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Supplementary Information

Multiplexed detection of nucleic acids using ^{19}F NMR chemical shift change based on DNA photo-cross-linking of 3-vinylcarbazole derivatives

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General

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 modified oligonucleotides

The phosphoramidite of ^{CNV}K was prepared following to previous reports. The modified oligonucleotides containing ^{CNV}K or ^{OMeV}K were prepared, according to standard phosphoramidite chemistry using DNA synthesizer (ABI 3400 DNA synthesizer, Applied Biosystems, CA). The ODN containing ^{OMeV}K was postmodified to ODN containing ^{OMeV}K, ^{OHV}K, or ^{NH2V}K in deprotection step. Synthesized ODN were detached from the support by soaking in concentrated aqueous ammonia for 1 h at room temperature. Deprotection was conducted 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 equipped with InertSustainTM C18 column Cosmosil ^{TM}5C18-AR-II column (5 μm, 10 × 150 mm, Nacalai tesque, Flow rate of 3.0 mL/min, 60°C) and lyophilized. Synthesis of ODN was confirmed by MALDI-TOF-MS. Other ODNs and miRNAs were purchased from Fasmac (Japan).

Synthesis ODN containing ^{TF}T

The phosphoramidite ^{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. Synthesized ODN were detached from the support and deprotected in 50 mM K_2CO_3 in MeOH for 8 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.

19F-NMR measurements

19F-NMR spectra were measured 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 fluorine. The solvent for 19F-NMR measurement was 10 mM Tris-HCl buffer (pH7.0) containing 100 mM NaCl and 10 μM trifluoroacetic acid (for internal standard, -75.6 ppm) and 10% D_2O.

Photoirradiation

The ODN containing ^{TF}T (20 μM) and ODN containing 3-vinylcarbazole derivatives (20 μM) in buffer solution (10 mM Tris-HCl buffer (pH7.0) containing 100 mM NaCl and 10 μM trifluoroacetic acid) 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.

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).

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.

Table S1 MALDI-TOF-MS analysis of ODN

Entry	Sequence(5'-3')	Calcd [M+H]+	Found
ODN (^{NH2V}K)	TGCA^{NH2V}KACGT	2823.55	2823.67
ODN (^{OHV}K)	TGCA^{OHV}KACGT	2824.54	2824.84
ODN (^{OMeV}K)	TGCA^{OMeV}KACGT	2838.55	2829.02
ODN (^{TF}T)	ACGTG^{TF}TGCA	2792.47	2792.91
ODN (T)	ACGTGTGCA	2738.50	2738.82

