Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

Quick and Reversible Photocrosslinking Reaction of 3-Cyanovinylcarbazole Nucleoside in DNA Triplex

Kenzo Fujimoto,*[a,b] Hiroki Yoshinaga,[a] Yasumasa Yoshio[a] and Takashi Sakamoto[a]

[a]School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahi-dai, Nomi, Ishikawa 923-1292, Japan.

[b]Research Center for Bio-Architecture, Japan Advanced Institute of Science and Technology, 1-1 Asahi-dai, Nomi, Ishikawa 923-1292, Japan.

General Method

Mass spectra were recorded on a Voyager-DE PRO-SF, Applied Biosystems. Irradiation was performed by UV-LED (366 nm, 1,600 mW/cm^2; ZUV, OMRON, Japan). CD spectra were measured on a JASCO J-720 spectropolarimeter. HPLC was performed on an InertSustain^{TM} C18 column (GL Science, 5 μm, 10 × 150 mm) or an InertSustain^{TM} C18 column (GL Science, 5 μm, 4.6 × 150 mm) with a JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 detector at 260 nm. Reagents for the DNA synthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite, and CPG support were purchased from Glen Research. Cy3-labeled ODNs and unmodified ODNs were purchased from Fasmac (Japan) and used without farther purification.

Preparation of ODNs

ODN sequences were synthesized by the conventional phosphoramidite method using an automated DNA synthesizer (3400 DNA synthesizer, Applied Biosystems, CA). ^{CNV}K phosphoramidite was prepared according to the literature method (Y. Yoshimura K. Fujimoto, *Org. Lett.*, 2008, **10**, 3227.). The coupling efficiency was monitored with a trityl monitor. The coupling efficiency of a crude mixture of ^{CNV}K showed a 97% yield. The coupling time of a crude mixture of ^{CNV}K was 999 sec. They were deprotected by incubation with 28% ammonia for 4 h at 65 ºC and purified on a Chemcobond 5-ODS-H column (10 x 150 mm) by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-25% CH_3CN, linear gradient (30 min) at a flow rate of 3.0 mL/min. Preparation of ODNs was confirmed by MALDI-TOF-MS analysis. MALDI-TOF-MS: calcd. 13476.24 for C_K2 [(M + H)$^+$], found 13479.07; calcd. 13476.24 for C_K4 [(M + H)$^+$], found 13480.17; calcd. 13476.24 for C_K7 [(M + H)$^+$], found 13476.54; calcd. 13476.24 for C_K11 [(M + H)$^+$], found 13473.19; calcd. 13668.35 for G_K34 [(M + H)$^+$], found 13668.76; calcd. 13668.35 for G_K38 [(M + H)$^+$], found 13668.84; calcd. 13668.35 for G_K40 [(M + H)$^+$], found 13663.28; calcd. 13668.84 for G_K43 [(M + H)$^+$], found 13664.04; calcd. 13702.33 for AT_{-2} [(M + H)$^+$], found 13703.79; calcd. 13702.33 for AT_{-1} [(M + H)$^+$], found 13701.18; calcd. 13718.33 for AT_0 [(M + H)+], found 13723.34; calcd. 13702.33 for AT_{+1} [(M + H)$^+$], found 13702.71; calcd. 13702.33 for AT_{+2} [(M + H)$^+$], found 13705.81; calcd. 13719.32 for GC [(M + H)$^+$], found 13719.04; calcd. 13668.35 for G_K27 [(M + H)$^+$], found 13666.69; calcd. 13735.39 for G_K27+43 [(M + H)$^+$], found 13732.40; calcd. 6756.24 for NFkB–TFO [(M + H)$^+$], found 6752.18.

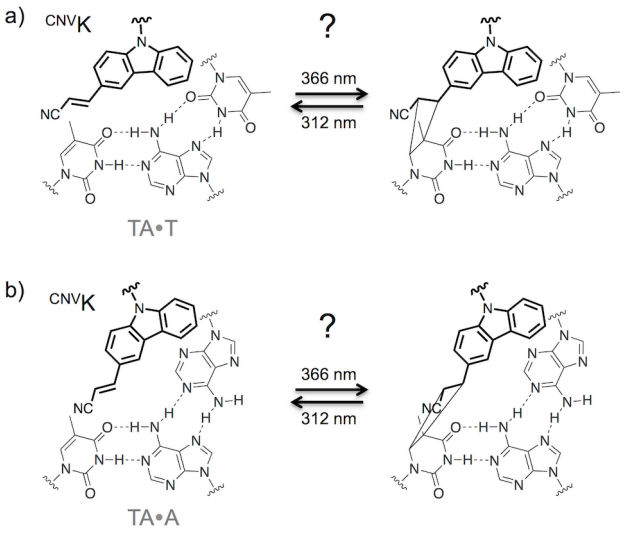

Figure S1. Schematic drawing of the reversible photocrosslinking reaction of ^{CNV}K in parallel triplex (a) and antiparallel triplex (b)

