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Electronic Supplementary Information

DNA-Mediated Reversible Capture and Release of Circulating Tumor Cells with Multivalent Dual–Specific Aptamer Coating Network

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Experimental Procedures

Materials. T4 DNA ligase, Phi29 DNA polymerase and dNTPs were purchased from New England Biolabs Co., Ltd. (Beijing, China). Exonuclease I and Exonuclease III were obtained from TaKaRa Biotechnology Co., Ltd. (Dalian, China). Bovine serum albumin was purchased from Sigma-Aldrich (Shanghai, China). DNA-BIND 1×8 Stripwell plates (N-oxysuccinimide modified surface) were obtained from Corning Incorporated (New York, USA). All DNAs were synthetized and purified (HPLC) by Sangon Biotechnology Co., Ltd. (Shanghai, China) and the sequences were shown in Table S1. All other chemical reagents used were analytical grade. Ultrapure water (18.2 MΩ resistivity, Milli-Q, Milli-pore) was used to prepare all aqueous solutions.

Table 1. Oligonucleotide sequences for RCA and cell capture.

Name	Sequence (5′-3′)
Ligation DNA	CCTTCATTCCACCATAG
Linear DNA	Phosphate-GAATGAAGGACAAAAAAAAAAGACCGAAG ATCACTAGTTGACAAAAAAAGAACACATGAAGATTGTAATGAAAAAACAGACTATGGTG
Amino-DNA	CAGACTATGGTGGAATGAAGGACTTTTT-C_7-NH_2
Sgc8c-contain DNA	GAACACATGAAGATTGTAATGTTTTATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA
Sgc4f-contain DNA	GACCGAAGATCACTAGTTGACTTTTATCACTTATAACGAGTGCGGATGCAAACGCCAGACAGGGGGACAG GAGATAAGTGA
rDNA-1	TCTAACCGTACAGTATTTTCCCGGCGGCGCAGGTCCTCTC
rDNA-2	TCACTTATCTCCTGTCCCCCTGTCTGGCGTTTGCATCCGCACTCGTTGCCGTCTC
cDNA-1	GAGAGGACCTGCGCCGCCGGGAAAATACTGTACGGTTAGA
cDNA-2	GAGACGGCAACGAGTGCGGATGCAAACGCCAGACAGGGGGACAGGAGATAAGTGA
Sgc8c unit	GTCCTTCATTCCACCATAGTCTGTTTTATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA
Sgc4f unit	GTCCTTCATTCCACCATAGTCTGTTTTATCACTTATAACGAGTGCGGATGCAAACGCCAGACAGGGGGACA GGAGATAAGTGA
Noncomplementary DNA-1	TGTCTACGGAACTGAAATTGCCATCCGCGCAGGTCCTCTC
Noncomplementary DNA-2	TCTACTTCCTCCACACGTCCACTCGTCCGATTCATCCCTCACACCTTGCCGTCTC

Apparatus. The UV-Vis absorption spectra were measured using a SH-1000 Spectrophotometer (Corona, Japan). The images of gel electrophoresis were captured by a ChemiDOC™ Touch Imaging System (Bio-Rad, USA). The fluorescent images of cells were performed on a inverted fluorescence microscopy (Nikon Ti-s, Japan).

Preparation of Circular DNA and RCA Products. The preparation of circular DNA was performed by mixing phosphorylated linear DNA (3 μM) and ligation DNA (4 μM) in 1×T4 DNA ligation buffer

(50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, pH 7.5) and then annealed at 90 ℃ for 5 min. After slowly cooled to room temperature, T4 DNA ligase (4.8 U/μL) was added into the mixture and the solution was incubated at 16 ℃ for 12 h. The reaction was terminated by heating at 65 ℃ for 10 min. Subsequently, Exonuclease I and Exonuclease III were added to degrade the unreacted DNA and hybridized ligation DNA at 37 ℃ for 2 h, followed by denatured at 85 ℃ for 15 min. To synthetize the RCA products, circular DNA (0.2 μM) was mixed with ligation DNA (0.3 μM) in 1×phi29 DNA reaction buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 4 mM DTT, pH 7.5) containing phi29 DNA polymerase (10 U), BSA (0.02 mg/mL) and dNTPs (0.05 mM). The solution was incubated overnight at 30 ℃ and then quenched at 65 ℃ for 10 min. The circular DNA and RCA products were characterized by electrophoresis analysis. Briefly, 12% denatured PAGE was prepared by using 30% acrylamide/bis-acrylamide gel solution (29:1), and gels were then stained by GelRed and visualized by imaging system.