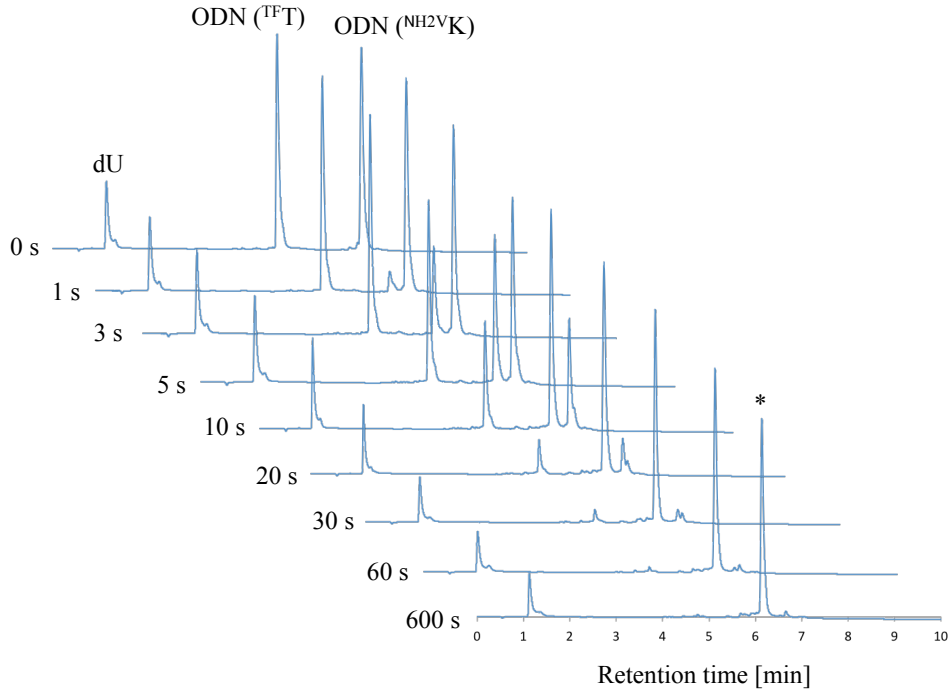

Figure S1. UPLC chromatograms of the duplex consisting of ODN (^{NH2V}K) and ODN (^{TF}T) after the indicated time period of 366 nm photoirradiation. [dsDNA] = 20 μM in 10 mM Tris-HCl buffer (pH7.0) containing 100 mM NaCl and 10 μM trifluoroacetic acid.

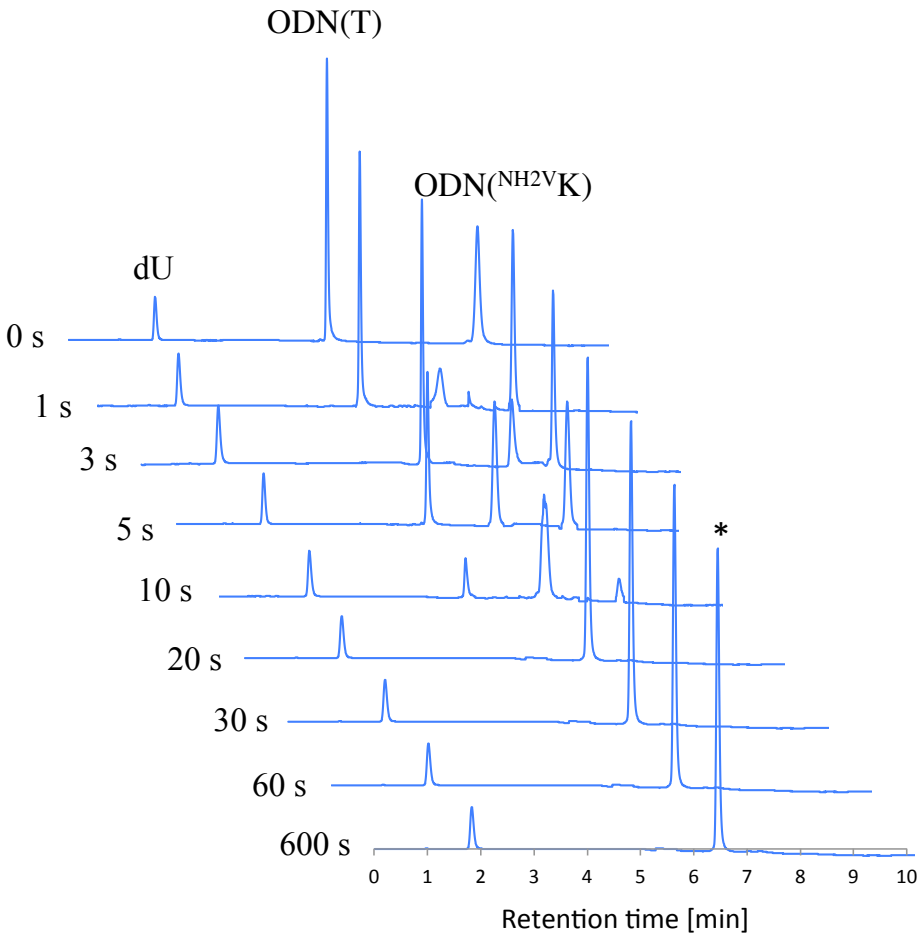

Figure S2. UPLC chromatograms of the duplex consisting of ODN (^{NH2V}K) and ODN(T) after the indicated time period of 366 nm photoirradiation. [dsDNA] = 20 μM in 10 mM Tris-HCl buffer (pH7.0) containing 100 mM NaCl and 10 μM trifluoroacetic acid.

