Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Alkyne-tethered oligodeoxynucleotides that allow simultaneous detection of multiple DNA/RNA targets using Raman spectroscopy

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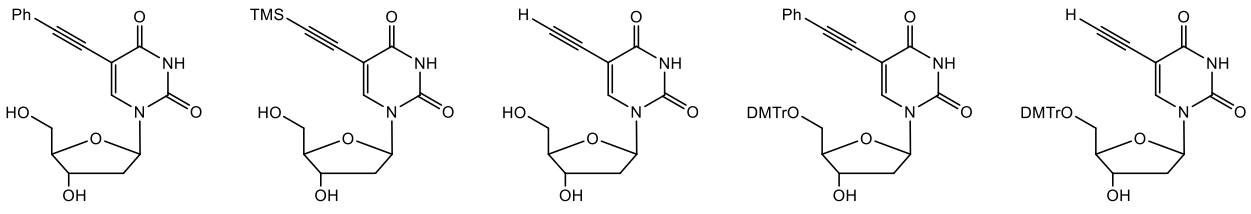
Materials

General. Reagents were purchased from Wako pure chemical industries, Tokyo chemical industries, Sigma Aldrich, nacalai tesque, GLEN RESEARCH and eurofins Genomics. MALDI-TOF mass spectrometry were recorded on an AXIMER-LNR spectrometer (SHIMADU). Raman spectrum measurement was carried out on a RENISHAW inVia Raman Microscope. DNA synthesis was performed with a 3400 DNA synthesizer (Applied Biosystems). Analytical and preparative High-performance liquid chromatographies were carried out with a D2000 HPLC system (HITACHI) and L7000 HPLC system (HITACHI) with a reversed phase column (inertsil ODS-3, GL Science Inc., ϕ 10 mm×250 mm, ϕ 4.6 mm×250 mm). The column eluents were monitored by the UV absorbance at a flow rate of 0.6 (analysis) or 3.0 (preparative) mL min^{-1} with a 0-30% (over 60 min), 30-100% (over another 30 min) gradient of ACN/TEAA buffer (100 mM, pH 7.0). Compounds $d^{Ph}U$,[1] $d^{TMS}U$,[2] $d^{H}U$,[1] DMTr-$d^{Ph}U$[1] and DMTr-$d^{H}U$[1] were synthesized by reported procedure. Human lung carcinoma cell line, A549, was purchased from JCRB cell bank (Japanese Collection of Research Bioresources Cell Bank). The cells were incubated using CO_2 incubator (MCO-18AIC, SANYO) at 37 ˚C in 5% CO_2.



$d^{Ph}U$ $d^{TMS}U$ $d^{H}U$ DMTr-$d^{Ph}U$ DMTr-$d^{H}U$

Synthesis of RP 1 (General procedure for the synthesis of ODNs). N,N-diisopropylethylamine (40 µl, 0.24 mmol) and 2-cyanoethyldiisoproryl-chlorophosphor-amidite (21 µl, 0.10 mmol) were added to compound (**^{Ph}U-DMTr**, 30 mg, 0.048 mmol) in anhydrous acetonitrile (595 µl) and

stirred for 2 h at room temperature. After the reaction, the mixture was filtered and placed on DNA synthesizer. After automated DNA synthesis, RP 1 was purified by reversed phase HPLC. The purity and concentration of the oligomers were determined by complete digestion with AP, P1 and phosphodiesterase I at 37 ˚C for 6 h. Identities of synthesized oligomers were identified by MALDI-TOF mass spectrometry (RP 1: $[M - H]^-$ calcd. 6784.2, found 6784.3,RP 2: $[M - H]^-$ calcd. 7810.3, found 7810.5).

