Supporting Information

Fluorescence Detection of Telomerase Activity in Cancer Cells Based on Isothermal Circular Strand-displacement Polymerization Reaction

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1. The fluorescence enhancement induced by the chemical synthesized DNA

To further proof the detection capability of the strategy, a chemical synthesized DNA strand containing the same sequence with the telomerase product was used for the control experiment. Through recognition and hybridization with the chemical synthesis DNA sequence, the conformational of the hairpin fluorescence probes changed and the obvious fluorescence intensity (Curve b), comparing to the control buffer (Curve a), was observed in Figure S1. In addition, with the proceeding of the multiple circles strand-displacement process, the fluorescence signal enhanced dramatically (Curve c).

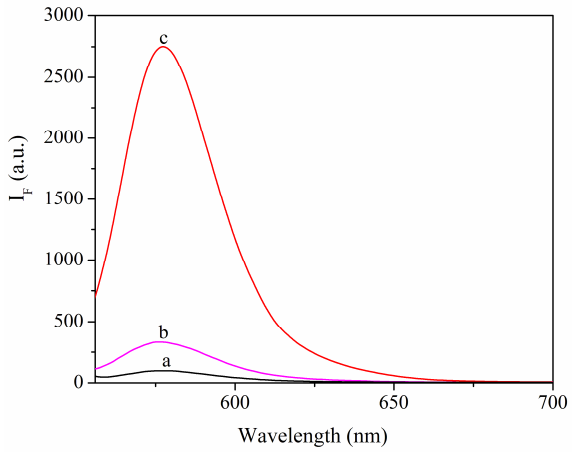


Figure S1. The fluorescence enhancement of 500 pM chemically synthesized telomerase product. The oncentrations of hairpin fluorescence probe, primer **1**, and primer **2** were 300 nM, 500 pM, and 300 nM, respectively.

2. Detection of telomerase activity of the commercial telomerase

As we all known, TS primer would be elongated to a long strand DNA with a repeat unit of TTAGGG in the presence of telomerase. The resultant long strand DNA would hybridize with the loop of the hairpin fluorescence probe and open the stems, restoring the fluorescence signal. As shown in Figure S2 and Figure S3, the fluorescence signals increased with the increasing of the concentrations of the commercial telomerase. With the process of elongation step (Step **1**) only, 2.5×10^{-15} g mL^{-1} telomerase activity could be detected (Figure S2A and Figure S2B). However, after the process of the multiple circles of the strand displacement amplification process (Step **2**), the fluorescence intensities enhanced dramatically, and as low as 2.5×10^{-19} g mL^{-1} telomerase activity could be detected (Figure S3A and Figure S3B).

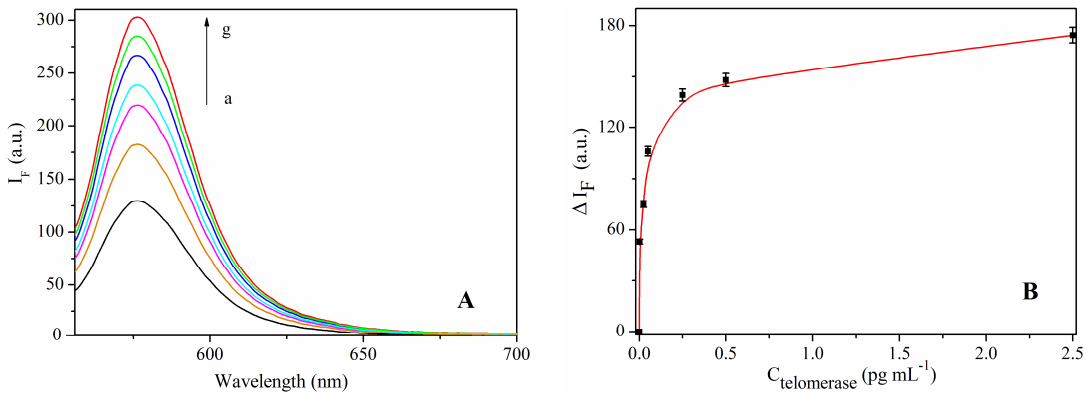


Figure S2. (A) Fluorescence intensities of sensing system to different concentrations of commercial telomerase before the strand displacement amplification: (a) 0, (b) 2.5×10^{-15}, (c) 2.5×10^{-14}, (d) 5.0×10^{-14}, (e) 2.5×10^{-13}, (f) 5.0×10^{-13}, and (g) 2.5×10^{-12} g mL^{-1}. (B) The relationship between the difference of fluorescence intensities and telomerase concentrations before and after the strand displacement amplification. The conditions are the same as in Figure S1.

