Dimerization of an aptamer generated from Ligand-guided Selection (LIGS) yields high affinity scaffold against B-cells

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Supporting information

Cell culture

Cell lines, including BJAB (Burkitt's lymphoma), Ramos (Burkitt's lymphoma), CA-46 (Burkitt's lymphoma) and SKLY-16 (B-cell lymphoma), were a generous gift from the David Scheinberg Lab, Memorial Sloan Kettering Cancer Center. These cells were cultured using HyClone RPMI 1640 medium supplemented with 100 units/mL penicillin–streptomycin and 10% fetal bovine serum (heat-inactivated; Invitrogen). MOLT-3 (acute lymphoblastic leukemia) and Toledo (diffuse large cell lymphoma) cell lines were purchased from American Type Culture Collection (ATCC) and were cultured using HyClone RPMI 1640 medium supplemented with 100 units/mL penicillin–streptomycin and 20% fetal bovine serum (heat-inactivated; Invitrogen). All cell lines were routinely assessed for the expression of appropriate CD markers to authenticate the cell line.

Cell Suspension Buffer (CSB)

All in vitro experiments were performed using a Cell Suspension buffer composed of HyClone DPBS, 4.5 g/L glucose, 10 mM $MgCl_2$, 100 mg/L tRNA, 1 g/L BSA, 200 mg/L salmon's sperm DNA and 100 mg/L Polyethylene glycol (Acros Organics; CAS: 25322-68-3).

DNA synthesis

All DNA reagents needed for DNA synthesis were purchased from Glen Research or ChemGenes. The dimeric R1.2 molecules were chemically synthesized by attaching a fluorophore at the 5'-end using standard solid phase phosphoramidite chemistry on an ABI394 DNA synthesizer (Biolytics) using a 1 μmol scale, and a Spacer

Phosphoramidite 18 (Glen Research) was used to tether the two monomeric R1.2 molecules. Three, five and seven spacers were used for DR1.2_3S, DR1.2_5S and DR1.2_7S, respectively. The completed DNA sequences were deprotected according to the base modification employed and purified using HPLC (Waters) equipped with a C-18 reversed phase column (Phenomenex/Waters/Thermo Fisher). The dimeric Random Control was purchased from IDT DNA Technologies.

Binding buffers

All dilutions of the stock solutions of the three dimeric aptamers were done using a DNA binding buffer composed of HyClone DPBS, 4.5 g/L glucose, 10 mM $MgCl_2$ and 100 mg/L tRNA (Sigma-Aldrich). All *in vitro* experiments were performed using a cell suspension buffer composed of HyClone DPBS, 4.5 g/L glucose, 10 mM $MgCl_2$, 100 mg/L tRNA, 1 g/L BSA and 200 mg/L salmon sperm DNA. The wash buffer was composed of HyClone DPBS with 10 mM $MgCl_2$ and 4.5 g/L glucose (Sigma-Aldrich).

Preparation of solutions

After the purification of DR1.2_3S, DR1.2_5S and DR1.2_7S, the concentration of the stock solutions was determined by using a UV-Vis spectrophotometer (Thermo). Then, 10 μM solutions of the three dimeric molecules were prepared by dilution of the respective stock solutions with the DNA binding buffer containing 550 μM KCl. These 10 μM solutions were then used to prepare 1 μM and 500 nM working solutions by using DNA binding buffer containing 0.2 M KCl for dilution. Random controls were prepared in a manner similar to that of each dimeric R1.2 molecule. All molecules were folded as described previously.

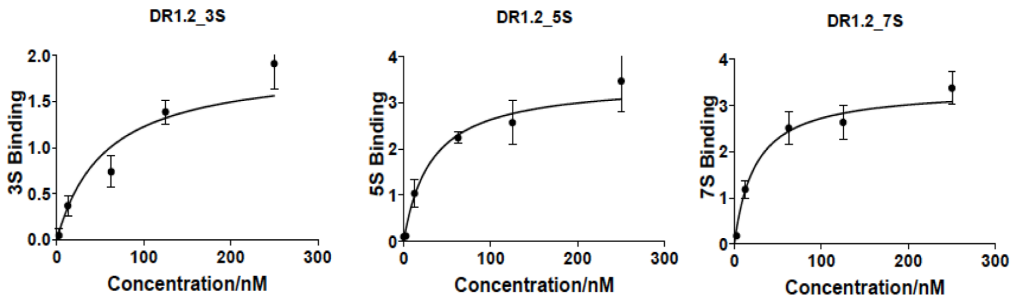

Figure 1. Affinity analysis of dimeric aptamer R1.2 constructs against BJAB cells at 4°C.

