Supplementary Information

Binding-responsive catalysis of Taq DNA polymerase for sensitive and selective detection of cell-surface proteins

Zhuxin Wang,[a] Yifei Li,[a,b] Peng Han,[a] Xiaoxia Mao,[a] Yongmei Yin[c,*] and Ya Cao[a,*]

[a] *Center for Molecular Recognition and Biosensing, Shanghai University, Shanghai 200444, China*

[b] *Shanghai Key Laboratory of Bio-Energy Crops, Shanghai University, Shanghai 200444, China*

[c] *Department of Oncology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China*

Experimental section

Chemicals and materials

Taq DNA polymerase and standard Taq buffer were obtained from New England Biolabs (Ipswich, MA). Biotin, avidin, bovine serum albumin (BSA), myoglobin (Mb), trypsin, hemoglobin (Hb), deoxynucleotide solution mixture (dNTPs) and ethylenediamine retra-acetic acid (EDTA) were purchased from Sigma Aldrich. Folate receptor (FR) was bought from Sino Biological Inc. (Beijing, China). Oligonucleotides were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China), and their sequences are listed in Table S1. All other reagents were of analytical grade and used without additional purification. Double-distilled water (18 MΩ cm) used to prepare all buffer solutions was obtained through a Direct-8 Millipore purification system.

Table S1 Sequences of oligonucleotides used in this work

Oligonucleotides	Sequence (from 5' to 3')
Taq aptamer	AGTCAGTCAGTCCAATGTACAGTATTGGACTGACTGACT
Aptamer probe I1	AGTCAGTCAG-(T-biotin)-CCAATGTACAGTATTGGACTGACTGACT
Aptamer probe I2	AGTCAGTCAGTCCAATG-(T-biotin)-ACAGTATTGGACTGACTGACT

Aptamer probe I3	AGTCAGTCAGTCCAATGTACAGTA-(T-biotin)-TGGACTGACTGACT
Aptamer probe I4	AGTCAGTCAGTCCAATGTACAGTATTGGACTGAC-(T-biotin)-GACT
Primer	GACGGGAAG
Hairpin probe	FAM-CCTCTCCGTGTCTTGTACTTCCCGTCAGAGAGG-Eclipse

Cell Culture and preparation

Cancer cell lines, MCF-7 (human breast adenocarcinoma), HeLa (human cervical carcinoma) and HCT-116 (human colon carcinoma), were ordered from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). The three types of cancer cells were grown respectively in RPMI1640, DMEM and McCoy's 5a medium containing 10% fetal bovine serum under standard cell culture conditions (37 °C in humidified air with 5% CO_2). Normal cell line, H9C2 (cardiac muscle cell), was kindly gifted from Prof. Junjie Xiao (School of Life Sciences, Shanghai University, Shanghai 200444, China) and was grown in DMEM medium containing 10% fetal bovine serum under standard cell culture conditions. In the exponential phase of growth, the cells were collected from the medium for following experiments. In brief, the cells were washed twice with phosphate buffered saline (PBS) and detached with trypsin-EDTA solution (0.25% trypsin and 1 mM EDTA) for 1 min at 37 °C. Afterward, the cells were harvested by centrifugation at 1000 rpm for 5 min, and re-suspended with a certain concentration, which was determined by hemocytometer counting (TC10^{TM}, Bio-Rad, USA).

Binding-responsive catalysis of Taq DNA polymerase

Binding-responsive catalysis of Taq DNA polymerase was performed in a 100-μL reaction mixture, which was concocted as part A and part B separately. Part A (total volume of 50 μL) was prepared by mixing 200 nM aptamer probe (biotin-labeled aptamer of Taq DNA polymerase), 1 mM dNTPs and a certain concentration of biotin receptor (avidin as a model) in standard Taq buffer at 30 °C for 1 h, followed by being treated with Taq DNA polymerase (100 U/mL as final concentration) for 30 min to

sustain aptameric interaction; whereas part B was manufactured by incubating 400 nM hairpin probe and 400 nM primer in 50 μL of standard Taq buffer at 30 °C for 1 h. After preparation, part A and part B were mixed together, allowing the catalysis of DNA polymerase to proceed at 30 °C for 1 h. Finally, the fluorescence spectrum of the resulting mixture was recorded on an F-7000 fluorescence spectrophotometer (Hitachi, Japan), with the excitation wavelength fixed at 460 nm.

