Electronic Supporting Information (ESI)

A Signal-on Split Aptasensor for Highly Sensitive and Specific Detection of Tumor Cells Based on FRET

Baoyin Yuan, ‡ Yu Zhou, ‡ Qiuping Guo, Kemin Wang,* Xiaohai Yang, Jun Wan, Yuyu Tan, Zhixiang Huang, Qin Xie, Xiayu Zhao*

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Biology, College of Chemistry and Chemical Engineering, Hunan University, Key Laboratory for Bio-Nanotechnology and Molecule Engineering of Hunan Province, Changsha, China 410082

* To whom correspondence should be addressed:

E-mail: kmwang@hnu.edu.cn.

E-mail: guoqping@126.com

Tel/Fax: 86-731-88821566.

EXPERIMENTAL SECTION

Materials and apparatus. All oligonucleotides were synthesized and purified by Sangon Biotechnology Company, Ltd. (Shanghai, China). Sequences of the oligonucleotides were listed in Table S1. The experimental containers were sterilized by vertical high-pressure steam sterilizer before use. Water was treated by the Milli-Q ultrapure water system (18.2 MΩ•cm, Millipore System Inc.). 0.01M pH 7.4 PBS was purchased from Bei-jing Dingguo Changsheng Biotechnology CO. LTD. (Beijing, China). The binding buffer contained 5 mM MgCl2, 4.5 g/L glucose, 1mg/mL BSA and 0.1 mg/mL yeast tRNA in PBS. Other chemicals were of analytical grade and used without further purification.

SMMC-7721, Bel-7404, QBC-939, L-02 cells used in the experiment were purchased from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), A549 cells were obtained from American Type Culture Collection, and MCF-7 cells were offered by our laboratory. The Cells were cultured in RPMI 1640 with 10% fetal bovine serum at 37 °C in a humidified incubator containing 5 wt %/vol CO2. Both subculture and pretreatment of cells were completed in the clean bench. The cell number was counted for three times by a hemocytometer, then calculated the average value.

Flow Cytometry Assays. Generally, a mixture of 100 nM probes were incubated with 100,000 cells in 200 μL binding buffer on ice for half an hour and then immediately analyzed with flow cytometer (FACScalibur, BD Biosciences) by counting 10,000 events. Bel-7404 cells were used as control. Samples with cell numbers ranging from 0 to

200,000 were used for the quantitative assay in binding buffer. Samples with cell numbers ranging from 0 to 20,000 were used for the quantitative assay in 20% human serum. For the mixed cells assay, samples were prepared by mixing target SMMC-7721 cells and non-target Bel-7404 cells in binding buffer in ascending ratios with a fixed total of 70,000 cells. After incubation, the samples were analyzed directly by using flow cytometry without any washing and separation steps.

Confocal imaging. To further confirm the performance of the split aptasensor, the cells were imaged by a laser scanning confocal microscope (Olympus, Japan). SMMC-7721 or Bel-7404 cells were cultivated overnight in culture dishes, and washed with PBS for three times. The cells were then incubated with the split aptamers (100 nM) in binding buffer on ice for 30 min. After incubation, the cells were imaged directly by using laser scanning confocal microscope without any washing and separation steps. The FRET fluorescence signal was collected by a 40× objective (fluorescence channel: EX 488 nm, EM 660 nm long-pass).

Table S1. All of the oligonucleotides used in this work.

Probe	**Sequence** (5'→3')
Split A	CGTCAGGTTGAGCTGAAGATCGTACCGTGAAGTCCGT-Cy3
Split B	Cy5-ACGGACTACCTGGCG
ZY11	TTGACTTGCCACTGACTACCTGGCGCATTGACGTCAGGTTGAGCTGAAG ATCGTACCGTGAAGTCAGTCGGTCGTCATC
a	FAM-ATTGAGCTGAAGATCGTACCGTGAATC
b	FAM-TGACGTCAGGTTGAGCTGAAGATCGTACCGTGAAACCTGGCGCAT
c	FAM-TTGACTTGCCACTGACTATTGAGCTGAAGATCGTACCGTGAAGTC AGTCGGTCGTCATC
d	FAM-TGACTACCTGCATTGACAGGTTGAGCTGAAGATCGTACCGTGAAG TA
e	FAM-TTGACTTGCCACTGACTTGAGCTGAAGATCGTACCGTGAACCTGG CGCATTGACGTCAGGTGTCAGTCGGTCGTCATC

Table S2. List of detection limit of some aptamer-based methods in cell detection.

