Electronic Supplementary Information

Chemiluminescence Imaging Array for the Detection of Cancer Cells by Dual-Aptamer Recognition and Bio-Bar-Code Nanoprobe-Based Rolling Circle Amplification

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EXPERIMENTAL SECTION

Materials and Apparatus

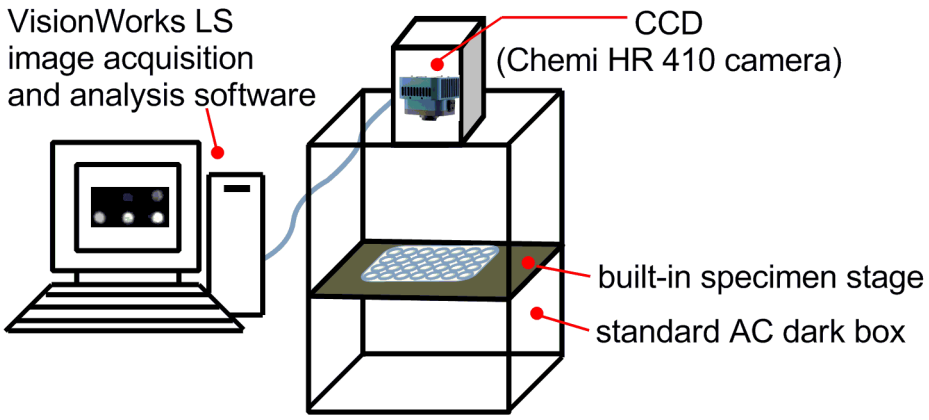
The circular DNA template and the other oligonucleotides were synthesized by Takara Biotechnology Co., Ltd. (Dalian, China) and Sangon Biotechnology Co., Ltd. (Shanghai, China), respectively. Their sequences are listed in Table S1. Klenow DNA polymerase was obtained from Fermentas (Canada). The mixture of deoxyribonucleoside 5'-triphosphates (dNTPs) solution was ordered from SBS Genetech Co., Ltd. (Beijing, China). Luminol standard powder (Aladdin Chemistry Co., Ltd，Shanghai, China) was prepared as 10 mM in 0.1 M NaOH and further stored in dark. *p*-iodophenol (PIP) was purchased from Aladdin Chemistry Co., Ltd.（Shanghai, China）, which needed to be purified by recrystallization before use. Double-distilled, deionized water was used throughout the experiments. All the regents provided were of analytical grade and used without further purification.

Table S1. DNA Sequence Used in This Work

Oligonucleotides name	Sequences (5' to 3')[a]
biotinylated aptamer TE02	biotin- TAGGCAGTGGTTTGACGTCCGCATGTTGGGAATAGCCACGCCT
thiolated aptamer TD05	SH-AACACCGGGAGGATAGTTCGGTGGCTGTTCAGGGTCTCCTCCCGGTG
primer	SH-GCATTATTGAGCACTAGCCTCCAG
detection probe	biotin-CCCAACCCGCCCTACCC
circular DNA template	GTGCTCAATAAAAACCCAACCCGCCCTACCCAAAACTGGAGGCTA

[a] Font colors correspond to the line colors in the schemes.

The chemiluminescence imaging was performed on a BioSpectrum 400 chemiluminescence imager (UVP, USA). The setup is shown in Scheme S1. UV-vis spectra were recorded on a Cary 50 UV-Vis-NIR spectrophotometer (Varian, USA). Atomic Force Microscope (AFM) image was taken with a Being Nano-Instruments CSPM-4000 (Benyuan, China).



Scheme S1. The schematic diagram of the instrumental setup of the BioSpectrum 400 chemiluminescence imager (UVP, USA).

Cell Culture

Ramos cells (human Burkitt's lymphoma) and MCF-7 (human adenocarcinoma) were cultured according to the American Type Culture Collection (ATCC), respectively. Each cell line was cultured in RPMI medium 1640 supplemented with 10% fetal bovine serum (FBS) and 100 IU/mL penicillin-streptomycin. The cell density was determined using a hemocytomer prior to each experiment. Then, a 1.0 mL suspension of approximate 1.0×10^6 cells dispersed in RPMI cell media buffer was centrifuged at 3500 rpm for 5 min and rinsed with phosphate-buffered saline (8.7 mM $Na_2HPO_4 \cdot 12H_2O$, 1.4 mM KH_2PO_4 , 2.7 mM KCl, and 136.7 mM NaCl) three times, which was finally redispersed in 1.0 mL of cell media buffer.