Detection of cell-surface biotin receptor via binding-responsive catalysis of Taq DNA polymerase

A typical experiment for the detection of biotin receptor on live cell surfaces was conducted as follows. First, a mixture of 10 μL of 1 μM aptamer probe I2 and 10 μL of different concentrations of cells was prepared and maintained at 37 °C to perform biotin/biotin receptor binding. Then, the mixture was incubated with Taq DNA polymerase (100 U/mL as final concentration) and dNTPs (1 mM as final concentration) in 30 μL of standard Taq buffer for 30 min. Afterward, the above mixture was further treated with 50 μL of standard Taq buffer containing 400 nM pre-incubated hairpin probe and primer. After 1 h duration, the fluorescence spectrum of the resulting mixture was collected with the excitation wavelength at 460 nm.

Results and Discussion

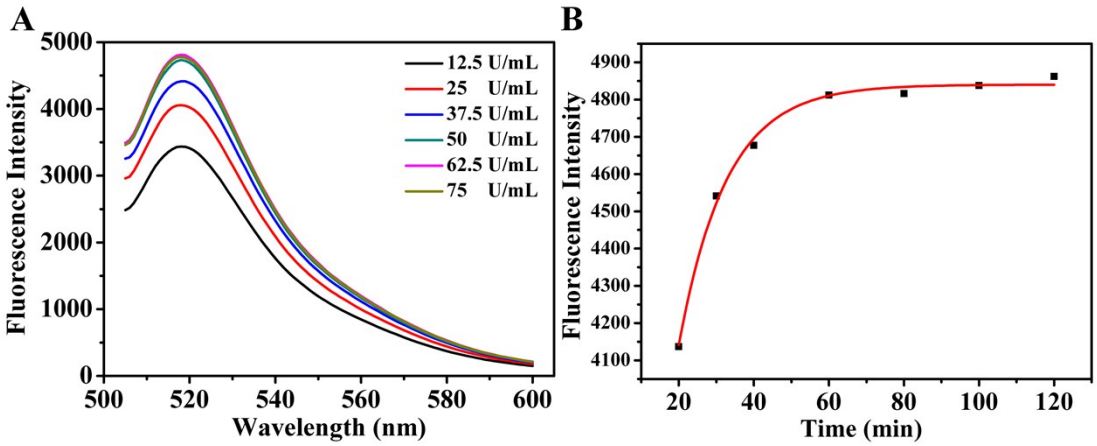

Fig. S1 (A) Effects of Taq DNA polymerase concentration on the fluorescence response of enzyme-catalyzed primer-extension process. (B) Effects of extension duration on the fluorescence response of enzyme-catalyzed primer-extension process performed with 50 U/mL Taq DNA polymerase.

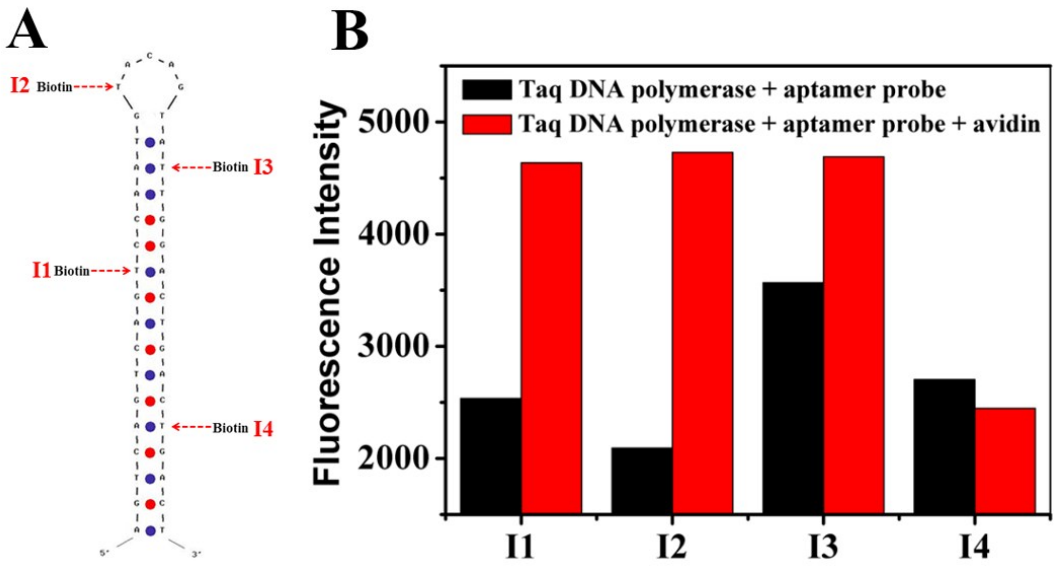

Fig. S2 (A) Structures of aptamer probes: location of biotin moiety on Taq aptamer. (B) Fluorescence peak intensities obtained upon analyzing 0 and 100 μg/mL avidin performed with varied kinds of aptamer probes.