Fabrication of the MA-RCA Network. Firstly, amino-modified oligonucleotide was diluted with coupling buffer (500 mM Na_2HPO_4-NaH_2PO_4, 1 mM EDTA, pH 8.5) and incubated with the N-oxysuccinimide functionalized plate at a final concentration of 1 μM. After overnight incubation at 4 ℃, the plate was washed with washing buffer (7 mM Tris-HCl, 0.17 mM NaCl, 0.05% Tween-20, pH 8.0) for three times. Then, 3% BSA using as the blocking solution was added and incubated at 37 ℃ for 1 h to block the unreacted areas and washed three times with washing buffer. The RCA product was introduced into the plate and incubated at 37 ℃ for 2 h. Subsequently, the plate was washed three times and further incubated with aptamers (2 μM) in hybridization buffer (20 mM Tris-HCl, pH 7.4, 500 mM NaCl, 20 mM $MgCl_2$) at 37 ℃ for 2 h and then washed as described above. To demonstrate the successful modification of the RCA products on the substrate, 100 μL SYBR Green I solution (1×) was introduced to stain RCA products and incubated for 20 min. After washing to remove excess dye, the fluorescent image was acquired using fluorescence microscopy.

Cell Culture and Cell Capture Assay. CCRF-CEM cell (CCL-119, T lymphoblast) and Ramos cell (CRL-1596, B lymphocyte) (ATCC, Manassas, VA) were cultured in RPMI-1640 medium (Hyclone) that supplemented with 10% fetal bovine serum (FBS, Hyclone) and 100 IU/mL penicillin-streptomycin at 37 ℃ with 5% CO_2. To evaluate the cell capture performance, CCRF-CEM cells were firstly prestained with Calcein AM (2 μM) at 37 ℃ for 20 min. After rinsing with PBS, cells were resuspended in binding buffer (1×DPBS containing 5 mM $MgCl_2$, 4.5 mg/mL glucose and 0.1% BSA).

Then, cells (5×10^4) were added onto the modified substrates and incubated at 37 ℃ in 5% CO_2 for 20 min. Subsequently, substrates were gently washed three times with washing buffer (1×DPBS containing 5 mM $MgCl_2$ and 4.5 mg/mL glucose) to remove the uncaptured cells. The fluorescent images of captured CEM cells were obtained by the inverted fluorescence microscope and the captured cell number was counted to calculate the cell capture efficiency.

Evaluation of the Specificity of Cell Capture. CCRF-CEM cells and Ramos cells were prestained with Calcein AM (2 μM) and Hoechst 33342 (10 μg/mL) at 37 ℃ for 20 min, respectively, and washed with PBS. The prestained CEM cells and Ramos cells were resuspended in binding buffer and then incubated on the substrates at 37 ℃ in 5% CO2 for 20 min, respectively. After gently washing with washing buffer, cells remaining on the substrate were imaged by the inverted fluorescence microscope and the cell number was counted to calculate the cell capture efficiency. For further demonstration of the specificity of the MA-RCA network, the prestained CEM cells (2.5×10^4) and Ramos cells (2.5×10^4) were mixed at a ratio of 1:1 in binding buffer. The cell mixture solution was incubated on the MA-RCA network at 37 ℃ in 5% CO_2 for 20 min. The substrates were imaged after washing for three times.

Cell Capture Experiment of Rare Cancer Cells. CEM cells prestained with Calcein AM (2 μM) with different numbers (10, 20, 50, 100, 200) were spiked into buffer or lysed blood samples (human whole blood treated with red blood cell lysing buffer), and then added onto the MA-RCA network, respectively. After 20 min incubation, the substrates were washed three times with washing buffer and the capture efficiency was calculated.