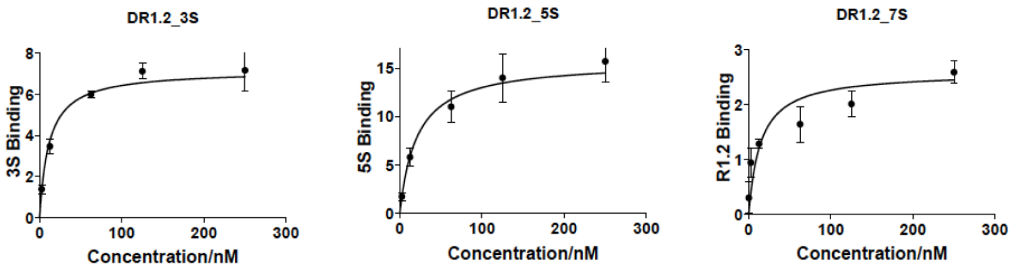

Figure 2. Affinity analysis of dimeric aptamer R1.2 constructs against BJAB cells at 25°C.

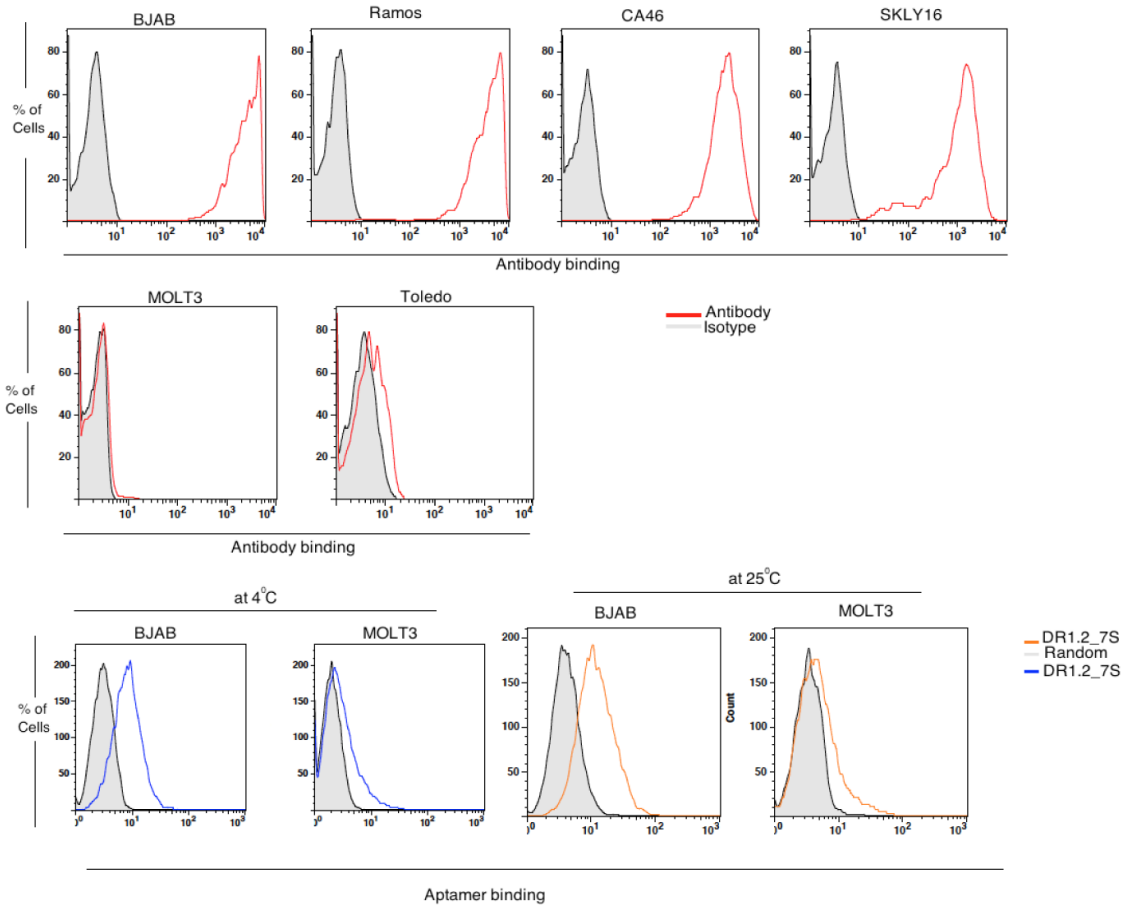

Figure 3. Analysis of specificity of DR1.2_7S at 4°C and 25°C against cell lines. Anti-IgM antibody binding was used as a positive control.