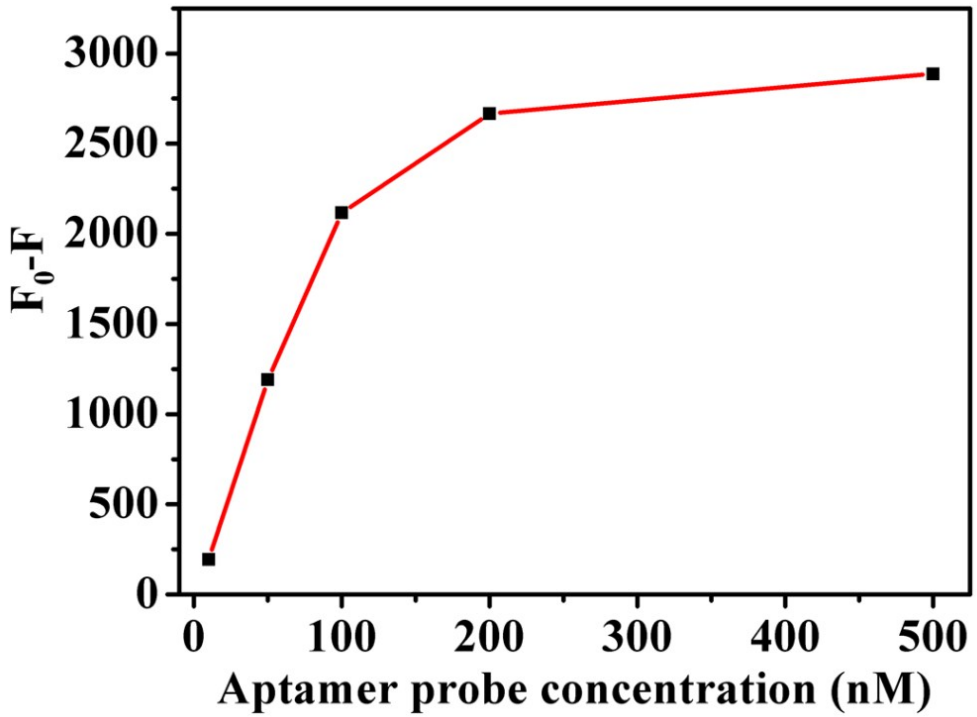

Fig. S3 Effects of aptamer probe concentration on the fluorescence response decrease (F_0-F) of detection system containing 0 μg/mL target avidin, where F and F_0 are the fluorescence peak intensities of detection system with and without aptamer probe.