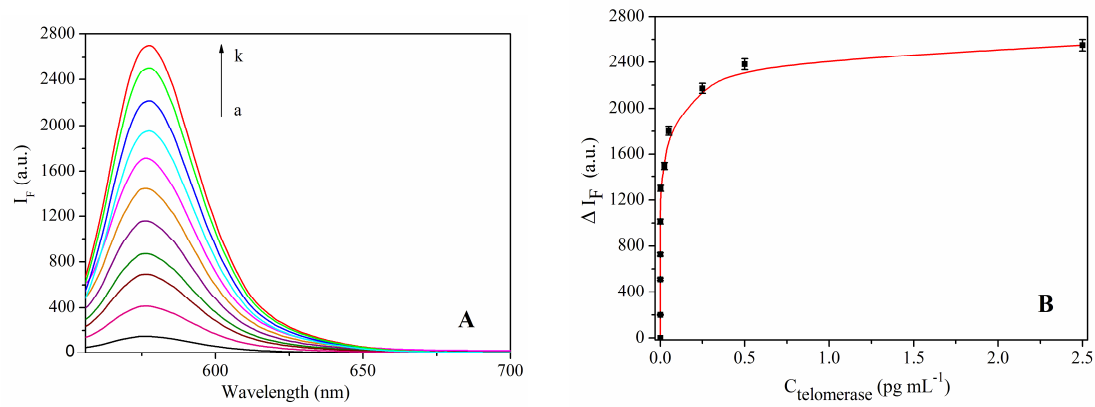


Figure S3. Fluorescence intensities of sensing system to different concentrations of commercial telomerase(A) and the relationship between the difference of fluorescence intensities and telomerase concentrations after the strand displacement amplification (B). The concentrations of the telomerase: (a) 0, (b) 2.5×10^{-19}, (c) 2.5×10^{-18}, (d) 2.5×10^{-17}, (e) 2.5×10^{-16}, (f) 2.5×10^{-15}, (g) 2.5×10^{-14}, (h) 5.0×10^{-14}, (i) 2.5×10^{-13}, (j) 5.0×10^{-13}, and (k) 2.5×10^{-12} g mL^{-1}. The conditions are the same as in Figure 1.

3. Comparison of the sensitivity of the present method to other techniques

This method and some other techniques for telomerase assays were summarized in Table S1. The sensitivity of this present work was found to be increased about 1-4 orders of magnitude than that of some methods and was comparable to that of Sato's work using bioluminescence detection. Because the procedure of cell culture and telomerase preparation could be done before the detection experiment and the telomerase extracted from Hela cells can be stored at -20°C for about two weeks with stability for further experiment, the total detection time of the two

methods is about 3 h and 6 h, respectively, which was competitive comparing to the methods with the similarly detection limit. Only need a fluorescence probe and some dNTPs, the direct detection of telomerase could be completed by the present method with step **1**, the low cost was another merit compared to other methods. The cost of the strategy with the multiple circles of the strand displacement amplification process was also tolerated due to its ultrasensitivity.

Table S1. Comparsion between the Current Method and Other Reported Techniques for Analysis of Telomerase Activity[a]

