Using aptamers evolved from cell-SELEX to engineer a delivery platform

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Oligonucleotide synthesis, purification and sequences

Aptamer Sgc8 modified with reaction platform sequence and nonspecific control DNA was synthesized at the 1umole scale using standard solid-state phosphoramidite chemistry using an ABI3400 DNA/RNA synthesizer (Applied Biosystems, Foster City, CA). Dithiol phosphoramidite (DTPA) which was used at the 5' end as the activator, Dabcyl-CPG, Fluorescein-dT, disulfide phosphoramidite; spacer phosphoramidite was purchased from Glen Research. The completed sequences were then deprotected in concentrated ammonium hydroxide at 65º C overnight. The solutions resulted from deprotection were precipitated in ethanol. The precipitates were then dissolved in 0.5ml of 0.1 M triethylammonium acetate (pH7.0) for further purification with high-pressure liquid chromatography using reverse-phase HPLC purification using a ProStar HPLC (Varian, Walnut Creek, CA) with a C18 column (Econosil, 5u, 250 × 4.6 mm, Alltech, Deerfield IL) of the DMT-on fraction. Next the DMT cleavage was done using 80% acetic acid followed by ethanol precipitation of the DNA. End product was quantified measuring the UV absorbance at 260nm.

Flowcytometric Analysis of aptamer binding and competition assay:

Since aptamer binding with its target depends on the 3-D folding of the DNA sequence, addition of extra bases to the aptamer may interfere with the binding with the cellular target. Therefore, modified Sgc8 aptamer with the platform sequences were analyzed for binding. In doing so, CEM cells were incubated with 250nM of FITC-labeled Sgc8 and random sequence at 4°C for 30 minutes in 300 uL of cell binding buffer [4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's PBS with calcium chloride and magnesium chloride; (Sigma), yeast tRNA 0.1 mg/ml; (Sigma)]. After incubation, the cells were washed to remove the unbound probe. Binding of FITC-Sgc8 was analyzed by flow cytometry to obtain the maximum binding. For competition assays, cells were incubated with equimolar mixtures of FITC-Sgc8 and Sgc8-P or Scg8-RP for 30 minutes at 4°C in 300 uL of cell binding buffer. After equilibration, cells were washed to remove the unbound probe and analyzed for the binding with Flow Cytometry using a FACScan cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) counting 20000 events.

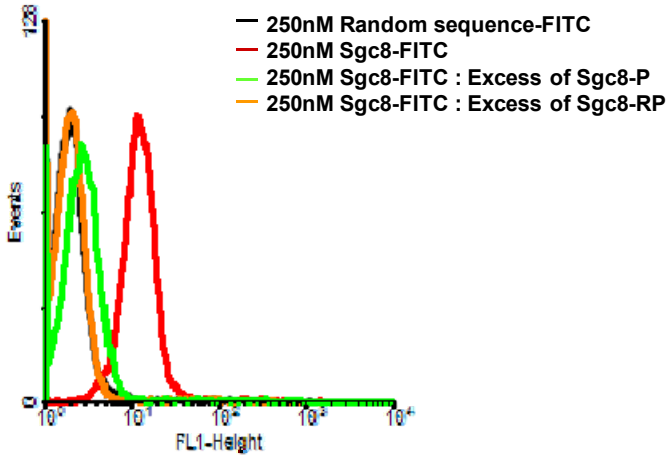

Figure S1. Flow cytometric analysis of binding of Sgc8tem with CEM cells. The fluorescence intensity of aptamer Sgc8-FITC decreases in the presence of a

10-fold excess of Sgc8tem and Sgc8-Random indicating that Sgc8 binding is retained after attachment of additional bases at the 5' end.

