

Reversible Photoswitching of a G-Quadruplex

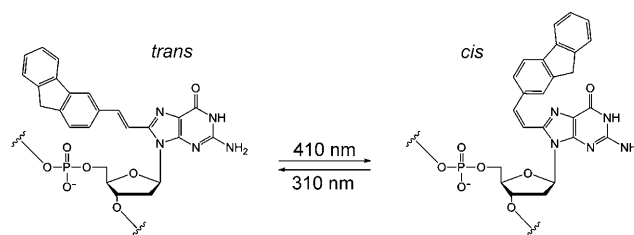
Shinzi Ogasawara* and Mizuo Maeda

G-quadruplexes are formed by stacked G-quartets, a planar association of four guanines, by Hoogsteen hydrogen bonding. They are found in guanine-rich sequences and are involved in several key biological events. For example, telomeric DNA consists of long (TTAGGG)_n repeats, and can fold into an intramolecular G-quadruplex that is required to stabilize the ends of chromosomes for proper replication and segregation of eukaryotic chromosomes.^[1] This G-quadruplex can also inhibit telomere elongation by telomerase, which is expressed in 85–90 % of tumor cells. Furthermore, G-quadruplexes in the promoter regions of *c-myc*,^[2] *RET*,^[3] *KRAS*,^[4] *c-kit*,^[5] *VEGA*,^[6] *HIF-1α*,^[7] and *bcl-2*^[8] or in the 5'-UTRs of *Zic-1*^[9] and *NRAS*^[10] mRNA may modulate transcription or translation. G-quadruplexes are also sometimes found in aptamers, which are small, structured single-stranded nucleic acids selected from vast combinatorial libraries using the SELEX method. They bind with high affinity and specificity to their target molecules and prevent their activity.^[11] For example, (GGGT)₄ or (GGGC)₄ can form G-quadruplexes in the presence of potassium ions, and can protect host cells from the cytopathic effects of human immunodeficiency virus type 1 (HIV-1) by binding to integrase.^[12] Similarly, anti-thrombin aptamers form intramolecular G-quadruplexes that bind to and inhibit thrombin, which plays an important role in thrombosis and hemostasis.^[13]

The possibility of regulating biological processes by controlling G-quadruplex formation with external stimuli is both exciting and challenging.^[14] The most promising external trigger is light, as it allows accurate and easy control of the location, dosage, and timing of stimulation.^[15]

Heckel and co-workers have developed a light-controlable anti-thrombin aptamer with a photoprotecting group that can be completely removed by photoirradiation.^[16] Unfortunately, however, such uncaging methods are irreversible.^[17] We recently developed a new strategy for photo-regulating nucleic acid structures using *cis-trans* photoisomerization of a photochromic nucleobase (PCN), which reversibly changes its photochemical and physical properties upon exposure to external light stimuli. This method permits efficient, reversible duplex regulation even at room temperature.^[18] Herein, we describe a novel method of photoregulating G quadruplexes by *cis-trans* photoisomerization of 8-fluorenylvinyl-2'-deoxyguanosine (^{8FV}G). ^{8FV}G can be photoisomerized highly efficiently from *trans* to *cis* by irradiation at

410 nm, and back to *trans* at 310 nm (Scheme 1). To show the potential applications of our method, we performed reversible photoregulation of the ability of G-quadruplex aptamers to bind with thrombin (Figure 1 a).



Scheme 1. Photoinduced *cis-trans* isomerization of 8-fluorenylvinyl-2'-deoxyguanosine (^{8FV}G).

The details of ^{8FV}G synthesis have been previously reported.^[18] Briefly, 8-bromo-2'-deoxyguanosine was converted into 8-vinyl-2'-deoxyguanosine by Stille cross-coupling with tributylvinyltin; the vinyl derivative was then subjected to a Herrmann palladacycle-assisted Heck olefination with 2-bromofluorene to generate *trans*-^{8FV}G. ^{8FV}G was incorporated into aptamers at various *syn*-guanine positions using standard automated DNA synthesis protocols. To optimize the number and positions of ^{8FV}G, we synthesized several aptamer variants Q2–Q13 (Table 1).