Method	Label	Detection modes	Detection time	Sensitivity data	Ref.
PAGE	Radioactive dGTP	Autoradio graphy	2	200-500 HeLa cells	1
electrochmical assay	Label-free	Electrochemistry	8	About 40 cells/μL	2
HPA assay	Acridiniumester labeled DNA probe	Chemiluminescence	3.5	100000 HeLa-60 cells	3
Catalytic beacons	Peroxidase-like DNAzyme	Spectroscopy	-[c]	500 HeLa cells	4
DNAzyme based assay	DNAzyme functionalid AuNPs	Chemiluminescence	16.5	1000 HeLa cells	5
Magnetic nanosensor imaging	DNA-functionalized magnetic nanoparticles	Magnetic resonance	2	10 amol of telomeric repeats	6
Electrochemical and QCM assay	Avidin-alkalinephosph atase conjugate	Electrochemistry	17	1000-5000 HeLa cells	7
Bioluminescent method	Label-free	Bioluminescence	6	5-10 cells	8
TRE chip-based assay	Label-free	SPR	-[d]	100 tumor cells in 10^5 background cells	9
ELISA	Alkaline phosphata-seantidigoxigenin	Chemiluminescence	28.5	100 amol of telomeric repeats	10
Magnetic bead basedECL assay	ECL Nanoprobes	Electrochemilum-inescence	27.5	500 HeLa cells	11
Polyvalent Oligonucleotide-AuNP Assay	Polyvalent oligonucleotide AuNP	Verigene ID light scattering reader system.	30	10 HeLa cells	12
Fluorescence Assay[b]	Fluorophore	Circular Strand-displacement Polymerization	3	4 HeLa cells	

[a] Some were adapted from ref 11; [b] This method; [c,d] Do not reported in their paper.

4. Detection of the amount of the Ramos cells.

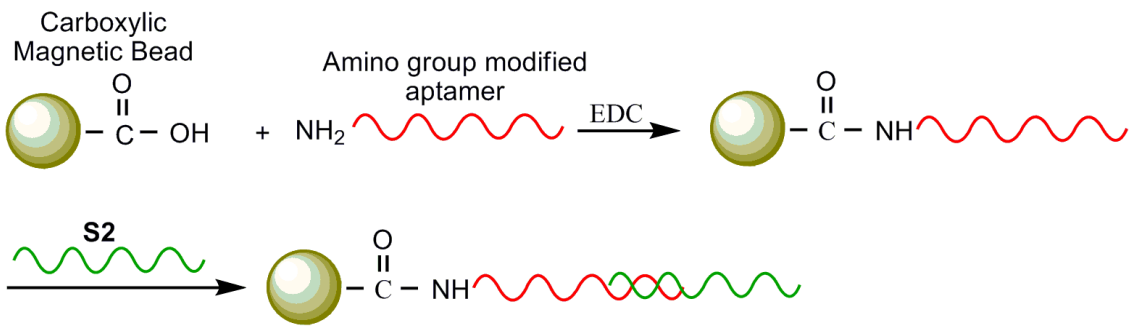
The assay for the direct detection of cancer cells has attracted great interests for its practical application in early and accurate cancer diagnosis, which will be a key for the effective and ultimately successful treatment of such diseases.

Aptamers are single-stranded nucleic acid which bind highly specifically with protein molecular or cellular targets, and thus are clear alternatives to long established antibody-based diagnostic or biotechnological products for research, diagnostics, and therapy.[13,14] Unlike monoclonal antibodies, aptamers have low molecular weights and can be chemically synthesized and readily modified.[15,16] An in vitro process identifying DNA sequences with strong affinities toward intact tumor cells referred to as tumor cell SELEX was used to select aptamers with high specificity toward target cancer cell lines by Tan's group. The aptamers selected were used to detection cancer cells due to their ability to distinguish one cell type from numerous other cell types. They have developed a novel two-nanoparticle assay with aptamers as the molecular recognition element for the rapid collection and detection one kind of leukemia cells and then multiple cancer cells.

To practically validate the isothermal strand displacement polymerization reaction used for the determination of telomerase activity in the manuscript, the amount of the Ramos cells was determined by combining the fluorescence assay and aptamer modified magnetic beads.

