

Supporting Information

Heterogeneity of osmium oxidation efficiency at consecutive thymines

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Experimental.

Materials. Oligodeoxynucleotides (ODNs) used in this study were purchased from Gene Design, Inc. or Operon Biotechnologies. All other chemicals were of the highest commercial grade and were used without further purification.

Osmium Oxidation of DNA. ODNs containing thymine were radiolabeled at 5'-terminus by reaction with the T4 polynucleotide kinase and [γ - 32 P]ATP. The 32 P-labeled ODN was incubated in a solution of 5 mM potassium osmate, 0.1 M potassium hexacyanoferrate(III), 0.1 M bipyridine, 1 mM EDTA in 0.1 M Tris-HCl buffer (pH = 7.7) and 10% acetonitrile (50 μ L total volume) at 0 °C for 0.25 – 5 min. The reaction was quenched by the addition of 900 μ L of ethanol. A reaction mixture was ethanol-precipitated with addition of 3 M sodium acetate (pH = 5.0), 10 μ g salmon sperm DNA, and cold ethanol. The precipitated ODN was washed with 80% cold ethanol and dried *in vacuo*. The obtained ODN was resolved in 10% piperidine (v/v), heated at 90 °C for 20 min, evaporated by vacuum rotary evaporation to dryness, and then resuspended in 80% formamide loading buffer (a solution of 80% formamide (v/v), 1 mM EDTA, 0.1% xlenecyanol, and 0.1% bromophenol blue). The resulting cleavage products were analyzed by electrophoresis on a 15% denaturing 19:1 acrylamide-bisacrylamide/7 M urea sequencing gel. The bands were visualized by autoradiography and quantified using Image Gauge version 4.01 software (FUJIFILM Corp.).

Simulation Analysis of Oxidative Cleavage. The time course of the oxidation of ODN(GTTG) was simulated based on the reaction scheme described in Figure 2a. The

concentration of each species at given time was defined by the following equations:

$$d[\text{GTTG}] / dt = -k_1[\text{GTTG}] - k_2[\text{GTTG}] \quad (\text{S1})$$

$$d[\text{GT}_{\text{Os}}\text{TG}] / dt = k_1[\text{GTTG}] - k_3[\text{GT}_{\text{Os}}\text{TG}] \quad (\text{S2})$$

$$d[\text{GTT}_{\text{Os}}\text{G}] / dt = k_2[\text{GTTG}] - k_4[\text{GTT}_{\text{Os}}\text{G}] \quad (\text{S3})$$

$$d[\text{GT}_{\text{Os}}\text{T}_{\text{Os}}\text{G}] / dt = k_3[\text{GT}_{\text{Os}}\text{TG}] + k_4[\text{GTT}_{\text{Os}}\text{G}] \quad (\text{S4})$$

The normalized band intensities at T_1 and T_2 on PAGE were estimated by the eqs 1 and 2.

$$[\text{Band intensity at } T_1] = \{[\text{GT}_{\text{Os}}\text{TG}] + [\text{GT}_{\text{Os}}\text{T}_{\text{Os}}\text{G}]\} / [\text{GTTG}]_{\text{initial}} \quad (1)$$

$$[\text{Band intensity at } T_2] = [\text{GTT}_{\text{Os}}\text{G}] / [\text{GTTG}]_{\text{initial}} \quad (2)$$

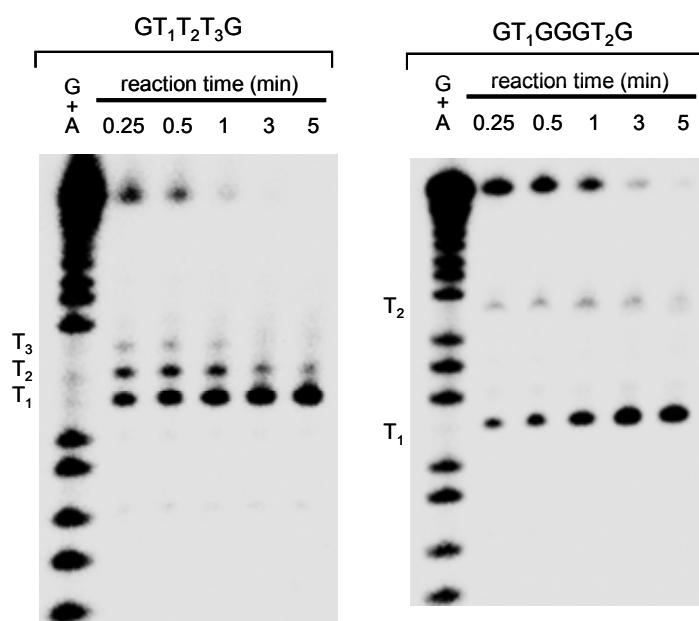


Figure S1. PAGE analysis of osmium oxidation of 5'-³²P-labeled ODN(GTTTGG) and ODN(GTGGGTG). G+A: Maxam-Gilbert sequencing lane.

