Electronic supplementary information

Real-Time Monitoring of DNA Hybridization Kinetics at Living Cell Surfaces

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Supporting Materials and Methods

1. Oligonucleotides and Chemicals: All DNA probes and targets in Table 1 purified by HPLC were purchased from Japan Bio Services Co., Ltd. Concentrations of the oligonucleotide were determined by measuring the absorbance at 260 nm at 90 °C using a Shimadzu 1700 UV/Vis spectrophotometer connected to thermo programmer. Single-strand extinction coefficients were calculated from mononucleotide and dinucleotide data using nearest neighbor approximation.

2. Cell cultures: HL60 cell lines were grown in RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 µg/mL). Cells were grown at 37 °C in 5% CO_2 atmosphere.

3. Immobilization and hybridization of DNA probe on HL60 cell surface: HL60 cells were washed twice with MC-buffer by centrifugation at 600 g for 3 min. Cells resuspended in MC-buffer at 4×10^5/100 µL were incubated with DNA probe with CholTEG moiety (at indicated concentration) at 25 °C for indicated time. After immobilization, cells were centrifuged (unless there is other description) and resuspended in MC-buffer. For hybridization of target DNA, cells were subsequently incubated with target DNA (at indicated concentration) at 25 °C for 10 min, and centrifuged at 600 × g for 3 min followed by resuspension in MC-buffer. Immobilization and hybridization of DNA probe were monitored by flow cytometry (FACS Aria II; BD Biosciences) using 488 nm excitation, and laser scanning microscopy (LSM 700; ZEISS) using 488 nm and 555 nm excitation for imaging of FAM and TAMRA, respectively.

4. Localization of immobilized DNA probe: HL60 cells were co-incubated with CytoRed (0.5 µM) (DOJINDO), which is a molecular probe for cellular cytosol, and Probe-1-Chol (0.5 µM) at 25 °C for 30 min. Fluorescence signals of CytoRed and Probe-1-Chol were analyzed by laser scanning microscopy using 555 nm and 488 nm excitation, respectively. Fluorescence intensities according to an axis, which crosses a cell, were calculated by ZEN 2009 image processing software (ZEISS).

LSM image and plot of fluorescence intensities in Figure S3 show accumulation of CytoRed and Probe-1-Chol at the center and peripheral region of cells, respectively. The results indicated that maximum Probe-1-Chol was localized on cell surface during 30 minutes incubation.

5. Kinetic analysis of DNA hybridization in solution: DNA probe (Probe-1-Chol, Probe-2-Chol, Probe-1, or Probe-2) was diluted in MC-buffer at 22.22 nM and pre-equilibrated to maintain the 25 °C temperature during experiment. An aliquot of 180 µL was injected into wells of a low-adsorbent 96-well plate containing 20 µL of complementary TAMRA-labeled target oligonucleotides at final concentration of 200 nM to 600 nM by using a Thermo Scientific Varioskan Flash fluorescence spectrometer equipped with automatic dispensers. Time course of fluorescence decrease in FAM intensity was monitored with 485 nm excitation and 525 nm emission. The fluorescence signals were recorded at every 100 ms until 60 sec. The first 10 data points were collected before the injection; the first data point collected after injection was assigned as zero (t=0) during data fitting. The instrument was pre-equilibrated at 25 °C to maintain the same temperature during experiment.

6. Kinetic analysis of DNA hybridization on cell surface: HL60 cells (4×10^6) were mixed with 0.2 µM DNA probe (Probe-1-Chol or Probe-2-Chol) in 200 µL of MC-buffer. Cells were incubated at 25 °C for 5-7 min to immobilize the DNA probe on cell surface. Cells were centrifuged, the supernatant was removed, and the cell pellet was resuspended in 1800 µL of MC-buffer. A 180 µL aliquot (4×10^5 cells) was injected into wells of a low-adsorbent 96-well plate as same as kinetic analysis in solution.

7. Calculation of rate constants: Data set of fluorescence signal obtained by injection of DNA probe or HL60 cells into a well, which does not contain the target DNA was subtracted from the data sets of various target DNA concentrations to normalize the fluoresce decrease depending on the hybridization. The following first order equation was used to fit the normalized data for determination of association and dissociation rate constants:

Equation-1 $$\mathbf{Y=A+ B \times (1-exp (-X \times t))}$$

where A is fluorescence intensity at time zero, B is amplitude of fluorescence change, and –X is apparent rate constant (k_{app}). k_{app} is equal to ($k_a\times$ initial concentration of target DNA + k_d), where k_a is association rate constant and k_d is dissociation rate constant. From linear plot of k_{app} depending on the initial concentration of the target DNA, k_a (slope) and k_d (y-axis intercept) were calculated.