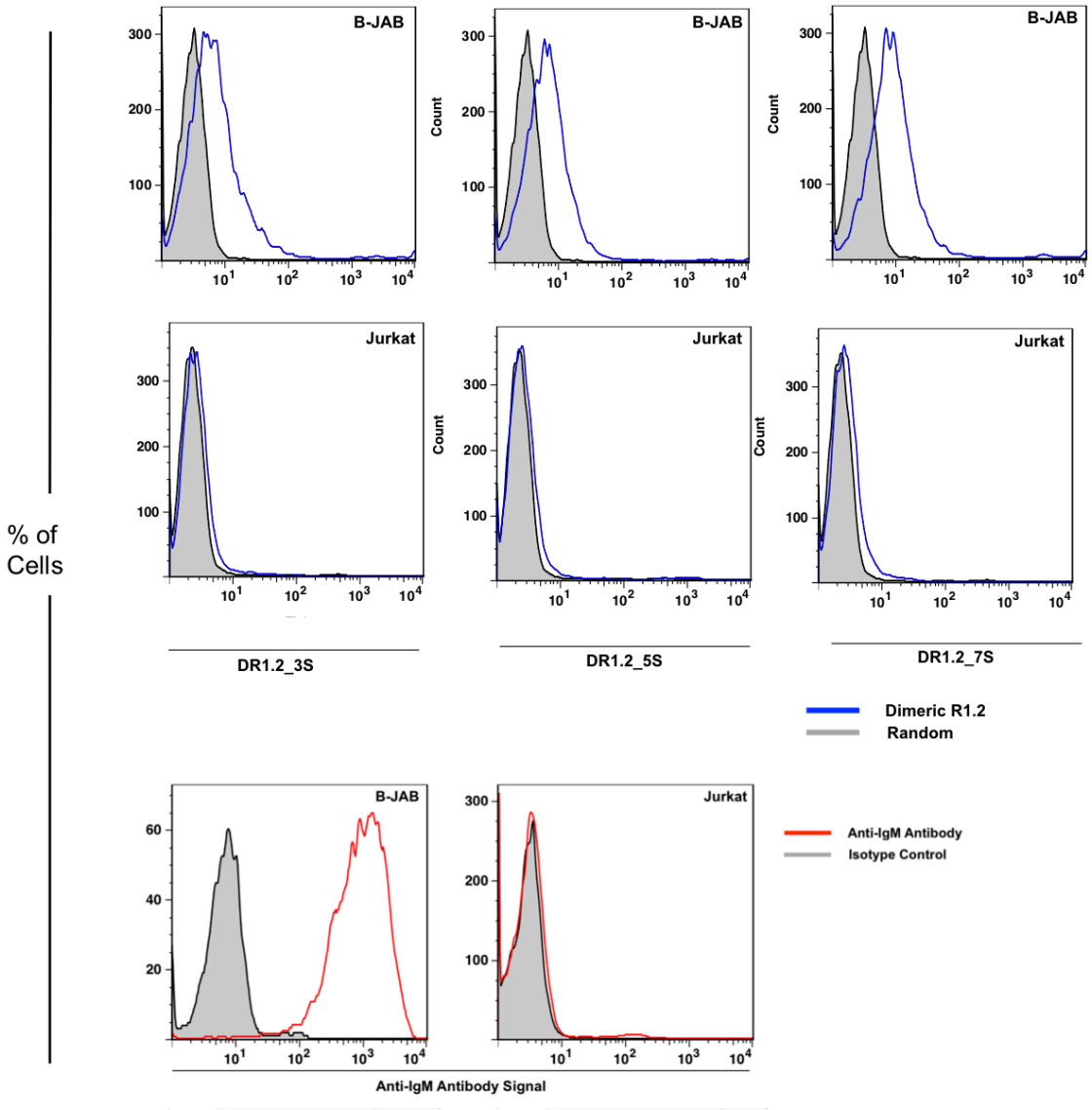

Figure 4. Analysis of specificity of DR1.2 analogues with 3, 5 and 7 Poly-Ethylene-Glycol spacers against mIgM positive BJAB and Jurkat.E6 cells at 4°C. Anti-IgM antibody binding was used as a positive control.