Target	Method	Limit of detection (cells per ml)	Ref.
CCRF-CEM	Fluorescence	590	1b
CCRF-CEM	Fluorescence	750	2a
SMMC-7721	Fluorescence	1000	6
CCRF-CEM	Colorimetric	200	2b
MCF-7	Electrochemical and enzyme amplification	12	2c
MCF-7	Raman signal	Single cell	2d
CCRF-CEM	Quartz crystal microbalance	8000	2e
SMMC-7721	Fluorescence	100	This assay

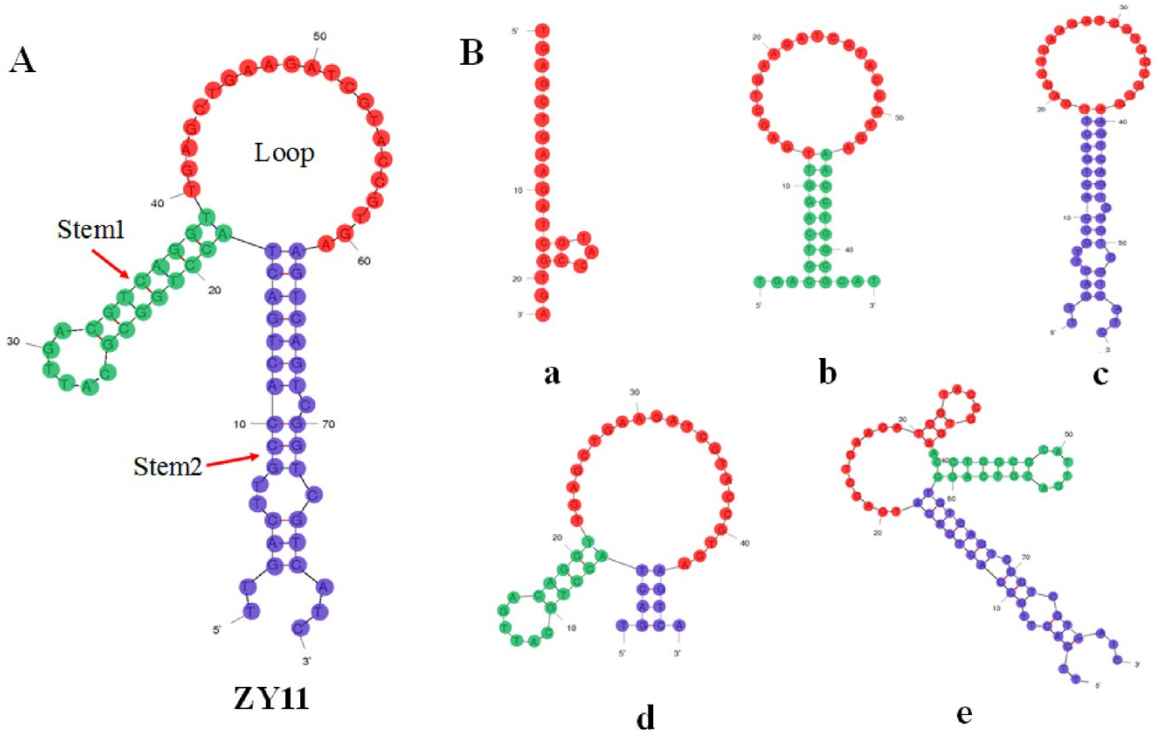

Figure S1. Optimization of the aptamer ZY11 sequence. (A) The structure of the aptamer ZY11 simulated by the Mfold software (http://mfold.rna.albany.edu/?q=mfold/dna-folding-form), consisting of Stem1 (green region), Stem2 (purple region) and Loop (red region). (B) The structures of a, b, c, d, e probes simulated by the Mfold after cut by five optimization strategies (a, Loop reserved; b, Loop and Stem 1 reserved; c, Loop and Stem 2 reserved; d, Loop reserved and two stems shortened; e, Loop reserved and two stems interchanged) respectively.