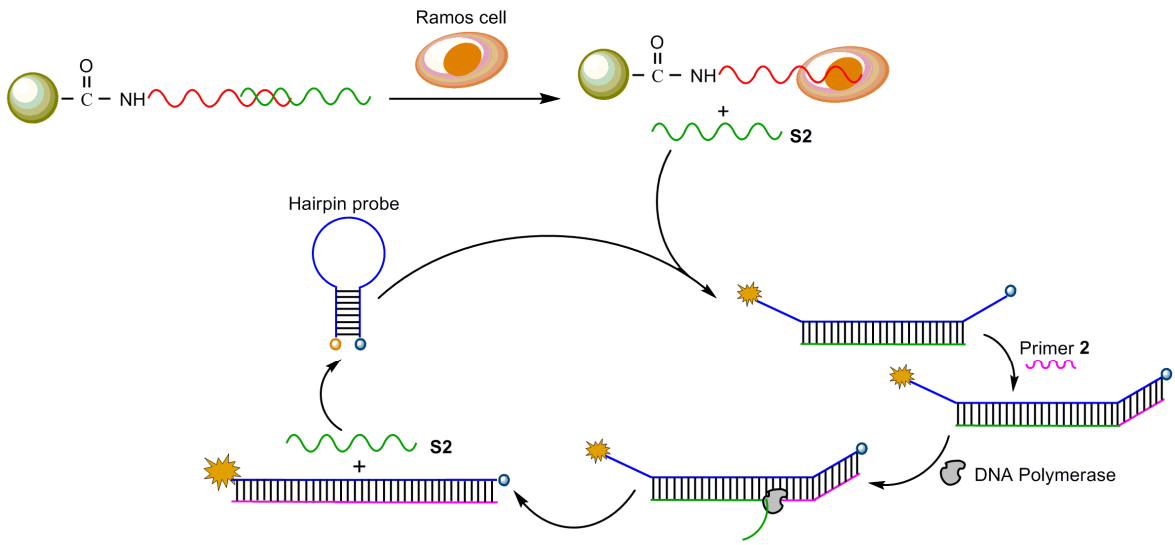
4.1 The detection process of the amount of Ramos cells

As shown in Scheme S1, the aptamer was immobilized to the surface of the magnetic bead through the linkage of an amidization reaction between carboxyl group on the surface of magnetic besd and amino group on the terminal of the aptamer.



Scheme S1. Schematic illustration of the immobilization procedure

Scheme S2 depicted the method for the amplified fluorescence assay of the Ramos cells. Magnetic beads were used as both the separation tool and the immobilization matrix of the aptamer of Romas cells and its partly complementary strand (**S2**). In the presence of the target cells, the aptamers recognized and conjugated with their targets on the surface of the cancer cells. **S2**, dehybrizing with aptamer and detaining at the solution after the magnetic separation, hybridized with the stem of the hairpin probe **2**, leading to the stem separation. Following this, primer **2** annealed with the opened stem and triggered a polymerization reaction in the presence of dNTPs/polymerase. With the process of primer **2** extension, **S2** was displaced by the polymerase with strand-displacement activity, after which a complementary DNA (c'DNA) was synthesized, forming a probe–c'DNA complex. Finally, to renew the cycle, the displaced **S2** hybridized with another probe, which triggered yet another polymerization reaction.



Scheme 2. Schemitic illustration of the design strategy for the fluorescence amplification assay of Ramos cells

4.2 Experimental section

About 1.0×10^6 Ramos cells dispersed in RPMI 1640 cell media buffer were centrifuged at 3500 rpm for 5 min, washed in cell media three times and were then redispersed in 1 mL cell media buffer. During all experiments, the cells were kept in an ice bath at 4 °C.[17]

30 μL of carboxyl-modified magnetic beads were transferred into a 1.5 mL Eppendorf tube. The magnetic beads were washed 3 times with 100 μL of 0.1 M imidazole buffer (pH 6.0) and then activated in 150 μL of 0.1 M imidazole buffer (pH 6.0) containing 0.2 M EDC with gentle shaking for 40 min. 100 μL 1 μM of aptamer was added into the activated beads and the mixture was