DNA-responsive Cell Capture/Release Experiments. CEM cells (5×10^4) were incubated on the MA-RCA network as described above. After successfully capturing, the substrates were then treated with the excessive release DNA-1 and release DNA-2 (3 μM), respectively. The substrates were gently washed with washing buffer for three times after 1 h incubation and the remaining CEM cells were imaged and counted. For comparison, enzyme treatment was tested to investigate the release of CEM cells. After CEM cells were captured on the MA-RCA network, DNase I solution (0.125 U/μL) was added and incubated for 45 min. Then, DNase I was removed and the substrates were washed with washing buffer. Images were captured by the inverted fluorescence microscope. The capture and release efficiency before and after DNase I treatment were calculated. For reversibly capture and release cells, the complementary DNA solution (3 μM for

each cDNA) and the release DNA (3 μM for each rDNA) solution were alternatively added, and the cells on the substrate were imaged by fluorescence microscope.

Live/Dead Cell Staining Assay. Live/Dead staining experiment was used to evaluate the cell viability before the cell capture and after the cell release. Calcein AM was used to indicate the living cells and propidium iodide (PI) was used to indicate the dead cells. Calcein AM/PI staining solution was prepared by mixing Calcein AM and PI at the final concentration of 2 μM and 4 μM, respectively. To evaluate the cell viability of the released cells, the released CEM cells were collected in a centrifugation tube and stained with Calcein AM/PI solution at 37 ℃ for 30 min. Then, fluorescence microscope was used to determine the cell viability. In addition, CEM cells before capture was similarly manipulated as described above as a control.

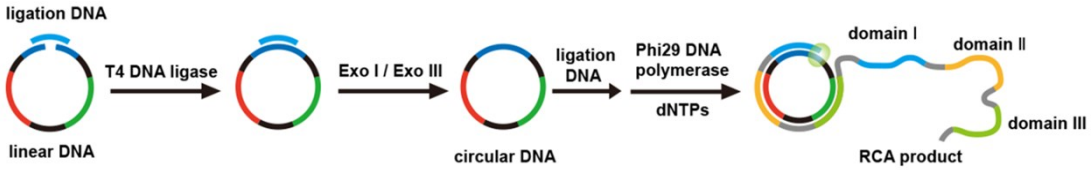

Figure S1. Synthesis of the long single-stranded DNA by rolling circle amplification. The RCA product has three repetitive functional segments: one binding site that is complementary to the amino-DNA (domain I), and two regions for attaching two different aptamer-contained sequences (domain II and III).

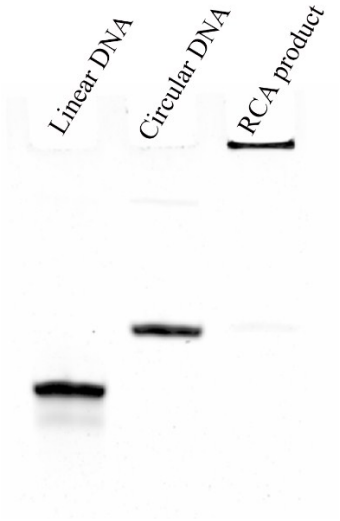

Figure S2. 12% denatured polyacrylamide gel electrophoresis characterization of the phosphorylated linear DNA (lane 1), circular DNA (lane 2) and RCA product (lane 3). The extremely low mobility of the band in lane 3 implies the successful formation of the RCA products after amplification reaction.

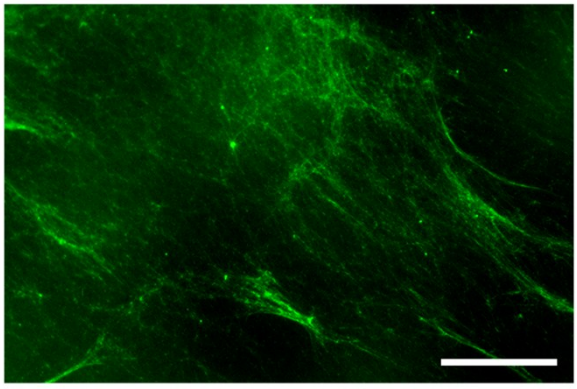

Figure S3. Fluorescent image of the DNA network on the substrate surface. The strong green fluorescence indicates the formation of a stretched DNA network on the substrate surface. Scale bar: 200 μm.

