Supporting Information

A Fluorescent Probe for 3′-Overhang of Telomeric DNA Based on Competition Between Two Interstrand G-Quadruplexes

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1. General experimental details

General experimental details

Each reaction was performed under anhydrous conditions under Ar or N_2. All reagents were purchased from Sigma–Aldrich, Fluka, Proligo, and Glen Research and used without additional purification. Most solvents were used without distillation, except for those for phosphoramidite synthesis. We obtained the MC, THF, DMF from distill tower. High-resolution fast atom bombardment (HRMS-FAB) mass spectra were recorded using a Jeol JMS700 HR mass spectrometer at the Korea Basic Science Center, Daegu, Korea. MALDI-TOF mass spectra were recorded at Bioneer, Taejon, Korea. ^{1}H NMR spectra were recorded using a FT-300MHz Bruker Aspect 3000 spectrometer. UV and fluorescence spectra were recorded using Cary 100 and Eclipse spectrometers (Varian). Samples for UV/fluorescence spectroscopy were prepared in a quartz cell (path length: 1 cm). DNA synthesis was performed using a Polygen 12 synthesizer. An Agilent high-performance liquid chromatography system (1100 Series) was used to purify the synthesized ODNs; Agilent, ZORBA X Eclipse XDB-C18, 4.6 × 150 mm; gradient elution: 0 min, A:B = 90:10; 10 min, A:B = 90:10; 20 min, A:B = 0:100; 25 min, A:B = 0:100; 30 min, A:B = 90:10; solution A, 0.1 M triethylammonium acetate (TEAA) buffer (pH 7.2)/MeCN = 95:5; solution B, 0.1 M TEAA buffer (pH 7.2)/MeCN = 1:1); flow rate: 2.5 mL/min; UV detection: 254 nm.

DNA sample preparation for fluorescence measurement

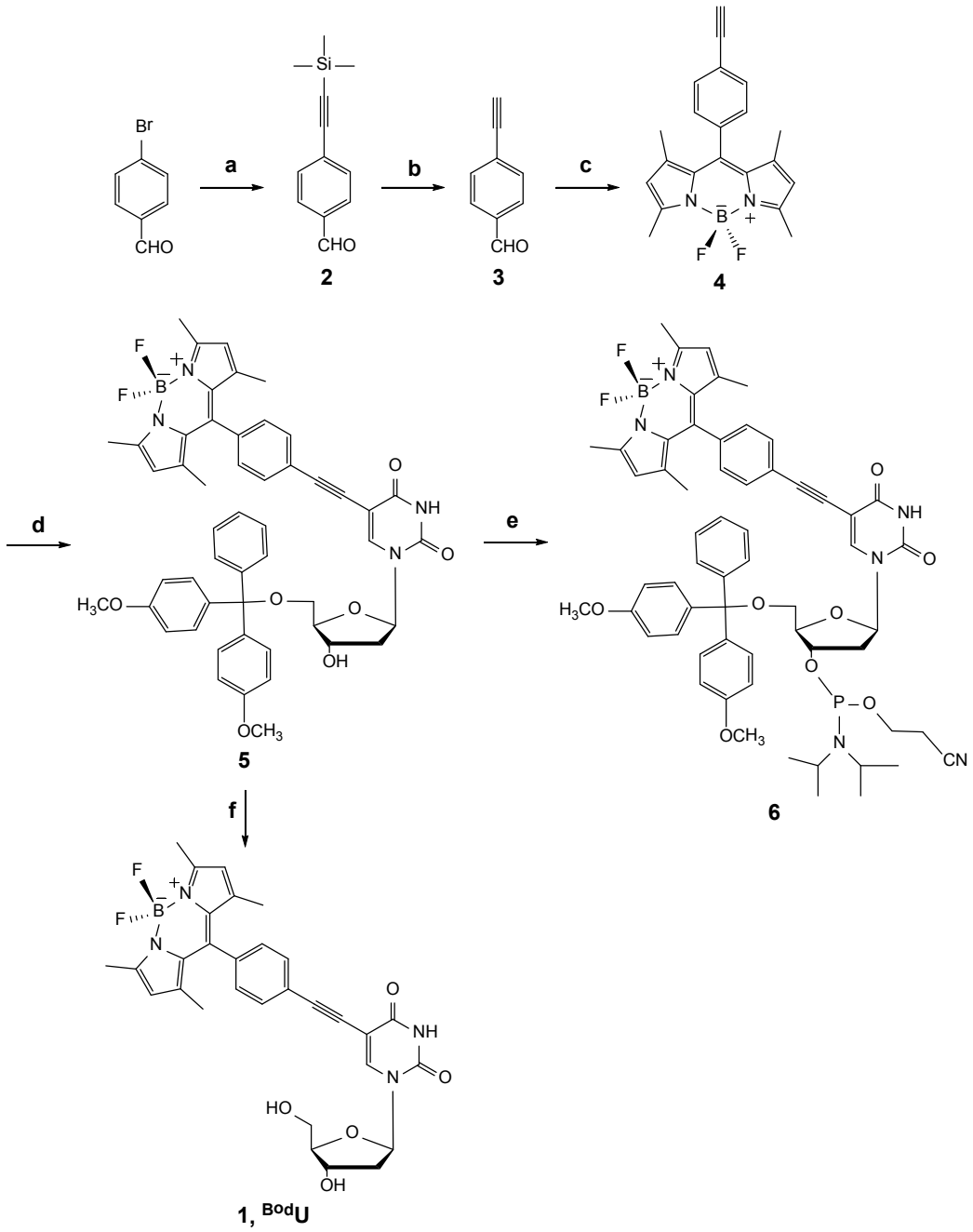
In 1.5 mL microtube, 10 μL of 150 μM **ODN-B** and a target ODN (1 equiv.) were added and mixed by vortex for 0.5 min. And 300 μL of 1 M KCl solution was added to the mixture. After addition of 50 μL of 200mM pH 7.2 Trizma buffer and water to make total 1 mL, solution was mixed by vortex for 3 min for fluorescence measurement. For annealed sample, we kept the sample at 90°C for 3 min. and cooling down at ambient condition for 3 h.

