Supplementary Material (ESI) for Molecular BioSystems

Supporting Information

Capturing cancer cells using aptamer-immobilized square capillary channels

Jennifer A. Martin, Joseph A. Phillips, Parag Parekh, Kwame Sefah, and Weihong Tan*

Center for Research at Bio/Nano Interface, Department of Chemistry and Shands Cancer Center, University of Florida Genetics Institute, University of Florida, Gainesville, FL 32611-7200, USA

*To whom correspondence should be addressed:

E-mail: tan@chem.ufl.edu; Tel/Fax: +1 352 846 2410.

Aptamer sequences

sgc8: 5′-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GAT TTT TTT TTT-3′-biotin

KDED2a-3: 5’-TGC CCG CGA AAA CTG CTA TTA CGT GTG AGA GGA AAG ATC ACG CGG GTT CGT GGA CAC GGT TTT TTT TTT T-3’-biotin

KCHA10: 5’-ATC CAG AGT GAC GCA GCA GGG GAG GCG AGA GCG CAC AAT AAC GAT GGT TGG GAC CCA ACT GTT TGG ACA CGG TGG CTT AGT TTT TTT TTT T-3’-biotin

Confocal images of aptamer immobilization

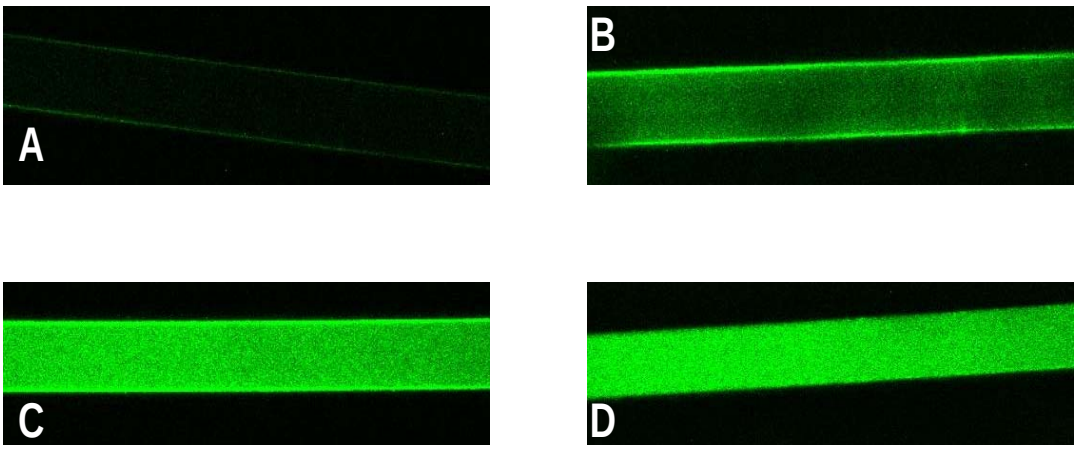

Figure S1. Confocal images when A) 0.5 μM, B) 25 μM, C) 50 μM, or D) 100 μM of FAM-sgc8-poly(T)$_{10}$-biotin was added.

Flow cytometry of HCT 116 cells in non-enzymatic buffer

The binding of aptamers with HCT 116 with and without non-enzymatic buffer was compared by flow cytometry. Cells (200 μL) were removed from a 1×10^6 cell/mL solution in BB or non-enzymatic buffer and diluted to 500 μL. Aliquots of 100 μL of each diluted cell solution were added to 8 total tubes. Each tube (except 1 control tube) was incubated with 250 nM DNA (library, KDED2a-3, and KCHA10) for 10 min. The cells were centrifuged at 970 rpm for 3 min, washed with 1.5 mL WB, and incubated with 100 μL streptavidin-PE-cy5.5 (channel 3) at a final dilution of 1:400 stock solution for 10 min. The cell solution was washed with 1500 μL WB, and the cell pellet was resuspended in 150 μL WB for analysis by flow cytometry. The results (Figure S2) show that the KCHA10 aptamer selected for HCT 116 shows similar binding to cells with and without non-enzymatic buffer (NEB). Results comparing library and KDED2a-3 binding in the presence of NEB were also similar to those in BB. Therefore, we concluded the NEB has minimal effects on aptamer/cell binding under the conditions utilized in this experiment.