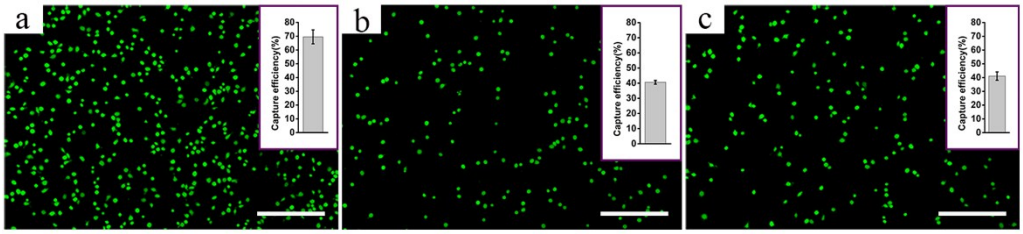

Figure S4. Comparison of the cell capture efficiency using a) dual aptamer-coated RCA network, b) sgc8c aptamer-coated RCA network and c) sgc4f aptamer-coated RCA network. CEM cells were prestained with calcein AM. Scale bars: 200 μm.

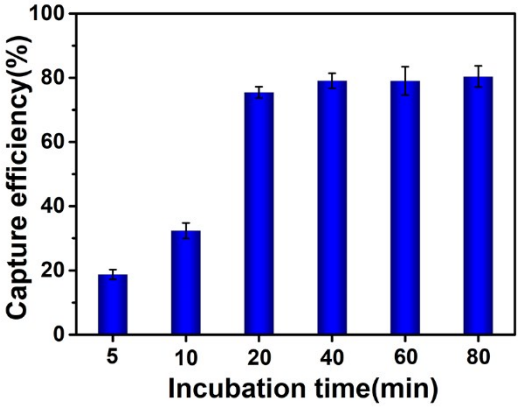

Figure S5. Cell capture efficiency of CEM cells incubated on the MA-RCA network at different incubation times.

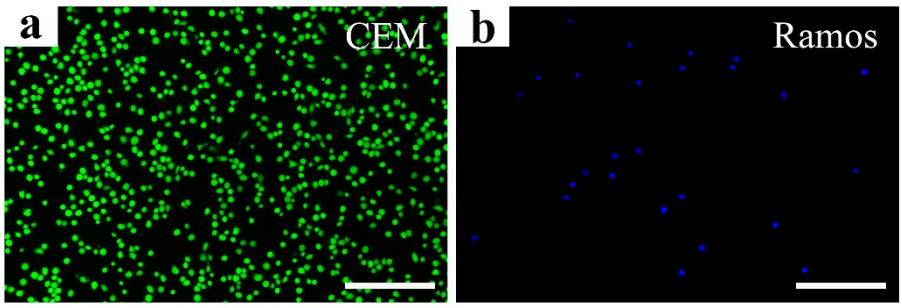

Figure S6. Capture specificity of the MA-RCA network. The fluorescent images of a) CEM cells and b) Ramos cells captured on the MA-RCA substrates, respectively. CEM cells were prestained with calcein AM and Ramos cells were prestained with Hoechst 33342. Scale bars: 200 μm.

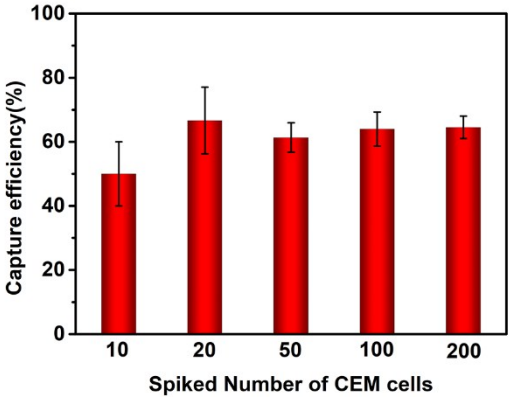

Figure S7. Capture efficiency of the MA-RCA network with different number of CEM cells spiked into lysed blood samples. The capture efficiency in lysed blood samples was lower than that in buffer due to the complex system of lysed blood samples.

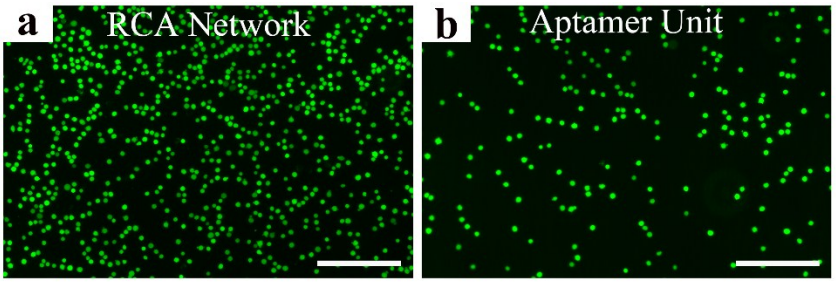

Figure S8. Comparison of the cell capture performance using a) the MA-RCA network and b) the aptamer units alone. Scale bars: 200 μm.