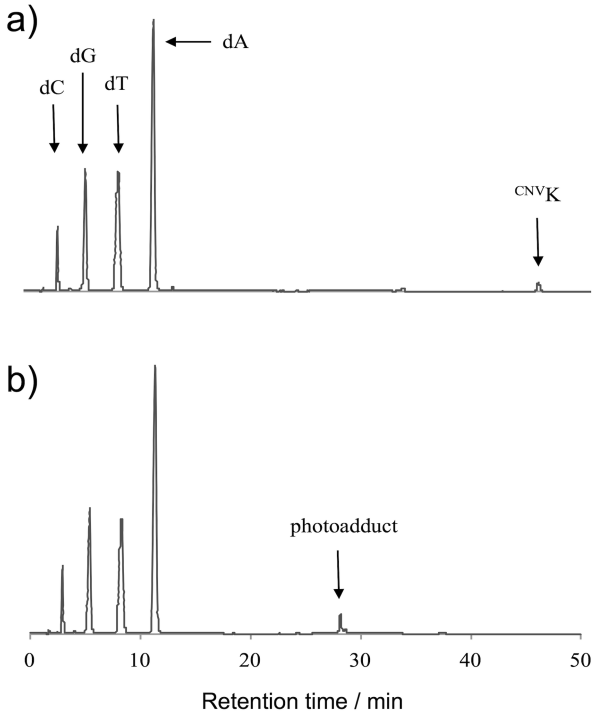

Figure S2. Reversed-phase HPLC analysis of the TFO (G_K40) after the nuclease and phosphatase treatment. (a) Before the 366 nm photoirradiation. (b) After the 10 sec of 366 nm photoirradiation at 0ºC. HPLC was performed with 50 mM aq. ammonium formate / MeCN gradient: 1–50% in 50 min on a reversed-phase ODS column.

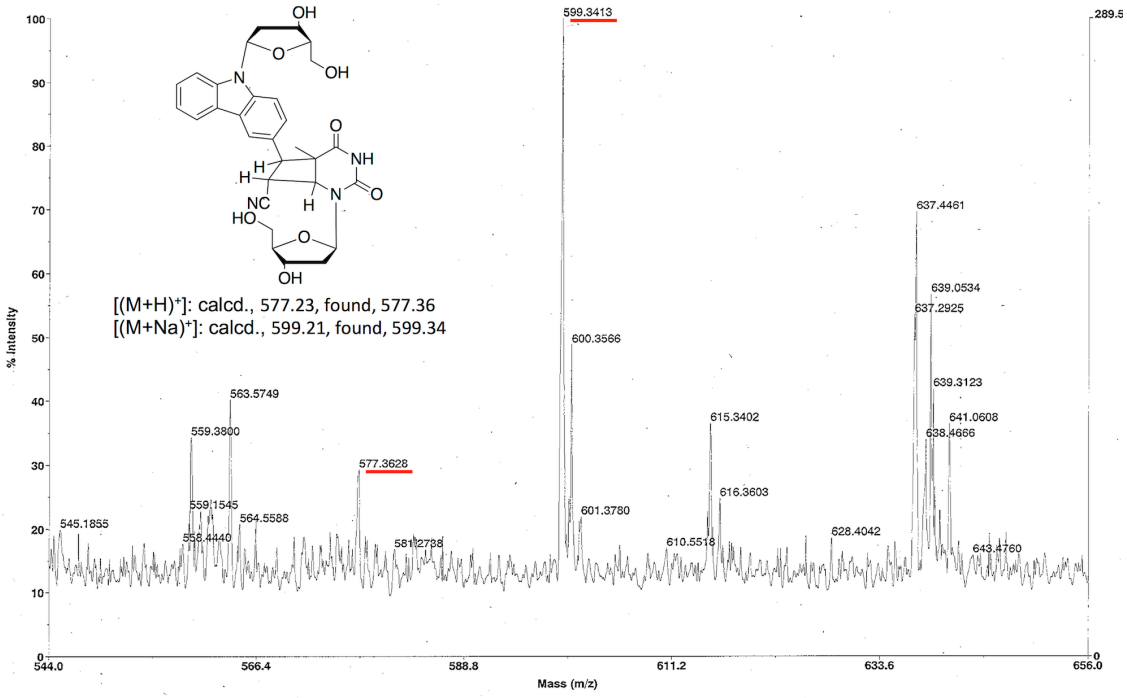

Figure S3. MALDI-TOF-Mass spectrum of the photoadduct purified by reversed-phase HPLC shown in Fig. S2

