Protocol update



Functionalized tetrahedral DNA frameworks for the capture of circulating tumor cells

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Functionalised tetrahedral DNA frameworks for the capture of circulating tumour cells

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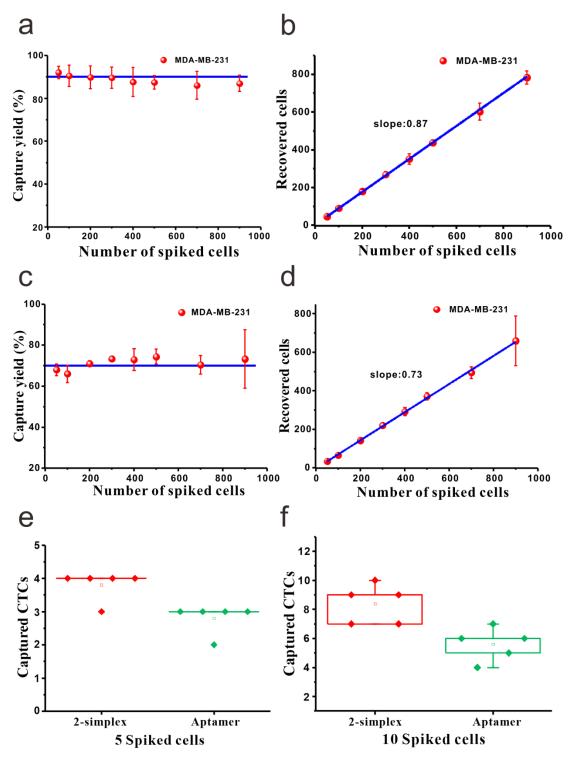
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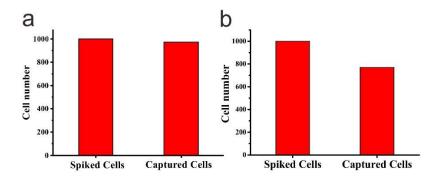
Supplementary Materials

- Figure 1 Capture yield and corresponding statistical analysis of spiked MDA-MB-231 cells captured by 2-simplex or aptamer in PBS
- Figure 2 Capture of MCF-7 cells spiked in fresh human whole blood using 2-simplex or aptamer
- Figure 3 Purity of captured cells in whole blood
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- Figure 5 Downstream experiments of isolated cells.
- Figure 6 Fluorescence microscopy imaging of captured cells from the mixture of MCF-7 cells and Ramos cells
- Figure 7 Generality verification of 2-simplex for different tumor cell lines

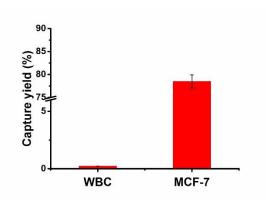


Supplementary Figure 1. Capture yield and corresponding statistical analysis of spiked MDA-MB-231 cells captured by 2-simplex or aptamer in PBS (a) Capture yields based on 2-simplex from PBS buffer spiked with various MDA-MB-231 cells. Each data point was repeated for at least 3 times. The error bars represent standard deviations of measurements within each experiment. (b) Regression analysis of capture efficiency for various target cell concentrations based on 2-simplex. The plot represents number of cells spiked versus number of cells recovered. (c) Capture yields based on aptamer from PBS buffer spiked with various MDA-MB-231 cells. Each data point was repeated in at least 3 times. The error bars represent standard deviations of measurements within

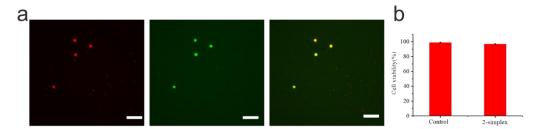
each experiment. (d) Regression analysis of capture efficiency for various target cell concentrations based on aptamer. The plot represents number of cells spiked versus number of cells recovered. (e) Cell recovery for MDA-MB-231 cells spiked into 1 mL of buffer at spike concentration of 5 cells per mL. (f) Cell recovery for MDA-MB-231 cells spiked into 1 mL of buffer at spike concentration of 10 cells per mL.