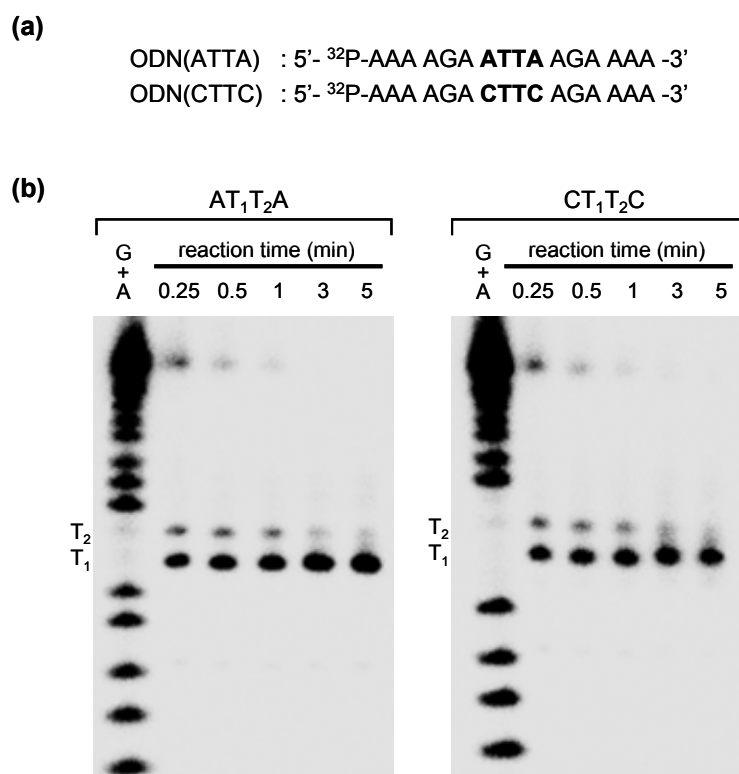


Figure S2. Osmium oxidation of DNAs containing thymines. (a) Sequences of ODN(XTTX) (X = A or C). (b) PAGE analysis of the oxidation products of ³²P-labeled ODNs.

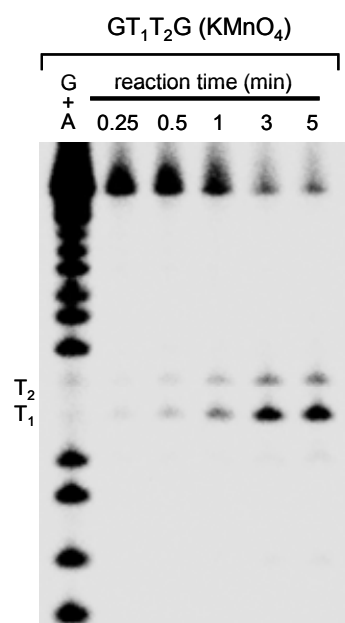


Figure S3. PAGE analysis of oxidation of 5'-³²P-labeled ODN(GTTG) with potassium permanganate.

