DNA Conformational Switches

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Reversible Photoswitching of a G-Quadruplex

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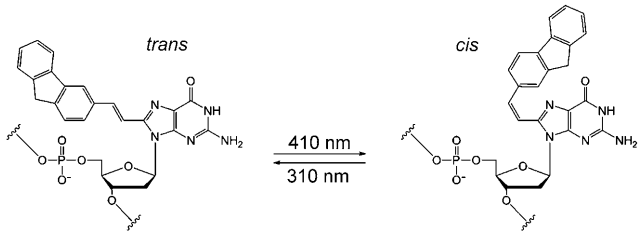
G-quadruplexes are formed by stacked G-quartets, a planar association of four guanines, by Hoogsteen hydrogen bonding. They are found in guanine-rich sequences and are involved in several key biological events. For example, telomeric DNA consists of long $(TTAGGG)_n$ repeats, and can fold into an intramolecular 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-controllable 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 photoregulating 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 1a).



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 Q**2**-Q**13** (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 (Q**1**) is characterized by a negative peak near 265 nm and a positive peak near 295 nm in the CD spectrum (Figure 1b, solid black line), which indicates an antiparallel quadruplex configuration.[13b] The modified aptamers Q**2**–Q**5**, 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 Q**6**, Q**8**, and Q**9**, 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 Q**7** and Q**10**, which also contain two ^{8FV}Gs, upon *cis–trans* photoisomerization (see Figure 1b, and the Supporting Information for Q**10**). 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

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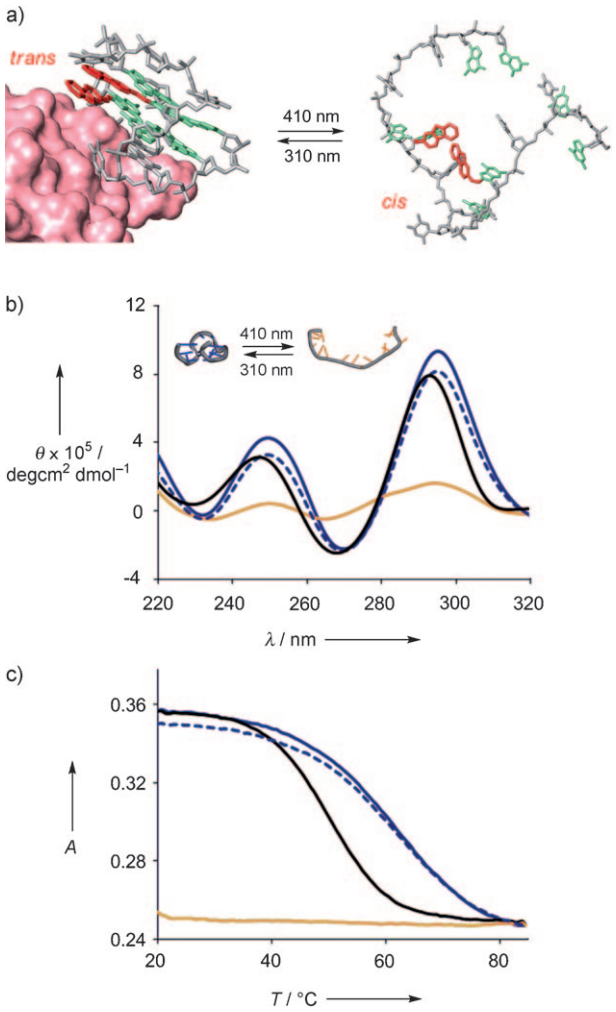

