Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Photo-cross-linking using trifluorothymidine and 3-cyanovinylcarbazole induced large shifted ^{19}F MR signal

Shigetaka Nakamura and Kenzo Fujimoto*

School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahi-dai, Nomi, Ishikawa, 923-1292 (Japan), E-mail: kenzo@jaist.ac.jp

Experiments

General

^{1}H NMR and 19F NMR spectrum were recorded on a Bruker AVANCE III 400 system and Bruker AVANCE III 500 system, respectively. Mass spectra were recorded on a Voyager PRO-SF, Applied Biosystems. HPLC was performed on a Chemcosorb 5-ODS-H column with JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 detector at 260 nm. Reagents for the DNA sysnthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite , and CPG support were purchased form Glen research.

Preparation of ^{CNV}K-modified oligonucleotides

The phosphoramidite of ^{CNV}K was prepared following to previous reports. The modified oligonucleotides containing ^{CNV}K were prepared, according to standard phosphoramidite chemistry using DNA sysnthesizer. Synthesized ODN were detached from the support by soaking in concentrated aqueous ammonia for 1 h at room temperature. Deprotection was conduced by heating the concentration aqueous for 4 h at 65°C concentrated aqueous ammonia was then removing it by speedvac, and the crude oligomer was purified by reverse phase HPLC and lyophilized.

Preparation of ^{TF}T-modified oligonucleotides

The pgosphoramidite of ^{TF}T was prepared according to a method reported in literature. The modified oligonucleotides containing ^{TF}T were prepared, according to standard phosphoramidite chemistry, on a DNA synthesizer using the phosphoramidite of ^{TF}T as shown in Table. Synthesized ODN were detached from the support and deprotected in 50 mM K_2CO_3 aq for 4 h at room temperature. Then it was neutralize by trichloroacetic acid and removing solvent by speedvac, and the crude oligomer was purified by reverse phase HPLC and lyophilized.

Photo-irradiation

The ODN containing ^{TF}T (20 μM) and ODN containing ^{CNV}K (20 μM) in buffer solution (100 mM NaCl, 50 mM sodium cacodylate) was -irradiated at 366 nm using UV-LED illuminator(OMRON Inc, 1600 mW) at 4°C and then it was analyzed HPLC and NMR measurement. Photo-splitting was performed at 50°C photo-irradiation at 312 nm using transilluminator (Funakoshi Inc).

HPLC analysis

The samples were analyzed with HPLC system (JASCO) with InterSustain^{TM} C18 (GL Science, 5 µm, 10 × 150 mm, elution was with 0.05 M ammonium formate containing 1-20% CH_3CN, linear gradient (40 min) at a flow rate of 1 mL/min)

UPLC analysis

The samples were analyzed with UPLC system (Aquity, Waters) equipped with BEH Shield RP18 column (1.7µm, 2.1 × 50 nm, elution was with 0.05M ammnonium formate containing 1-10% CH_3CN, linear gradient (10min) at a flow rate of 0.4 mL/min, 60°C).

Enzymatic digestion, HPLC and MALDI-TOF-MS analysis

The enzymatic digestion was carried out the treatment of snake venom phosphatase (0.2 U), P1 nuclease (1 U), ans calf intestine alkaline phosphatase (20 U) in 50 mM Tris-HCl buffer (pH 9.0) containing 1 mM $MgCl_2$ and 0.1 mM $ZnCl_2$ and 1 mM Spermidin at 37°C for 5 h. After the purification of photoadduct by reversed phase HPLC with InterSustain^{TM} C18 (GL Science, 5 µm, 10 × 150 mm, elution was with 0.05 M ammonium formate containing 1-50% CH_3CN, linear gradient (50 min) at a flow rate of 3 mL/min), the molecular masses were analyzed with a MALDI-TOF-Mass soectrometer (Voyager DE-Pro-SF, Applied Biosystems).

^{19}F-NMR measurement

^{19}F-NMR spectrums were recorded on a Bruker AVANCE III 500 instrument with a 5 mm probe head (PA BBO 500S2 BBF-H-D-05 Z) at 470 MHz for ^{19}F. The solvent for ^{19}F-NMR measurement was 10 mM Tris-HCl buffer (pH 7.6) containing 100 mM NaCl and 10 µM trifluoroacetic acid (for an internal standard, 75.6 ppm) and 10% (v/v) D_2O. Acquisition is 8192 times.