Table S1. Association rate constants (k_a) of probes with and without CholTEG in solution in the absence and presence of 20 μM of β-cyclodextrin

β-cyclodextrin μM	Base pairing Probe + Target	In solution $k_a \times 10^5$ / M^{-1} s^{-1}	Base pairing Probe + Target	In solution $k_a \times 10^5$ / M^{-1} s^{-1}
0	Probe-1 + Tar-1-15	1.93	Probe-1-Chol + Tar-1-15	0.90
20	Probe-1 + Tar-1-15	1.95	Probe-1-Chol + Tar-1-15	1.36

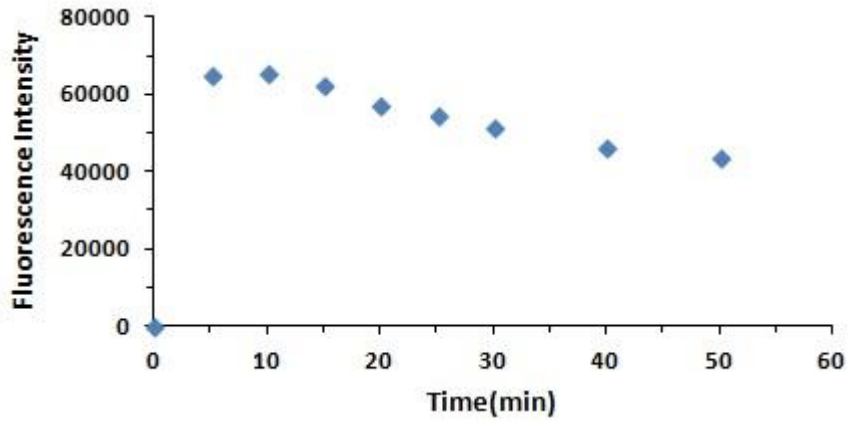

Figure S1. Flow cytometer analysis for optimization of immobilization time. HL60 cells were incubated with Probe-1-Chol (0.5 μM) at 25 °C. An aliquot of 20 μL was removed at given times and directly diluted into 180 μL of MC-buffer without centrifugation. Fluorescence intensities were measured by flow cytometry.

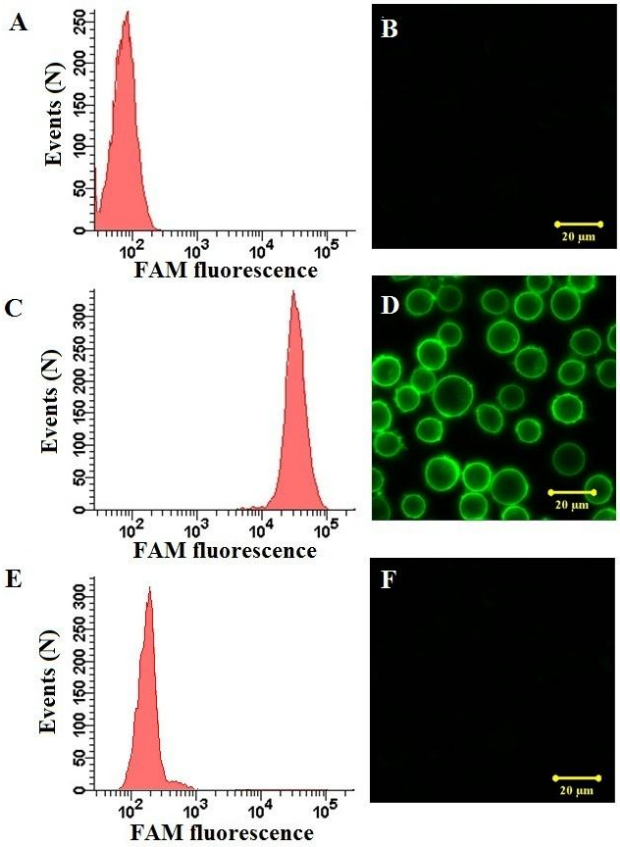

Figure S2. Flow cytometer analyses and LSM images of HL60 cells incubated with DNA probes. (A) and (B) HL60 cells incubated without DNA. (C) and (D) HL60 cells incubated with 0.5 μM Probe-1-Chol at 25 °C for 5 min. (E) and (F) HL60 cells incubated with 0.5 μM Probe-1 at 25 °C for 10 min.

