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Materials and Methods:

DNA synthesis, labeling, purification and quantification: All DNA synthesis reagents were purchased from Glen Research, and all DNA probes (see sequences in Table S1) were synthesized on an ABI3400 DNA/RNA synthesizer (Applied Biosystems, Foster City, CA, USA). FITC, Quasar570, or Cy3 were synthesized on the 3'-end of these DNA probes, unless otherwise specified. DNA sequences were deprotected in AMA (ammonium hydroxide/40% aqueous methylamine 1:1) at 65°C for 30 min, followed by purification with reversed-phase HPLC (ProStar, Varian, Walnut Creek, CA, USA) on a C-18 column using 0.1 M triethylamine acetate (TEAA, Glen Research Corp.) and acetonitrile (Sigma Aldrich, St. Louis, MO) as the eluent. The collected DNA products were dried and detritylated by dissolving and incubating DNA products in 200 µL 80% acetic acid for 20 minutes. The detritylated DNA product was precipitated with NaCl (3 M, 25 µL) and ethanol (600 µL). UV-Vis measurements were performed with a Cary Bio-100 UV/Vis spectrometer (Varian) for probe quantification.

Self-assembly of aptamer-tethered DNA nanodevices: Aptamer-trigger probes, M1 and M2, were first snap-cooled (heated at 95 °C for 2 min, incubated on ice for 2 min, then left at room temperature for 1 h). They were then mixed (molar ratio of aptamer: M1: M2=1 µM: 10 µM: 10 µM) in Dulbecco's PBS (Sigma) supplemented with 5 mM $MgCl_2$ and left at room temperature for 24 h. The formation of nanodevices was confirmed using agarose gel (3%) electrophoresis. To construct different types of fluorescent DNA nanodevices, M1 and M2 with or without the corresponding chemically-modified fluorophores were utilized.

Agarose gel electrophoresis. Formation of aptamer-tethered nanodevices was confirmed by agarose gel (3%) electrophoresis (100 V, 50 min), stained with Ethidium Bromide (EB), followed by imaging under UV irradiation.

Fluorescence spectrometry: The fluorescence of EvaGreen (Biotium, Inc.) and Cy3 was monitored in Dulbecco's PBS buffer (Sigma) supplemented with Mg^{2+} (5 mM) on a Fluorolog-Tau-3

spectrofluorometer (Jobin Yvon, Edison, NJ). The excitation wavelength was 440 nm or 488 nm, as specified. The slit width was 5 nm.

In the study of FRET nanodevices, the aptND concentration was 20 nM, unless specified otherwise. FRET efficiency was calculated based on the fluorescence quenching of donor (EG), according to **Equation 1**:

$$\text{FRET efficiency} = 1 - F_{DA}/F_D \qquad \textbf{(Eq. 1)}$$

where the fluorescence intensity ratio of EG was calculated in the presence (F_{DA}) and absence (F_D) of acceptor Cy3.

The signal-to-noise ratio was calculated according to **Equation 2**:

$$S/N = ((F_{Cy3}/F_{EG})_{DA})/((F_{Cy3}/F_{EG})_{D}) \qquad \textbf{(Eq. 2)}$$

where relative fluorescence intensity ratio of acceptor to donor (F_{Cy3}/F_{EG}) is calculated in the presence ($(F_{Cy3}/F_{EG})_{DA}$) and absence ($(F_{Cy3}/F_{EG})_{D}$) of acceptor Cy3.

Cell culture: Cell lines CCRF-CEM (Human T-cell ALL) and Ramos (Human B-cell Burkitt's lymphoma) were obtained from the American Type Culture Collection (Manassas, VA). The cells were cultured in RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum (FBS) (heat inactivated, GIBCO) and 100 IU/mL penicillin-streptomycin (Cellgro) at 37 °C in a humid atmosphere with 5% CO_2. The cell density was determined using a hemocytometer and evaluated prior to each experiment.