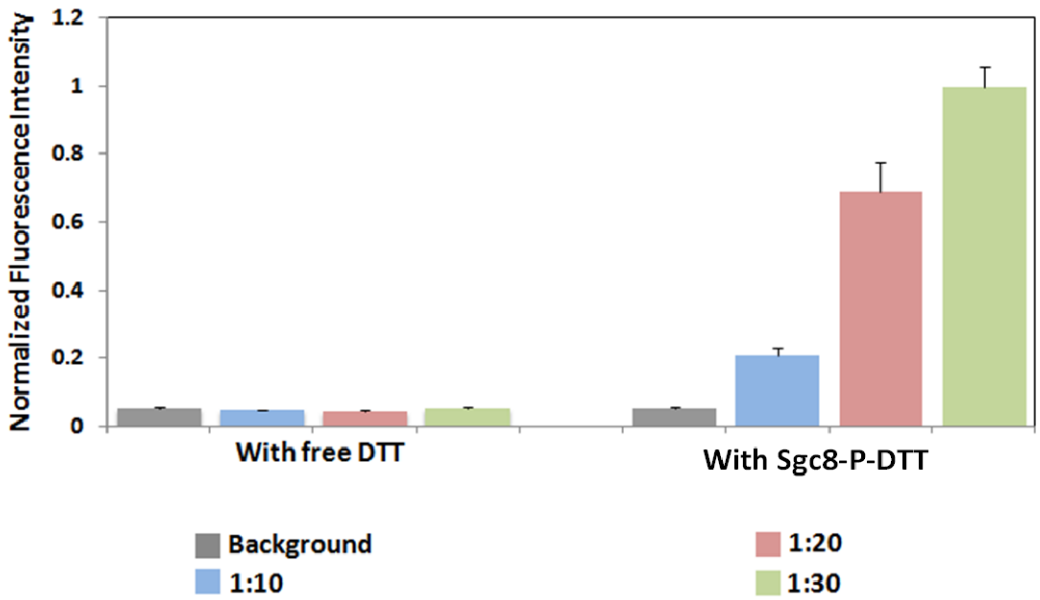

Figure S2. Analysis of disulfide bond reduction of cDNA probe using DTT bound to aptamer platform and free DTT. The fluorescence intensities were normalized against highest fluorescence shift observed for Sgc8-P-DTT at 1:30 ratio.

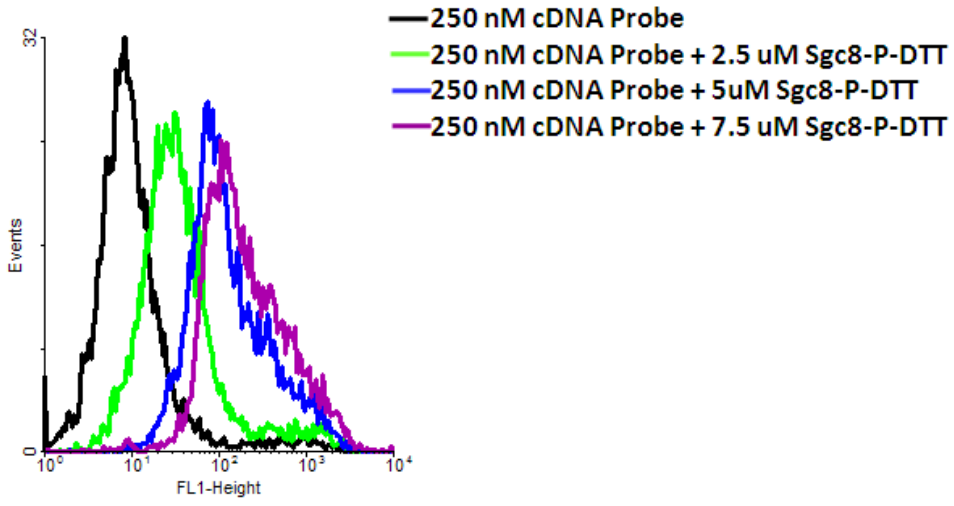

Figure S3. Histograms from the flow Cytometric analysis of disulfide reduction varying the ratios of Sgc8-P-DTT with cDNA probe.