WST-1 assay

ODN-B was added to the cells. Cells were incubated at 37°C under 5% CO_2 for 24 h. The medium was removed after incubation. With DPBS buffer (100 mL), the cells were washed three times followed by addition of WST-1 reagent (1 mg mL-1 of WST-1 dissolved in phenol red free medium, Roche; 100 mL) to each well. The mixtures were incubated at 37°C for 2 h. After that, absorbance was measured at a wavelength of 450 nm using a microplate reader (Asys) to check cell viability (relative to control cells).

2. Synthesis of ^{Bod}U

Scheme S1 Synthesis of ^{Bod}U



Reagents and conditions: (a) TMSA (1.2 eq), TPP (0.05 eq), $PdCl_2$ (0.01 eq), $Cu(OAc)_2$ (0.01 eq), TEA, reflux, 6 h, 97%; (b) K_2CO_3 (10% mol), MeOH, r.t., 2 h, 96%; (c) 1. 2,4-dimethylpyrrole (2.1 eq), TFA (2 drops), DCM, r.t., 20 h.; 2. *p*-chloranil (1 eq), DCM, 2 h; 3. TEA (6 eq), $BF_3{\cdot}OEt_2$ (18 eq), DCM, 6 h, 28%; (d) $Pd(PPh_3)_2Cl_2$ (0.05 eq), $CuOAc_2$ (0.01 eq), 5-iodo-2′-deoxyuridine (1 eq), DIPEA (4 eq), DMF, 50 °C, 6 h, 85%; (e) CEP-Cl (1.5 eq), NMM (3 eq), DCM, r.t., 45 min, 94%; (f) aq. 80% AcOH, r.t., 1 h, 90%.

3. Synthetic procedures and compound characterization data

The syntheses of **2** and **3** were performed using previously reported procedures[1]

4,4-Difluoro-1,3,5,7-tetramethyl-8-(4-ethynylphenyl)boradiazaindacene (4)

2,4-Dimethylpyrrole (1.0 mL, 9.7 mmol) was added to a solution of **3** (494 mg, 3.8 mmol) in anhydrous DCM (40 mL) and then the mixture was stirred at room temperature for 15 min. Next, TFA (2 drops) was added and the mixture stirred for 20 h; the color of the mixture changed to red. *p*-Chloranil (970 mg, 3.8 mmol) in anhydrous DCM (200 ml) was added and the mixture stirred for 2 h. This solution was concentrated and the residue dissolved in anhydrous DCM (15 mL). Anhydrous triethylamine (3.2 mL, 23.1 mmol) was added and then the mixture was stirred at room temperature for 30 min. Next, $BF_3{\cdot}OEt_2$ (8.7 mL, 69.5 mmol) was added dropwise to the flask at 0 °C. The mixture was warmed to room temperature, stirred for 6 h, and then the reaction was quenched through the addition of water (100 mL). The organic phase was washed with water and concentrated. The solid residue was purified chromatographically (SiO_2; DCM/hexane = 1:4) to give compound **4** (370 mg, 28%); δ_H (300 MHz, $CDCl_3$) 1.39 (s, 6H, CH_3), 2.55 (s, 6H, CH_3), 3.18 (s, 1H, acetylene) 5.98 (s, 2H, Bodipy-H), 7.25 (d, J = 7.8 Hz, 2H, PhH), 7.61 (d, J = 8.1 Hz, 2H, PhH); δ_C (75 MHz, $CDCl_3$) 14.7, 14.8, 78.8, 83.1, 121.6, 123.2, 128.4, 130.0, 131.3, 132.6, 133.0, 135.7, 140.7, 143.1, 156.0; δ_F (280 MHz, $CDCl_3$, $CFCl_3$) –146.1; HRMS-FAB: calcd for $C_{21}H_{19}BF_2N_2$ (M^+), m/z 348.1609; found 348.1612.