We initially performed a photoisomerization of synthetic aptamers in a mixture containing 5 μM aptamer, 10 mM potassium phosphate (pH 7.0), and 100 mM KCl at room temperature, and evaluated how this affected the structure of quadruplexes using circular dichroism (CD) spectroscopy and UV-spectroscopy-monitored thermal denaturation analysis. In the presence of potassium ions, the native thrombin aptamer (Q1) is characterized by a negative peak near 265 nm and a positive peak near 295 nm in the CD spectrum (Figure 1 b, solid black line), which indicates an antiparallel quadruplex configuration.^[13b] The modified aptamers Q2–Q5, which contain a single ^{8FV}G group, exhibited a normal antiparallel configuration when ^{8FV}G was in both the *trans* and *cis* forms (see Supporting Information). In contrast, although structural changes induced by *trans-cis* isomerization were observed in Q6, Q8, and Q9, which contain two ^{8FV}Gs, the thermal stability of the quadruplexes in the *trans* form was very low. Surprisingly, we observed a drastic conformational change in Q7 and Q10, which also contain two ^{8FV}Gs, upon *cis-trans* photoisomerization (see Figure 1 b, and the Supporting Information for Q10). These aptamers exhibited a typical antiparallel quadruplex CD signature, with a negative peak at 270 nm and a positive peak at 295 nm when ^{8FV}G was in the *trans* form. However, these peaks were lost

[*] Dr. S. Ogasawara, Prof. Dr. M. Maeda
Bioengineering Laboratory, RIKEN
2-1 Hirosawa, Wako, Saitama 351-0198 (Japan)
Fax: (+81) 48-462-4658
E-mail: o_shinji@riken.jp

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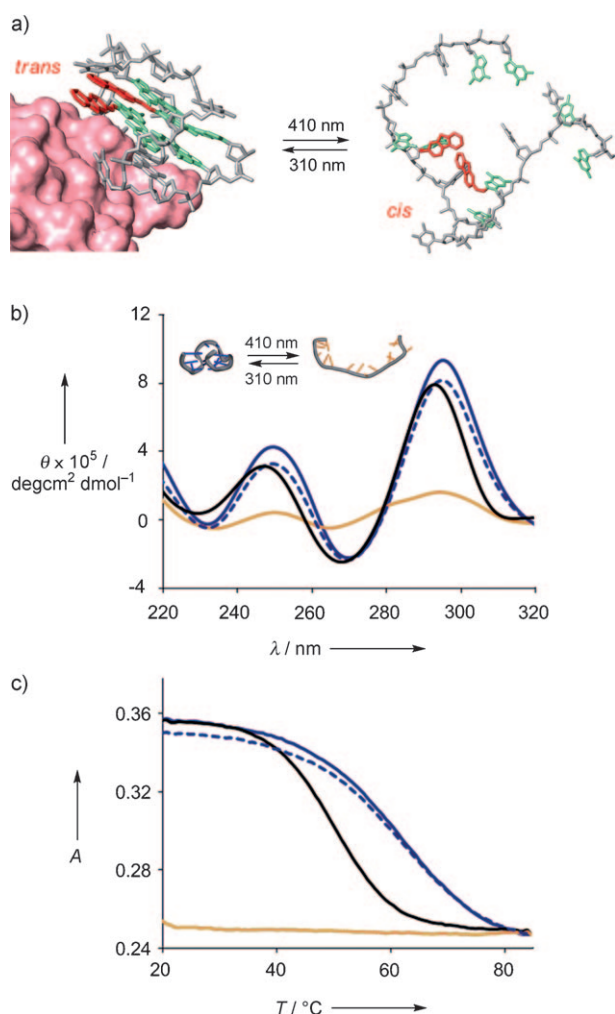


Figure 1. a) Photoregulation of the thrombin aptamer G-quadruplex by *cis-trans* photoisomerization of photochromic nucleobase ^{8FV}G. Fluorene red, guanine green, thrombin pink. b,c) CD spectra (b) and melting curves (c) of photoresponsive G-quadruplex Q7. Black line: Q1 (native aptamer as control); blue line: Q7 before irradiation (*trans* form); orange line: after irradiation at 410 nm for 2 min (*cis* form); broken blue line: subsequent irradiation at 310 nm for 1 min.

Table 1: The oligodeoxynucleotides (ODNs) used in this study.

Entry	Sequences ^[a] (X = ^{8FV} G)
Q1	5'-d (GGTTGGTGTGGTTGG) -3'
Q2	5'-d (XGTTGGTGTGGTTGG) -3'
Q3	5'-d (GGTTXGTTGTGGTTGG) -3'
Q4	5'-d (GGTTGGTGTGGTTXG) -3'
Q5	5'-d (GGTTGGTGTGGTTXG) -3'
Q6	5'-d (XGTTGGTGTGGTTGG) -3'
Q7	5'-d (GGTTXGTGTGGTTGG) -3'
Q8	5'-d (GGTTGGTGTGGTTXG) -3'
Q9	5'-d (GGTTXGTGTGGTTXG) -3'
Q10	5'-d (XGTTGGTGTGGTTXG) -3'
Q11	5'-d (XGTTXGTGTGGTTGG) -3'
Q12	5'-d (XGTTXGTGTGGTTGG) -3'
Q13	5'-d (XGTTXGTGTGGTTXG) -3'

