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Supporting Information

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DNA logic gates based on structural polymorphism of telomere DNAs responding to chemical input signals

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Experimental

Oligodeoxynucleotides lacking attached fluorescence probes were chemically synthesized on a solid support using the standard phosphoramidite method.[4] HPLC-grade fluorescence-labeled oligodeoxynucleotides were purchased from Takara Bio Co., Ltd. (Kyoto, Japan) or Greiner Japan Co., Ltd. (Tokyo, Japan). Prior to the measurements, the oligodeoxynucleotides were heated to 90°C, gently cooled at a rate of 1.0°C/min, and incubated for 1 h at the desired temperature.

CD spectra were collected for 50 μM total strand concentration of DNA at 4°C in a 0.1-cm path length cuvette with a J-820 spectropolarimeter (JASCO, Hachioji, Japan). The CD spectra were obtained by taking the average of at least three scans between 200 and 350 nm. The temperature of the cell holder was regulated by a PTC-348 temperature controller (JASCO), and the cuvette-holding chamber was flushed with a constant stream of dry N_2 gas to avoid condensation of water on the cuvette exterior. Before the measurement, the sample was heated to 90°C, gently cooled at a rate of 1.0°C min^{-1}, and incubated at 4°C for 1 h.

ITC analysis of duplex formation was carried out using a MicroCal VP-ITC isothermal titration calorimeter (MicroCal, MA, USA). The titration of 17 μM C-strand on 2.0 μM G-strand was carried out at 10°C in buffers containing 20 mM Li^+ and 50 mM Tris-HCl (pH 8.0); 20 mM K^+ and 50 mM Tris-HCl (pH 8.0); 20 mM Li^+ and 50 mM MES (pH 5.0); or 20 mM K^+ and 50 mM MES (pH 5.0). Before the measurement, the sample was heated to 90°C, gently cooled at a rate of 1.0°C min^{-1}, and incubated at 10°C for 1 h.

Fluorescence spectra were measured for 1.0 μM total strand concentration of DNA at 4°C using a FP-6500 spectrofluorometer (JASCO) with a 0.3-cm path length quartz cell. The excitation wavelength was 504 nm. Normalization of the fluorescence intensities

was performed as follows: F = (fluorescence intensity at each condition) / (fluorescence intensity at i_1=0 and i_2=0), where F is the relative fluorescence intensity at each condition. Before the measurement, the sample was heated to 90°C, gently cooled at a rate of 1.0°C min^{-1}, and incubated at 4°C for 1 h.

