Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Evaluation of DNA-mediated electron transfer using a hole-trapping nucleobase under crowded conditions

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Materials

All oligonucleotides, including both ^{Py}U-containing oligonucleotides and 2-fluoro-O_6-nitrophenyldeoxyinosine (Glen Research) -containing oligonucleotides on a solid support, were purchased from Japan Bio Services Co., Ltd. Diaza(1,3)bicyclo[5.4.0]undecane (DBU) and triethylamine were purchased from Sigma-Aldrich. Cyclopropylamine was purchased from Wako Pure Chemical Industries, Ltd. Crotalus adamanteus venom phosphodiesterase I (Worthington) and alkaline phosphatase, calf intestinal (Promega) were used for the enzymatic digestion of oligonucleotides. PEG1540 (average molecular weight: 1350-1650) was purchased from Wako Pure Chemical Industries, Ltd. PEG1540 stock solutions (64 w/v%) were stored in a refrigerator under argon atmosphere to prevent degradation. SYBR® Green I Nucleic Acid Gel Stain (×10,000) in DMSO was purchased from Takara Bio. Aliquots of dsDNA samples were prepared by annealing an equimolar amount of each desired DNA complement. The samples were heated at 90 °C for 3 minutes, then cooled slowly. Before each experiment, a concentrated PEG solution was added to a DNA sample solution. All aqueous solutions utilised ultrapure water (Komatsu Electronics, UL-pure).

Synthesis of ^{cp}G-containing strands

^{cp}G-containing strands were synthesised according to a previous report.[1] 2-Fluoro-O_6-nitrophenyldeoxyinosine-containing strands (42mer), containing a 5'-dimethoxy group and on a solid support, were treated with a 1 M DBU solution in acetonitrile for 10 min at room temperature, then washed with acetonitrile, treated with a 1% solution of triethylamine in acetonitrile, and again washed with acetonitrile. The oligonucleotides were incubated for 18 h in 6 M aqueous cyclopropylamine solution at 60 °C. Deprotection and cleavage form the resin of the nucleotides were simultaneously performed during incubation. The cleaved oligonucleotides were dried and resuspended in ultrapure water. The solutions were purified by size exclusion chromatography (Nap5 Columns, Sephadex G-25, Cytiva). After concentration *in vacuo*, the solution containing oligonucleotides was purified by reversed-phase HPLC (JASCO, Gulliver) on a COSMOSIL 5C_{18}-MS-II column (10×150 mm; Nacalai Tesque) (DMT ON). The oligonucleotides obtained from HPLC were dried again, detritylated in 80% acetic acid, and repurified by reversed-phase HPLC (DMT OFF). HPLC profiles for ^{cp}G-containing oligonucleotide are shown in Fig. S1 and S2. The aimed products were characterised by mass spectrometry (Japan Bio Services Co., Ltd.) and HPLC analysis after enzyme digestion.

CD spectral measurements

CD measurements were carried out on a JASCO J-720W spectropolarimeter (Japan Spectroscopic Co., Ltd.) using a one-drop measurement unit (1 mm path length).

Melting-temperature measurements

The melting temperatures (T_m) of the duplexes were measured using a JASCO V-730BIO spectrophotometer with a temperature control attachment. Absorption at 260 nm of equimolar DNA complements (4.0 μM in 100 mM NaCl, 50 mM Tris-HCl buffer pH7.4) was measured every 0.5 ºC/min from 20 °C to 80 °C.

Photooxidation experiments

Aliquots (4.0 μM for dsDNA, 100 mM NaCl, 50 mM Tris-HCl, pH 7.4, total volume 30 μL) were prepared for irradiation. DNA solutions were irradiated under air with a Xe lamp (300 W; Asahi Spectra Co. Ltd.; MAX-303) through UV cutoff filter (LUX350). The irradiated solutions were filtered using Amicon membrane NMWL of 3 kDa (Merck) to remove PEG. Filtrated DNA samples were digested by incubation with both alkaline phosphatase and phosphodiesterase I at 37 ºC overnight in order to yield the free nucleosides, and the digested samples were analysed by reversed-phase HPLC monitored at 254 nm. HPLC analyses were performed with a JASCO Extrema chromatography system using a Chemcobond 5-ODS-H column (4.6×150 mm; Chemco Plus Scientific Co., Ltd.) eluted with 0.05 M ammonium acetate buffer containing acetonitrile (gradient: 2-14 % over 30 min). The percentage decomposition of ^{cp}G was determined using dC as an internal standard for all HPLC traces. Irradiation experiments were performed at least three times and the results were averaged.

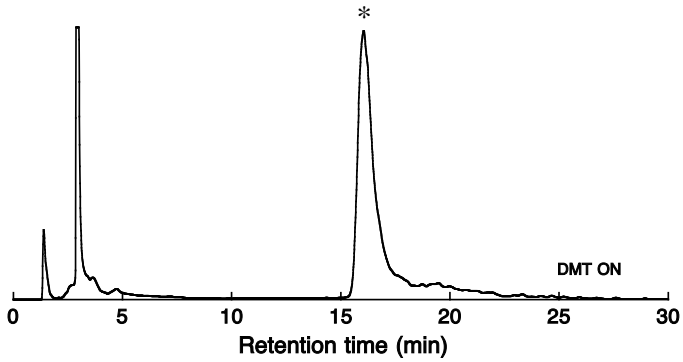
Confocal laser scanning microscopy

DNA condensates in 40% (w/v) PEG solutions containing 100 mM NaCl were incubated with 10 μM SYBR Green I in pH 7.4 Tris-HCl buffer (50 mM) before measurements. Images of liquid crystalline DNA were collected with an LSM710 confocal laser scanning microscope (Carl Zeiss).