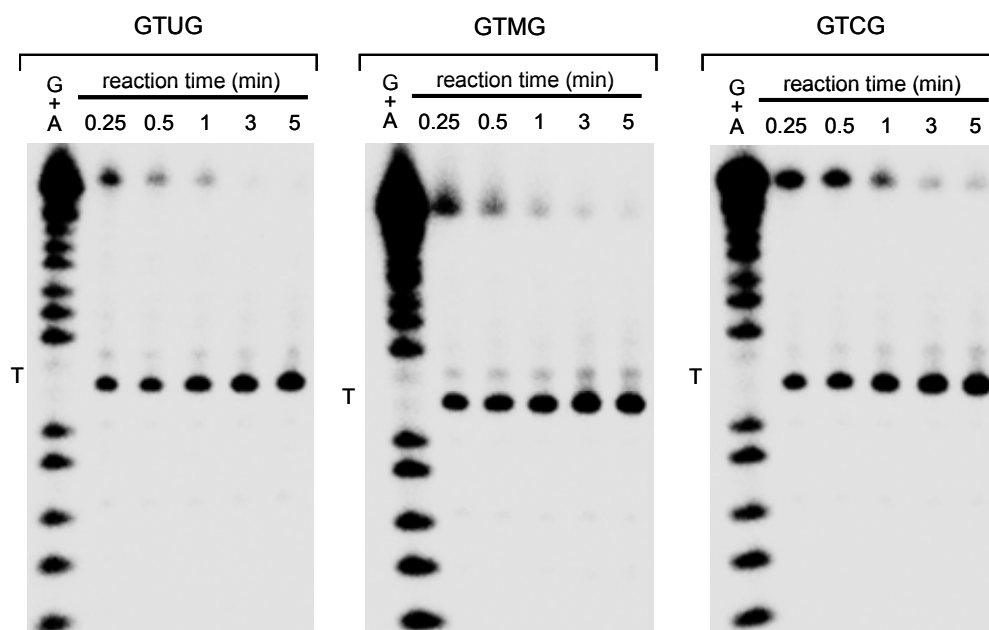


Figure S4. PAGE analysis of osmium oxidation of 5'-³²P-labeled ODN(GTPyG) (Py = U, M, or C).

■sequences

(a) pUC19(1085-1124): 5'-³²P-GTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGGCTGTGTGCA-3'

(b) pUC19(1275-1335): 5'-³²P-GAAGTGGTGGCCTAAC TACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTG-3'

(c) pFOS1(518-562) : 5'-³²P-GATAACACTGCGGCCAAC T TACTTCTGACAACGATCGGAGGACCG-3'

■conditions

5 mM K₂OsO₄, 0.1M bipyridine, 0.1 M K₃Fe(CN)₆, 0.1 M Tris-HCl(pH7.7), 1 mM EDTA, 10% MeCN.
 temp.: 0 °C, reaction time: (b) 20 s, (c) 10 s, (d) 5 s.

■PAGE analysis: 15% polyacrylamide / 7 M urea sequencing gel in 1 x TBE buffer, 1900 V x 70 min.