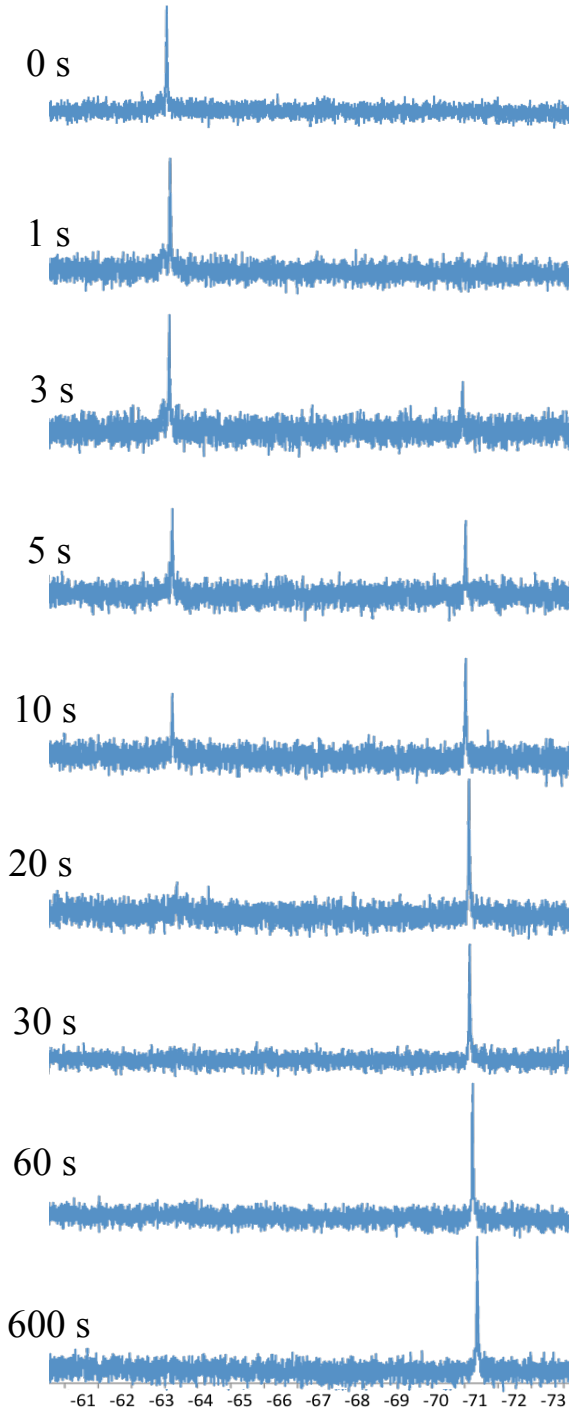

Figure S3. 19F NMR spectra of the duplex consisting of ODN (^{NH2V}K) and ODN(^{TF}T) after the indicated time period of 366 nm photoirradiation. [dsDNA] = 20 μM in 10 mM Tris-HCl buffer (pH7.0) containing 100 mM NaCl and 10 μM trifluoroacetic acid.