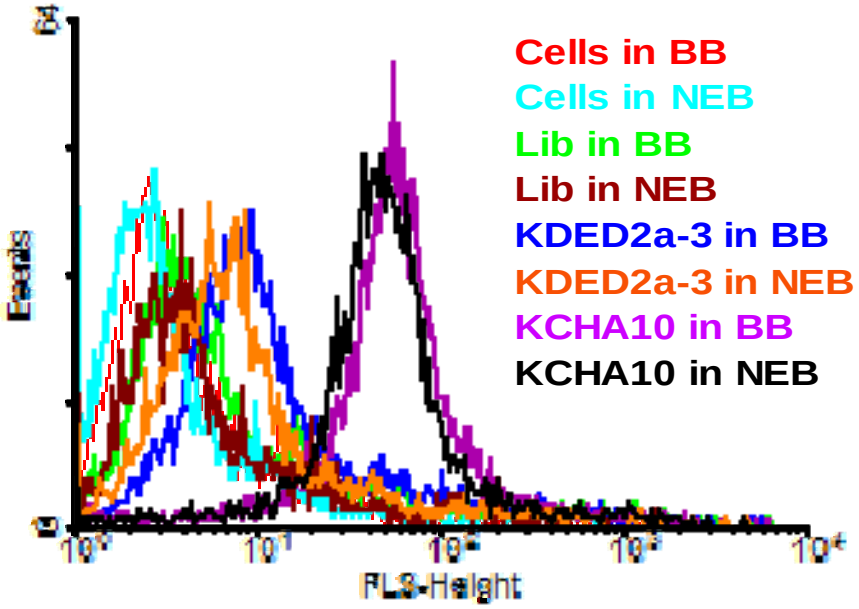

Figure S2. Flow cytometry comparison of aptamer binding with and without non-enzymatic buffer.

Cell staining

CEM cells (1.7×10^6 cells) were diluted in 2 mL WB. CellTracker Green (2 µL; Invitrogen) was incubated with cells for 45 min at room temperature, according to manufacturer's instructions. The cells were centrifuged at 970 rpm for 3 min to remove excess dye, resuspended in 1 mL WB, and incubated for 30 min at 37°C. The cell solution was centrifuged at 970 rpm for 3 min, and the supernatant liquid was discarded. The pellet was washed with 1 mL of BB2, centrifuged at 970 rpm for 3 min, then resuspended in 100 µL BB. This cell solution was kept on ice until needed.

Removal of red blood cells

Red blood cells were removed from human whole blood (Innovative Research) by separation by Ficoll-Paque (GE Healthcare). Ficoll-Paque is a density gradient centrifugation medium that separates blood into 4 main layers: the top layer consists of the plasma, followed by the buffy coat, the Ficoll layer, and the red blood cells concentrated at the bottom of the tube. The buffy coat contains the white

blood cells and platelets, and is the layer collected and combined with the CEM cells in this experiment. This red blood cell removal is essential so the high concentration of red blood cells do not clog the capillary.

The Ficoll-Paque blood separation was carried out according to manufacturer's instructions. Briefly, 3 mL whole blood was slowly added to 3 mL Ficoll in a 15 mL centrifuge tube. The layers were centrifuged at 1200 × g at 4°C with the rotor brake off. The buffy coat was removed and centrifuged at 1800 rpm for 5 min. The supernatant liquid was discarded and the cells were washed with 3 mL PBS buffer. The washed cell pellet was combined with 1 mL red blood cell lysis buffer (LB-150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA in distilled H_2O (pH 7.3) filtered with 0.45 μm filter (Nalgene)) and incubated for 15 min. The supernatant liquid was removed and the pellet was washed twice with WB (all centrifugations at 1800 rpm for 3 min). The final pellet was resuspended in 3 mL BB2 to simulate the concentration of white blood cells in whole blood.