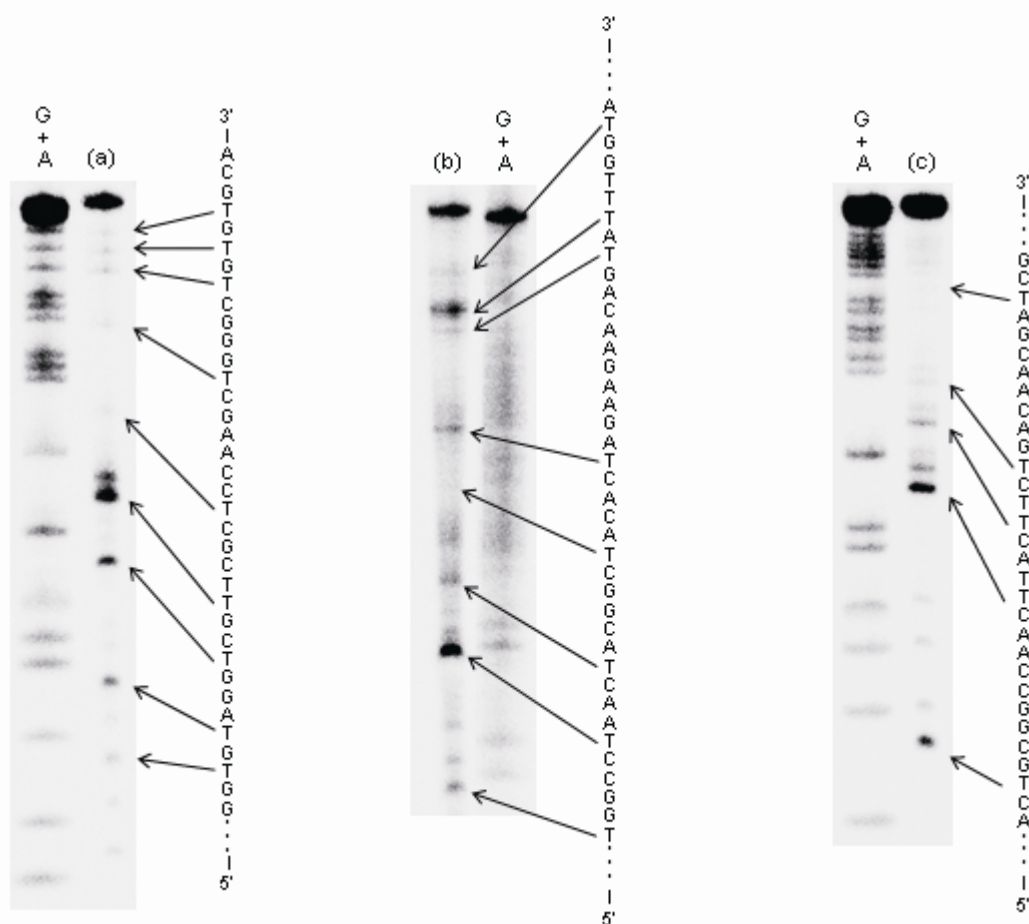


Figure S5. PAGE analysis of osmium oxidation of wild-type DNA strands.

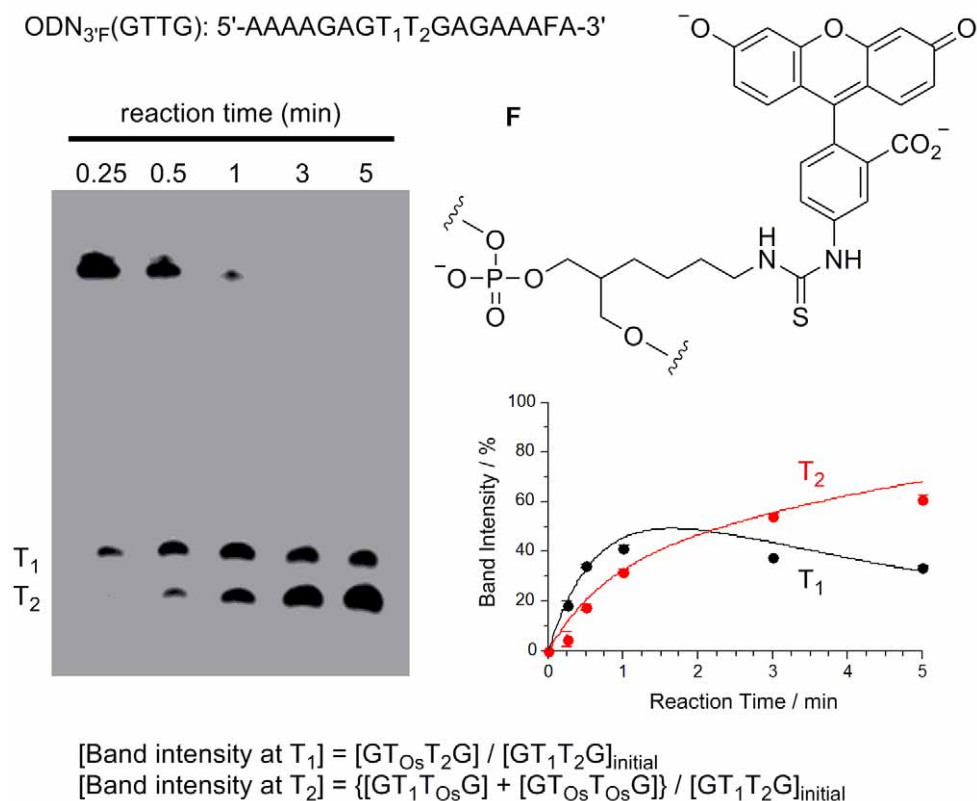


Figure S6. PAGE analysis of osmium oxidation of 3'-fluorescein-labeled ODN_{3'F}(GTTG). The ODN (50 μM) was incubated at 0 °C for 0.25–5 min in a solution of 5 mM potassium osmate, 0.1 M potassium hexacyanoferrate(III), 0.1 M bipyridine, 1 mM EDTA in 0.1 M Tris-HCl (pH = 7.7), and 10% acetonitrile, and followed by hot piperidine treatment (90 °C, 20 min). Solid lines in the chart (lower right) show the predicted time-course of band intensities obtained by simulation. $\alpha = 1.80$, $r^2(T_1) = 0.951$, $r^2(T_2) = 0.966$.