3′-Dimethoxytrityl-5-[4-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3*a*,4*a*-diaza-*s*-indacyl)phenyl]ethynyl-2′-deoxyuridine (5)

Diisopropylethylamine (0.52 mL, 3.00 mmol) was added to a solution of **4** (420 mg, 1.21 mmol), $Pd(PPh_3)_2Cl_2$ (35 mg, 0.05 mmol), $CuOAc_2$ (2 mg, 0.01 mmol), and 5-iodo-2′-deoxyuridine (656 mg, 1.00 mmol) in anhydrous DMF (20 mL) and then the mixture was stirred for 5 h at 45–55 °C. The solvent was evaporated and the residue purified chromatographically (SiO_2; MeOH/DCM, 1:20) to give **5** (740 mg, 85%); δ_H (300 MHz, DMSO-d_6, Me_4Si) 1.33 (s, 6H, Bodipy-CH_3), 2.28–2.31 (m, 2H, 2′-H), 2.44 (s, 6H, Bodipy-CH_3), 3.21–3.22 (m, 2H, 5′-H), 3.67 (s, 6H, OCH_3), 3.98–3.99 (m, 1H, 3′-H), 4.35 (m, 1H, 4′-H), 5.38 (d, J = 4.5 Hz, 1H, 3′-OH), 6.15 (s, 1H, 1′-H), 6.18 (s, 2H, Bodipy-H), 6.84–6.87 (m, 4H, PhH), 7.14–7.18 (m, 3H, PhH), 7.25–7.32 (m, 8H, PhH), 7.41–7.43 (m, 2H, PhH), 8.19 (s, 1H, 6-H), 11.8 (s, 1H, NH); δ_C (75 MHz, $CDCl_3$, Me_4Si) 14.1, 14.2, 40.1, 54.9, 55.0, 63.6, 70.4, 83.2, 85.3, 86.0, 86.2, 91.3, 98.3, 113.2, 121.5, 123.1, 126.7, 127.6, 127.9, 128.0, 129.6, 129.7, 130.4, 131.9, 133.9, 135.4, 135.6, 141.0, 142.6, 1 14.1, 14.2, 54.9, 55.0, 63.6, 70.4, 83.2, 85.3, 86.0, 86.2, 91.3, 98.3, 113.2, 121.5, 123.1, 126.7, 127.6, 127.9, 128.0, 129.6, 129.7, 130.4, 131.9, 133.9, 135.4, 135.6, 141.0, 142.6, 143.3, 144.7, 149.3, 155.1, 158.1, 161.4; δ_F (280 MHz, $CDCl_3$, $CFCl_3$) –145.0; HRMS-FAB: calcd for $C_{51}H_{47}BF_2N_4O_7$ (M^+) m/z 876.3506; found: 876.3510.

5-[4-(4,4-Difluoro-1,3,5,7-tetramethyl-4-bora-3*a*,4*a*-diaza-*s*-indacyl)phenyl]ethynyl-2′-deoxyuridine (^{Bod}U, 1)

A solution of **5** (30 mg, 0.034 mmol) in 80% aqueous AcOH (10 mL) was stirred for 1 h and then concentrated. The residue was purified chromatographically (SiO_2; MeOH/DCM, 1:15) to obtain **1** (18 mg, 90%); δ_H (300 MHz, DMSO-d_6, Me_4Si) 1.39 (s, 6H, Bodipy-CH_3), 2.15–2.19 (m, 2H, 2′-H), 2.45 (s, 6H, Bodipy-CH_3), 3.61–3.66 (m, 2H, 5′-H), 3.81–3.82 (m, 1H, 3′-H), 4.25–4.28 (m, 1H, 4′-H), 5.19 (t, J = 4.8 Hz, 1H, 5′-OH) 5.28 (d, J = 4.5 Hz, 1H, 3′-OH), 6.14 (t, J = 6.6 Hz, 1H, 1′-H), 6.20 (s, 2H, Bodipy-H), 7.43 (d, J = 8.4 Hz, 2H, PhH), 7.65 (d, J = 8.4 Hz, 2H, PhH), 8.44 (s, 1H, 6-H), 11.7 (s, 1H, NH); δ_C (75 MHz, $CDCl_3$, Me_4Si) 14.2, 40.1, 60.8, 69.9, 83.8, 84.9, 87.6, 91.2, 97.9, 121.5, 123.3, 128.5, 130.5, 132.1, 134.2, 141.0, 142.6, 144.2, 149.4, 155.2, 161.4; δ_F (280 MHz, $CDCl_3$, $CFCl_3$) –143.5; HRMS-FAB: calcd for $C_{30}H_{29}BF_2N_4O_5$ (M^+): m/z 574.2199; found: 574.2194.

5-[4-(4,4-Difluoro-1,3,5,7-tetramethyl-4-bora-3*a*,4*a*-diaza-*s*-indacyl)-phenyl]ethynyl-2′-deoxyuridine Phosphoramidite (6)