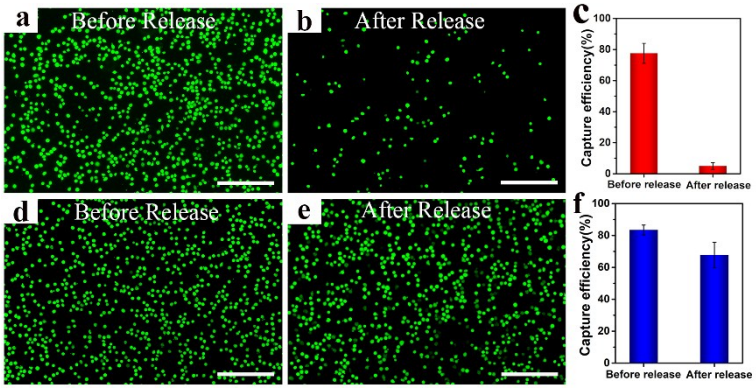

Figure S9. DNA-responsive capture and release of CEM cells. Fluorescent images of captured CEM cells on the MA-RCA network: a) before and b) after adding the release DNAs. c) Quantitative evaluation of the cell-release effect after the release DNAs were treated. d, e) Fluorescent images of trapped CEM cells on the MA-RCA network: d) before and e) after adding the noncomplementary DNAs. f) Quantitative evaluation of the cell-release effect after the noncomplementary DNAs were treated. CEM cells were prestained with calcein AM (green). scale bars: 200 μm.

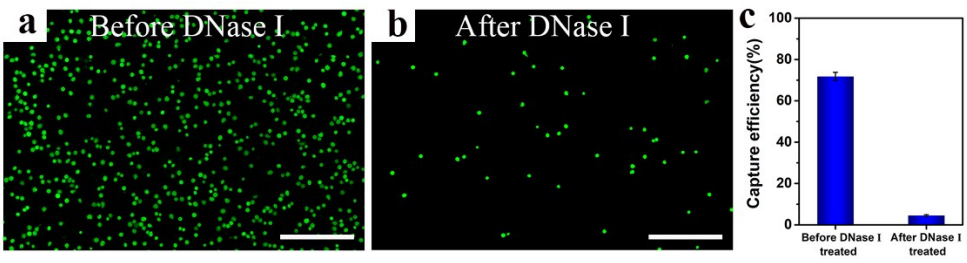

Figure S10. Enzymatic digestion-triggered cell release using DNase Ⅰ. The fluorescent images of CEM cells on the MA-RCA network a) before and b) after DNase Ⅰ treated. c) Quantitative evaluation of the cell-release effect after DNase Ⅰ was treated. Scale bars: 200 μm.

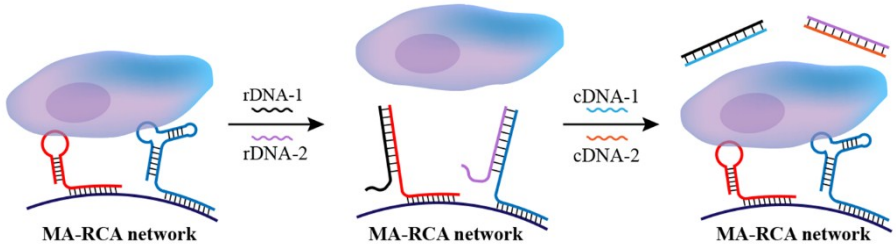

Figure S11. Illustration of the reversible capture and release of CTCs based on DNA assembly. rDNA-1 and rDNA-2 are partly complementary to the sgc8c aptamer and sgc4f aptamer, respectively, and open up the structures of aptamers to release CTCs. cDNA-1 and cDNA-2 are fully complementary to rDNA-1 and rDNA-2, respectively, and facilitate the MA-RCA network to capture CTCs again. In the absence of the complementary DNAs, aptamers are inactive because of the formation of the aptamer-rDNA complexes. Once the cDNAs are added, the rDNAs can be removed from the complexes and hybridize with the cDNAs via toehold-mediated strand displacement, and then facilitate the MA-RCA network to regain the cell-binding capacity.