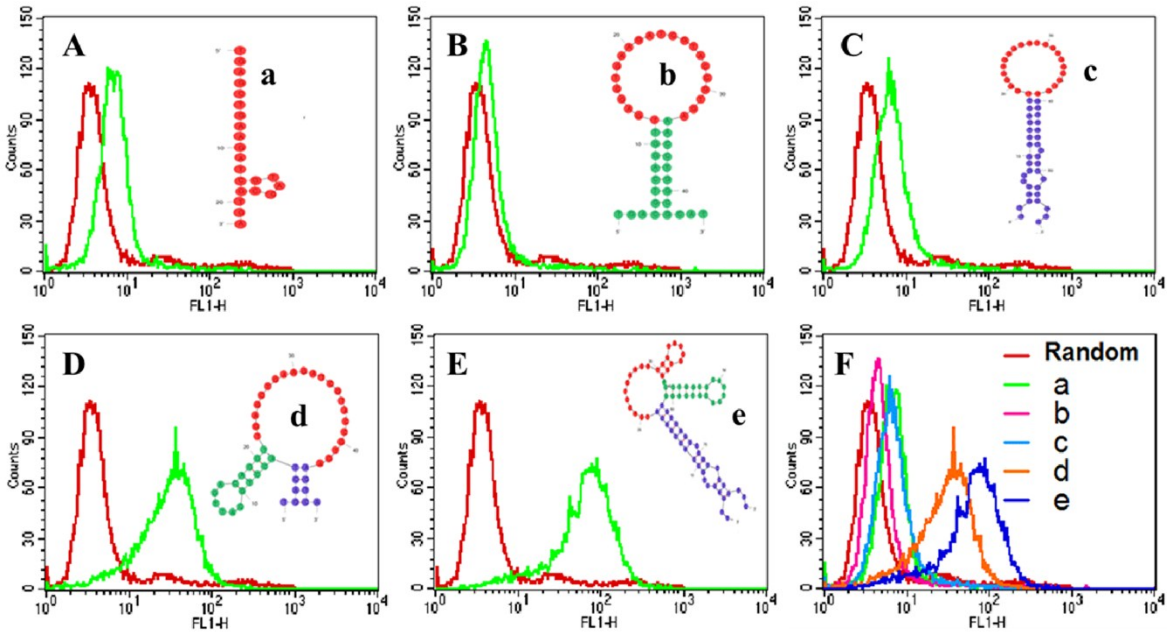

Figure S2. Investigation of binding ability of the cut probes (a, b, c, d, e) to target SMMC-7721 cells by using flow cytometry. In figure A, B, C, D, E, red line represent SMMC-7721 cells incubated with random probe, green line represent SMMC-7721cells incubated with the cut probe a, b, c, d, e respectively. Figure F is the overlay of figure A, B, C, D, E. The results showed that probe a, b, c can not bind to target cells, but probe d, e can bind to target cells well. Probes (a, b, c, d, e) were labeled with FAM fluorescent group. The fluorescence signal was determined by counting 10000 events in channel 1 (EX 488 nm, EM 515-545 nm) for FAM.