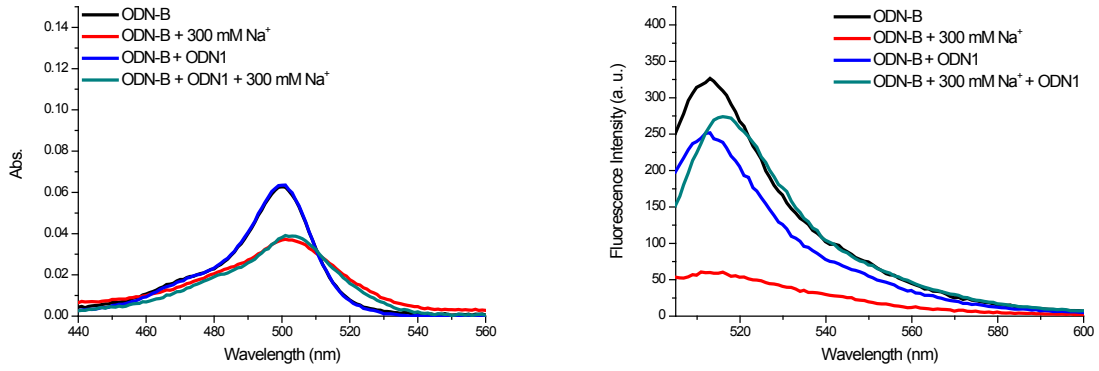
A solution of **5** (280 mg, 0.32 mmol) in anhydrous DCM (10 mL) and stirred for 10 min and then 4-methylmorpholine (0.1 mL, 1.00 mmol) was added and the mixture stirred for 30 min. 2-Cyanoethyldiisopropylchlorophosphoramidite (0.11 mL, 0.5 mmol) was added and then the mixture was stirred for 30 min. At this point, two spots for isomeric phosphoramidites appeared (TLC); the organic solution was quickly washed with water and DCM containing 2% of TEA then concentrated. The residue was dissolved in a small amount of DCM and then hexane was added; the solid was filtered off and the precipitation process repeated several times. To remove residual TEA, EtOAc was added (10 mL) and evaporated. Finally, MeCN was added (10 mL) and evaporated to give **6** (322 mg, 94%); δ_F (280 MHz, $CDCl_3$,$CFCl_3$) –147.5; δ_P (120 MHz, $CDCl_3$, 85% H_3PO_4) 148.6, 149.0; HRMS-FAB: calcd for $C_{60}H_{64}BF_2N_6O_8P$ (M^+) m/z 1076.4584; found: 1076.4580.

4. Photophysical and T_m data for ^{Bod}U nucleoside and oligonucleotides

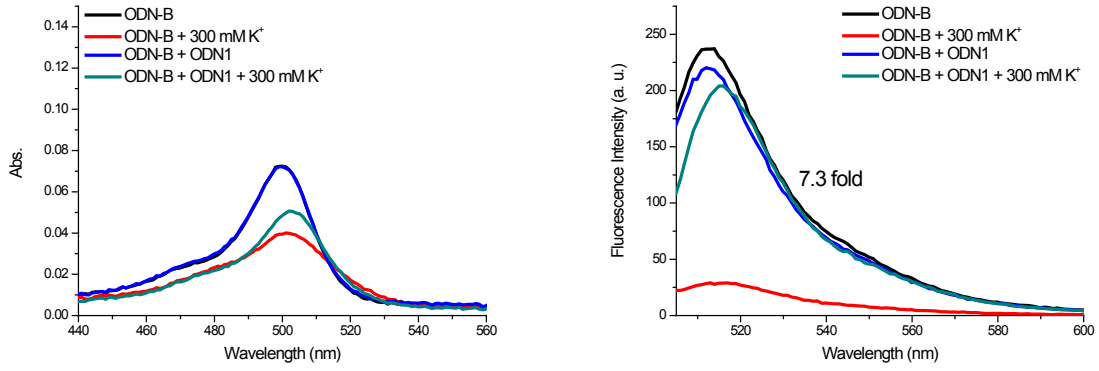
Table S1 Photophysical data for the fluorescent nucleoside ^{Bod}U

Solvent	Absorption Max.[a] (nm)	Emission Max.[a] (nm)	Extinction Coefficient [a]	φ_F [a b]
$CHCl_3$	504	517	16,332	0.44
MeOH	499	510	16,383	0.33
MeCN	499	511	15,753	0.32
Water	504	516	N. A.	N. A.

[a] Measured at 30 μM at 298 K. [b] Fluorescence quantum yield measured relative to fluorescein (φ_F = 0.79) in 0.1 M NaOH.

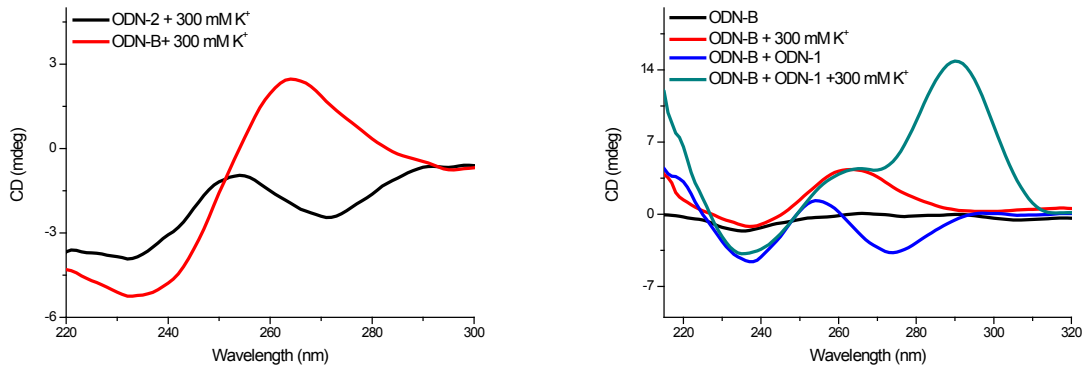


(a) (b)