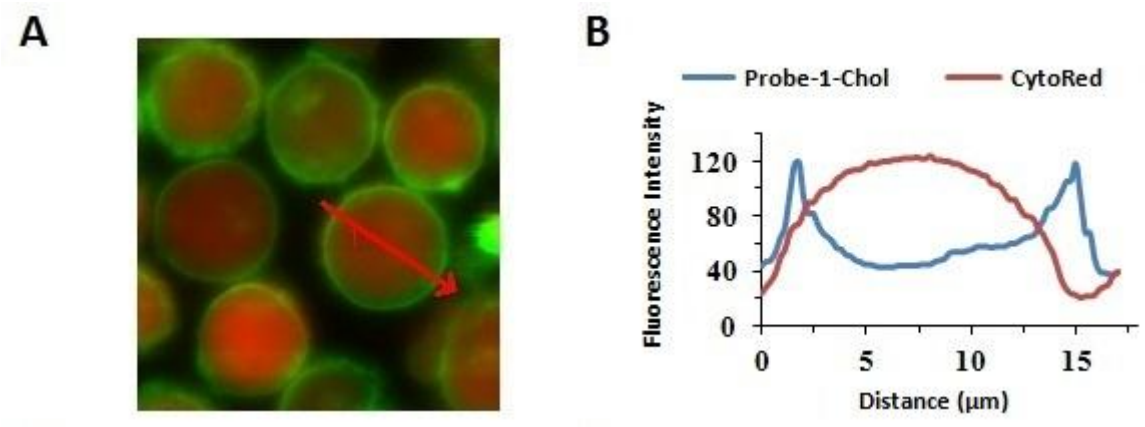

Figure S3. Localization of DNA probe at plasma membrane on cell surface A) LSM image of HL60 cells co-incubated with Probe-1-Chol and CytoRed (0.5 μM each) in MC-buffer at 25 °C for 30 minutes. B) Fluorescence intensity profiles were plotted across the red line shown in panel A.