Denaturing PAGE analysis

Polyacrylamide gel electrophoresis (PAGE) was performed with 15% polyacrylamide containing 8 M urea. After the electrophoresis (150V-60min), the gel was stained by SYBRgold for 30 min, then fluorescent image was taken by a luminescent image analyzer (LAS3000, Fujifilm).

MicroRNA detection procedure based on ^{19}F chemical shift coupled with HCR

Each hairpin probe was hated to 90% for 3 min and then allowed to cool to 37°C for 1h before use. Then 10 nM miRNA was incubated at 37°C for 24 h with 10 μM each hairpin probes in 50 mM cacodylate buffer containing 100 mM NaCl. After incubation, the photo-irradiation at 366 nm was performed at 37°C for 10 s.

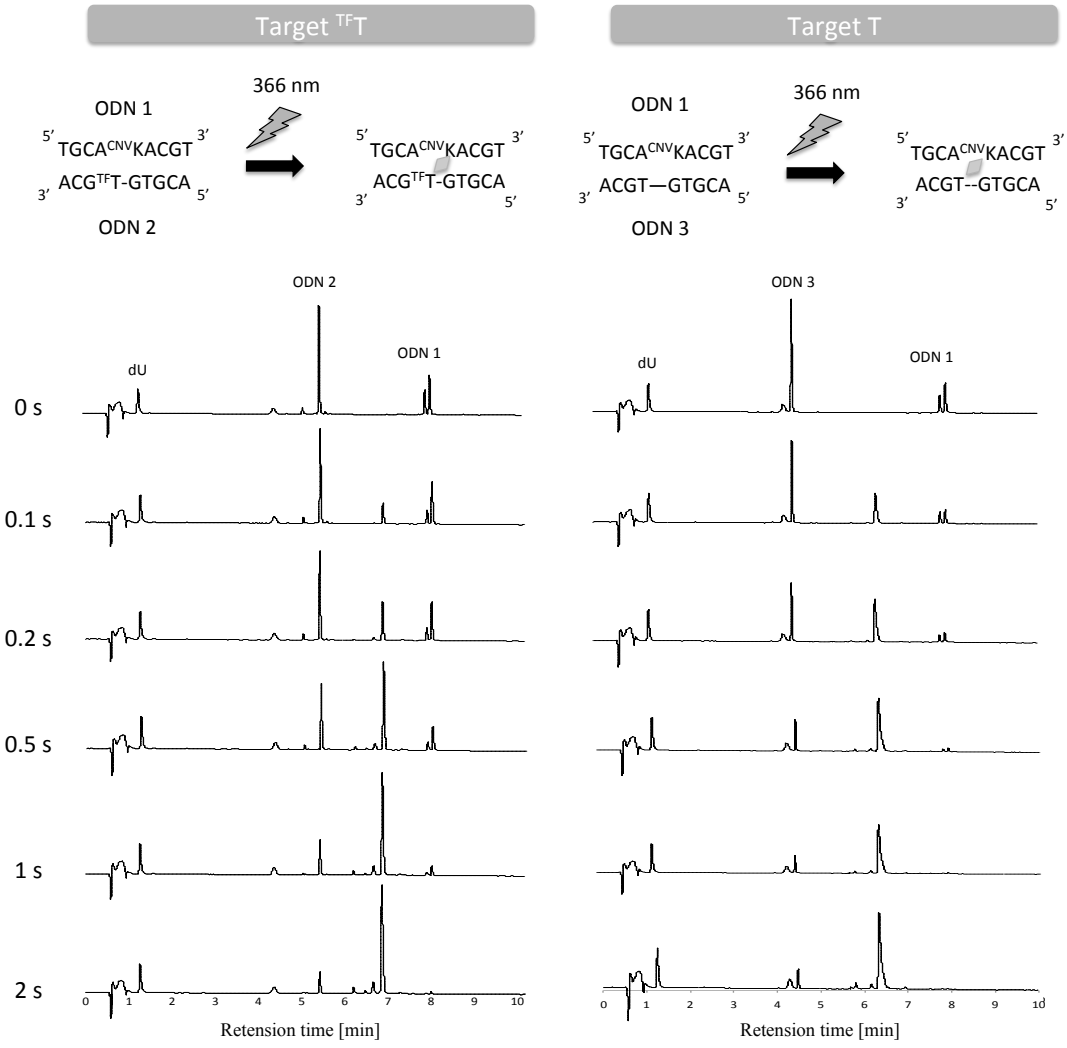

Fig. S1 The UPLC chromatogram of the photo-reactivity of ^{CNV}K and ^{TF}T or T.