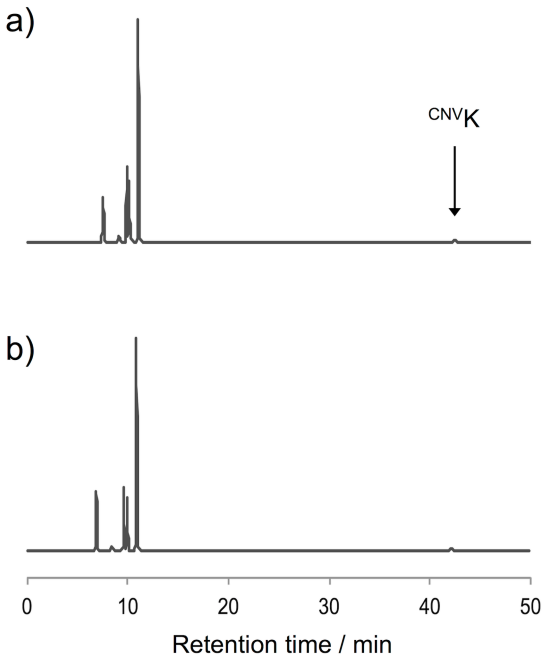

Figure S4. Reversed-phase HPLC analysis of the TFO (C_K7) after the nuclease and phosphatase treatment. (a) Before the 366 nm photoirradiation. (b) After the 10 sec of 366 nm photoirradiation at 0ºC. HPLC was performed with 50 mM aq. ammonium formate / MeCN gradient: 0–50% in 50 min on a reversed-phase ODS column.

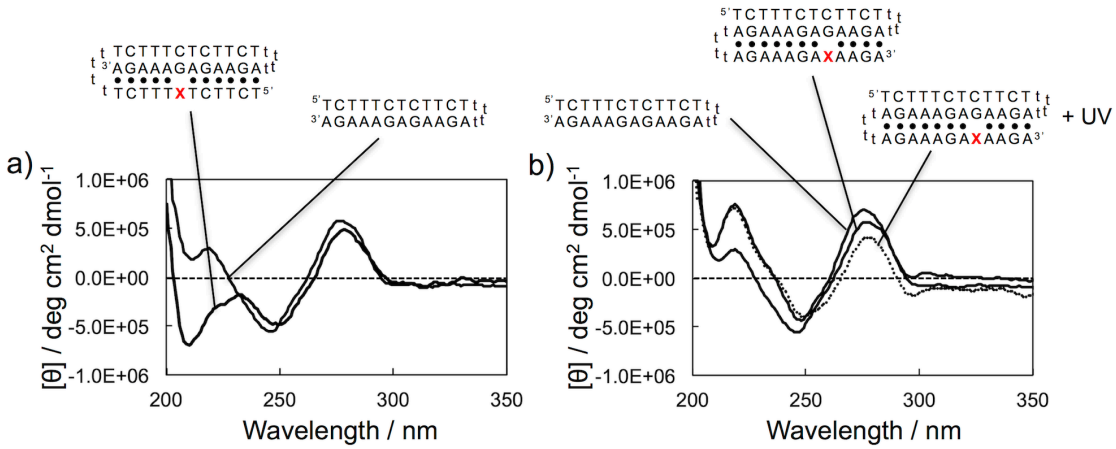

Figure S5. Circular dichroism spectra of intramolecular TFOs, C_K7 (a) and G_K40 (b). [TFO] = 2 μM in 50 mM Acetate buffer (pH 5.0) containing 100 mM NaCl (for C_K7) or in 50 mM Na-cacodylate buffer (pH 7.4) containing 100 mM NaCl (for G_K40).