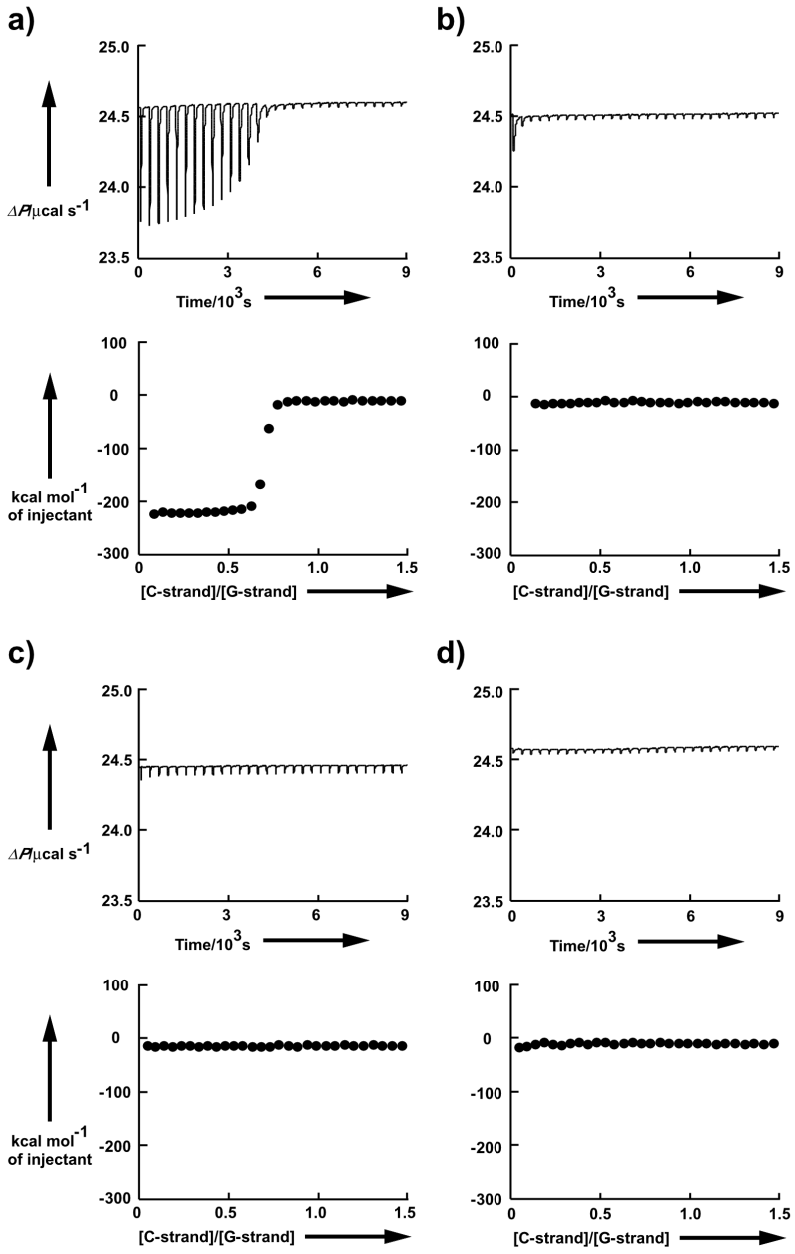

Figure S1. ITC profiles of $d(G_4T_4)_3G_4$ and $d(C_4A_4)_3C_4$. The profiles were measured at 10°C and in the presence of (a) 20 mM Li^+ and 50 mM Tris-HCl (pH 8.0), (b) 20 mM K^+ and 50 mM Tris-HCl (pH 8.0), (c) 20 mM Li^+ and 50 mM MES (pH 5.0), or (d) 20 mM K^+ and 50 mM MES (pH 5.0).

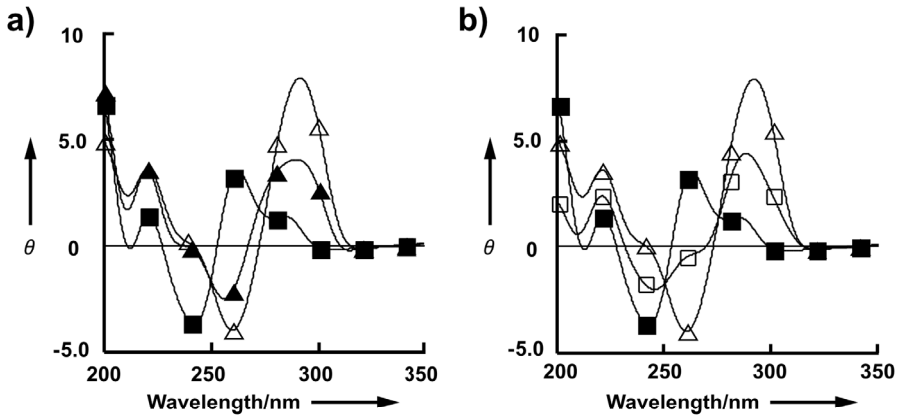

Figure S2. a) CD spectra (θ in 10^5 deg cm^2 dmol^{-1}) of the 1:1 mixture of $d(G_4T_4)_3G_4$ and $d(C_4A_4)_3C_4$ in the presence of 20 mM Li^+ and 50 mM Tris-HCl (pH 8.0) (filled squares); 20 mM Li^+, 100 mM K^+, and 50 mM Tris-HCl (pH 8.0) (filled triangles); or 20 mM Li^+, 100 mM K^+, and 50 mM Tris-HCl (pH 5.0) (open triangles). b) CD spectra (θ in 10^5 deg cm^2 dmol^{-1}) of the 1:1 mixture in the presence of 20 mM Li^+ and 50 mM Tris-HCl (pH 8.0) (filled squares); 20 mM Li^+ and 50 mM Tris-HCl (pH 5.0) (open squares); or 20 mM Li^+, 100 mM K^+, and 50 mM Tris-HCl (pH 5.0) (open triangles). All measurements were carried out at 4°C with a total strand concentration of 50 μM.

After the addition of excess of K^+ (100 mM) to the duplex in the presence of 20 mM Li^+ at pH 8.0 and annealing, the CD spectrum of the mixture indicated that the G- and C-strands fold into G-quadruplexes and random coils, respectively (Fig. S2a). Next, the G- and C-strands were converted to G-quadruplexes and i-motifs, respectively, by lowering the pH (to pH 5.0) with HCl and annealing (Fig. S2a). Similarly, lowering the pH to 5.0 converted the duplex to a random coil and i-motif (Fig. S2b). Furthermore, the structural conversion from a random coil and an i-motif to a G-quadruplex and i-motif was induced by the addition of 100 mM K^+ (Fig. S2b). These results indicate that the continuous structural conversions of the G- and C-strands can be induced by changes in the surrounding conditions.