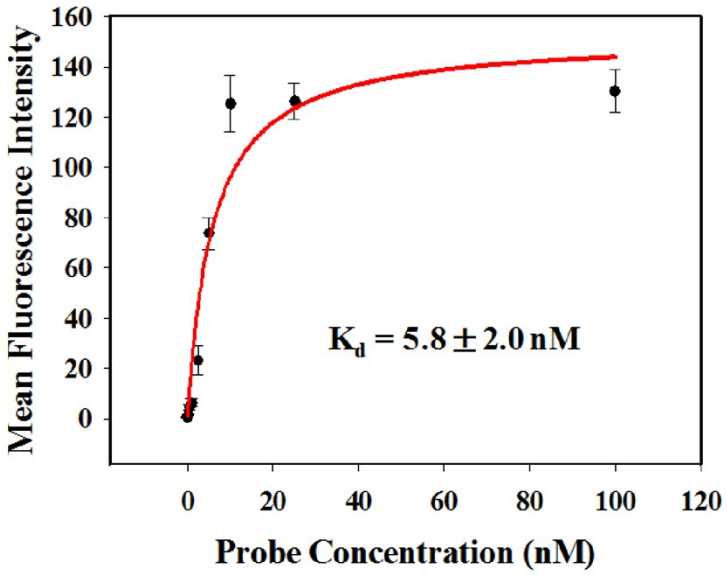

Figure S3. The dissociation constant of the split aptamers for target SMMC-7721 cells. Flow cytometry was used to analyze the binding affinity of the split aptamers to SMMC-7721 cells. By fitting the dependence of mean fluorescence intensity of cell/aptamer complex on probe concentration with the equation $Y=Bmax\ X / (K_d + X)$, the dissociation constant (K_d) of the aptamer-cell binding was determined. The mean fluorescence intensity was determined by counting 10000 events in channel 3 (EX 488 nm, EM 660 nm long-pass) for FRET signal using flow cytometry. All the error bars represent standard deviations of three repeated measurements.

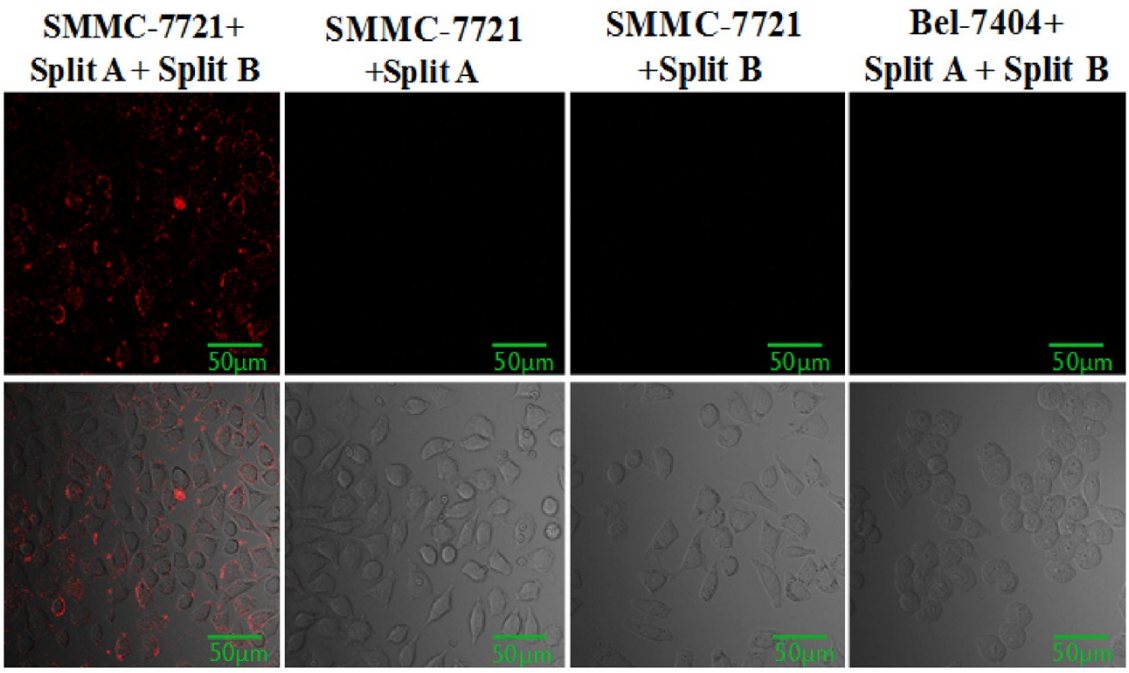

Figure S4. Laser scanning confocal microscope images of SMMC-7721 cells or Bel-7404 cells incubated with different probes. The upper are fluorescence images, the lower are the overlays of the fluorescence channel and the bright-field channel. The FRET fluorescence signal was collected by a 40× objective (fluorescence channel: EX 488 nm, EM 660 nm long-pass). Scale bar is 50 μm.

