Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2021

Supporting Information For

Programming DNA Cascade Circuits on Live Cell Membranes for Accurate Cancer Cell Recognition and Gene Silencing

Xiangnan Wang, Lan Liu, Han Wu, Zhenkun Wu,* Li-Juan Tang and Jian-Hui Jiang *

State Key Laboratory of Chemo/Bio-Sensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China

E-mail address: tomwu@hnu.edu.cn
E-mail address: jianhuijiang@hnu.edu.cn

Chemicals and Materials

All oligonucleotides used in this study were purchased from Sangon Biotech Co., Ltd (Shanghai, China). The sequences were shown in Table S1. Cell lines CCRF-CEM (CCL-119), Ramos (CRL-1596) and HeLa lines (CCL-2) were obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Washing buffer was Dulbecco's PBS supplemented with 4.5 g/L glucose and 5 mM $MgCl_2$. Binding buffer was washing buffer replenished with 1 mg⁄mL BSA, 0.1 mg⁄mL yeast tRNA and 10% fetal bovine serum.

RPMI 1640 cell culture medium, fetal bovine serum (FBS, heat inactivated) and penicillin-streptomycin were purchased from Thermo Scientific HyClone (MA, USA). Yeast tRNA, bovine serum albumin (BSA) and Dulbecco's phosphate buffered saline were purchased from Sigma Aldrich (St. Louis, Mo, USA). LysoTracker Green DND-26 was purchased from Invitrogen (Carlsbad, CA). All other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solutions were prepared using ultrapure water that was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) with an electrical resistance >18.25 MΩ.

Gel electrophoresis analysis

All the samples were incubated in 30 μL 1× PBS buffer at 37 °C for 1 h. The concentrations of DNA probes are 1 μM. Then 2 μL 6× loading buffer was added into 10 μL of each sample for gel electrophoresis experiments. Gel electrophoresis analysis was carried out on 3% (w/w) agarose gels in 0.5× TBE buffer at a constant potential of 110 V for 45 min. Finally, the gels were stained with 0.5 μg/mL GoldView and 0.5 μg/mL ethidium bromide. All gels were imaged and analyzed using Tanon 4200SF gel imaging system (Tanon Science & Technology Co., Ltd., China).

Fluorescence measurements in buffer solution

100 nM probe A, 100 nM TAMRA-BHQ2 labelled probe B and 100 nM probe C were incubated with Initiator I in 1× PBS buffer at 37 °C for 2 h. The fluorescence of TAMRA were measured at room temperature in a quartz cuvette on a FS5 spectrometer (FS5, England). The excitation wavelength was 550 nm. The excitation and emission slits were 5.0 nm under a PMT voltage of 950 V.

100 nM probe A, 100 nM TAMRA-BHQ2 labelled probe B and 100 nM probe C were mixed with 100 nM Initiator I in 1× PBS buffer, then directly subjected to real-time fluorescence measurements at 37°C. The dynamic fluorescence response of DNA cascade reaction was recorded using Tecan Finite M1000 (Tecan, Switzerland).

The structure of sgc4f-I was optimized as following procedures: 5 μM aptamer sgc4f and 5 μM initiator I were annealed at 95°C for 5 min, then the mixture was slowly cooled to room temperature over 3 h for further use. Then, 100 nM sgc4f-I, 100 nM probe A, 100 nM TAMRA/BHQ2 labelled probe B and 100 nM probe C in 1× PBS buffer were incubated with CEM or HeLa cells (1x 10^5) at 37 °C for 2 h. Finally, the fluorescence of TAMRA were measured at room temperature in a quartz

cuvette on a FS5 spectrometer (FS5, England). The excitation wavelength was set at 550 nm. The excitation and emission slits were all set at 5.0 nm under a PMT voltage of 950 V.

To explore the stability of reconstructed DNA nanodevices, 1 μM I_{16}, 1 μM probe A, 1 μM BHQ2 labelled probe B and 1 μM TAMRA labelled probe C were pre incubated for 1h in 1× PBS buffer，and then the DNA nanodevices were incubated in cell lysate which were collected by thermal shock. The dynamic fluorescence was recorded using Tecan Finite M1000 (Tecan, Switzerland).

Cell culture and confocal Imaging

Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C in an incubator containing 5% CO_2.

