
4	5'-SH-TTT TTT ACA CAG GGT AGG GTT GGG CGG GAT GGG GGA TCG AAC ACC GGG AGG GGG TAG GGC GGG TTG GGT ACC CTG TGT TTT TTT-SH-3
5	5'-CCA CCG AAC TAT CCT CCC GGT GTT CGA TCC-3'
6	5'-AAC ACC GGG AGG ATA GTT CGG TGG CTG TTC AGG GTC TCC TCC CGG TG-3'

[a] The NEase recognition site of the target is underlined. Oligonucleotides **1'** has a single mismatch at different positions of the NEase recoginition site, and **1"** is a noncomplementary target DNA sequence. The mismatched positions are highlightened in italic.

Apparatus. The CL measurements were performed by using a BPCL ultraweak luminescence analyzer (Institute of Biophysics Academic Sinica, Beijing, China). The sample cell was a 2-mL quartz cuvette. Transmission electron microscopy (TEM) images were carried on a JEM-2000EX/ASID2. Magnetic Fe_3O_4-Au core-shell nanoparticles (Fe_3O_4@Au) were obtained from Shaanxi Lifegen Co., Ltd.

Preparation of MB-Fe_3O_4@Au Conjugates. The oligonucleotide-Fe_3O_4@Au conjugates were synthesized by adding ~10 μL of the commercialized Fe_3O_4@Au solution (~ 5 mg/ml) to 300 μL of 1.0×10^{-8} M dithiol-modified molecular beacon (MB). Thiol-modified DNA was activated with TCEP (10 mM) for 1 h at room temperature before attaching to Fe_3O_4@Au. After shaking gently for 16 h at room temperature, the MB-Fe_3O_4@Au conjugates were "aged" in the solution (0.3 M NaCl, 10 mM Tris-acetate, pH 8.2) for another 48 h. Excess reagents were removed by magnetic field. Following removal of the supernatant, the resulting precipitate was washed with 300 μL of 10 mM pH 7.4 phosphate buffer containing 0.01 M NaCl, recentrifuged, and then redispersed in 10 mM pH 7.4 phosphate buffer containing 0.01 M NaCl for further use.

Cancer Cell Culture. Ramos cells (target cells) and CEM cells (control cells) were cultured in cell flasks separately according to the instructions from the American Type Culture Collection. The cell line was grown to 90% confluence in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS) and 100 IU/mL penicillin-streptomycin at 37 ^{o}C, followed by culturing cells in a humidified atmoshpere with 5% CO_2. The cell density was counted on a hemocytomer prior to each experiment. Then, a 1.0 mL suspension of ~8.0×10^6 cells dispersed in RPMI 1640 cell media buffer was centrifuged at 3500 rpm for 5 min and washed with phosphate-buffered saline (18.6 mM phosphate, 4.2 mM KCl, and 154.0 mM NaCl) five times and resuspended in 1.0

mL cell media buffer.

CL Measurements. The detection of DNA (Scheme 1A in the main text) was performed in 300 μL of solution containing **2** (10 nM), **3** (10 nM) and different concentrations of target DNA **1** in 2 μL of NEBuffer. For the improved strategy (Scheme 1B in the main text), 200 μL of MB-Fe_3O_4@Au conjugates was reacted with 100 μL of target DNA **1** with different concentrations. Upon incubation at 58 ^oC for 5 min, 1 μL of NEase was added to the solution. After standing for 2 h at 37 ^oC, the resulting mixtures were heated at 80 ^oC for 20 min to deactivate the NEase, followed by the addition of hemin (1.0×10^{-8} M), luminol (1.0×10^{-6} M). The CL reaction was triggered by injecting 100 μL of H_2O_2 (7.5×10^{-4} M) with a syringe through a septum after the CL analyzer began to record at 10 s. The kinetics of CL signals between 0 to 30 s were recorded and the peak heights of the emission curves were measured by means of a photon counting unit.

For cancer cell assays (Scheme 2 in the main text), the Ramos cells aptamer **5** (10 nM) and its complementary oligonucleotides **6** (10 nM) were previouly hybridized in 300 μL of 10 mM phosphate buffer containing 0.01 M NaCl (pH 7.4) at 37 ^oC for 1 h to form the duplex structure (**II**). 10 μL of target cells of different concentrations was added to the solution containing duplex structure (**II**) and MB-Fe_3O_4@Au conjugates followed by incubating the mixture for 1 h at 37 ^oC. The following procedures were the same as DNA detection.

Supplementary Results and Discussion

Sensitivity of the Original Strategy of DNA Amplified Detection.

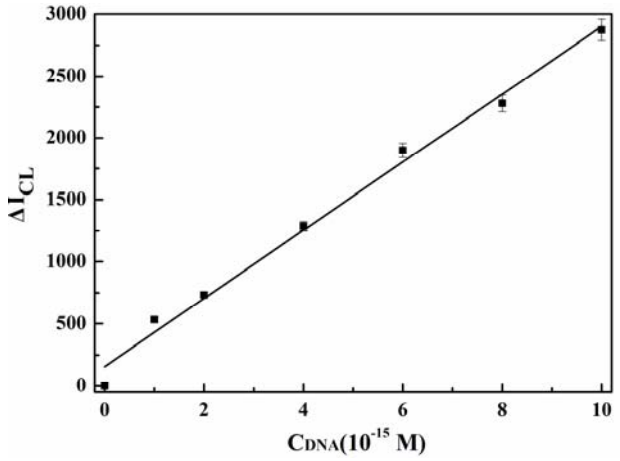

Fig. S1 The calibration curve of peak height verus the concentration of target DNA from 1.0 × 10^{-16} to 1.0 × 10^{-14} M obtained by the strategy of Scheme 1A in the main text.