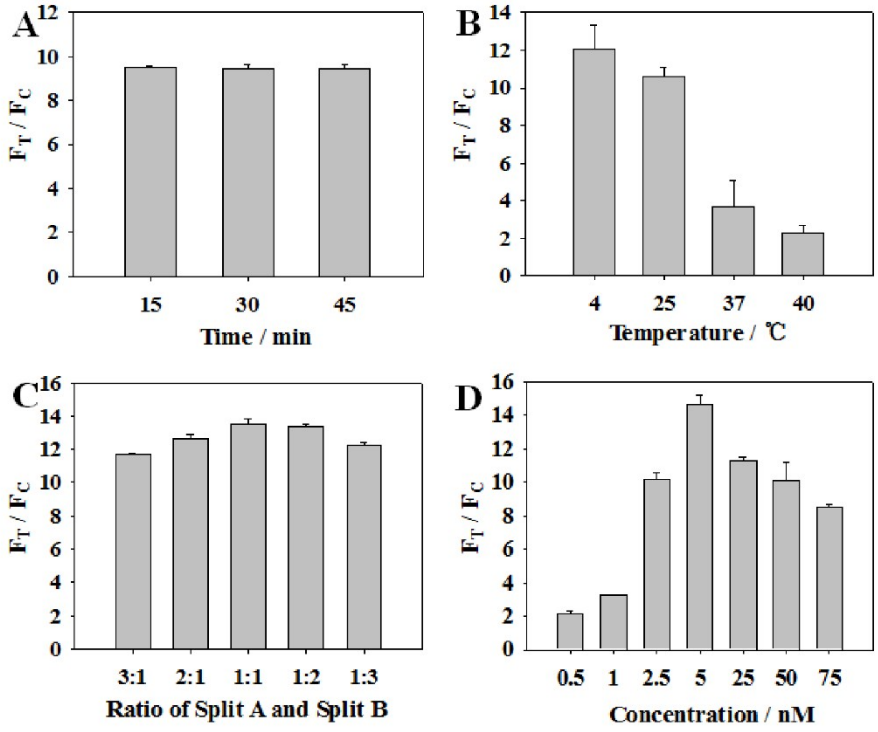

Figure S5. The effect of different experimental conditions for detection of target cells using the split aptasensor. (A) Different incubation time, incubation temperature: 4 ^{o}C, probe ratio: 1:1, 5 nM split probes. From the results, we found that incubation time do not significantly affect the probes binding to targets because of rapid assembly of the split aptamers. Taking account of time-saving, we chose 15 min as the best incubation time. (B) Different incubation temperature, incubation time: 15min, probe ratio: 1:1, 5 nM split probes. The results indicated that 4 ^{o}C was the best incubation temperature because the aptamer was screened at 4 ^{o}C. (C) Different probe ratio, incubation time: 15 min, incubation temperature: 4 ^{o}C, 5 nM split probes. The best ratio of 1:1 probably attributed to the split aptamer consisting of a SplitA and a SplitB. (D) Different probe concentration, incubation time: 15 min, incubation temperature: 4 ^{o}C, probe ratio: 1:1. The best concentration of 5nM probably attributed to the high affinity of the split

aptamers (K_d = 5.8 nM). The error bars indicated the standard deviations of three experiments.