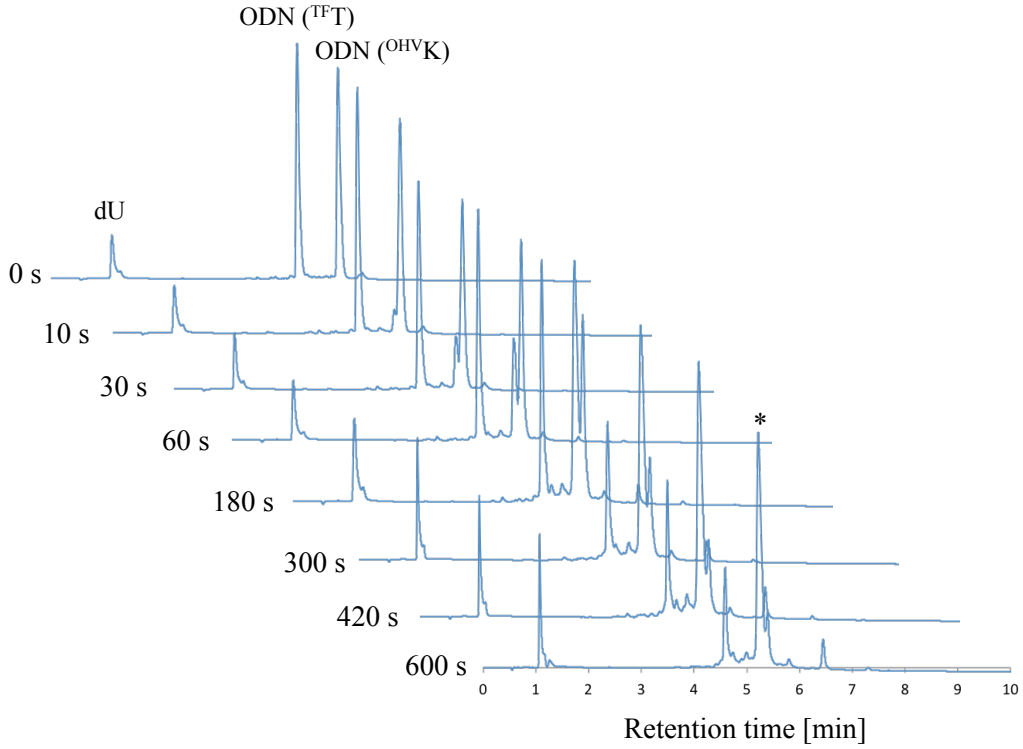

Figure S4. UPLC chromatograms of the duplex consisting of ODN (^{OHV}K) and ODN (^{TF}T) after the indicated time period of 366 nm photoirradiation. [dsDNA] = 20 μM in 10 mM Tris-HCl buffer (pH7.0) containing 100 mM NaCl and 10 μM trifluoroacetic acid.

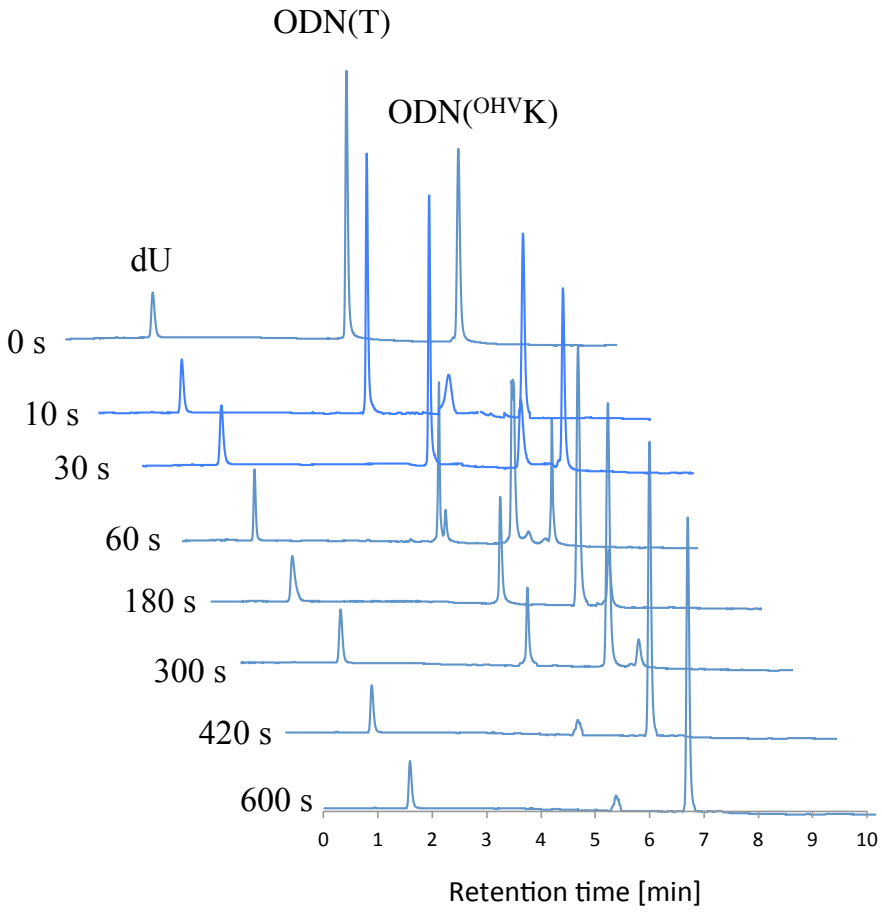

Figure S5. UPLC chromatograms of the duplex consisting of ODN (^{OHV}K) and ODN (T) after the indicated time period of 366 nm photoirradiation. [dsDNA] = 20 μM in 10 mM Tris-HCl buffer (pH7.0) containing 100 mM NaCl and 10 μM trifluoroacetic acid.