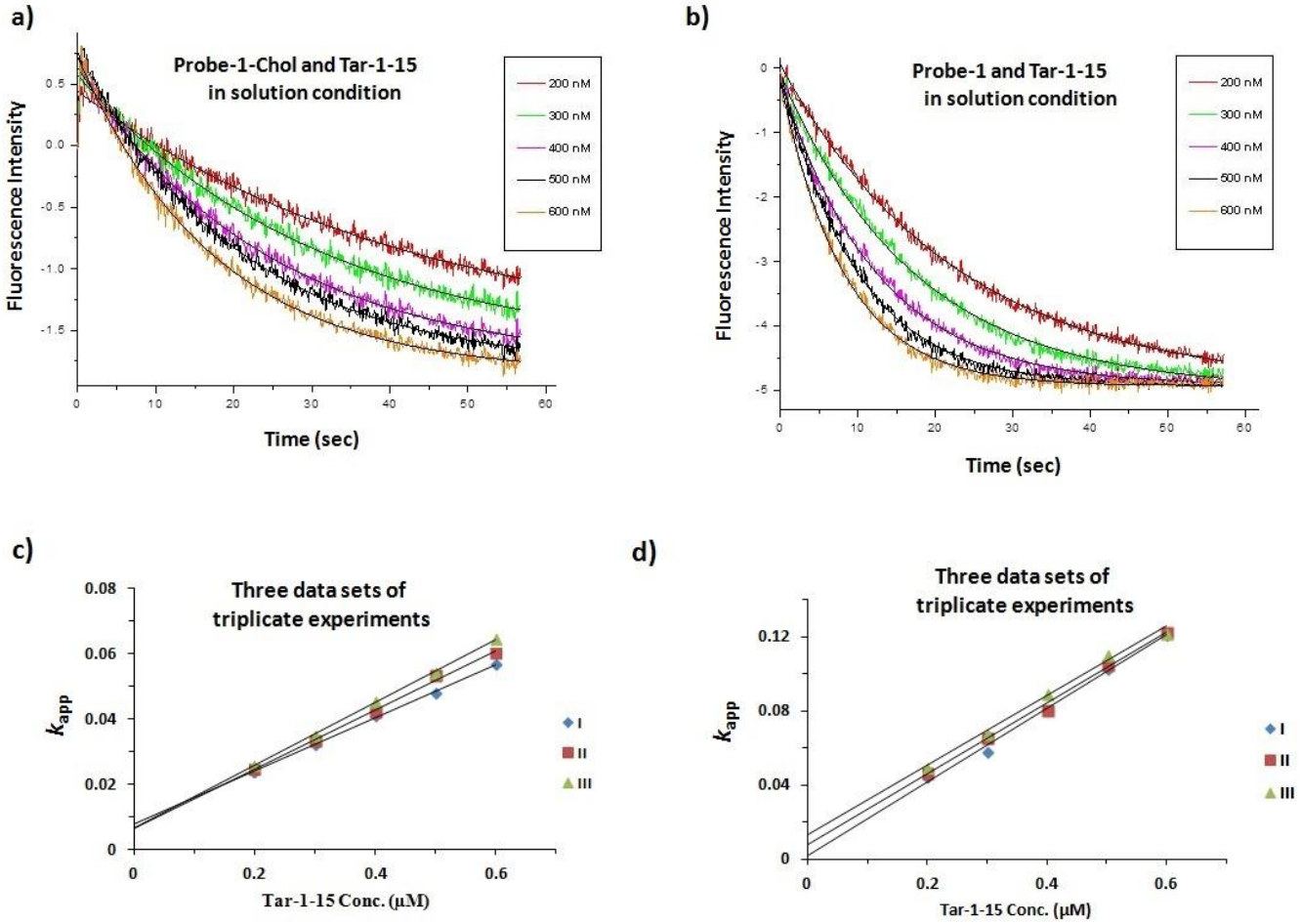

Figure S4. Example of pseudo first-order fits of in solution hybridization data of with and without CholTEG labeled probes. a) and b) kinetics data from analysis of Probe-1-Chol and Probe-1 (final concentration of 20 nM) association with various concentrations of Tar-1-15, respectively. Theoretical curves fitted in the pseudo first-order equation are shown in black. c) and d) k_{app} values for Probe-1-Chol and Probe-1 calculated from the fitting were plotted on the initial concentrations of Tar-1-15, respectively. Black line shows fitted linear line for each data sets (data from triplicated experiments are shown).

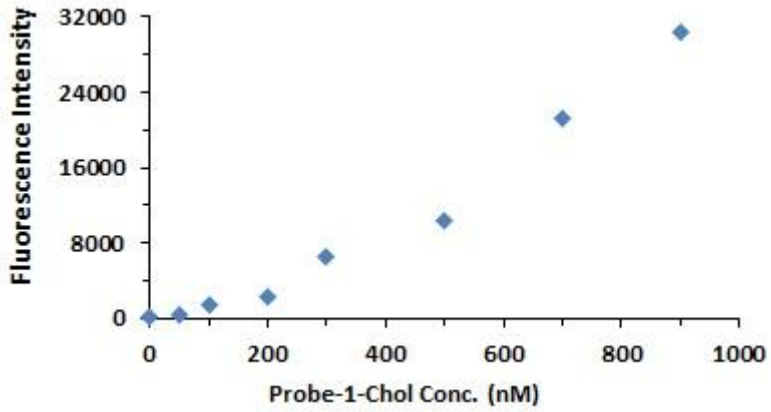

Figure S5. Fluorescence intensities on the cell surface depending on concentration of DNA probe. HL60 cells were incubated with various concentrations of Probe-1-Chol ranging from 50 nM to 900 nM at 25 °C for 5 minutes washed and diluted ten times before analyzing fluorescence intensities by flow cytometery.

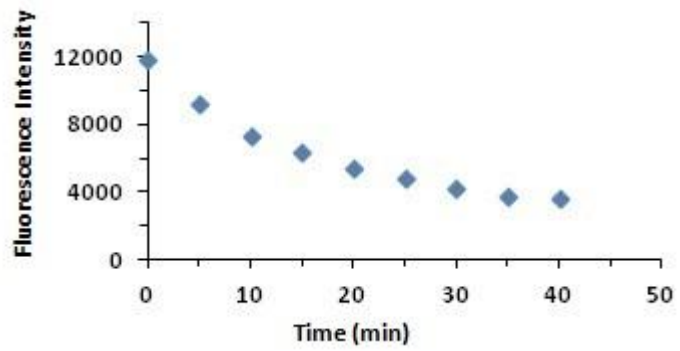

Figure S6. Stability of immobilized DNA probe on the cell surface. HL60 cells were incubated with Probe-1-Chol (0.2 µM) for 5-10 minutes at 25 °C and then centrifuged to remove supernatant. The cell pellet was resuspended in 10 times volume of the original volume of MC-buffer used for immobilization. Fluorescence intensity of cells was analyzed by flow cytometer at given times after the resuspention.