CCRF-CEM cells and Ramos cells were centrifuged at 1000 rpm for 3 min to wash twice with the washing buffer, then were re-dispersed in the binding buffer. HeLa cells were washed twice with the washing buffer, and then were cultured in the binding buffer. 300 nM sgc4f-I_{16}, 300 nM probe A, 300 nM TAMRA/BHQ2 labelled probe B and 300 nM probe C were incubated with the cells at 37 °C for 2 h. The cells were then subjected to confocal microscopic for observation. For the single-aptamer-based DNA circuits, 300 nM probe SR-A, 300 nM TAMRA/BHQ2 labelled probe SR-B and 300 nM probe SR-C were incubated with the cells at 37 °C for 2 h. The cells were then subjected to confocal microscopic for observation. All fluorescence images were acquired using Nikon TI-E+A1 SI confocal laser scanning microscope (Japan).

For flow cytometry assay, 300 nM sgc4f-I_{16}, 300 nM probe A, 300 nM FITC/BHQ1 labelled probe B and 300 nM probe C were incubated with the cells at 37 °C for 2 h. Cells were collected and finally re-dispersed in 1x PBS buffer for flow cytometry analysis using FACScan cytometer (BD Biosciences).

Cellular uptake mechanism

CEM cells were pre-treated in the culture medium containing 0.1% NaN_3 for 30 min. Then, cells were incubated with 300 nM sgc4f-I_{16}, 300 nM probe A, 300 nM TAMRA/BHQ2 labelled probe B, and 300 nM probe C for 2 h. The cells were then subjected to confocal microscopic for observation. All fluorescence images were acquired using Nikon TI-E+A1 SI confocal laser scanning microscope (Japan).

To explore the internalization and localization of DNA circuits, 300 nM sgc4f-I_{16}, 300 nM probe A, 300 nM TAMRA/BHQ2 labelled probe B (Cy3 labelled probe B), and 300 nM probe C (Cy5 labelled probe C) were incubated with CEM cells for 2 h. Cells were followed by stained with 50 nM LysoTracker Green for 30min. The cells were then subjected to confocal microscopic for observation. All fluorescence images were acquired using Nikon TI-E+A1 SI confocal laser scanning microscope (Japan).

To explore the persistence of DNA nanodevices in live CEM cells. 300 nM sgc4f-I_{16}, 300 nM probe A, 300 nM Cy3 labelled probe B, and 300 nM Cy5 labelled probe C were incubated with CEM cells for 2 h, and then washed with PBS buffer. The cells were then subjected to confocal microscopic to chase the FRET signal at intervals.

Cell viability assay

CEM cells were seeded on a 96-well microplate with 100 μL RPMI 1640 medium supplemented with 10% fetal bovine serum at 37 °C for 24 h. Then, CEM cells were centrifuged at 1000 rpm for 3 min to wash twice with the washing buffer, followed by incubated with different concentrations of DNA probes in binding buffer for 2 h. Thereafter CEM cells were centrifuged at 1000 rpm for 3 min to remove the unreacted DNA probes and continued to culture for an additional 24 h in medium. After cell medium removed, 10 μL of the MTS stock solution diluted with 100 μL fresh RPMI 1640 medium was added per well and incubated for 1 h, then directly subjected to measure the absorbances at 490 nm of each well.

DNAzyme mediated VEGF mRNA cleavage in solution

20 μM DNA enzyme strand (Dz_{VEGF}) and 18 μM substrate strand (S_{VEGF}) were annealed at 95°C for 5 min, then the mixture was slowly cooled to room temperature over 3 h to form the DNAzyme. 4 μM DNAzyme were incubated with different concentration of Mg^{2+} (0, 2 mM, 5 mM, 10 mM, 12 mM), and then were analyzed by gel electrophoresis.

100 nM DNAzyme-TAMRA/BHQ2 were incubated with different concentration of Mg^{2+} (0, 2 mM, 5 mM, 10 mM, 12 mM), the fluorescence signals were recorded using Tecan Finite M1000 (Tecan, Switzerland).

RT-PCR quantification of mRNA expression

For RT-PCR analysis, cells ($1x10^6$) were seeded in a 60-mm dish and incubated for 24 h. Then cells were incubated with DNA circuits in binding buffer. After 4 h incubation, the culture medium was replaced by another 2 mL fresh culture medium with 5 mM Mg^{2+}. After 18 h incubation, cells were washed with 1× PBS. Total cellular RNA was extracted from cells using the RNeasy Mini Kit (Qiagen, USA) according to the protocol. The cDNA samples were prepared with a Revert Aid Premium Reverse Transcriptase kit (Thermo Scientific) according to the indicated protocol. The cDNA samples were diluted 10 times before use. RT-PCR analysis of cDNA was performed with SybrGreen Fast qPCR Master Mix (ABI, USA) on an ABI Stepone Plus qPCR instrument. These primer sequence of housekeeping gene and target gene listed below.