Flow cytometric analysis: Experiments were performed in buffer containing 4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's PBS (Sigma). The binding abilities of aptamers or preformed aptamer-tethered DNA nanodevices were determined by incubating these probes or nanodevices (final aptamer or aptamer equivalent concentration: 50 nM) with the corresponding cells (2×10^5) on ice for 30 min, followed by washing twice with buffer (1 mL) and then suspending in buffer (200 μL). The resultant cell solutions

were subjected to flow cytometric analysis on a FACScan cytometer (BD Immunocytometry Systems) or on a BD FACSVerse flow cytometer. The molar ratio of EG/aptND for flow cytometric analysis of cells with label-free fluorescent nanodevices and FRET nanodevices was 20/1. Data were analyzed with the FlowJo or FCSExpress software. Random DNA sequences (lib) of the same corresponding concentrations were used as negative controls. The S/N was calculated according to **Equation 3**:

$$S/N= ((G_{Cy3}/G_{EG})_{DA})/((G_{Cy3}/G_{EG})_{D}) \quad \textbf{(Eq. 3)}$$

where relative geometric fluorescence intensity ratio of acceptor to donor (G_{Cy3}/G_{EG}) is calculated in the presence ($(G_{Cy3}/G_{EG})_{DA}$) and absence ($(G_{Cy3}/G_{EG})_{D}$) of acceptor Cy3, and geometric fluorescence intensities G_{Cy3} and G_{EG} were the corresponding net fluorescence intensities (with background fluorescence intensity of cells subtracted).

Confocal microscopy imaging: Experiments were performed in buffer containing 4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's PBS (Sigma). Cellular fluorescent images were collected on the FV500-IX81 confocal microscope (Olympus America Inc., Melville, NY) with 60x oil immersion objective (NA=1.40, Olympus, Melville, NY) and the Fluoview analysis software, or on a Leica TCS SP5 confocal microscope (Leica Microsystems Inc., Exton, PA) with a 63x oil immersion objective and Leica Confocal Software. Cells (2×10^5, 200 µL) were incubated on ice with aptamers or preformed aptamer-tethered DNA nanodevices, followed by two washes with 1 mL washing buffer at 4 °C and resuspension in 200 µL binding buffer before observation. A volume of cell suspension (100 µL) was dropped on poly-d-lysine-coated 35 mm glass bottom dishes (Mat Tek Corp.), and fluorescence intensities were observed after a 3-min settling time.

***In situ* self-assembly of nanodevices on target cell surfaces**: Aptamer-tethered capture probes (100 nM) were first incubated with target cells on ice for 0.5 h. The resultant cells were washed twice with washing buffer (1 mL 4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's PBS (Sigma Aldrich)) and then resuspended in 100 µL binding buffer (with yeast tRNA (0.1 mg/mL) (Sigma Aldrich) and BSA (1

mg/mL) (Fisher Scientific) in the washing buffer). Probes P1, P2 or M1, M2 (1 μM each) were introduced into the resultant cell solution at different time points to allow *in situ* self-assembly (room temperature) for a specified time, and the assembly of all groups was terminated by washing cells at the same time (twice) with washing buffer (1 mL) and resuspending in binding buffer (100 μL). For nanodevice assembly in cell mixtures, biotinylated TDO5 was introduced 30 min before termination of assembly, followed by washing away excess probes and further incubation with streptavidin-PE-Cy5.5 for 20 min, prior to washing and resuspension. All the resultant samples were subjected to flow cytometric analysis or confocal microscopic observation of cell fluorescence intensities using the same instrument settings.

Supplementary Tables

Table S1. Sequences of DNA probes (purple: aptamer sequences; black: linker; red: capture or trigger probe; sky blue and green: sequences complementary to those in the same colors in the corresponding sequence pairs). Sequences were designed using website nupack.org.[1]