[a] Q1 is the aptamer control. Underlined positions contain syn-glycosidic bonds in the aptamer.

after photoisomerization to the *cis* form by irradiation at 410 nm (103 mW cm^{-2}) for 2 min, indicating that Q7 and Q10 cannot form a quadruplex when ^{8FV}G adopts the *cis* form. Following subsequent irradiation at 310 nm (81 mW cm^{-2}) for 1 min, the antiparallel quadruplex CD signature was observed once again. We also found structural changes of the quadruplex in thermal denaturation experiments (see Figure 1c, and the Supporting Information for Q10). The melting temperature T_m of the *trans* form is 60.5°C , which is 10.2°C higher than that of the native aptamer Q1, whereas that of the *cis* form could not be determined because hypochromicity owing to denaturation was not observed, suggesting that the quadruplex was not formed. However, a melting curve indicating quadruplex formation reappeared upon *cis-trans* isomerization by subsequent irradiation at 310 nm. The CD spectra of Q11, Q12, and Q13 in the *trans* form showed a small negative peak near 240 nm and a positive peak near 290 nm, with a shoulder at 260 nm (see the Supporting Information). These spectra are similar to previously reported results, indicating a mixed parallel/antiparallel quadruplex.^[19] Destabilization of quadruplexes, when ^{8FV}G is in the *cis* form, is probably due to steric hindrance of the fluorene moiety with its neighboring nucleobase and backbone. The three-dimensional structures of Q7 and Q10 (see Supporting Information) suggest an explanation for why they effectively alternate between a stable antiparallel quadruplex and a non-structured state by *cis-trans* photoisomerization of ^{8FV}G. In both Q7 and Q10, the two fluorene moieties of ^{8FV}G are directed in the same direction, and they are probably stacked when ^{8FV}G is in the *trans* form, thereby stabilizing the quadruplex. In contrast, when ^{8FV}G is photoisomerized to the *cis* form, quadruplex formation is likely prevented by the steric barrier between the two fluorene moieties.

Based on the above results, we selected Q7 as a photocontrollable aptamer, and performed an electrophoretic mobility shift assay to investigate the interaction between the *trans* or *cis* form of Q7 and thrombin (Figure 2). Q7 was photoisomerized before addition of thrombin. The *trans* form of Q7 was photoisomerized to the *cis* form in phosphate-buffered saline (PBS; pH 7.4) containing $5 \mu\text{M}$ Q7 and 10 mM MgCl_2 at room temperature by irradiating at 410 nm for 2 min, with $> 99\%$ conversion as determined by the peak area in HPLC analysis (see Supporting Information). Conversely, photoisomerization to the *cis* form was carried out by irradiating at 310 nm for 1 min, with 90% conversion. Before irradiation, binding of the *trans* form of Q7 (lane 3) to thrombin resulted in a strong band shift (lane 4), suggesting that the presence of ^{8FV}G in the aptamer did not affect the interaction between the aptamer and thrombin. This was expected, as the aptamer associates with thrombin through the TGT loop or TT loop^[20] and not through the G-quartet. After photoisomerization to the *cis* form by 410 nm irradiation (lane 5), the quadruplex was disrupted and binding was completely suppressed (lane 6). After subsequent irradiation at 310 nm, the *cis* form was isomerized back to the *trans* form (lane 7), and Q7 was able to bind thrombin once again (lane 8).

Finally, reversible switching between binding and dissociation of aptamer Q7 and thrombin was repeated for two

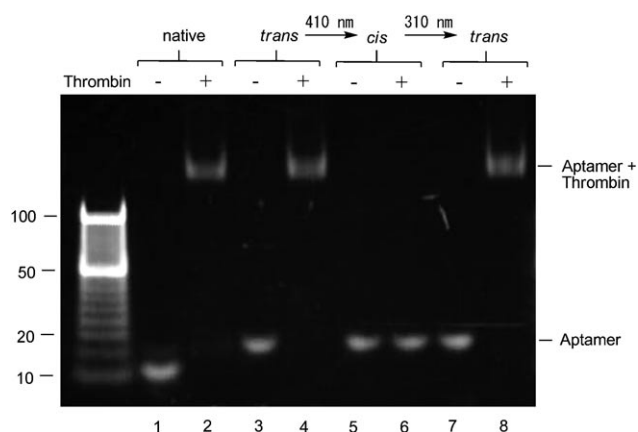


Figure 2. Electrophoretic mobility shift analysis showing reversible photoregulation of the interaction between thrombin and aptamer Q7. Lane 1: Q1 (native aptamer as control); 2: Q1 + thrombin; 3: before photoirradiation (*trans* form); 4: lane 3 + thrombin; 5: after photoirradiation at 410 nm for 2 min (*cis* form); 6: lane 5 + thrombin; 7: subsequent irradiation at 310 nm for 1 min (*trans* form); 8: lane 7 + thrombin. The scale on the left is determined from a 5-base ladder size marker.