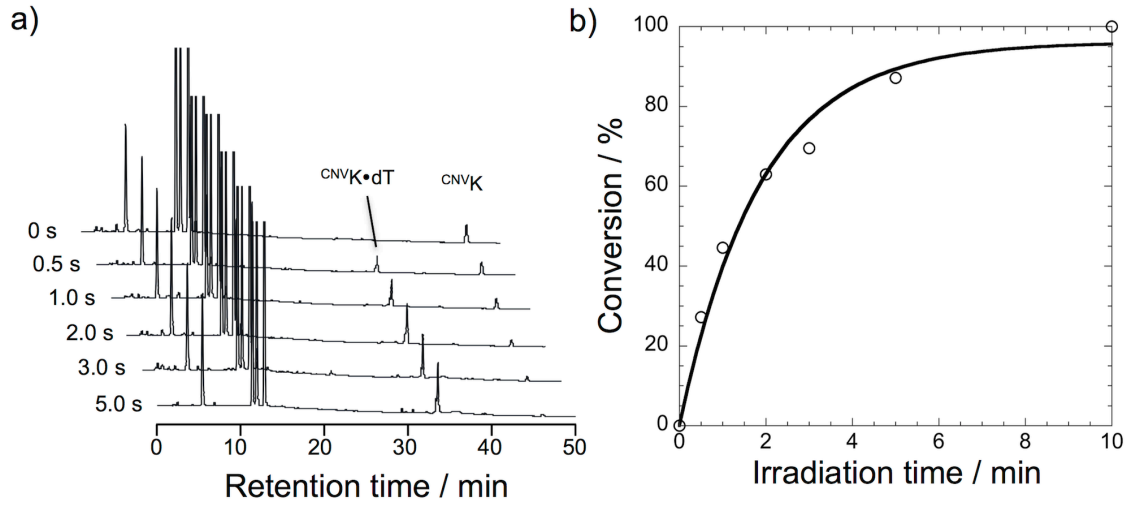

Figure S6. Reversed-phase HPLC analysis of G_K34 after the nuclease and phosphatase treatment. HPLC was performed with 50 mM aq. ammonium formate / MeCN gradient: 0–50% in 50 min on a reversed-phase ODS column.

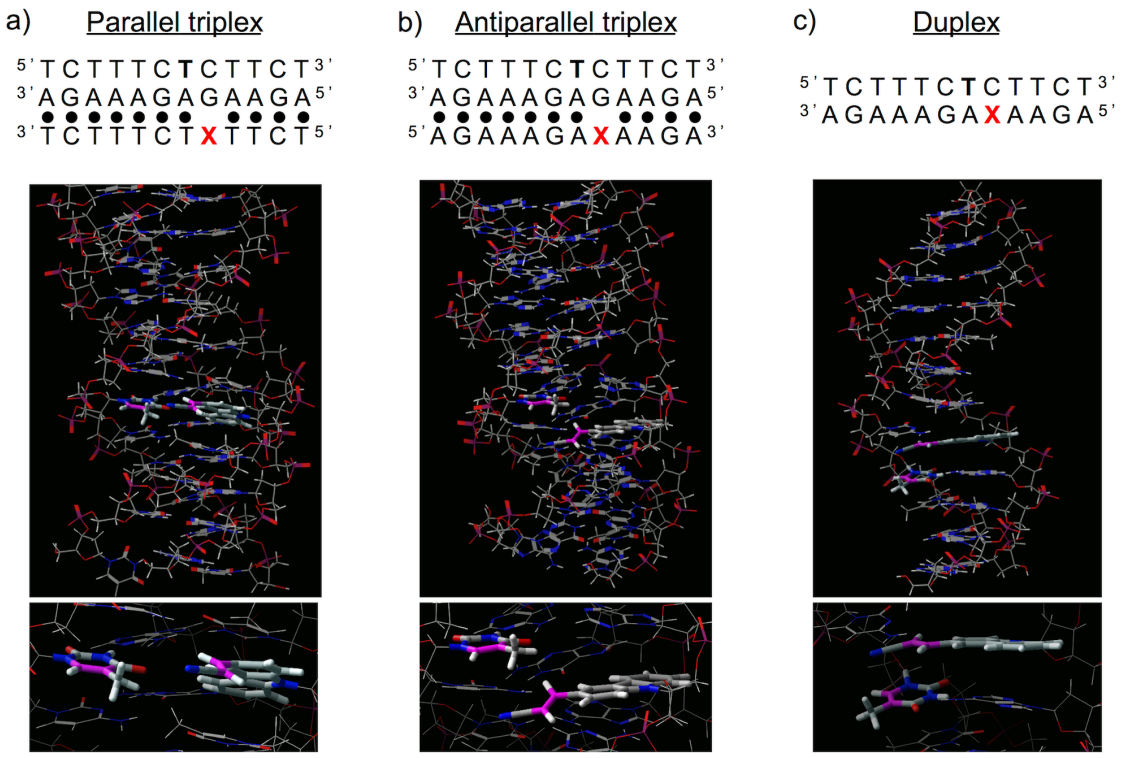

Figure S7. Predicted energy minimized structures of parallel triplex (a), antiparallel triplex (b) and duplex (c) containing ^{CNV}K. Bold nucleobases were presented with tubular form in the structure models.