β-actin forward, 5'-TAGTTGCGTTACACCCTTTCTTG-3';
β-actin reverse, 5'-TCACCTTCACCGTTCCAGTTT-3'.
VEGF forward, 5’-CGAGACCCTGGTGGACATCTT-3’;
VEGF reverse, 5’- TGGCCTTGGTGAGGTTTGATC -3’.

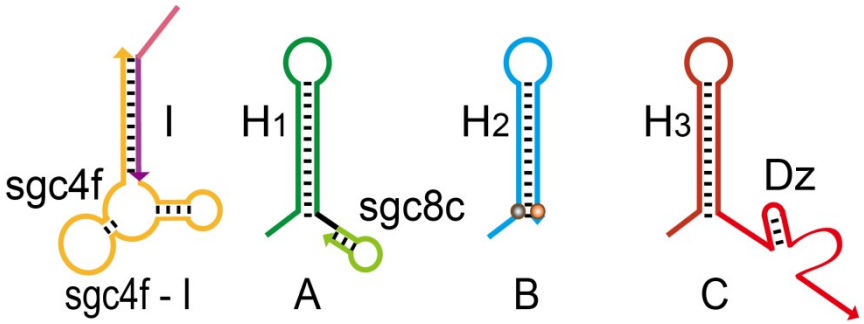
Western blotting analysis

For western blotting analysis, cells ($1x10^6$) were seeded in a 60-mm dish and incubated for 24 h. Then cells were incubated with the DNA circuits in binding buffer. After 4 h incubation, the culture medium was replaced by another 2 mL fresh culture medium with 5 mM Mg^{2+}. After 36 h incubation, cells were washed with 1× PBS.

Cells were lysed in 60 μL of lysis buffer (50 mM HEPES, pH 7.6, 250 mM NaCl, 0.1% NP-40, 5 mM EDTA, 1 mM PMSF, 10 mM N-ethylmaleimide, and 2 μg each of aprotinin, bestatin, and leupeptin/mL) for 20 min. The lysates were clarified by microcentrifugation for 20 min. Total cellular proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. Protein detection was performed using infrared fluorescent-conjugated secondary antibodies on a ChemiDoc XRS+ with image Lab software (Bio-RAD).

Table S1. Sequences of synthesized oligonucleotides for construction dual-aptamer-based logic cascade circuits.

Name	Sequences (5'-3')
Sgc4f	ATC ACT TAT AAC GAG TGC GGA TGC AAA CGC CAG ACA GGG GGA CAG GAG ATA AGT GA
I_{15}	GTC CAC CTC TCA CTT ATC TCC TGT
I_{16}	GTC CAC CTC TCA CTT ATC TCC TGT C
I_{17}	GTC CAC CTC TCA CTT ATC TCC TGT CC
I_{18}	GTC CAC CTC TCA CTT ATC TCC TGT CCC
A	ACA GGA GAT AAG TGA GAG GTG GAC GCT ATC CTA CCA GTC CAC CTC TCA CTT ATC AAA AAT CTA ACT GCT GCG CCG CCG GGA AAA TAC TGT ACG GTT AGA
B	TGA GAG GTG GAC TGG TAG GAT AGC CTT ATC TCC TGT GCT ATC CTA CCA GTC CAC
B-TAMRA/BHQ2	TAMRA-TGA GAG GTG GAC TGG TAG GAT AGC CTT ATC TCC TGT GCT ATC CTA CCA GTC CAC-BHQ2
B-FITC/BHQ1	FITC-TGA GAG GTG GAC TGG TAG GAT AGC CTT ATC TCC TGT GCT ATC CTA CCA GTC CAC-BHQ1
B-Cy3	TGA GAG GTG GAC TGG TAG GAT AGC CTT ATC TCC TGT GCT ATC CTA CCA G/T-Cy3/C CAC
B-BHQ2	TGA GAG GTG GAC TGG TAG GAT AGC CTT ATC TCC TGT GCT ATC CTA CCA G/T-BHQ2/C CAC
C	TGGTAG GAT AGC ACA GGA GAT AAG GTC CAC CTC TCA CTT ATC TCCTGT GCT ATC TCT TTG GTT GTC AGC GAC TCG AGG CAT TCA C
C-Cy5	Cy5-TGGTAG GAT AGC ACA GGA GAT AAG GTC CAC CTC TCA CTT ATC TCCTGT GCT ATC TCT TTG GTT GTC AGC GAC TCG AGG CAT TCA C
C-TAMRA	TAMRA-TGGTAG GAT AGC ACA GGA GAT AAG GTC CAC CTC TCA CTT ATC TCCTGT GCT ATC TCT TTG GTT GTC AGC GAC TCG AGG CAT TCA C
$Sgc4f_{control}$	TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTG GGA CAG GAG ATA AGT GA
H1	ACA GGA GAT AAG TGA GAG GTG GAC GCT ATC CTA CCA GTC CAC CTC TCA CTT ATC
H2	TGA GAG GTG GAC TGG TAG GAT AGC CTT ATC TCC TGT GCT ATC CTA CCA GTC CAC
H3	TGG TAG GAT AGC ACA GGA GAT AAG GTC CAC CTC TCA CTT ATC TCC TGT GCT ATC
Dz_{VEGF}	TCT TTG GTT GTC AGC GAC TCG AGG CAT TCA C
S_{VEGF}	GUG AAU GCA GAC CAA AGA
S_{VEGF}-TAMRA/BHQ2	TAMRA-GUG AAU GCA GAC CAA AGA-BHQ2