Name	Sequence (5'-3')
Sgc8	ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA
TDO5	AACACCGTGGAGGATAGTTCGGTGGCTGTTCAGGGTCTCCTCCGGTG
Sgc8-trigger	GACCCTAAGCATACATCGTCCTTCATTTTATCTAACTGCTGCGCCGCCGGGAA AATACTGTACGGTTAGA
TDO5-trigger	GACCCTAAGCATACATCGTCCTTCATTTTAACACCGTGGAGGATAGTTCGGT GGCTGTTCAGGGTCTCCTCCGGTG
M1	ATGAAGGACGATGTATGCTTAGGGTCGACTTCCATAGACCCTAAGCATACAT
M2	GACCCTAAGCATACATCGTCCTTCATATGTATGCTTAGGGTCTATGGAAGTC
Sgc8-trigger (FRET)	TGCTGCTGCTGCTGCTGCACGACGTTTATCTAACTGCTGCGCCGCCGGGAAA ATACTGTACGGTTAGA
TDO5-trigger (FRET)	TGCTGCTGCTGCTGCTGCACGACGTTTAACACCGTGGAGGATAGTTCGGTGG CTGTTCAGGGTCTCCTCCGGTG
M1 (FRET)	CGTCGTGCAGCAGCAGCAGCAGCAACGGCTTGCTGCTGCTGCTGCTGC
M2 (FRET)	TGCTGCTGCTGCTGCTGCACGACGGCAGCAGCAGCAGCAGCAAGCCGT
Sgc8-cap	GACCCTAAGCATACATCGTCCTTCATTTTATCTAACTGCTGCGCCGCCGGGAA AATACTGTACGGTTAGA
TDO5-cap	GACCCTAAGCATACATCGTCCTTCATTTTAACACCGTGGAGGATAGTTCGGT GGCTGTTCAGGGTCTCCTCCGGTG
P1	ATGAAGGACGATGTATGCTTAGGGTCCCGACCTCGACCTACAGAGACCACAG
P2	GACCCTAAGCATACATCGTCCTTCATCTGTGGTCTCTGTAGGTCGAGGTCGG

Supplementary Figures

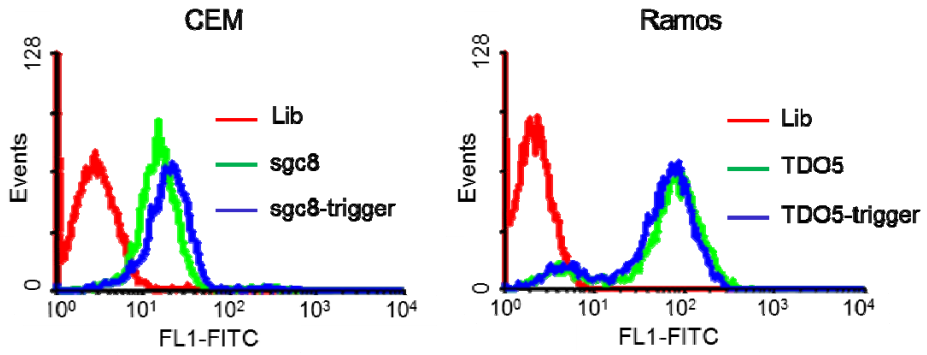

Figure S1. Flow cytometric results verifying that sgc8-trigger and TDO5-trigger maintained selective binding abilities to target CEM and Ramos cells, respectively, but not to the corresponding nontarget cells (Ramos cells for sgc8; CEM cells for TDO5). Unmodified sgc8 and TDO5 were utilized as positive controls, and Lib (random sequences) was used as a negative control. All probes were labeled with FITC.