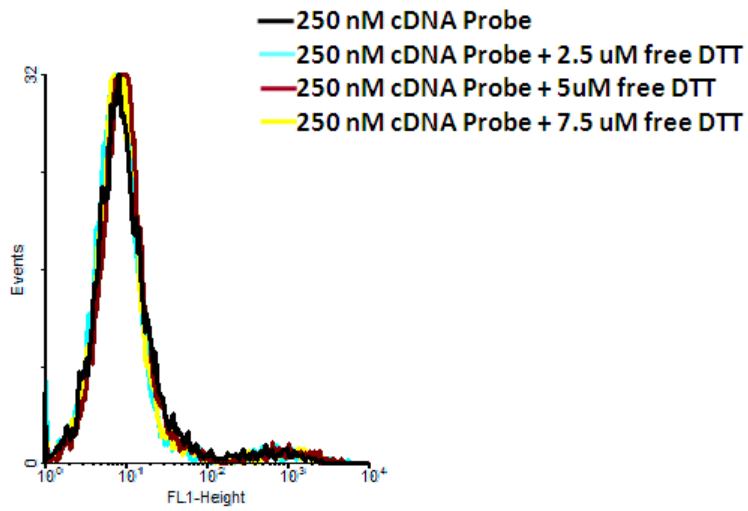

Figure S4. Histograms from the flow Cytometric analysis of disulfide reduction varying the ratios of free DTT with cDNA probe.

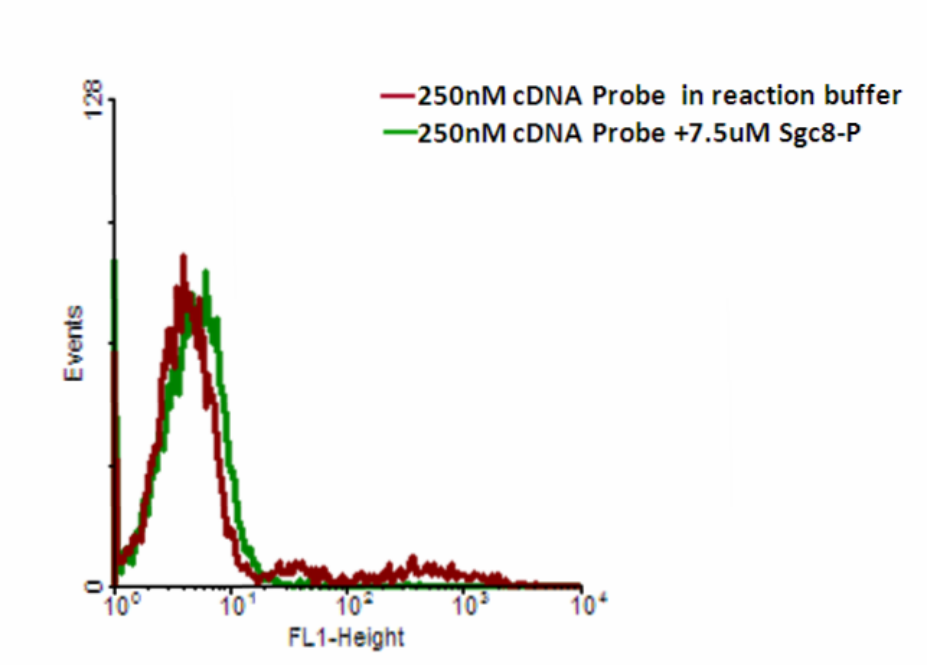

Figure S5. Histograms from the flow Cytometric analysis showing cDNA probe does not internalized into the cells during the reaction.

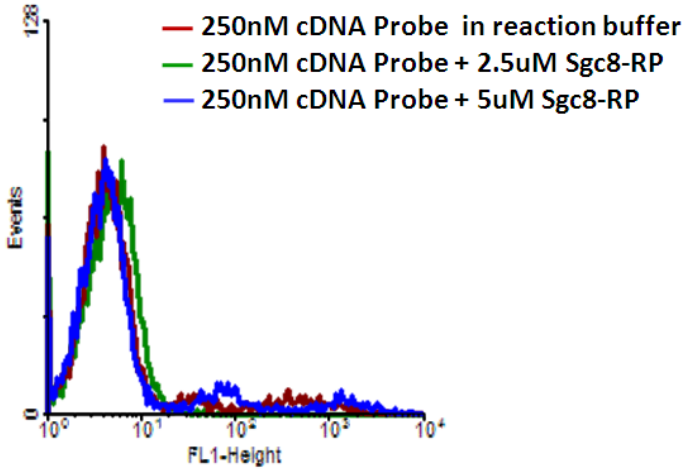

Figure S6. Histograms from the flow Cytometric analysis showing disulfide cleavage by DTT attached to aptamer is specific. Randomized DNA platform sequence does not hybridize with cDNA probe thus, the FRET pair remains intact.