Scheme S1 The detailed structure of four kinds of probes: sgc4f-I, A, B and C. Sgc4f-I probes contained two functional domains: the sgc4f aptamer for receptor targeting and initiator I that can trigger the DNA cascade reaction. Probes A were composed of the hairpin structure H1 and sgc8c aptamer for PTK7 recognition and specific delivery. Probe C also included two functional domains: the hairpin H3 and the DNAzyme domain that can cleave the target intracellular mRNA for gene regulation. Moreover, H1, H2 and H3 were designed as the reactants for DNA cascade reaction. To generate fluorescence signals, H2 were modified with TAMRA fluorophores and BHQ2 quenchers.

Table S2. Sequences of synthesized oligonucleotides for construction single-aptamer-based DNA circuits.

Name	Sequences (5'-3')
SR-A	GTT AGA TTG CAA GCC GAT CTC AAC TAC GCT TGC AAT CTA ACT GCT GCG CCG CCG GGA AAA TAC TGT ACG GTT AGA
SR-B	GCA AGC GTA GTT GAG ATC GAT CTA ACC TCA ACC GAT CTC AAC TAC
SR-B-TAMRA/BHQ2	TAMRA-GCA AGC GTA GTT GAG ATC GAT CTA ACC TCA ACC GAT CTC AAC TAC-BHQ2
SR-C	GTA GTT GAG ATC GGT TGA GGT TAG ATG CTT GCA ATC TAA CCT CAA CCG ATC TCA TTG TCA GCG ACT CGT GGG TAC TC

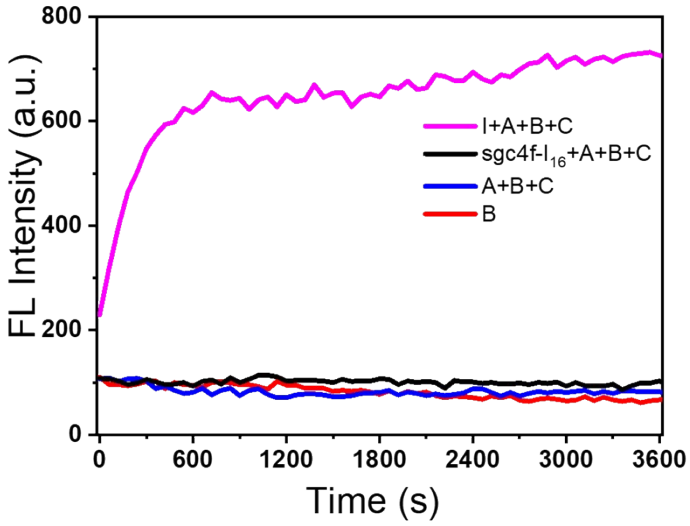


Fig. S1 Real-time fluorescence response of DNA cascade reaction.

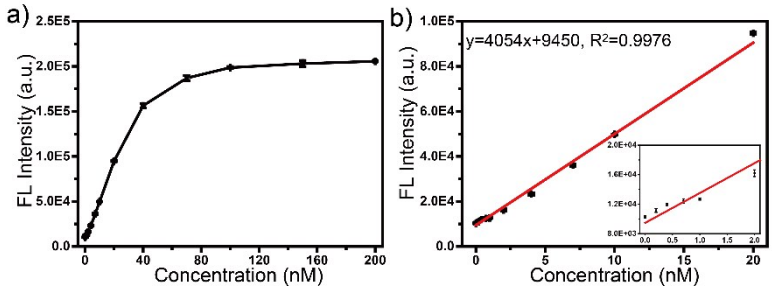


Fig. S2 (a) Plots of fluorescence intensities at 582 nm versus different concentrations of initiator I. (b) The linear relationship of fluorescence intensities at 582 nm versus different concentrations of initiator I. The inset figure shows the linear region from 0 to 2 nM. Error bars indicated SDs across three repetitive assays.