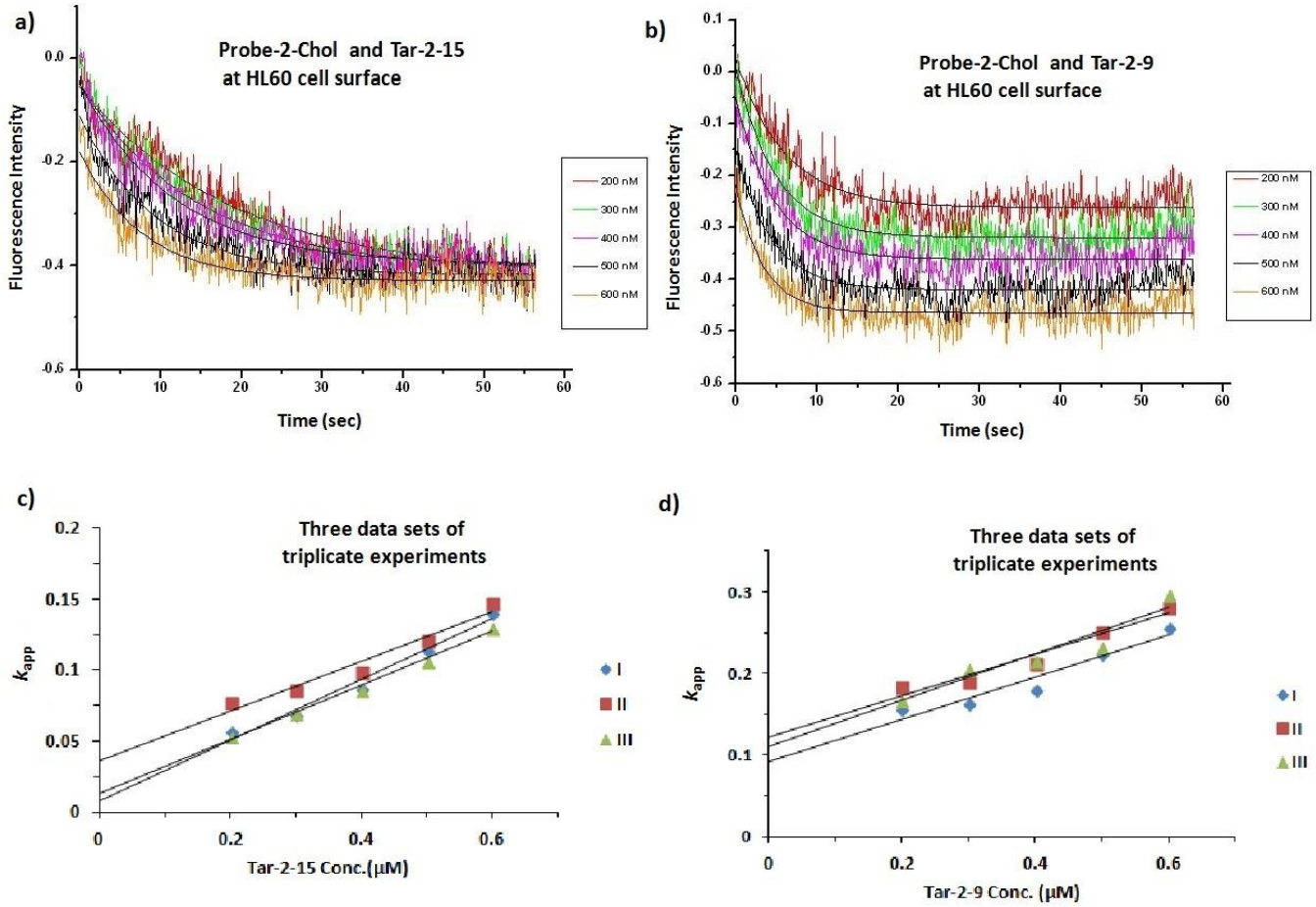

Figure S7. Example of pseudo first-order fits of at cell surface hybridization data. a) and b) kinetics data from analysis of Probe-2-Chol association with various concentrations of Tar-2-15 and Tar-2-9, respectively. Theoretical curves fitted in the pseudo first-order equation are shown in black. c) and d) k_{app} values for Probe-2-Chol calculated from the fitting were plotted on the initial concentrations of Tar-2-15 and Tar-2-9, respectively. Black line shows fitted linear line for each data sets (data from triplicated experiments are shown).