To test the feasibility of our system for DNA detection, we first prepared various concentrations of a 18-mer single-stranded target DNA that contained the recognition sequence (-GATCC-) of a NEase (Nt.AlwI). The quasi-circular structure **I**, with a linker sequence complementary to that of the target DNA was then added to each of these solutions. Fig. S1 shows the calibration curve of peak height verus the concentration of target DNA. As the concentrations of the target DNA increased, the CL signals were enhanced. A linear range from 1.0 × 10^{-15} to 1.0 × 10^{-14} M was achieved with an equation of I = 274.4880C + 156.5529 (I is the CL intensity; C represents the concentration of target DNA, 10^{-15} M; n = 7, R = 0.9959) and a detection limit of 8.6 × 10^{-16} M.

Selectivity of the Improved Strategy of DNA Amplified Detection.

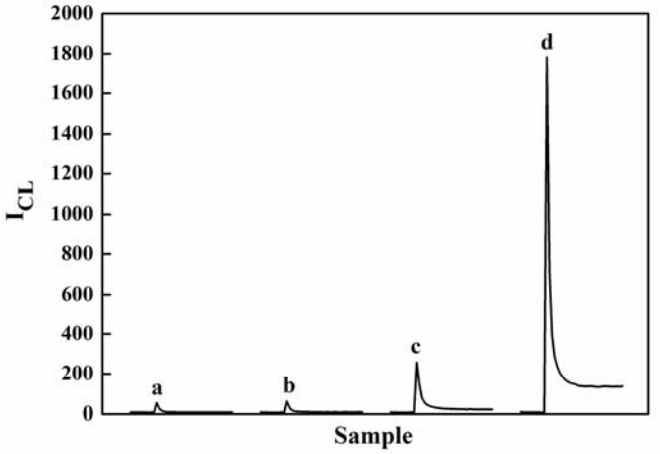

Fig. S2 CL signals of luminol-H_2O_2 system catalyzed by HRP-mimicking DNAzyme formed by NEase assisted strand cleavage and signal amplification with different target DNA. (a) blank, (b) noncomplementary sequences, (c) single-base mismatched sequences, and (d) complementary sequences. All the concentrations of target DNA are 4.0×10^{-14} M.

To evaluate the selectivity of the detection system, the effect of base mismatch was studied in the presence of 4.0×10^{-14} M target DNA with single-base mismatched DNA sequences and noncomplementary sequences. Fig. S2 shows that CL signals were significantly weaker than that of the complementary squences which were not significantly different from the blank. We proposed that this significant sequence specificity is attributed to the requirement of full complementary between the target DNA and the linker strand at the NEase recognition site and sufficient complementarity beyond the NEase recognition site to allow efficient hybridization.

Generality of the Improved Strategy of DNA Amplified Detection for Target DNA with Varied Lengths

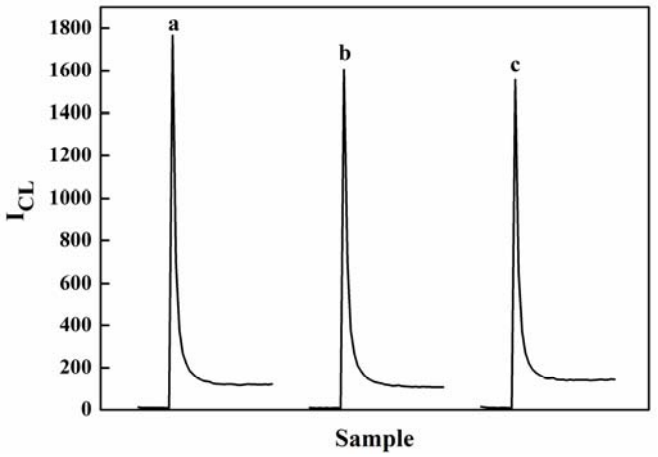

Fig. S3 CL intensities of target DNA sequences of different length containing NEase recognition sites: (a) 18-mer, (b) 36-mer, and (c) 72-mer. All the concentrations of target DNA are 4.0×10^{-14} M. DNA sequences: 18-mer: 5'-CCT CCC GGT GTT CGA TCC-3'; 36-mer: 5'-GAA CAG CCA CCG AAC TAT CCT CCC GGT GTT CGA TCC-3'; 72-mer: 5'-GCA AAA TCT AAA AGA GCA CCA CCG GGA GGA GAC CCT GAA CAG CCA CCG AAC TAT CCT CCC GGT GTT CGA TCC-3'.

From Fig. S3, the improved amplification strategy demonstrates remarkable generality for target DNA with varied lengths. In principle, the length of the dithiol-modified MBs structure could increase with the increase of the base number of the target strand. However, for relatively long-stranded MBs, immobilization and instability become significant challenges. Practically in our sytem, a fixed set of MBs can be readily extended to detection of long single-stranded DNA sequences that contains NEase recognition site. As shown in Fig. S3, CL detection of a range of varied length target DNA sequence (18-mer, 36-mer, and 72-mer) was achieved without the requirement of longer MBs.