The Preparation of Aptamer TE02-Coated Microarray

Firstly, the coating solution was prepared by diluting streptavidin to the concentration of 2 μg/ml with 0.05 M Na_2CO_3-$NaHCO_3$ buffer (pH 9.6). For each well of the microtiter plate, a 100 μL of the coating solution was introduced, followed by drying at 37 ^oC overnight to obtain the pre-coated microtiter plate. Then, the PBST buffer containing 0.01 M PBS and 0.05% (v/v)

Tween-20 was used to washed the microtiter plate for five times to remove the unbound streptavidin. It is worth to note that this treatment should last for 1 min to ensure the complete washing. After another drying at 37 ^{o}C for 2 h, the as-prepared streptavidin-coated 96-well microtiter plate was encapsulated and stored at 2~5 ^{o}C for further use. The aptamer TE02-coated microarray was further prepared by adding 10 μL of 1.0 × 10^{-7} M aptamer TE02 to each well, followed by incubation for 40 min at room temperature and washing with PBS for three times.

Synthesis of AuNPs

AuNPs were prepared by the method of reduction of tetrachloroauric acid ($HAuCl_4$) with trisodium citrate.[S1] Briefly, 100 ml of 0.01% $HAuCl_4$ solution filtrated through 22 μm porous membrane was heated to boiling with vigorous stirring and reflux, followed by rapid addition of 3.0 ml of 1% trisodium citrate solution dropwise. The solution maintained stirring and boiling for 30 min with the color gradually from gray yellow to deep red, indicating the formation of AuNPs. The heating was then stopped. After cooling down the resulting colloidal suspension to room temperature with stirring for 20 min, the AuNPs with an average diameter of 15 nm were obtained. The as-prepared AuNPs was characterized by AFM.

Fabrication of Bio-Bar-Code AuNP Probe

This step is one of the most essential procedures in the assay. Firstly, 10 μL of 1.0 × 10^{-7} M thiolated aptamer TD05 (thiol-TD05) and 10 μL of 1.0 × 10^{-6} M thiolated primer DNA were activated by 10 μL of 1.0 × 10^{-5} M and 10 μL of 1.0 × 10^{-4} M mild reducing agent DL-dithiothreitol (DTT), respectively, which were mixed and introduced to the as-prepared AuNP colloid (the original volume of AuNP solution is 1.3 mL). After incubating at 37 ^{o}C for 16 h with gently shaking in dark, the mixture was subjected to “aging” by the addition of 1.0 M NaCl up to a

concentration of 50 mM and reacted at room temperature for 6 h. Additional 1.0 M NaCl was continued adding to a final concentration of 100 mM and further reacted at room temperature for another 6 h. After centrifugating at 10000 rpm for 50 min, the precipitation of bio-bar-code AuNPs was further reacted with 10 μL of 1.0×10^{-6} M circular DNA template at 25 ^{o}C for 1 h. The resulting nanoprobes were centrifugated at 10000 rpm for 30 min, redipersed in 10 μL of 0.02 M Tris-HCl buffer, and stored at 4 ^{o}C before use.

Cell Assay on CLIA

In a typical experiment for the detection of cancer cells, 10 μL of sample solution containing a desired amount of Ramos cells in 0.01 M PBS buffer and 10 μL of the as-prepared bio-bar-code AuNP probe were added to the sample application aptamer TE02-coated well. After incubation for 30 min to allow the binding of cells on microwell surface via aptamer TE02 recognition and the recognition of cell membrane protein by aptamer TD05 on AuNP probes. The excess probes were removed by washing with 200 μL of 0.01 M PBS buffer for three times. The RCA reaction was initiated by adding 6.5 μL of 10 mM dNTPs, 2 μL of Klenow DNA polymerase buffer, 1.5 μL of 10 U/μL Klenow DNA polymerase, followed by reacting at 37 ^{o}C for 1 h in water bath. A 10 μL of 1.0×10^{-5} M biotinylated detection probe DNA and a 5 μL of 2.0×10^{-5} M streptavidin-HRP were then added and incubated at 25 ^{o}C for 1 h. After washing with PBS for four times, 50 μL of 10 mM luminol, 50 μL of 300 mM H_2O_2, and 50 μL of 10 mM PIP were added to each sample well and incubated for 10 min. The CL images were simultaneously recorded by using a cooled CCD camera. The spots were automatically quantified by VisionWorks LS image acquisition and analysis software of chemiluminescence imager BioSpectrum 400 (UVP, USA). The CL intensity of each spot was calculated as the mean pixel within a circle of a given diameter around each spot

center. In the case of Ramos cells detection in human blood, the desired amount of Ramos cells was spiked into 1:10 diluted blood and applied to the sample application well. Other procedures were the same as those described above.