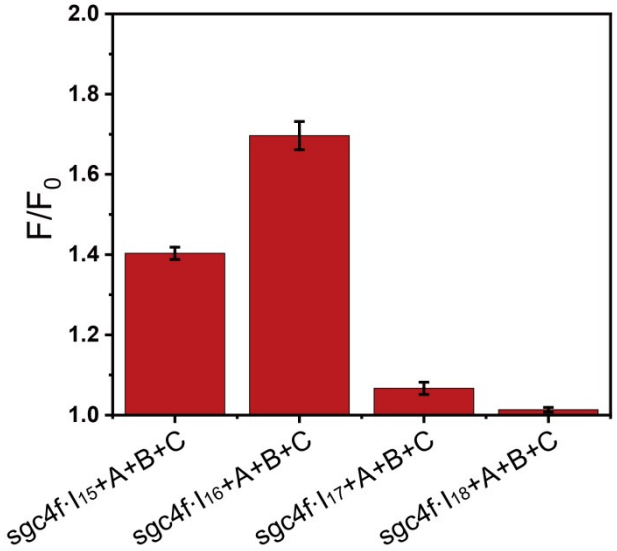


Fig. S3 Structure optimization of sgc4f-I probe. F represent the fluorescence of DNA cascade circuits incubated with CEM cells ($1x10^5$ cells); F_0 represent the fluorescence of DNA cascade circuits incubated with HeLa cells ($1x10^5$ cells). Error bars indicated SDs across three repetitive assays.

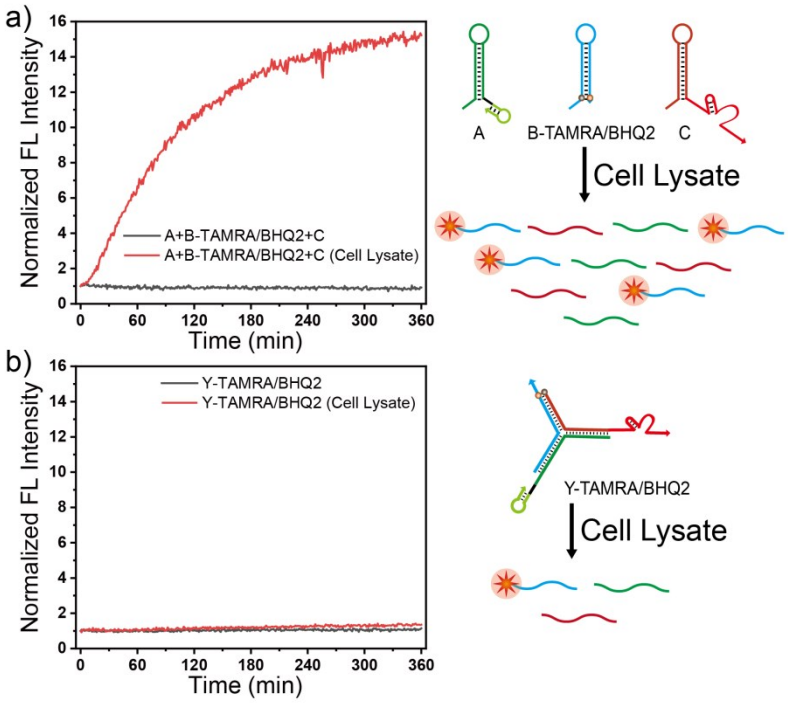


Fig. S4 Stability analysis of 100 nM Y-TAMRA/BHQ2 probe and 100 nM hairpin B-TAMRA/BHQ2 probe in cell lysate.

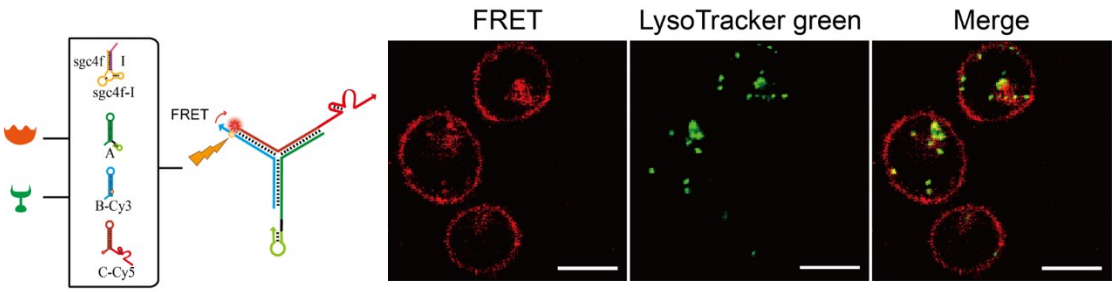


Fig. S5 FRET imaging of CEM cells incubated DNA cascade circuits for 2 h followed by staining with LysoTracker Green. Scale bar: 10 μm.