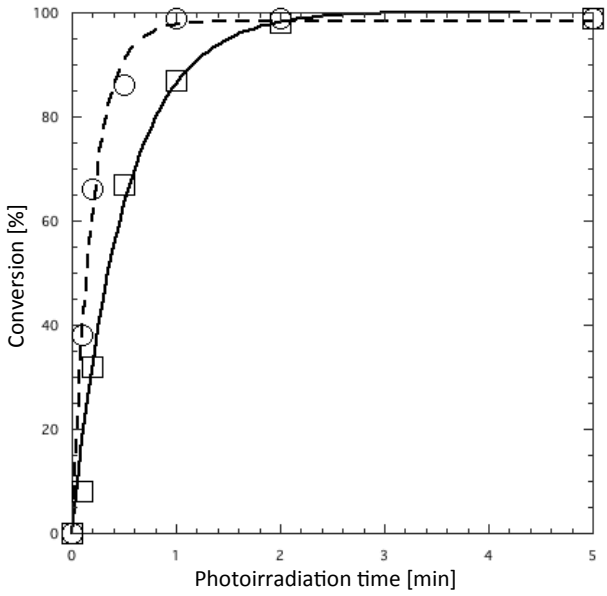

Fig. S2 The time course of photo-cross-linking between ^{CNV}K and ^{TF}T or T. (Solid line : Target ^{TF}T, Dot line : Target T)

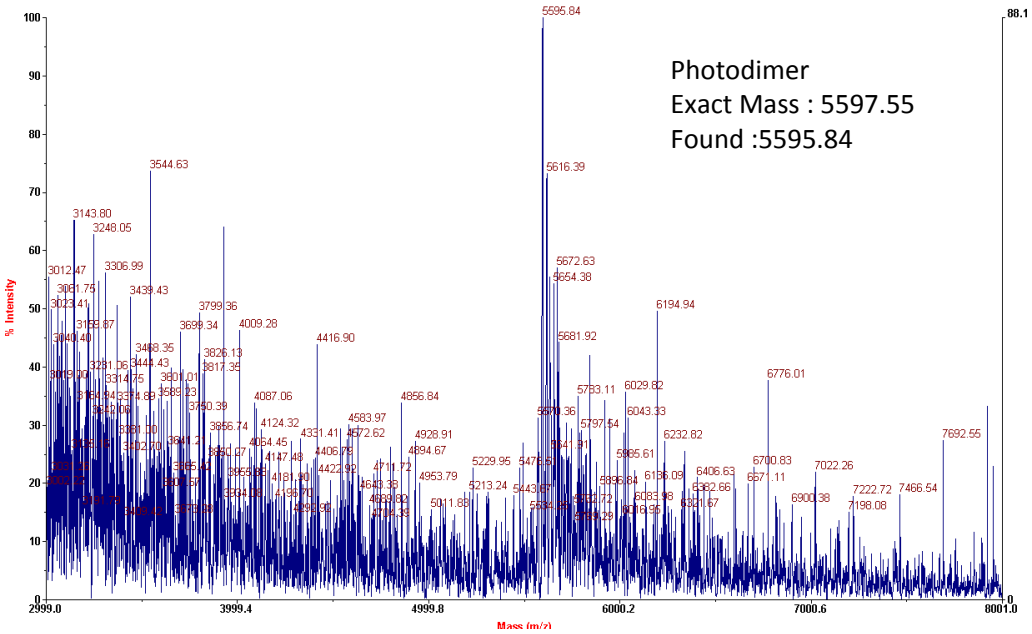

Fig. S3 The mass spectrum of photodimer consisting of ODN 1 and ODN 2.