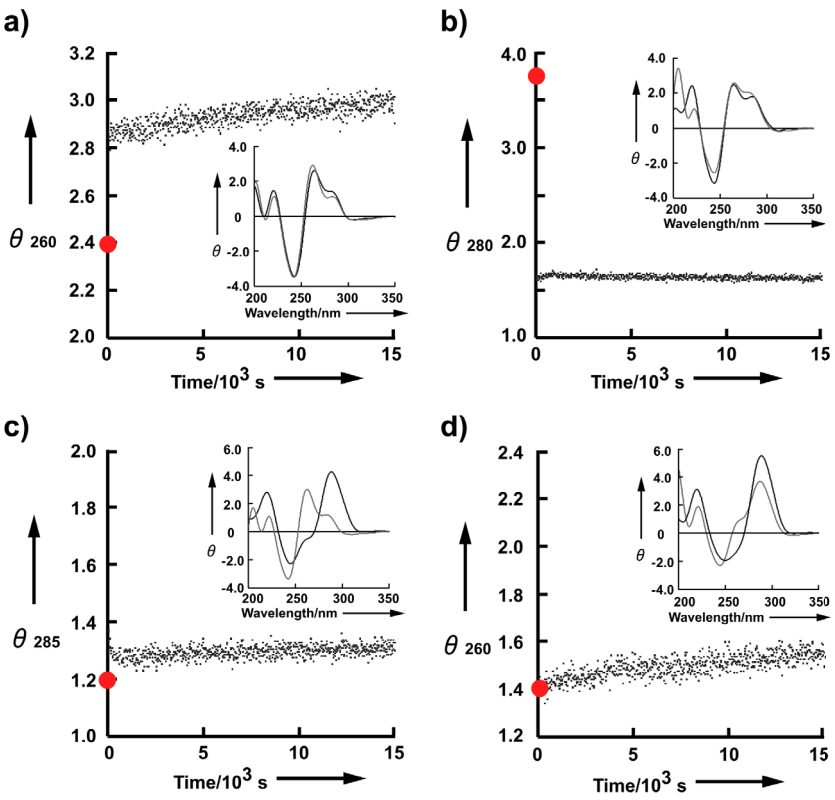

Figure S3. a) Time course (θ in 10^5 deg cm^2 dmol^{-1}) of the structural conversion of the mixture from a G-quadruplex and random coil of the C-stand to a duplex of the G- and C-stands. The conversion was induced by adding an excess of LiCl (final concentrations of Li^+ and K^+ were 1 M and 37 mM, respectively). b) Time course of the structural conversion from the G-quadruplex and the i-motif to the G-quadruplex and random coil of the C-strand. The conversion was induced by adjusting the pH to 8.0 with KOH (final concentrations of Li^+ and K^+ were 89 and 105 mM, respectively). c) Time course of the structural conversion from a duplex of G- and C-strands to a random coil of the G-strand and the i-motif. The conversion was induced by adjusting the pH to 5.0 with HCl (final concentrations of Li^+ and K^+ were 938 and 35 mM, respectively). d) Time course of the structural conversion from a random coil of the G-strand and an i-motif to a G-quadruplex and an i-motif. The conversion was induced by adding an excess of KCl (final concentrations of Li^+ and K^+ are 94 and 62 mM, respectively). To clarify the initial point of each time course, the initial point is highlighted with a red circle. Insets: CD spectra of the mixture after the reaction and annealing (gray line) and that after the annealing in the presence of 20 mM Li^+ at pH 8.0 (a), 20 mM K^+ at pH 8.0 (b), 20 mM Li^+ at pH 5.0 (c), or 20 mM K^+ at pH 8.0 (d) (black line). All measurements were carried out after annealing at a total strand concentration of 50 μM in a buffer containing 50 mM MES at 37°C.