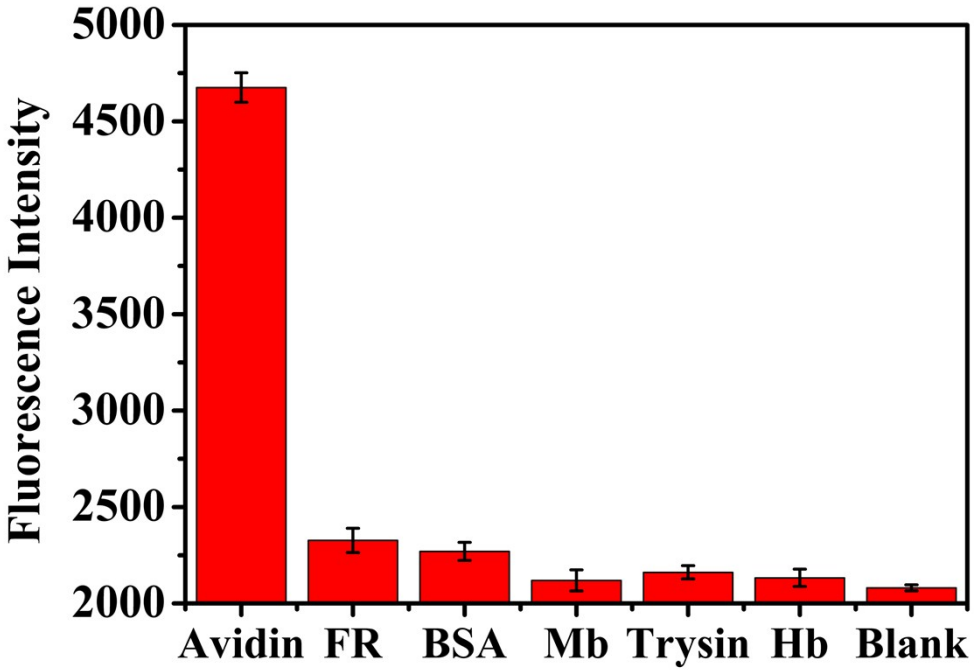

Fig. S4 Fluorescence peak intensities obtained upon analyzing different proteins.

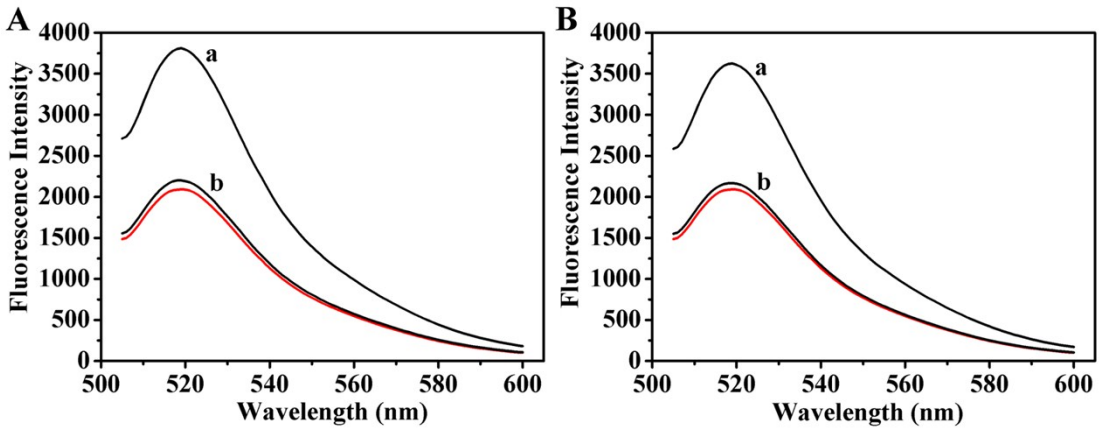

Fig. S5 (A) Fluorescence spectra for detecting biotin receptor on the surface of HeLa cells (a) with or (b) without Taq DNA polymerase and aptamer probe. (B) Fluorescence spectra for detecting biotin receptor on the surface of MCF-7 cells (a) with or (b) without Taq DNA polymerase and aptamer probe. The red line is the fluorescence response for blank control.

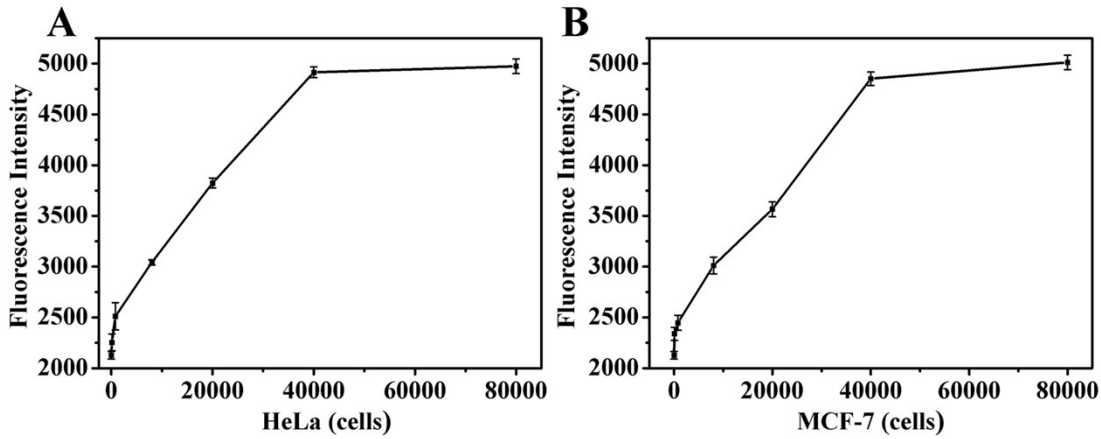

Fig. S6 The resulting calibration curve for the detection of biotin receptor on the surface of (A) HeLa cells and (B) MCF-7 cells of varying amounts.