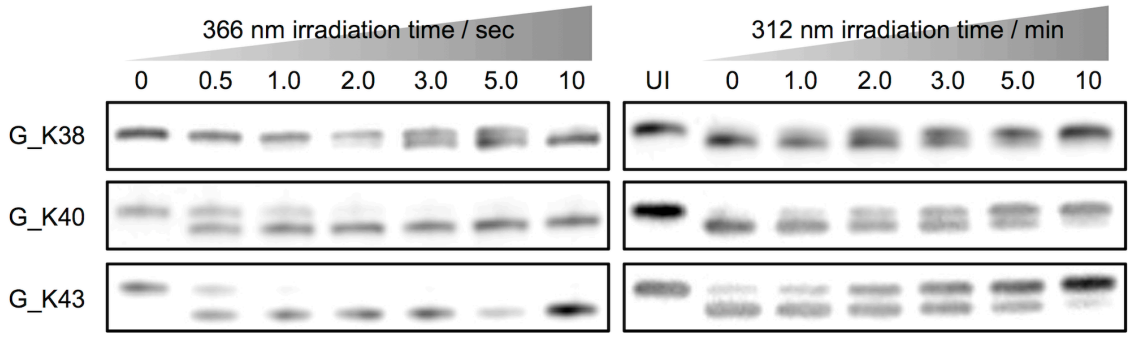

Figure S8. Denaturing PAGE analysis of the intramolecular TFOs after the different time periods of photoirradiation. Electrophoresis was preformed on a polyacrylamide gel containing 12% acrylamide, 25% formamide and 7.0 M urea (160 V, 90 min) in 1x TBE buffer. After the electrophoresis, the gel was stained with SYBR® Gold and imaged by a gel imager (LAS 3000). [TFO] = 1 μM in 50 mM Na-cacodylate buffer (pH 7.4) containing 100 mM NaCl. 366 and 312 nm irradiation was performed at 0ºC and 60ºC, respectively.

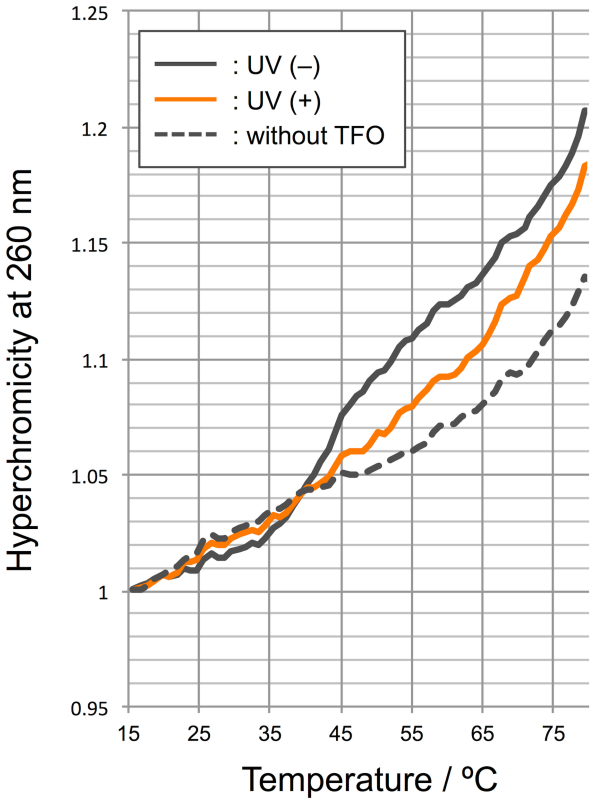

Figure S9. UV melting profiles of the intermolecular triplex before and after the photocrosslinking. [NFkB–TFO] = [dsDNA] = 2 μM in 10 mM Na- cacodylate buffer (pH 7.2) containing 100 mM NaCl and 10 mM $MgCl_2$. Photoirradiation was performed for 2 sec at 37ºC.