cycles in the mixture of Q7 and thrombin by alternating irradiation at 410 nm for 5 min and 310 nm for 2 min. After each irradiation, 2 μ L aliquots of the reaction mixture were sampled and incubated for 10 min at room temperature, and then run on a gel. As shown in Figure 3, the cycles showed complete reversibility with no side reactions. Interestingly, when Q7 was isomerized to the *cis* form, it completely dissociated from thrombin, even when pre-formed thrombin–aptamer complexes were present.

In summary, we have successfully developed a new method for photoregulation of G-quadruplex formation using *cis*–*trans* photoisomerization of the photochromic nucleobase 8FV G. Our photo-controllable quadruplexes can be switched between a very stable quadruplex state and a non-structured state in a straightforward and reversible

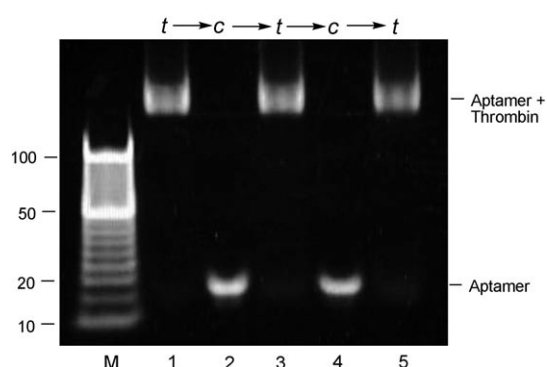


Figure 3. Electrophoretic mobility shift analysis showing reversible cycles between binding and dissociation of thrombin and aptamer Q7. The reaction mixture containing thrombin and aptamer was photoirradiated. Lane 1: before photoirradiation; 2: after photoirradiation at 410 nm for 5 min; 3: subsequent irradiation at 310 nm for 2 min; 4: subsequent irradiation at 410 nm for 5 min; 5: subsequent irradiation at 310 nm for 2 min. *t* = *trans* form, *c* = *cis* form. The scale on the left is determined from a 5-base ladder size marker (M).

fashion by alternately irradiating with monochromatic 410 nm and 310 nm light. To demonstrate the potential applications of this technology, we have shown that binding between a G-quadruplex aptamer and thrombin can be reversibly photoregulated. To the best of our knowledge, this is the first example of controlling G-quadruplex formation reversibly with light. In principle, this opens the possibility of also regulating non-antiparallel quadruplexes^[21] by light. We plan to apply this photoregulation technique to important biological events involving G-quadruplexes, such as gene expression, as well as to the construction of photo-controllable molecular machines.

Experimental Section

Photoisomerization of 8FV G-containing G-quadruplexes was performed in a mixture containing 5 μ M ODNs, 10 mM potassium phosphate (pH 7.0) and 100 mM KCl at room temperature using a 300 W xenon lamp (MAX-302, ASAHI Spectra Co. Ltd), which can extract a specific wavelength with a 10 nm peak width at half-height by employing an adequate bandpass filter (M.C.310, M.C.410, ASAHI Spectra Co. Ltd). The T_m values were determined by monitoring the absorbance at $\lambda = 295$ nm on a JASCO model V-530 spectrophotometer equipped with a programmable temperature controller using a 1 cm path length cuvette. The absorbance of the samples was monitored from 10 $^{\circ}$ C to 85 $^{\circ}$ C with a heating rate of 1 $^{\circ}$ C min $^{-1}$. From these profiles, first derivatives were calculated to determine the T_m value. The T_m values were obtained by taking the average of at least three data points. CD experiments utilizing a JASCO J-720WI spectropolarimeter were measured over the wavelength range 200–320 nm at 25 $^{\circ}$ C in a 1 cm path length cuvette. Each trace is the average of the 3 scans at 50 nm min $^{-1}$, with a 1 s time constant, 0.1 nm step resolution, and 1 nm bandwidth.

The reaction mixture containing 5 μ M aptamer, 10 mM MgCl $_2$, and 250 μ M bovine α -thrombin (Sigma) was incubated for 10 min at room temperature in phosphate-buffered saline (PBS; pH 7.4) after photoirradiation. Electrophoretic mobility shift analysis was performed on a 10% native polyacrylamide gel in TB buffer (89 mM Tris-HCl, 89 mM boric acid) with 10 mM KCl, and run for 20 min with a field of 80 V at room temperature. The gel was subsequently stained by SYBR gold (Invitrogen Co.).

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