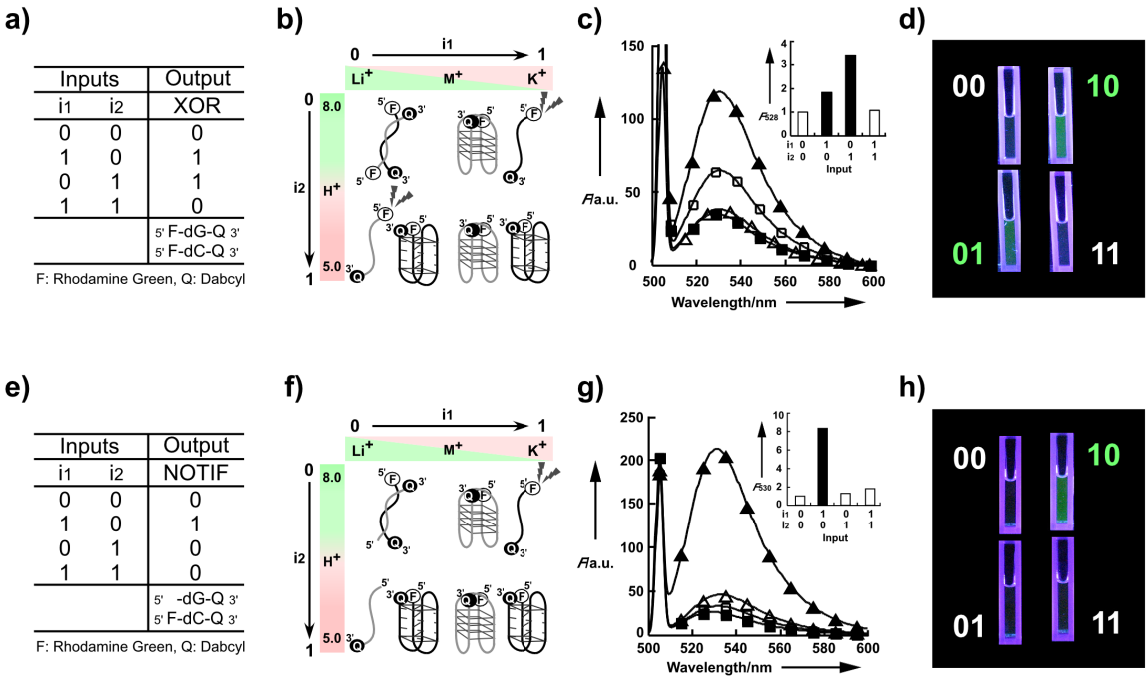

Figure S4. The truth table and molecular design of the XOR (a) and NOTIF (e) logic gates. Schematic illustration of the XOR (b) and NOTIF (f) functions of a 1:1 mixture of G- and C-strands and its response to M^+ and H^+. F and Q indicate Rhodamine Green and Dabcyl, respectively. Gray and black lines in the scheme indicate the G- and C-rich strands, respectively. Fluorescence spectra of the XOR (c) and NOTIF (g) logic gates in the presence of 20 mM Li^+ at pH 8.0 (filled squares), 20 mM K^+ at pH 8.0 (filled triangles), 20 mM Li^+ at pH 5.0 (open squares), or 20 mM K^+ at pH 5.0 (open triangles). All measurements were carried out at a total strand concentration of 1 μM and at 4°C. Inset: normalized fluorescence intensities at 528 nm for the XOR logic gate and at 530 nm for the NOTIF logic gate at 4°C and (i_1=0, i_2=0), (i_1=1, i_2=0), (i_1=0, i_2=1), or (i_1=1, i_2=1). Fluorescence images of the XOR (d) and NOTIF (h) logic gates. All measurements were carried out at room temperature, with a total strand concentration of 1 μM and (i_1=0, i_2=0), (i_1=1, i_2=0), (i_1=0, i_2=1) or (i_1=1, i_2=1).

Inputs		Outputs						
i_1	i_2	AND	OR	NAND	NOR	XOR	XNOR	NOTIF
0	0	0	0	1	1	0	1	0
1	0	0	1	1	0	1	0	1
0	1	0	1	1	0	1	0	0
1	1	1	1	0	0	0	1	0
		$^{5'}$A-dG-D$^{3'}$ $_{5'}$D-dC-A$_{3'}$	-dG-Q F-dC-	Q-dG-F F-dC-Q	-dG-D A-dC-	F-dG-Q F-dC-Q	A-dG-D A-dC-D	-dG-Q F-dC-Q

A : acceptor D : donor
F : fluorescence Q : quencher

Figure S5. The truth table and molecular design of the various logic gates (AND, OR, NAND, NOR, XOR, XNOR, and NOTIF). dG, G-strand; dC, C-strand; A, fluorescence acceptor; D, fluorescence donor; F, fluorophore; Q, quencher. For example, to perform an AND function, the mixture should fluoresce only when the duplex is formed. For the AND function, fluorescence acceptors should be attached to 5'-end of the G-strand and the 3'-end of the C-strand, whereas fluorescence donors should be attached to 3'-end of the G-strand and the 5'-end of the C-strand. This design leads to highest efficiency of fluorescence resonance energy transfers between two couples of the acceptor and the donor only when the G- and C-strands fold into the duplex.