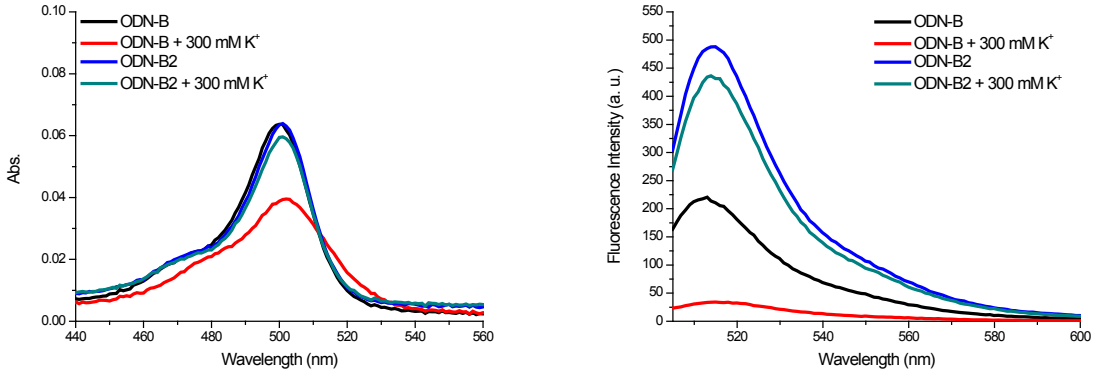
(c) (d)

Figure S1 UV absorption and fluorescence emission spectra of **ODN-B** in the presence of (a, b) Na^+ and (c, d) K^+ ions. (1.5 μM **ODN-B**, 10 mM trizma buffer, pH 7.2, total volume of sample: 1 mL, 20 °C, excitation wavelength: 500 nm).



(a) **(b)**

Figure S2 CD spectra of (a) **ODN-B** and **ODN-2** in the presence of K^+ ions and (b) **ODN-B** in the presence and absence of **ODN-1** and K^+ ions. (5 μM **ODN-B**, 10 mM trizma buffer, pH 7.2, total volume of sample: 1 mL, 20 °C)



(a) (b)

Figure S3 (a) UV absorption and (b) fluorescence emission spectra of **ODN-B** and **ODN-B2** in the presence and absence of K^+ ions. (1.5 μM **ODN-B,** 10 mM trizma buffer, pH 7.2, total volume of sample: 1 mL, 20 °C, excitation wavelength: 500 nm)

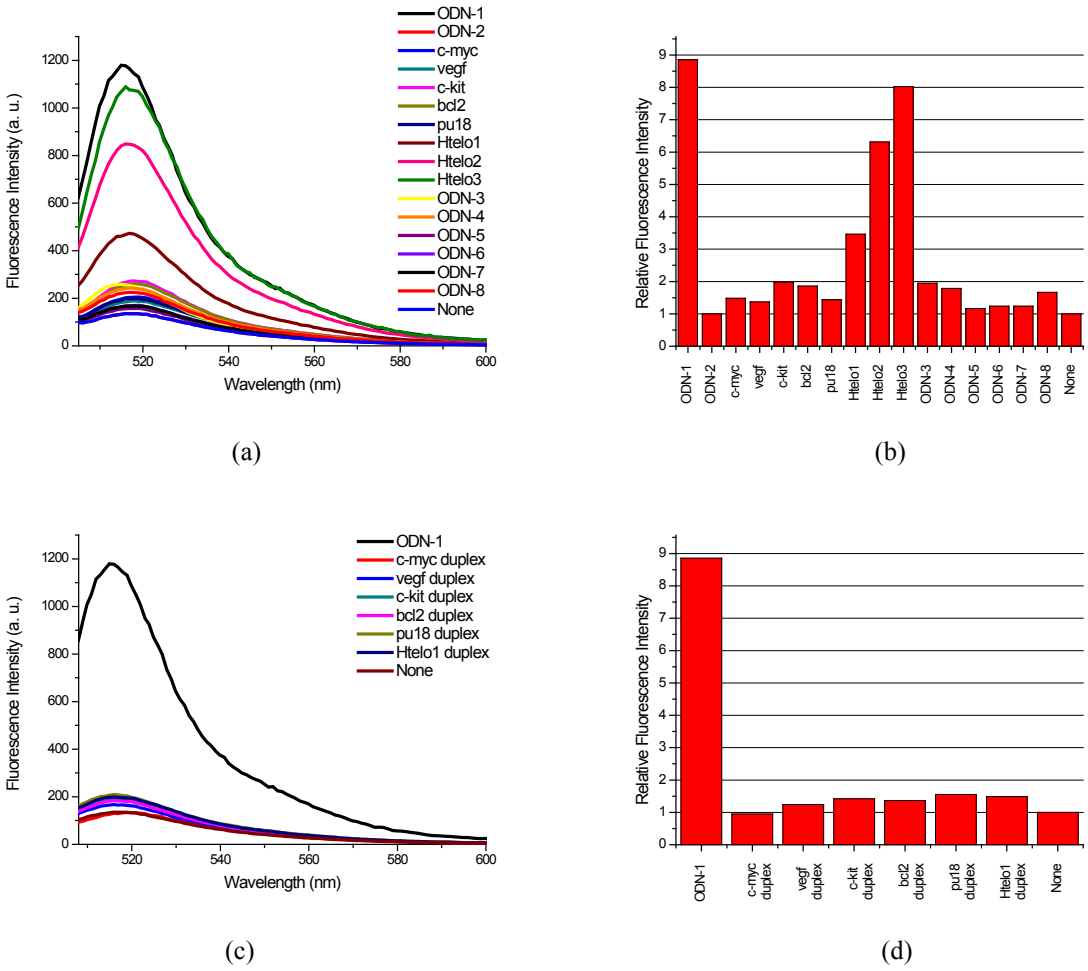


Figure S4 (a), (c) Fluorescence enhancements upon the formation of (3+1) intermolecular G-quadruplexes in presence of a variety of G-quadruplex–forming sequences, their complementary sequences (C-rich sequences), and their duplexes; (b), (d) relative fluorescence intensities. (1.5 μM **ODN-B,** 10 mM trizma buffer, pH 7.2, total volume of sample: 1 mL, 20 °C, excitation wavelength: 500 nm)