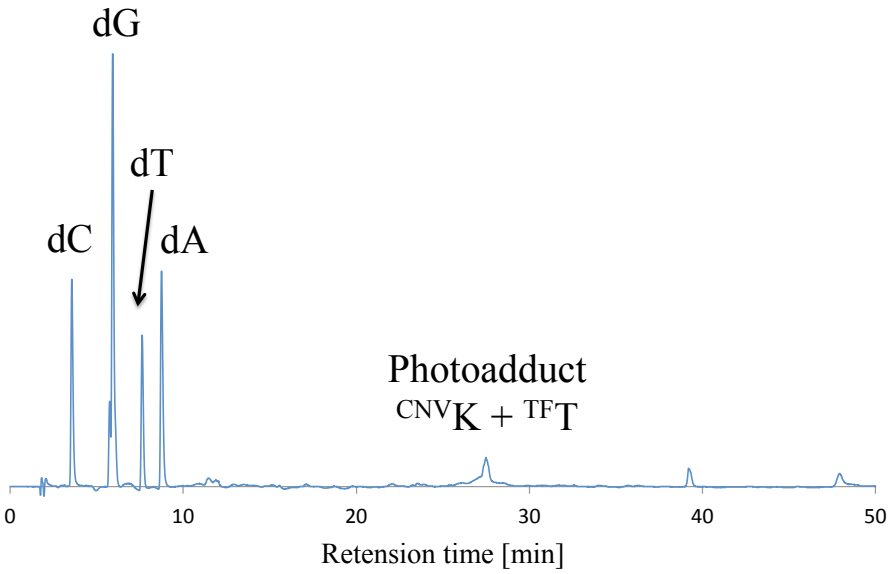

Fig. S4 Reversed-phase HPLC chromatogram of the product the nuclease and phosphatase treatment of photodimer consisting ODN 1 and ODN 2

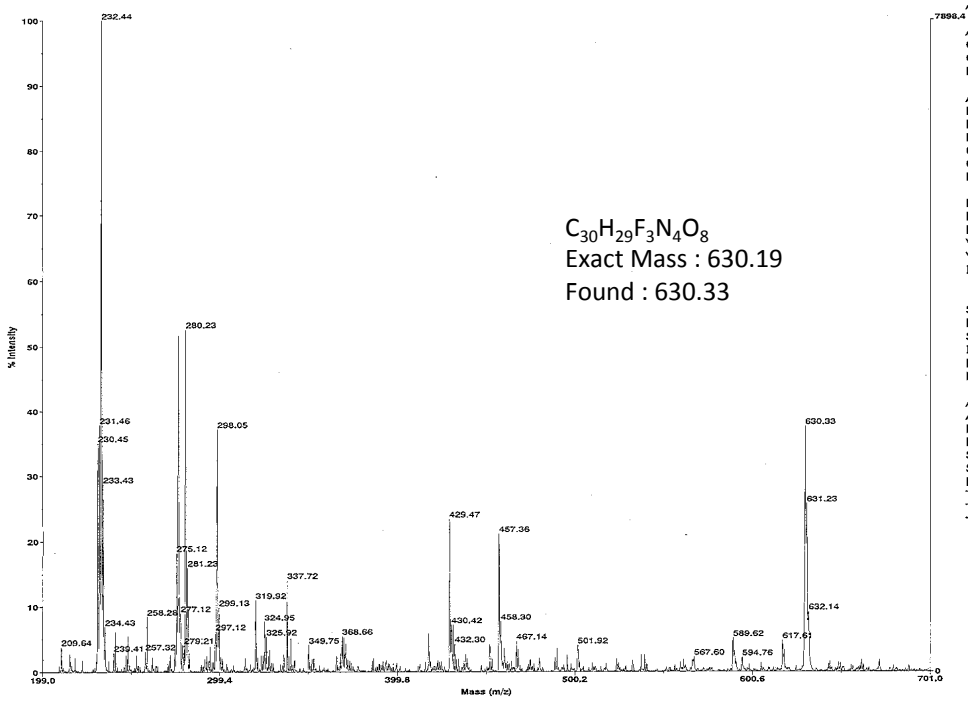

Fig. S5 The mass spectrum of photoadduct $^{CNV}K+^{TF}T$

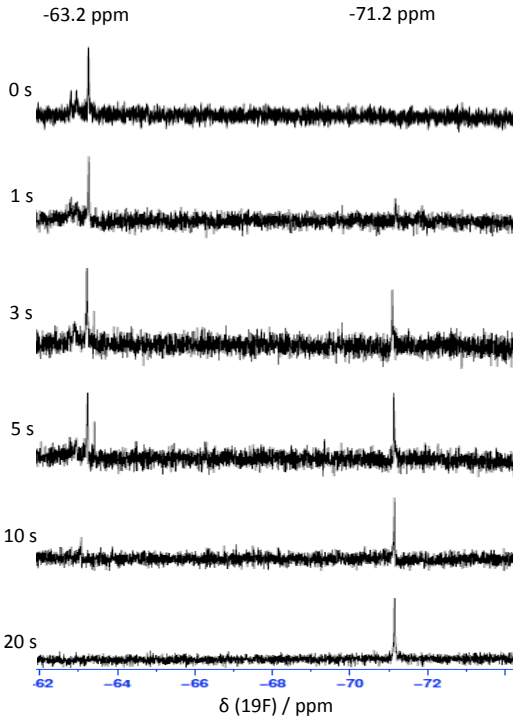

Fig. S6 ^{19}F-NMR spectra of the photo-cross-linking between ODN 1 and ODN 2 with photoirradiation at 366 nm for 0, 1, 3, 5, 10, and 20s.