Control Experiment

To confirm the role of the fabricated bio-bar-code nanoprobe for Ramos cell detection, control experiment was carried out by using only primers to construct AuNPs which further performed the RCA to obtain HRP-labeled nanoprobes (Fig. S1, pathway a). The results showed that no signal was observed in this case because there was no recognition site between the nanoprobes and target cells (Fig. S1, a). Thus, aptamer TD05 played an important role in not only fabricating bio-bar-code nanoprobe to amplify signal, but also recognizing target cell to improve specificity. In addition, there was no nonspecific absorption of HRP-labeled bio-bar-code AuNP probe on the surface of target cells.

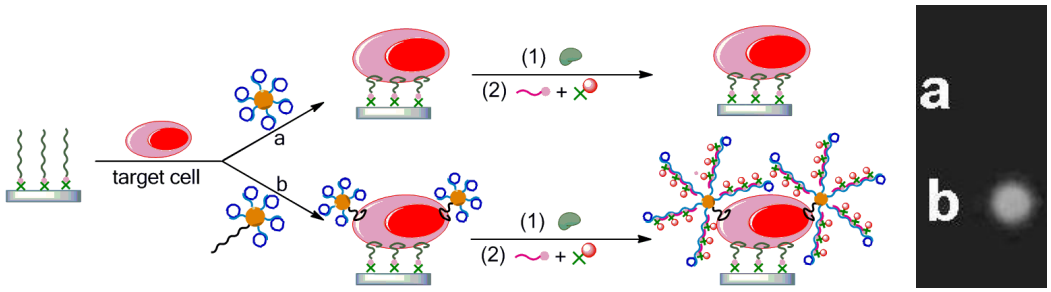


Fig. S1 Schematic representation and CCD results of the control experiment by using only primers to construct the AuNP probe. The amounts of Ramos cells are 6000 cells for each sample.

Calculation of the Number of Aptamer TD05 and Primers per ~15 nm-AuNP Surface

The UV-vis absorbance calibration curves of standard DNA solution of aptamer TD05 (from 1.0×10^{-8} M to 1.0×10^{-7} M) and primers (from 1.0×10^{-7} M to 1.0×10^{-6} M) are shown in **Fig. S2**.

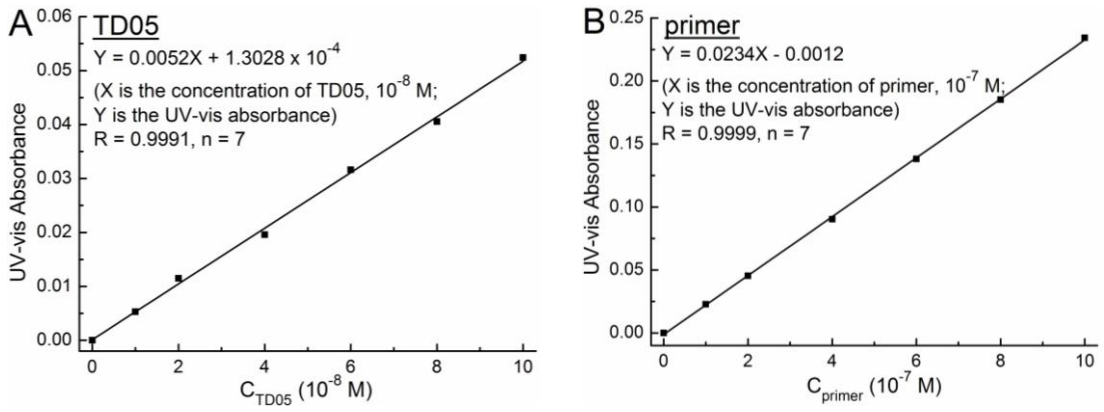


Fig. S2. UV-vis absorbance calibration curves of standard DNA solution of (A) aptamer TD05 and (B) primers.