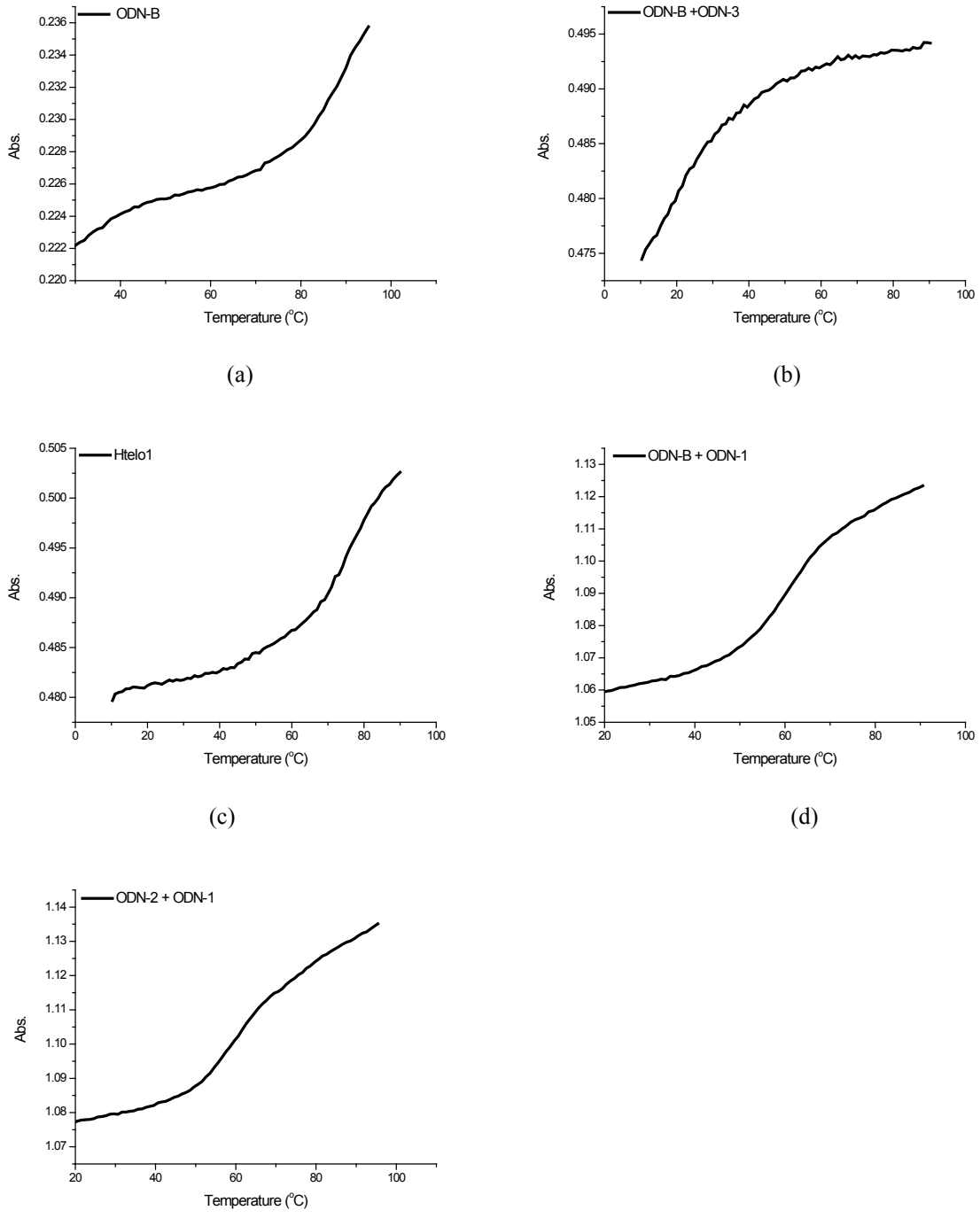
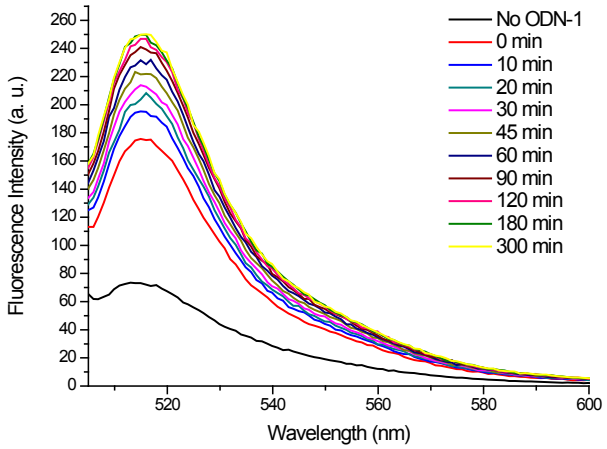
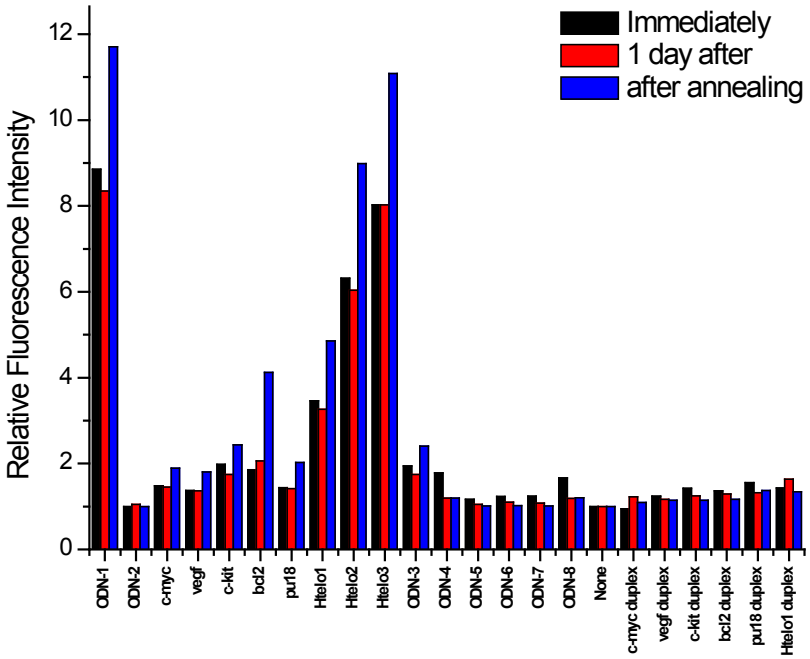