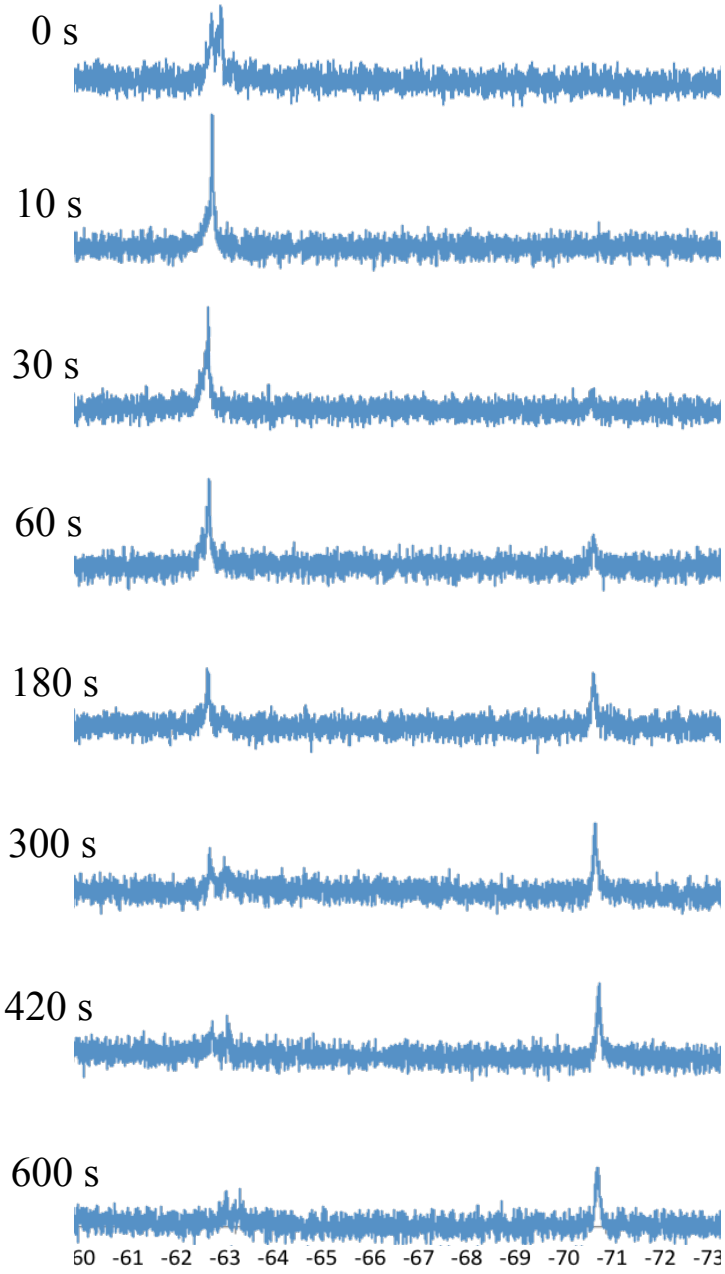

Figure S6. 19F NMR spectra of the duplex consisting of ODN 1 (^{OHV}K) and ODN (^{TF}T) after the indicated time period of 366 nm photoirradiation. [dsDNA] = 20 μM in 10 mM Tris-HCl buffer (pH7.0) containing 100 mM NaCl and 10 μM trifluoroacetic acid.

Table S2. The sequence of hairpin probe

Entry	Sequnece(5'-3')
Hairpin-^{CNV}K-a	TCTGGTAACGATGTTGAAA^{CNV}KCTACATCGTTACCAGACAG^{TF}TGTTA
Hairpin-^{CNV}K-b	AGG^{TF}TTTCAACATCGTTACCAGATAACA^{CNV}KTGTCTGGTAACGATGT
Hairpin-^{OHV}K-a	GTTGGCTAAGTTCTGCGCA^{OHV}KTAGCAGAACTTAGCCACTG^{TF}TGAA
Hairpin-^{OHV}K-b	TA^{TF}TTGCGCAGAACTTAGCCACTTC^{OH}KCAGTGGCTAAGTTCTGC
Hairpin-^{NH2V}K-a	AGTAGTTGTGCTGTTACA^{NH2V}KGCAACTGCTCAAACTACTACC^{TF}TCA
Hairpin-^{NH2V}K-b	GCA^{TF}TGTAACAGCACAAACTACTTGA^{NH2V}KGTAGTAGTTTGTGCTGTT

Table S3 The sequence of target miRNA

Entry	Sequnece(5'-3')
miRNA 200a	UAACACUGUCUGGUAACGAUGU
miRNA let7i	UGAGGUAGUAGUUUGUGCUGUU
miRNA 27a	UUCACAGUGGCUAAGGUUCUGC