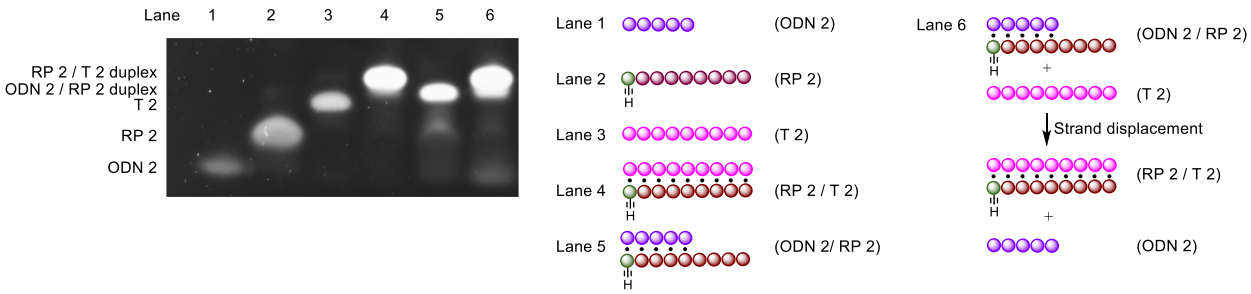


Figure S1. Electrophoretic mobility shift assay to identify strand displacement reaction of ODN 2 / RP 2 duplex by T 2 target. ODNs bearing alkyne tags (RP 2) were hybridized with their complementary strand (ODN 2). Then, T 2 was added to the sample and the resulting sample was loaded into 10% polyacrylamide gel. Lane 1, single stranded ODN 2; lane 2, single stranded RP 3; lane 3, single stranded T 2; lane 4, RP 2 / T 2 duplex; lane 5, ODN 2 / RP 2 partial duplex; lane 6, Addition of T 2 to ODN 2 / RP 2 partial duplex to form RP 2 / T 2 duplex and single stranded ODN 2.

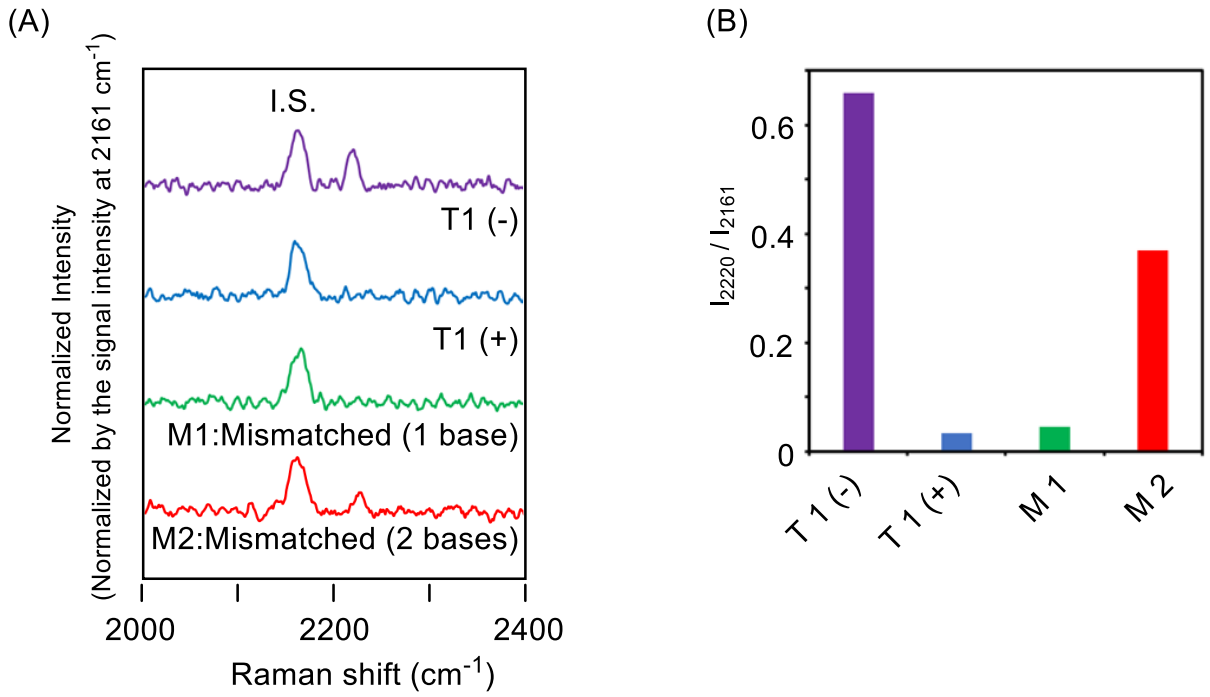


Figure S2. Change of Raman signal by strand displacement reaction of ODNs. The $d^{TMS}U$ (3 mM) was used as internal standard (I.S.). The Raman spectra were measured using 532 nm excitation. (A) Raman spectra of G-ODN 1 / RP 1 (0.1 μM) before or after addition of T 1, M 1 or M 2 (purple: without addition of target, blue: 20 μM T 1, green: 20 μM M 1, red: 20 μM M 2). (B) Relative intensity of the Raman signal of G-ODN 1 / T 1 in the presence of target. The data of signal intensity were obtained from Figure S2A.