Figure S5 UV melting curves of (a) tetramolecular G-quadruplex of **ODN-B**, (b) duplex of **ODN-B** and **ODN-3**, (c) **Htelo1**, (d) (3+1) interstrand G-quadruplex of **ODN-B** and **ODN-1**, and (e) (3+1) interstrand G-quadruplex of **ODN-2** and **ODN-1**. Their melting temperature was recorded at 89, <20, 76, 62, 60 °C, respectively. (1.5 μM of samples; 10 mM trizma buffer, pH 7.2; total volume of sample: 1 mL)



(a)



(b)

Figure S6 (a) Fluorescence intensity when **ODN-1** was added to tetramolecular G-quadruplex. In spite of high thermal stability of G-quadruplex, we could see the fluorescence enhancement in ambient condition; (b) measured relative fluorescence intensity (1) after sample preparation, (2) 1 day after sample preparation, and (3) after annealing. We observed almost same relative fluorescence intensity for all three cases. These data implies that our system is under thermodynamic control showing fast response (1.5 μM **ODN-B**; 10 mM trizma buffer, pH 7.2; total volume of sample: 1 mL; 20 °C; excitation wavelength: 500 nm).

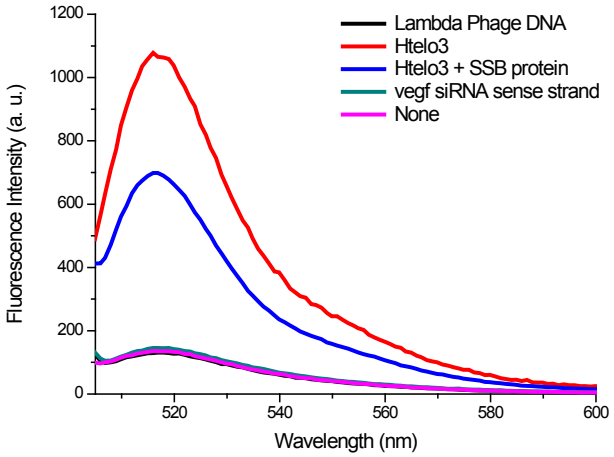


Figure S7 Fluorescence enhancements by several biomolecules such as lambda phage DNA, Single strand binding protein, sense strand RNA sequence (containing mRNA sequence). As a full-length DNA extracts, lambda phage DNA (from Sigma–Aldrich) exhibited no fluorescence signal enhancement. Vegf siRNA sequence, **RNA-1**, also showed no enhancement because of absence of GGG repeat.

Usually, 3′-overhang of telomeric DNA may form G-quadruplexes[2] or T-loop structures[3]. In long length of telomeric DNA, 3′-overhang of telomeric DNA invade subterminal telomeric repeat and form D-loop structure. Single strand form of the 3′-overhang of telomeric DNA, on the other hand, can bind to single-stranded binding protein (SSB, protection of telomeres 1 (POT1) protein for 3'-overhang) in cells. Thus, to see influences of SSB on our system, we treated **Htelo3** with single strand binding protein[4] (from USB) and checked the fluorescence with general procedure. We also observed fluorescence enhancement even in the presence of SSB. (for lambda phage DNA: 1.5 μM **ODN-B**, 10 mM trizma buffer, pH 7.2; total volume of sample: 1 mL; 20 °C; excitation wavelength: 500 nm; 6.8 μg of lambda phage DNA, 20 μM EDTA; for SSB: 1.5 μM **ODN-B**, 10 mM trizma buffer, pH 7.2; total volume of sample: 1 mL; 20 °C; excitation wavelength: 500 nm; 4.9 μg of SSB; 200 μM NaCl; 0.1 μM EDTA; 1μM DTT; 0.05% glycerol)