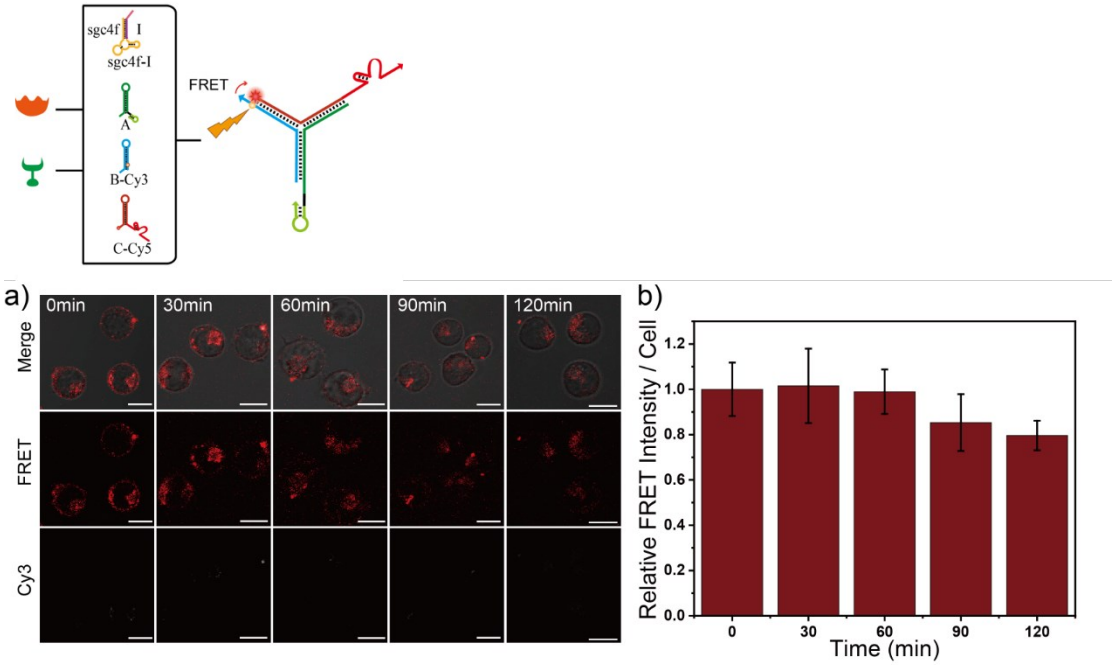


Fig. S6 Persistence of DNA nanodevices in live CEM cells. (a) FRET images of monitoring persistence of the reconstructed DNA nanodevices in live CEM cells. (b) Mean FRET signal intensity of cells from (a). Scale bar: 10 μm.

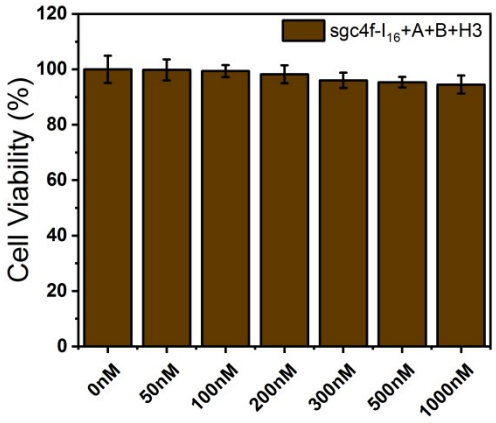


Fig. S7 Cell viability of CEM cells treated with various concentrations of probes.

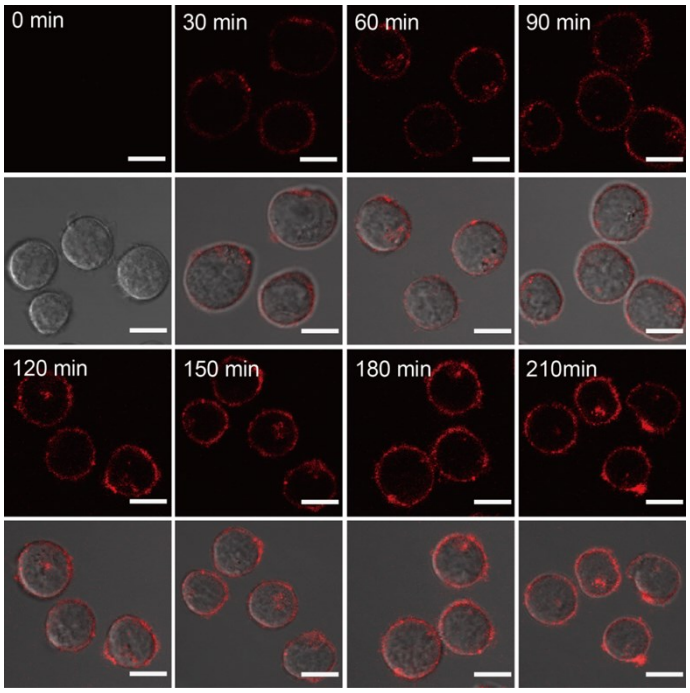


Fig. S8 Time-lapse confocal images of CEM cells incubated with DNA cascade circuits. Scale bar: 10 μm.