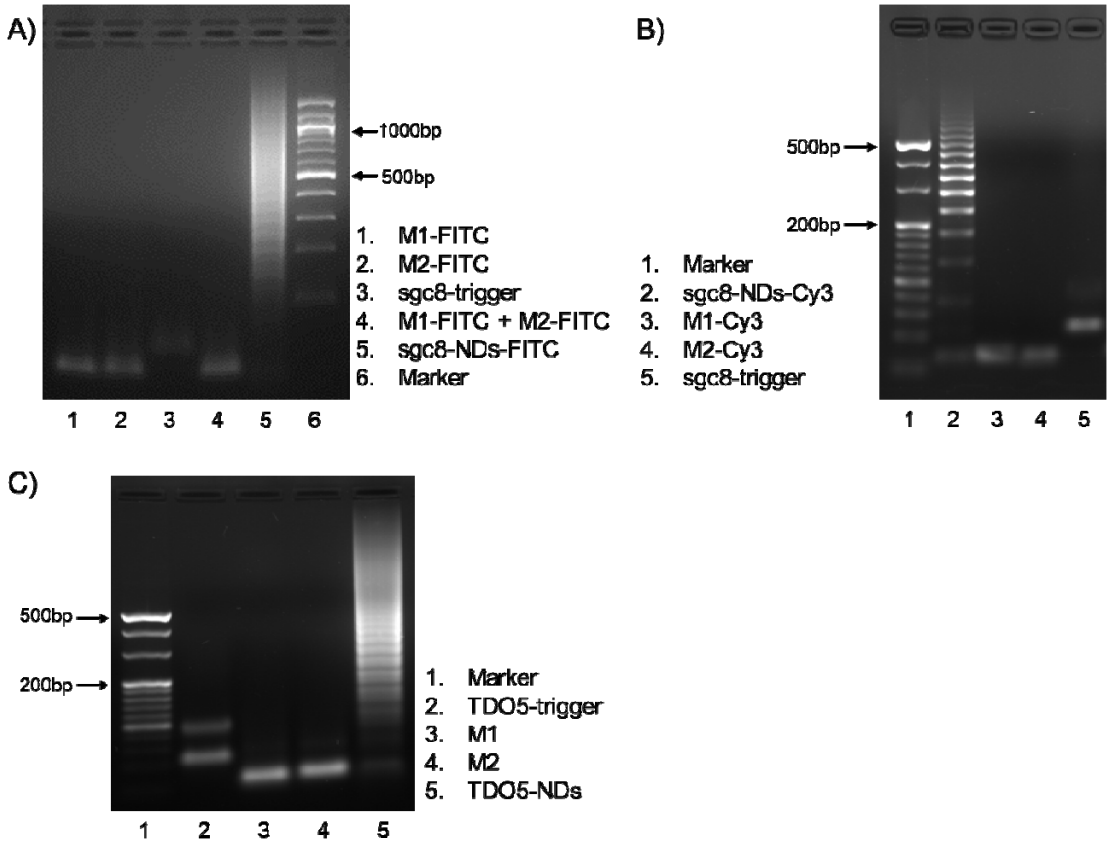

Figure S2. Agarose gel electrophoresis images verifying the self-assembly of A) sgc8-NDs-FITC, B) sgc8-NDs-Cy3, and C) TDO5-NDs upon the initiation of the corresponding chimeric aptamer-trigger probes. The upper band in lane 2 of C) presumably resulted from the dimerization of the TDO5-trigger.