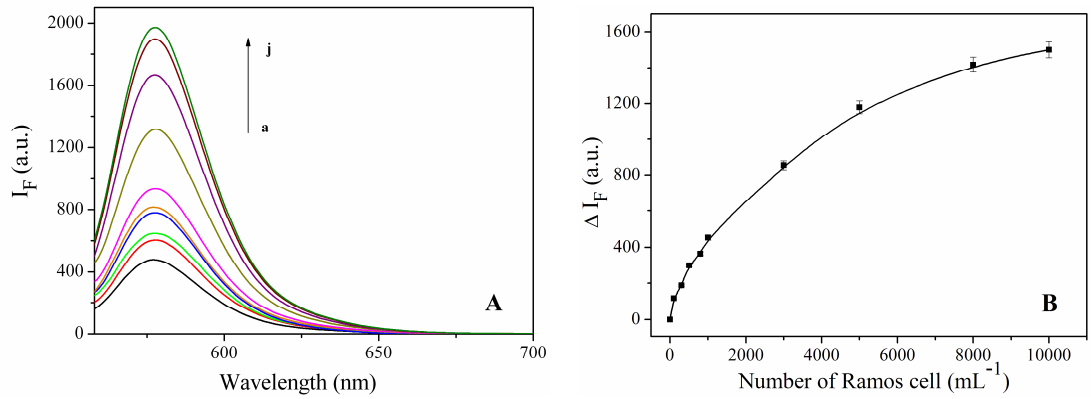
incubated at 37 °C for 12 h with gentle shaking. The resultant aptamer-magnetic bead conjugates were washed 3 times with 100 μL of wash buffer (5 mM Tris-HCl, pH 8.0, 0.17 M NaCl, 0.05% Tween 20) and resuspended in 100 μL of buffer (20 mM Tris-HCl, pH 8.0, 0.5 M NaCl). Then 100 μL 1 μM DNA (**S2**), which was partly complementary to the aptamer, was added to the solution and incubated at 37 °C for 1.5 h with gentle shaking. The aptamer-**S2**- magnetic bead conjugates were washed and resuspended in buffer before use.

Magnetic extraction was carried out by adding specified amounts of aptamer-**S2**-magnetic beads to each 300 μL cell sample followed by incubating the mixture for 2 h at 37 ^{o}C. And then a magnetic field was introduced to the sample container. After the magnetic separation, the supernatant containing released **S2** strand which was proportional to the amount of target cells was decanted using a pipette. The separated solution was stored at 4 °C for further usage.

The solution containing the **S2** strand released from the aptamer-**S2**-magnetic beads in the presence of Ramos cells was incubated with hairpin fluorescence probe **2** to open the stems of which. Then primer **2** was added to the mixture and incubated at 37 °C for 1 h. Then 4 U polymerase Klenow Fragment and 100 μM dNTPs were added to the mixture. After incubation at 37 °C for 2 h, the fluorescence intensities were recorded immediately. The total volume of the reaction mixture was 600 μL and the final concentrations of hairpin fluorescence probe and primer **2** were both 300 nM.

4.3 The sensitivity of the detection assay

By using the amplified detection method based on isothermal strand displacement polymerization reaction, fluorescence signals increased with the increasing of the amount of Ramos cell ranging from 1.0×10^2 to 1.0×10^4 cells mL^{-1} (Figure S4). A series of eleven duplicate measurements of 200 cells mL^{-1} were used for estimating the precision, and the relative standard deviation (RSD) was 2.9 %, showing good reproducibility. Due to the high sensitivity of the design protocol, as low as 100 cells mL^{-1} Ramos cell could be detected.



Firure S4. Dose-responses (A) and the relationship (B) between the fluorescence intensities and the different concentrations of the Ramos cells: 0, 100, 300 ,500, 800, 1000, 3000, 5000, 8000 and 10000 cells mL^{-1}.

This method and some other techniques for cancer cell assays were summarized in Table S2. Comparing to other methods listed in Table S2, the current protocol is one of the most sensitive assay for the determination of cancer cells.

Table S2 Sensitivity for cells determination by different ways.

Assay format	Detection system[b]	Detection range/ cell mL^{-1}	Detection limit /cell mL^{-1}	Ref.
Direct	colorimetric	CEM: 6.6×10^2-1.3×10^5	300	18
ACNPs [a]	fluorescence	CEM: 5×10^3-2×10^5	1250	19
Enzyme-linked	DPV[b]	K562: 5×10^4-1×10^7	1.0×10^4	20
Electrodeposition	Impedance	K562: 5×10^3-5×10^7	1.0×10^3	21
cytosensor	DPV	BGC: 1×10^3-1×10^7	620	22
Self-assembled monolayer	ASV[b]	K562: 1×10^2-1×10^7	-[c]	23
Circular Strand-displacement Polymerization [d]	Fluorescence	Ramos: 1×10^2-1×10^4	100	

[a] ACNP*s:* aptamer-conjugated nanoparticles.

[b] DPV: differential pulse voltammetry. ASV: anodic stripping voltammetric.

[c] Do not reported in their paper.

[d] This work.

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