Figure 1. a) Photoregulation of the thrombin aptamer G-quadruplex by *cis–trans* photoisomerization of photochromic nucleobase ^{8FV}G. Fluorene red, guanine green, thrombin pink. b,c) CD spectra (b) and melting curves (c) of photoresponsive G-quadruplex Q**7**. Black line: Q**1** (native aptimer as control); blue line: Q**7** 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)
Q**1**	5′-d(GGTTGGTGTGGTTGG)-3′
Q**2**	5′-d(**X**GTTGGTGTGGTTGG)-3′
Q**3**	5′-d(GGTT**X**GTGTGGTTGG)-3′
Q**4**	5′-d(GGTTGGTGT**X**GTTGG)-3′
Q**5**	5′-d(GGTTGGTGTGGTT**X**G)-3′
Q**6**	5′-d(**X**GTTGGTGT**X**GTTGG)-3′
Q**7**	5′-d(GGTT**X**GTGT**X**GTTGG)-3′
Q**8**	5′-d(GGTTGGTGT**X**GTT**X**G)-3′
Q**9**	5′-d(GGTT**X**GTGTGGTT**X**G)-3′
Q**10**	5′-d(**X**GTTGGTGTGGTT**X**G)-3′
Q**11**	5′-d(**X**GTT**X**GTGTGGTTGG)-3′
Q**12**	5′-d(**X**GTT**X**GTGT**X**GTTGG)-3′
Q**13**	5′-d(**X**GTT**X**GTGT**X**GTT**X**G)-3′

[a] Q**1** 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 Q**7** and Q**10** 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 Q**10**). 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 Q**1**, 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 Q**11**, Q**12**, and Q**13** 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 Q**7** and Q**10** (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 Q**7** and Q**10**, 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 Q**7** as a photocontrollable aptamer, and performed an electrophoretic mobility shift assay to investigate the interaction between the *trans* or *cis* form of Q**7** and thrombin (Figure 2). Q**7** was photoisomerized before addition of thrombin. The *trans* form of Q**7** was photoisomerized to the *cis* form in phosphate-buffered saline (PBS; pH 7.4) containing 5 μM Q**7** 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 Q**7** (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 surpressed (lane 6). After subsequent irradiation at 310 nm, the *cis* form was isomerized back to the *trans* form (lane 7), and Q**7** was able to bind thrombin once again (lane 8).

Finally, reversible switching between binding and dissociation of aptamer Q**7** and thrombin was repeated for two

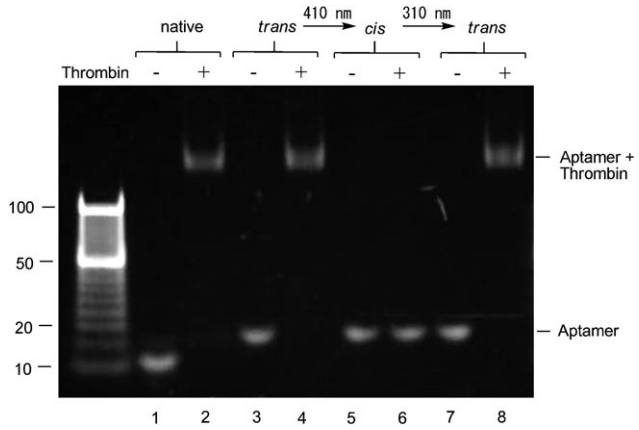

Figure 2. Electrophoretic mobility shift analysis showing reversible photoregulation of the interaction between thrombin and aptamer **Q7**. Lane 1: **Q1** (native aptamer as control); 2: **Q1** + thrombin; 3: before photoirradiation (*trans* form); 4: lane 3 + thrombin; 5: after photoirradiation at 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 μL 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 thombin–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 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.

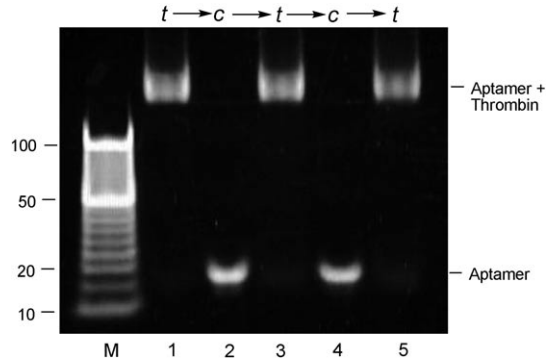

Figure 3. Electrophoretic mobility shift analysis showing reversible cycles between binding and dissociation of thrombin and aptamer **Q7**. The reaction mixture containing thrombin and aptamer was photoirradiated. Lane 1: before photoirradiation; 2: after photoirradiation at 410 nm for 5 min; 3: subsequent 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).

Experimental Section

Photoisomerization of ^{8FV}G-containing G-quadruplexes was performed in a mixture containing 5 μM 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 °C to 85 °C with a heating rate of 1 °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 °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 μM aptamer, 10 mM $MgCl_2$, and 250 μM 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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