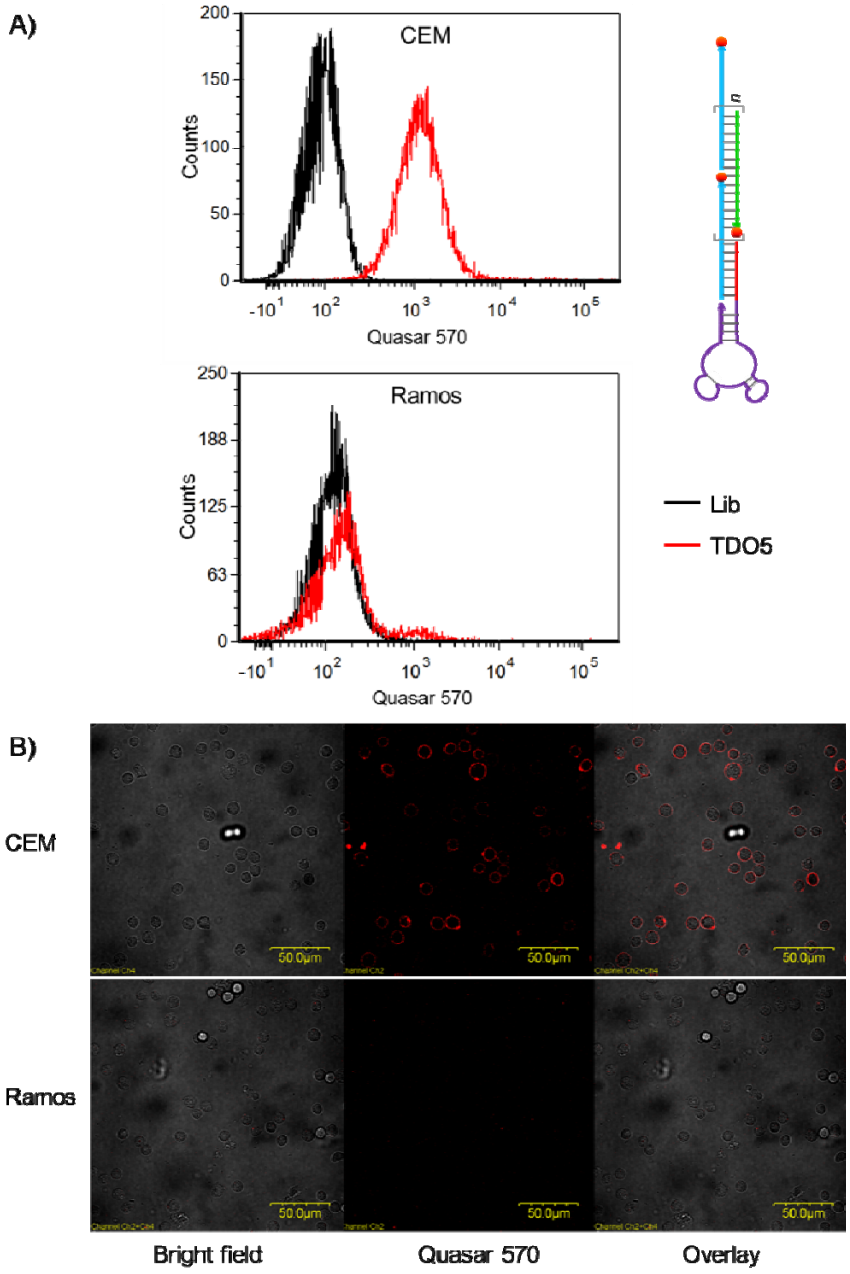

Figure S3. Anchoring of Quasar 570-labeled sgc8-NDs specifically on target CEM cell surfaces, but not on nontarget Ramos cells, as demonstrated by flow cytometric study (A) and confocal microscopy study (B) (Scale bar: 50 μm).

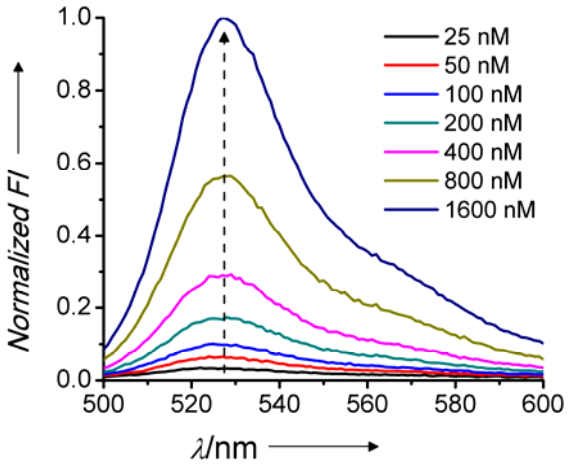

Figure S4. Fluorescence enhancement of fluorogenic EvaGreen (EG) upon intercalation into DNA aptNDs (Final aptND concentration: 20 nM aptamer equivalent; Ex: 440 nm).

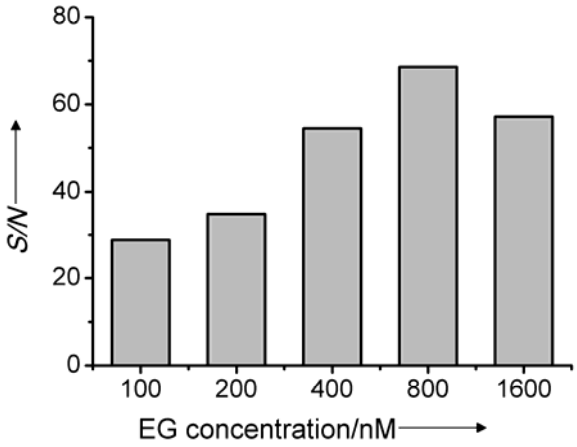

Figure S5. S/N of label-free fluorescent aptNDs for a series of different EG concentrations and aptNDs with a constant aptamer equivalent concentration. An EG/nanodevices molar ratio of 40/1 was used in subsequent studies, unless specified otherwise (Ex: 440 nm).