Take the calculation of the surface coverages of TD05 on AuNP as example. 2 mL of 1.0×10^{-7} M activated TD05 was added to AuNP colloids (the original volume of AuNP solution is 10 ml). After incubation as mentioned in "Fabrication of Bio-Bar-Code AuNP Probe", the number of aptamer TD05 immobilized on AuNPs was quantitatively calculated from the UV-vis absorbance difference at 260 nm before and after its immobilization as follows.

UV-vis absorbance of the supernatant before immobilization: 0.05241

Concentration of TD05 before immobilization: 1.0×10^{-7} M

UV-vis absorbance of the supernatant after immobilization**:** 0.02569

Concentration of TD05 after immobilization: 4.94×10^{-8} M

Moles of total TD05 immobilized on the as-prepared AuNPs: $(1.0 \times 10^{-7} - 4.94 \times 10^{-8}) \times 2 \times 10^{-3} = 1.01 \times 10^{-10}$ mole

Similarly, the number of primers immobilized on AuNPs was quantitatively calculated as follows.

UV-vis absorbance of the supernatant before immobilization: 0.23425

Concentration of primers before immobilization: 1.0×10^{-6} M

UV-vis absorbance of the supernatant after immobilization**:** 0.11357

Concentration of primers after immobilization: 4.92×10^{-7} M

Moles of total primers immobilized on the as-prepared AuNPs: $(1.0 \times 10^{-6} - 4.92 \times 10^{-7}) \times 2 \times 10^{-3}$ $= 1.02 \times 10^{-9}$ mole

The moles of the as-prepared AuNPs were determined by assuming that all nanoparticles were spherical, all $HAuCl_4$ was reacted and the density of nanoparticles was the same as bulk gold.[s2]

The concentration of Au^{3+} in $HAuCl_4 \cdot 4H_2O$: (0.01g / 411.85 g/mol) / 100 ml = 2.42806×10^{-4} M

The amount of Au atoms per AuNP: $(4/3\pi R^3)/(4/3\pi r^3) = (15/0.268)^3 = 175335.31$

The concentration of AuNP solution: 2.42806×10^{-4} M / 175335.31 = 1.385×10^{-9} M

The moles of the as-prepared AuNPs used: 1.385×10^{-9} M × 10 ml = 1.385×10^{-11} mole

Table S2. Quantitive Determination of the surface coverages of TD05 and primers on AuNPs

AuNPs (mol)	TD05 (mol)	primer (mol)	ratio of AuNPs/TD05/primer
1.385×10^{-11}	1.01×10^{-10}	1.02×10^{-9}	1/7.3/73.6

Table S3. Comparison between the proposed strategy and other reported methods for cancer cells detection based on aptamer recognition

Cancer Cells	Detection Method	Detection Limit	Ref.
Ramos	aptamer-nanoparticle strip biosensor	800 cells	S3
CCRF-CEM	fluorescence imaging	250 cells	S4
CCRF-CEM	colorimetric detection	90 cells	S5
MCF-7	electrochemical detection	100 cells	S6
Ramos	electrochemiluminescence detection	210 cells/mL	S7
Ramos	electrochemiluminescence detection	126 cells/mL	S8
MCF-7	fluorescence detection	500 cells/mL	S9
CCRF-CEM	fluorescence detection	4000 cells/mL	S10
MCF-7	electrochemical detection	100 cells/mL	S11
CCRF-CEM	quartz crystal microbalance	8000 cells/mL	S12
Ramos	chemiluminescence imaging	163 cells	[a]

[a]This method.

Selectivity.

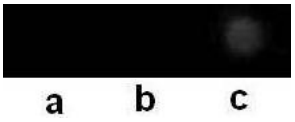


Fig. S3 Microwell array CCD images of (a) blank, (b) MCF-7 cells, and (c) Ramos cells. The amounts of cells are 1000 cells for each sample.

Detection of Ramos Cells in Human Blood

Table S4. CL Intensity of Blood Samples Spiked with and without Romas Target Cells

Sample	Blood sample (cells/mL)	Added target cells (cells/mL)	Detected cells (cells/mL)	Recovery (%)
1	- [a]	200	183	91.5
2	-	2000	1789	89.4
3	-	20000	18927	94.6

[a] No CL response

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