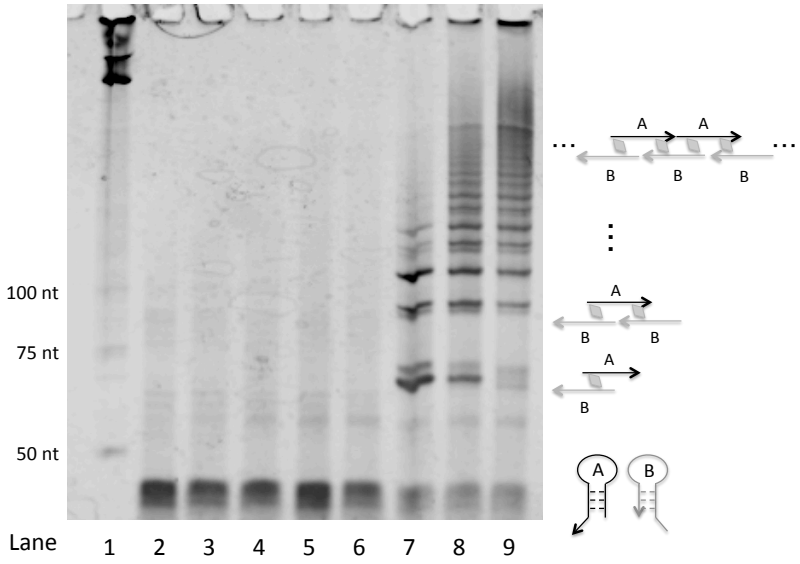

Fig. S7 Denaturing PAGE analysis of photo-cross-linking coupled with HCR. Lane 1; 25 bp ladder maker; Lane 2 to Lane 5; photo-irradiation at 366 nm for 0, 1, 5, or 10 s without miRNA200a; Lane 6 to Lane 9; photo-irradiation at 366 nm for 0, 1, 5, or 10s with miRNA200a

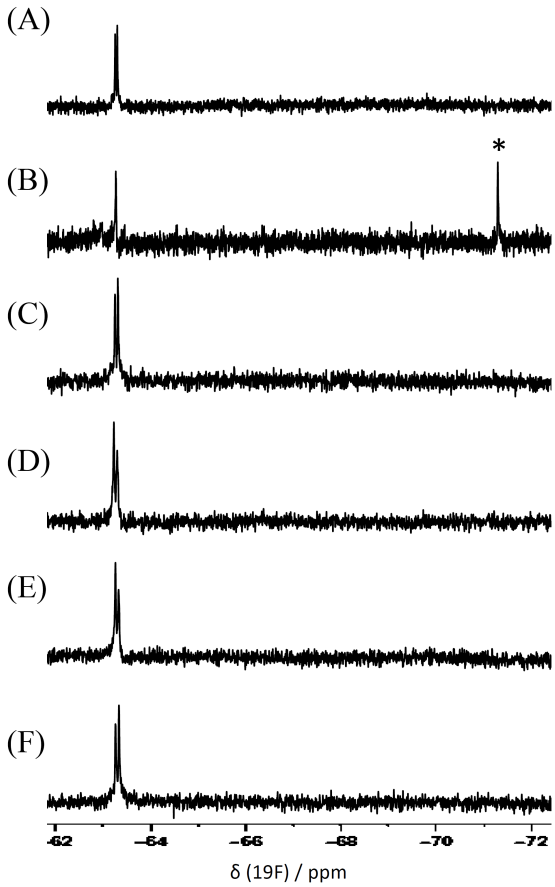

Fig. S8 ^{19}F-NMR spectrum of the triggered in the presence of (A) 200a without photoirradiation as well as (B) 200a, (C) 200b, (D) 200c, (E) 141, (F) 429 with photoirradiation at 366 nm for 10 s