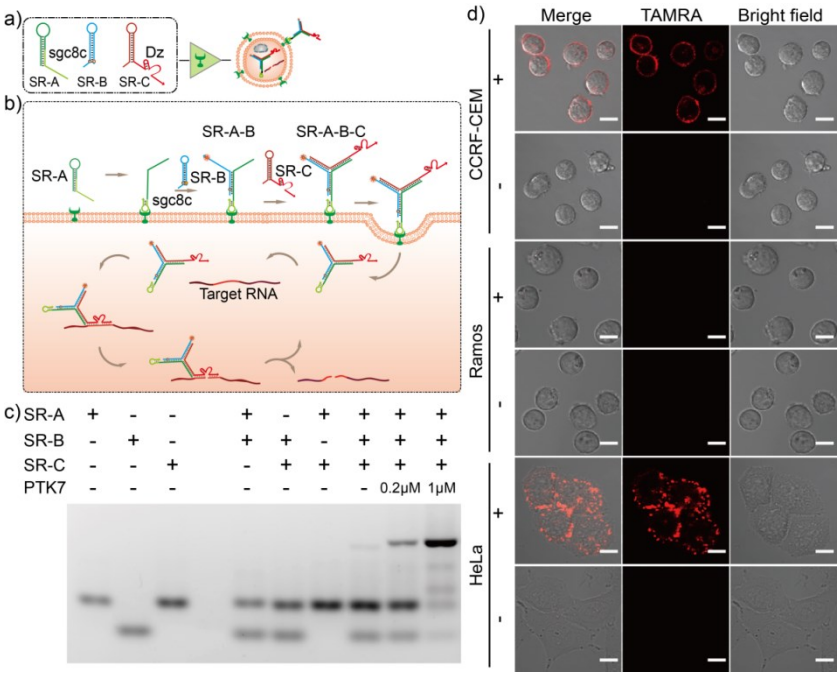


Fig. S9 Single-aptamer-based DNA circuits for cell recognition. (a) and (b) Illustration of single-aptamer mediated DNA circuits for cell identification and gene silencing in living cells. (c) Gel electrophoresis image of single-aptamer-based DNA circuits. (d) Confocal images of CEM, Ramos, HeLa cells after incubation with 300 nM SR-A, 300 nM SR-B-TAMRA/BHQ2 and 300 nM SR-C for 2 h. Scale bar: 10 μm.

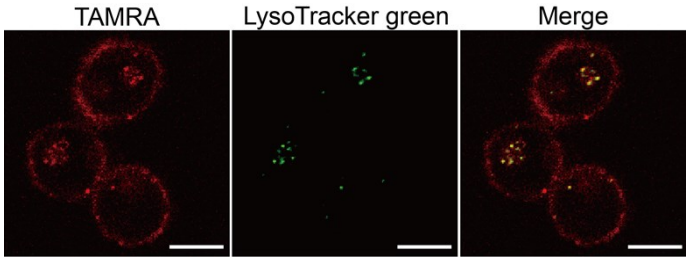


Fig. S10 Confocal images of CEM cells incubated DNA cascade circuits for 2 h followed by staining with LysoTracker Green. Scale bar: 10 μm.

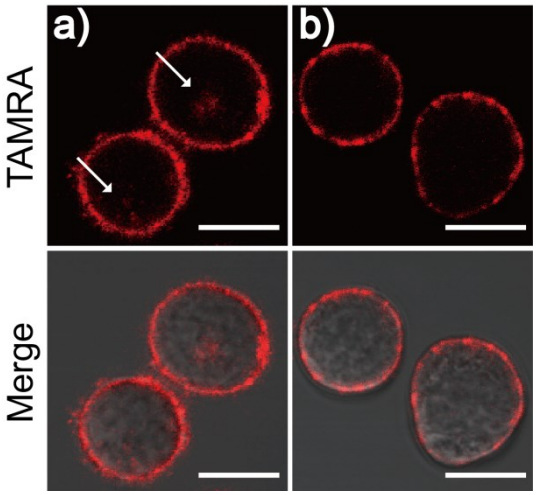


Fig. S11 Confocal images of CEM cells incubated with DNA cascade circuits for 2 h. (a) in the reaction buffer, (b) in the reaction buffer containing 0.1% sodium azide. Scale bar: 10 μm.