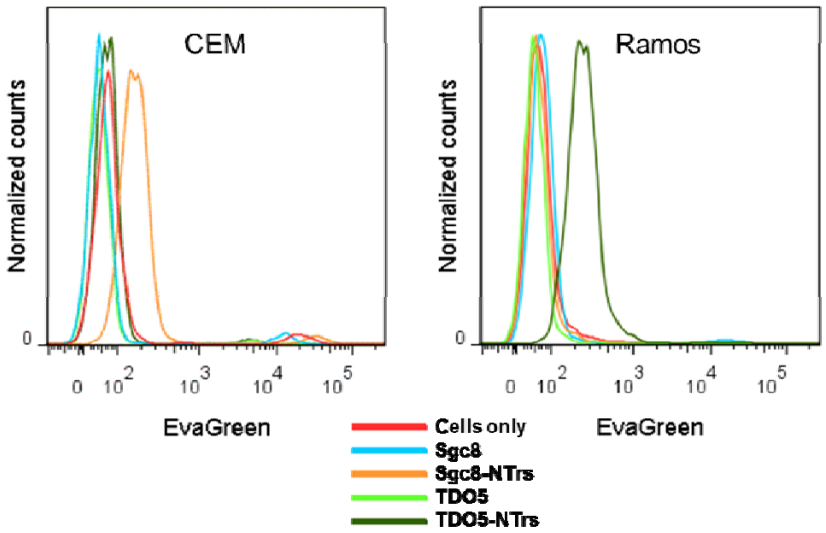

Figure S6. Flow cytometric results indicating the selective anchoring of label-free fluorescent sgc8- and TDO5-NDs on the corresponding target cell surfaces. These label-free fluorescent NDs were built by intercalation of a fluorogenic dsDNA intercalating dye, EvaGreen, into aptNDs.

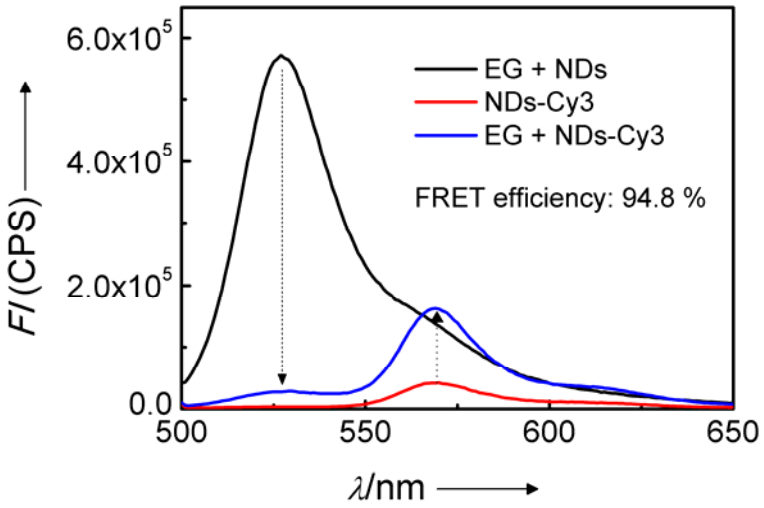

Figure S7. Fluorescence spectrometric results verifying energy transfer from physically-associated EG in the duplex boxcars to chemically-labeled Cy3 on M1 and M2 on the linear aptNDs platform. FRET efficiency was calculated as 94.8% (Ex: 488 nm).

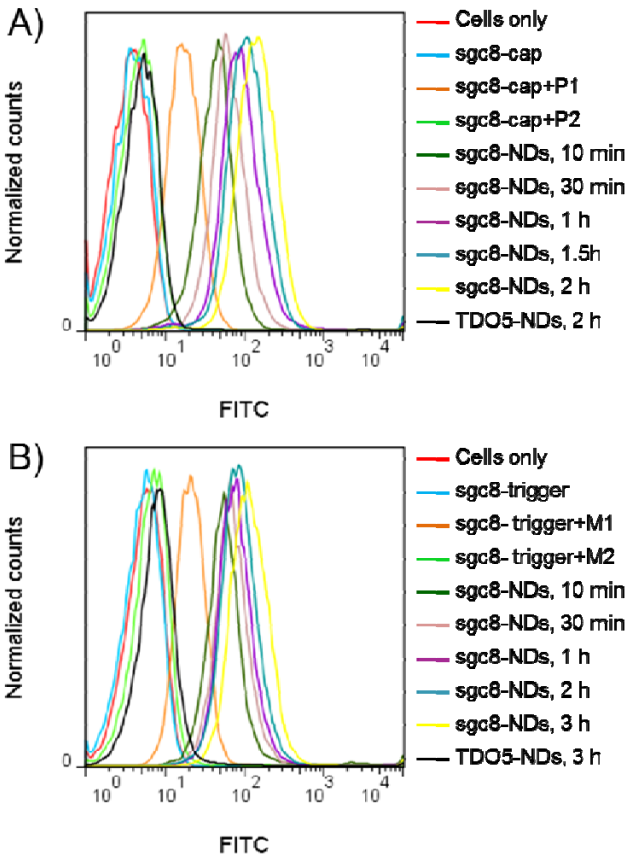

Figure S8. Color graphs of flow cytometric data shown in Figure 3A,B.