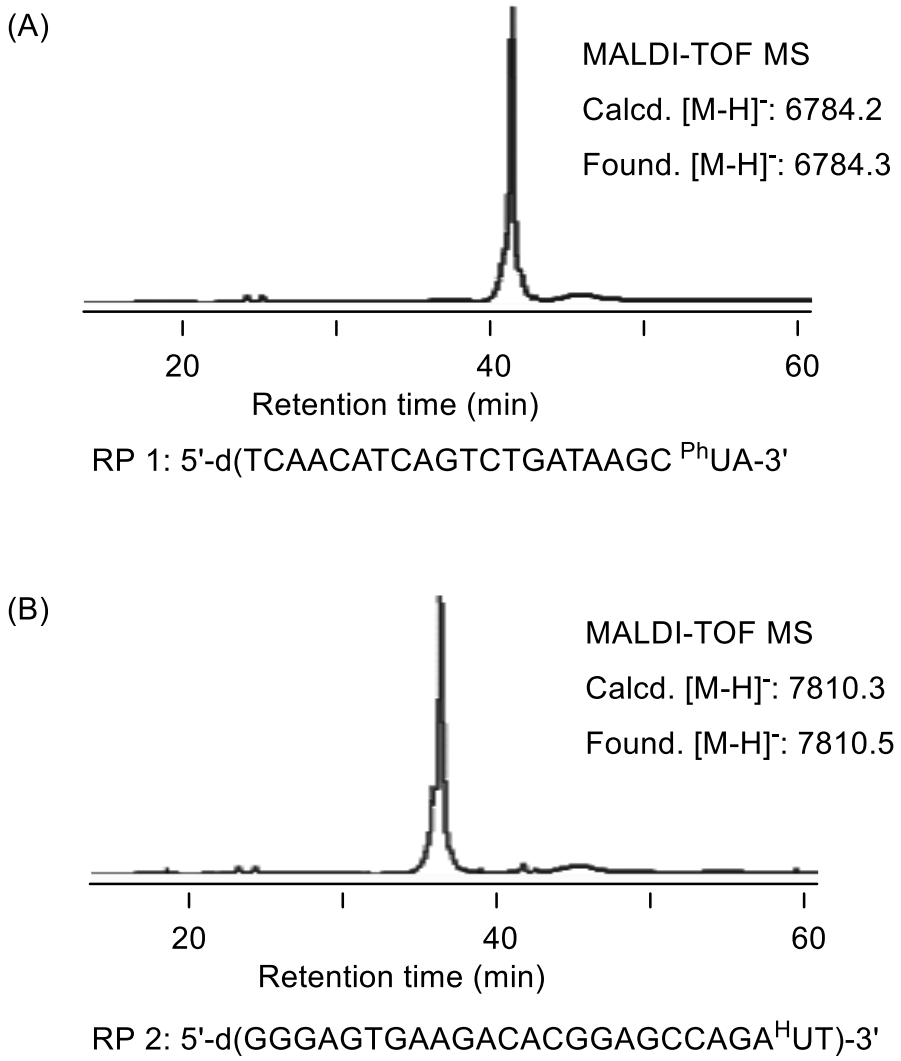


Figure S3. Characterization of ODNs synthesized in this study. HPLC chromatograms and MALDI-TOF MS data for RP 1 (A) and RP 2 (B). HPLC gradients: (A) MeCN in 0.1 M TEAA buffer from 0% to 30% over 60 min. (B) MeCN in 0.1 M TEAA buffer (pH 7.0) from 0% to 30% over 60 min.

References

(1) Itaya, R.; Idei, W.; Nakamura, T.; Nishihara, T.; Kurihara, R.; Okamoto, A.; Tanabe, K. Changes of C≡C triple bond vibration that disclosed non-canonical cytosine protonation in i-motif-forming oligodeoxynucleotides. *ACS Omega* **2021**, 6, 31595–31604.

(2) MSlavíčková, M.; Janoušková, M.; Šimonová, A.; Cahová, H.; Kambová, M.; Šanderová, H.; Krásný, L.; Hocek, M. Turning off transcription with bacterial RNA polymerase through CuAAC click reactions of DNA containing 5-ethynyluracil. *Chem. Eur. J.* **2018**, *24*, 8311−8314.