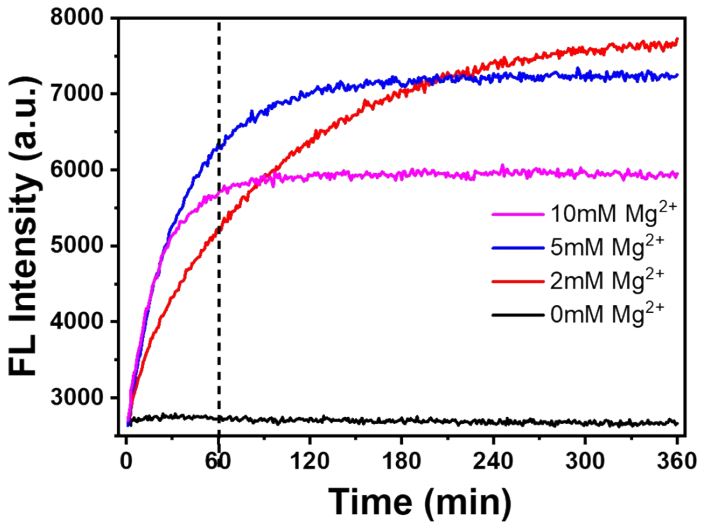


Fig. S12 Real-time fluorescence responses of DNAzyme mediated target strand cleavage with different concentrations of Mg^{2+} in 1x PBS.

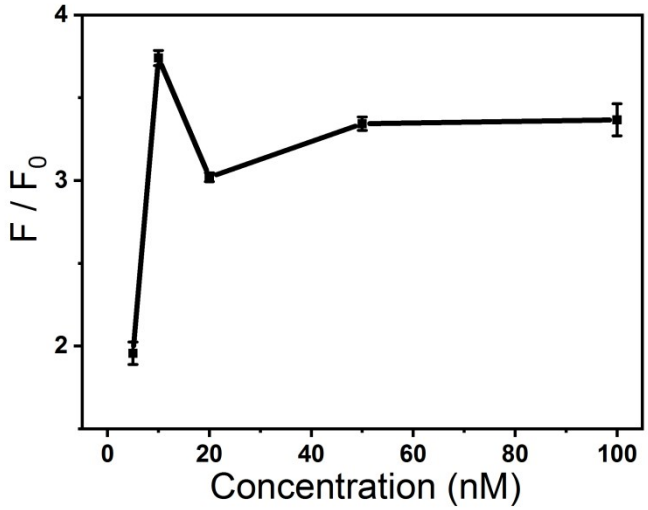


Fig. S13 Fluorescence responses of DNAzyme cleavage to different VEGF mRNA concentrations. The fluorescence was obtained by incubating 100 nM DNAzyme, 5 mM Mg^{2+} and different concentrations of VEGF mRNA mimics (5 nM, 10 nM, 20 nM, 50 nM and 100 nM) in 1x PBS for 1 h. F_0 and F represent the fluorescence signals of the DNAzyme in the absence and in the presence of Mg^{2+}, respectively. Error bars are standard deviations of three repetitive experiments.

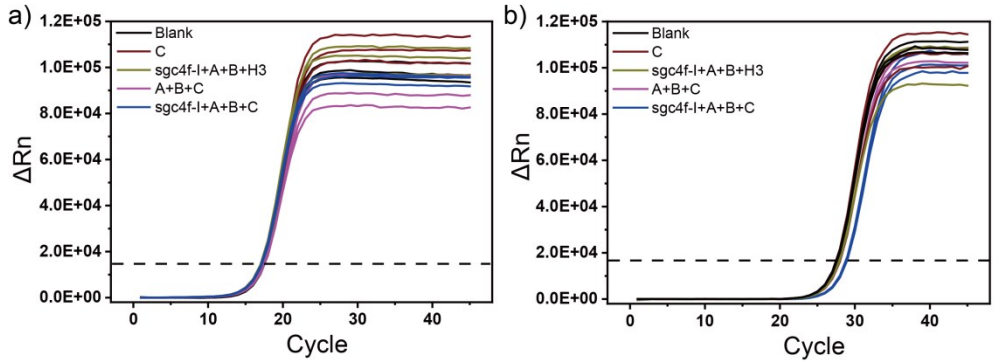


Fig. S14 RT-PCR analysis of VEGF mRNA expression in CEM cells. (a) Real-time fluorescence curves of internal reference gene β-actin. (b) Real-time fluorescence curves of target gene VEGF.