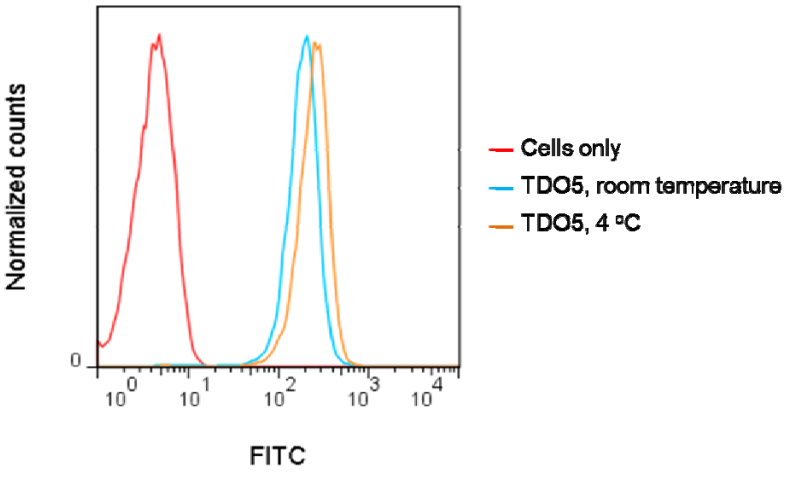

Figure S9. Flow cytometric results verifying the ability of aptamer TDO5 to specifically label Ramos cells at room temperature. TDO5 was labeled with FITC.

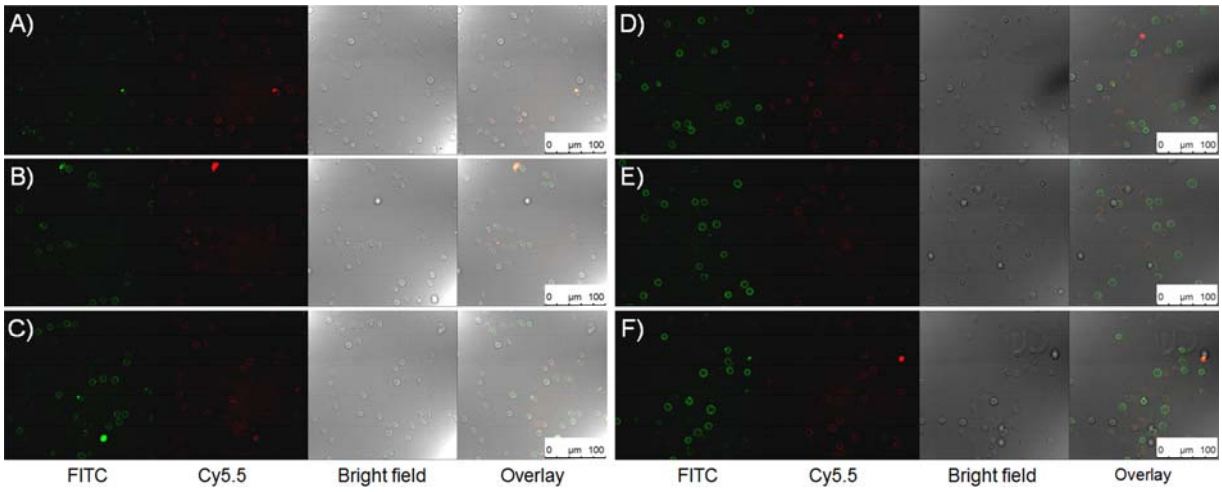

Figure S10. Confocal microscopy images displaying the progressive assembly of fluorescent nanodevices (sgc8-NDs) through HCR for 0.5 h (A), 1 h (B), 1.5 h (C), 2 h (D), 2.5 h (E) and 3 h (F), *in situ* on surfaces of target CEM cells in a cell mixture also containing nontarget Ramos cells, which were labeled with Cy5.5-conjugated TDO5. (Scale bar: 100 μm; M1, M2: labeled with FITC)

References

[1] J. N. Zadeh, C. D. Steenberg, J. S. Bois, B. R. Wolfe, M. B. Pierce, A. R. Khan, R. M. Dirks, N. A. Pierce, *J. Comput. Chem.* **2010**, *32*, 170-173.