Supplementary Figure 2. Capture of MCF-7 cells spiked in fresh human whole blood using 2-simplex (a) or aptamer (b). 1000 pre-stained MCF-7 cells were spiked into 1 mL of whole blood, which was collected from healthy volunteer.



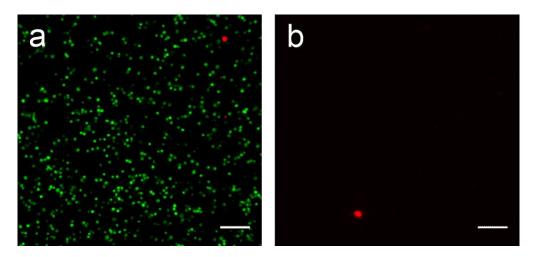
Supplementary Figure 3. Purity of captured cells in whole blood. 200 MCF-7 cells were spiked into 1 mL of whole blood, which was collected from healthy volunteer. Quantification of captured MCF-7 cells and non-specifically captured WBC using 2-simplex. Error bars represent the mean \pm s.d. of triplicates



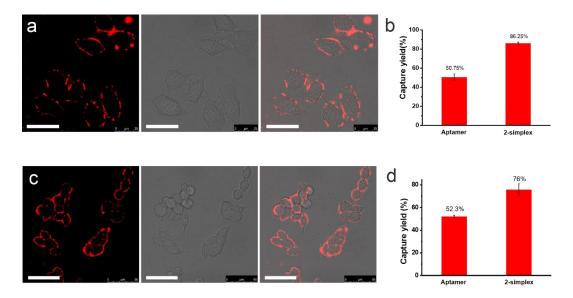
Supplementary Figure 4. Cell viability characterization of captured MCF-7 cells. (a) Fluorescence microscopy imaging of live cells and dead cells after capture. From left to right: all the spiked cells were stained with membrane dye DiI before capture (red); the captured cells were stained with Calcein AM (green); the live cells showed red-green fluorescence after merged (yellow). Scale bar: $100 \ \mu m$. (b) Cell viability analysis after magnetic separation with spiking of $400 \ MCF-7$ cells into $0.2 \ mL$ of PBS buffer.



Supplementary Figure 5. Downstream experiments of isolated cells. (a) From left to right: microscopy imaging results show that captured MCF-7 cells are well attached in the flask wall, 20×10^{10} magnification; initially cultured as a single large colony after 2 days, 20×10^{10} magnification; then formed large colonies by day 7, 10×10^{10} magnification. (b) Using cDNA processed by RT-PCR as sample, results of agarose gel electrophoresis show that captured MCF-7 cells express CK19 mRNA and EGFR mRNA. (From left to right: lane 1, Captured MCF-7 cells; lane 2, Original MCF-7 cells; lane 3, 20 bp DNA ladder.)



Supplementary Figure 6. Fluorescence microscopy imaging of captured cells from the mixture of MCF-7 cells (red labeled) and Ramos cells at ratio 1:1000, using 2-simplex before capture (a) and after capture (b). Scale bar, $100~\mu m$.



Supplementary Figure 7. Generality verification of 2-simplex for different tumor cell lines. (a) Confocal images of Her2 aptamer-based 2-simplex binding to HerG2 cells. Cy5 dye was labeled on the TDF framework of 2-simplex. From left to right: fluorescent field image, bright field image and merged image. Scale bar, 25 μ m. (b) Capture efficiency of HepG2 cells using Her2 aptamer and TDF-based 2-simplex. 200 pre-stained HepG2 cells were spiked into 1 mL of PBS buffer. (c) Confocal images of PTK7 aptamer-based 2-simplex binding to HCT 116 cells. Cy5 dye was labeled on the TDF framework of 2-simplex. From left to right: fluorescent field image, bright field image and merged image. Scale bar, 50 μ m. (d) Capture efficiency of HCT 116 cells using PTK7 aptamer and TDF-based 2-simplex. 200 pre-stained HCT 116 cells were spiked into 1 mL of PBS buffer.