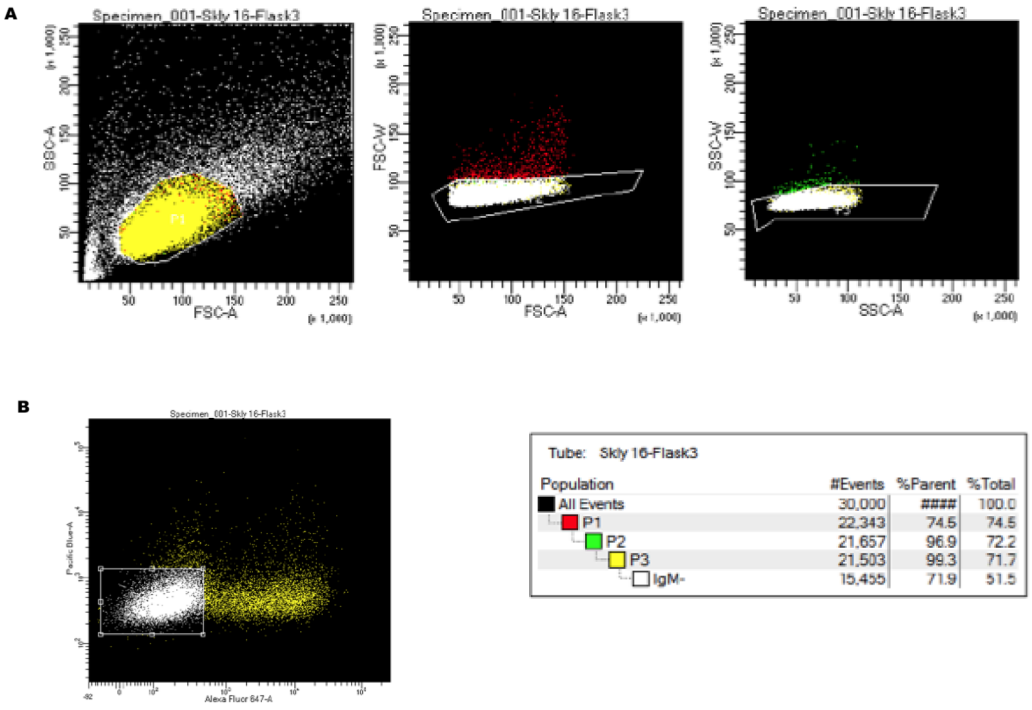

Figure 5. Evaluation of expression of mIgM of CRISPER/CAS9 treated SKLY16. Cells with correct morphology was gated (A). Cells negative for mIgM expression were gated, sorted and collected (B).

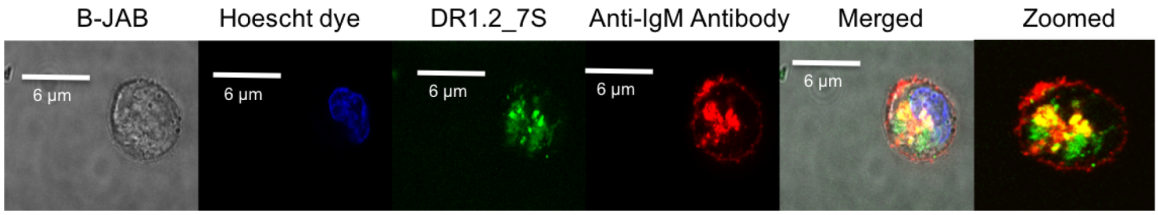

Figure 6. Rapid internalization of DR1.2_7S and anti-IgM antibody into BJAB cells. Internalized anti-IgM antibody and DR1.2_7S co-localizes into a subcellular compartment.