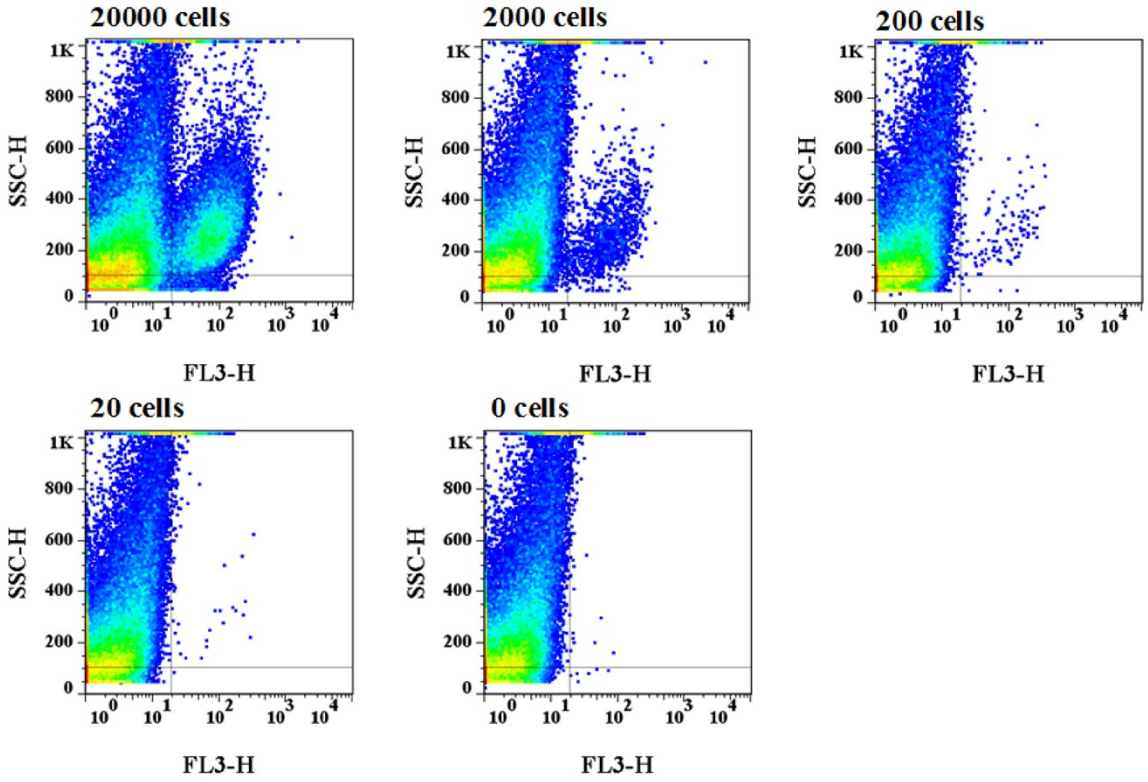

Figure S6. Flow cytometry assays of SMMC-7721 cells with cell number ranging from 0 to 20,000 in 200 μL binding buffer containing 20% human serum by the split aptasensor. Target cells appeared in the upright (UR) window, and serum fragments appeared in the left window. The result showed that the labeled events appearing in the UR window decreased corresponding to the decreased cell number in the sample.

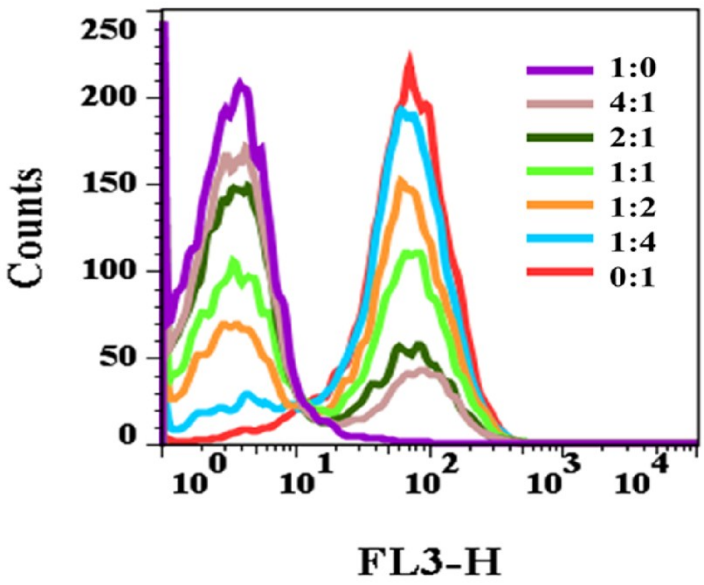

Figure S7. Flow cytometry assay of cell mixtures (SMMC-7721 and Bel-7404), using the split aptasensor. Artificial complex samples were made by mixing Bel-7404 cells and SMMC-7721 cells with different concentration ratios. In each sample, the total number of cells was 70,000 and the final volume was 200 μL. The ratio of Bel-7404 to SMMC-7721 is as follows: purple line, 1:0; brown line, 4:1; deep green line, 2:1; brilliant green line, 1:1; orange line, 1:2; blue line, 1:4; red line, 0:1, respectively. The FRET signal was collected by counting 10000 events in channel 3 (EX 488 nm, EM 660 nm long-pass) using flow cytometry.