5. Stability and cytotoxicity of ODN-B

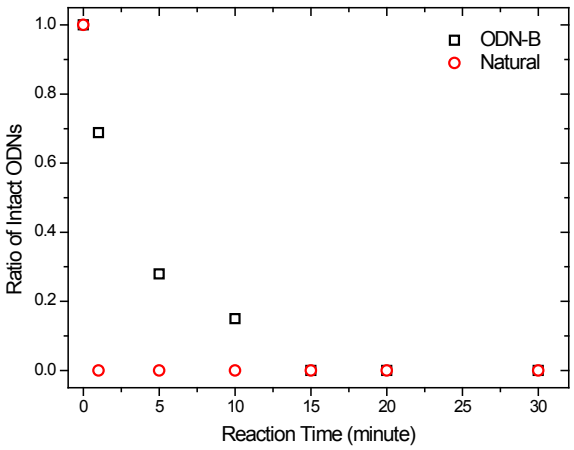


Figure S8 Nuclease resistance of **ODN-B** against Crotalus Adamanteus Venom Phosphodiesterase (CAVP). Reaction condition: 1.5 μM of **ODN-B**, 0.4 μg of CAVP, pH 8.0 100mM trizma buffer, 37°C, total volume 120 μL, 10mM $MgCl_2$, reaction time 0~30 min. Reaction mixture was quenched by keeping in 90°C for 5 min. Remaining intact **ODN-B** was analyzed by HPLC. We observed that **ODN-B** has more resistance against CAVP than natural oligonucleotides.[5] There are other nucleic acids with different type of backbone such as 2'-O-methyl oligoribonucleotide[6], chimeric phosphorothioate DNA[7,8], chimeric LNA[9,10], and methylborane Phosphine DNA chimera[11]. Comparing with chimeric phosphorothioate DNA[5], However, our **ODN-B** showed lower resistance than that (15 min. of degradation time for 1.5 μM **ODN-B** sample).

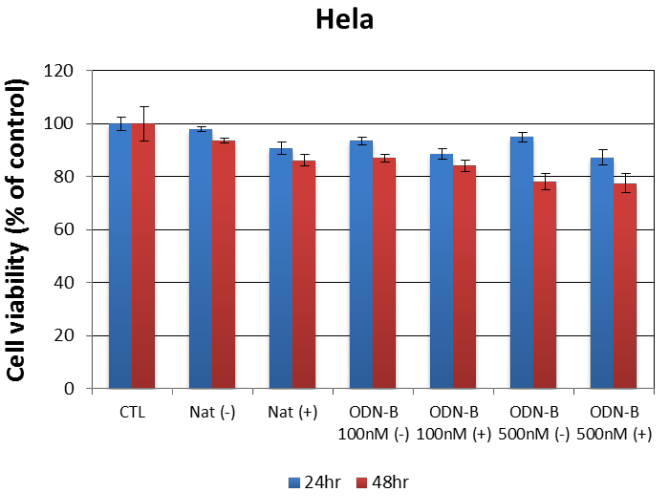


Figure S9 WST-1 assay of ODN-B. ODN-B exhibited no cytotoxicity in Hela cell (Nat : Natural ODN-2 (TGGGTT), (-) : without Lipofectamine™ 2000, (+) : without Lipofectamine™ 2000)

5. MALDI-TOF MS Data for ODNs

Table S2 Oligonucleotide sequences and their MALDI-TOF MS and ESI-MS data

Name	Sequence	Calcd. Mass (m/z)	Found Mass (m/z)
ODN-B	5′ ^{Bod}UGGGTT	2170.0415	2169.0014
ODN-B2	5′ ^{Bod}UTTTTT	2095.0415	2094.8082
ODN-1	5′ T$(GGGTTA)_3$T	5352.6288	5352.6632
ODN-2	5′ TGGGTT	1838.0215	1839.9505
ODN-3	5′ CCC TAA CCC TAA CCC TAA CCC T	6506.0503	6506.2653
ODN-4	5′ TTC CCC ACC CTC CCC ACC CTC A	6432.8503	6432.6461
ODN-5	5′ CCC GCC CCC GGC CCG CCC	5032.9931	5032.8980
ODN-6	5′ CCC CTC CCT CGC GCC CGC CCG	6200.4860	6200.4716
ODN-7	5′ CCC GCC CAA TTC CTC CCG CGC CC	6802.6146	6802.5774
ODN-8	5′ CCC CAC CCT CCC CAC CCT	5221.7931	5221.6598
c-myc	5′ TGA GGG TGG GGA GGG TGG GGA A	7042.2503	7042.2666
vegf	5′ GGG CGG GCC GGG GGC GGG	5702.9931	5702.1215
c-kit	5′ GGG CGG GCG CGA GGG AGG GG	6370.1217	6370.1258
bcl2	5′ GGG CGC GGG AGG AAT TGG GCG GG	7292.0146	7292.8792
pu18	5′ AGG GTG GGG AGG GTG GGG	5781.7931	5781.2284
Htelo1	5′ A$(GGGTTA)_3$GGGT	7271.4146	7271.0071
Htelo2	5′ A$(GGGTTA)_7$GGGT	14908.9578	14904.3395
Htelo3	5′ A$(GGGTTA)_{11}$GGGT	22546.5	22551.0[a]
RNA-1	5′ GGA GUA CCC UGA UGA GAU CdTdT	6709.0660	6711.0467

[a] Measured by ESI-MS(Qtrap, negative)

7. References

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