Reference

1. T. T. Williams, C. Dohno, E. D. A. Stemp and J. K. Barton, *J. Am. Chem. Soc.* 2004, **126**, 8148-8158

(a)



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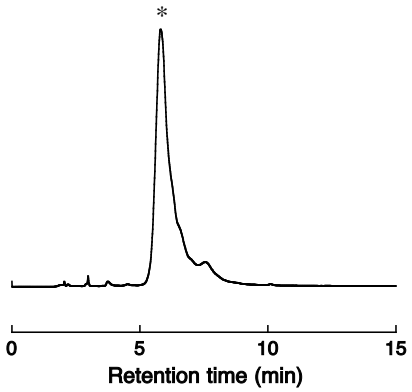
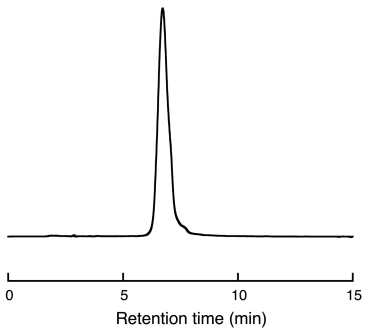


Fig. S1 HPLC profile of crude reaction mixture. (a) ^{cp}G-containing oligonucleotides (5'-TAT TAA GAA AGA TGA TTA AAA AA ^{cp}G GTA GTG AAT AAA TTG AAA -3') DMT ON and (b) DMT OFF. HPLC analysis was carried out on a COSMOSIL 5C_{18}-MS-II column eluted with 0.05 M ammonium acetate buffer containing acetonitrile. Gradient: 7-25 % over 28 min for (a) and 5-14 % acetonitrile over 18 min for (b). The largest peaks (*) were collected.

(a)



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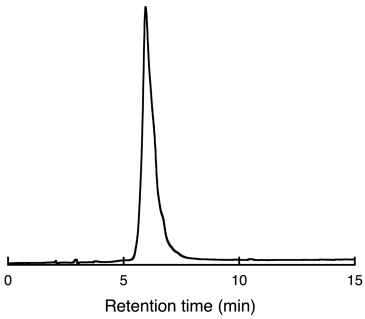
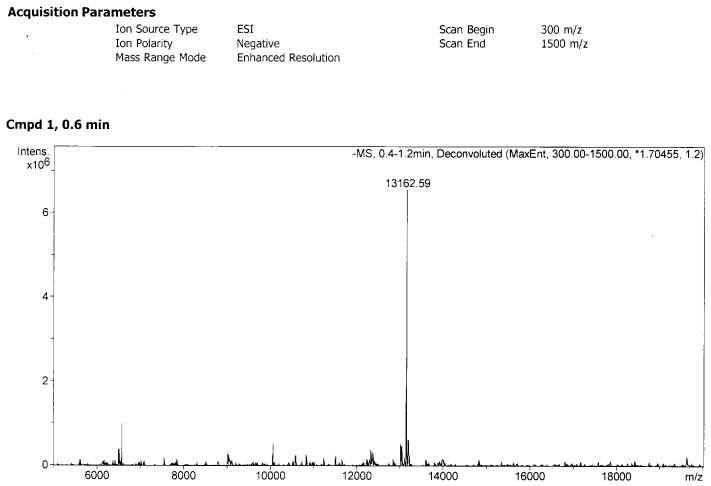


Fig. S2 HPLC profile of purified ^{cp}G-containing oligonucleotides, (a) 5'- TAT TAA GAA AGA TGA TTA AAA AA ^{cp}G GTA GTG AAT AAA TTG AAA -3' for PyU-cpG1 and (b) 5'- TAT TAA GAA AGA TAA TTA AAA AA ^{cp}G ATA GTG AAT AAA TTG AAA -3' for PyU-cpG2.

(a)



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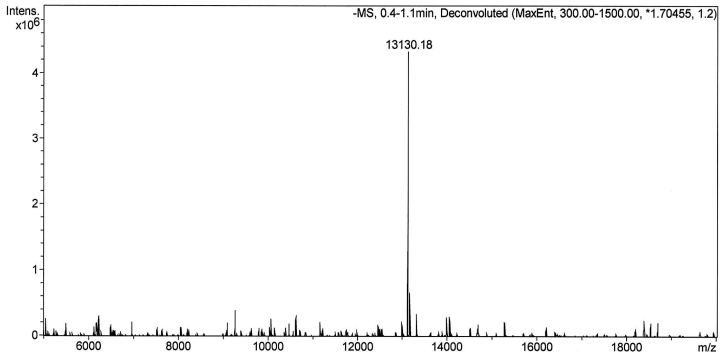
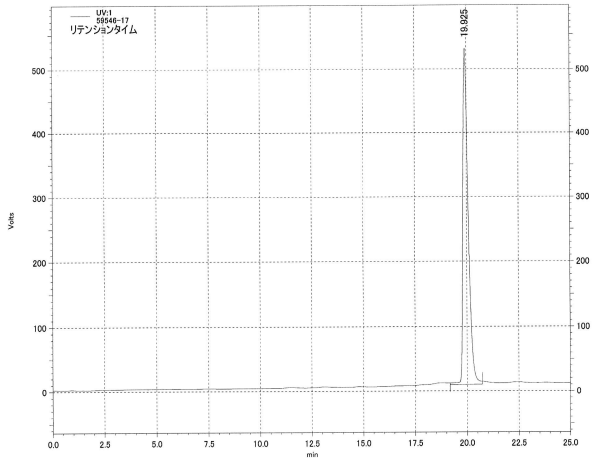


Fig. S3 Mass spectra of ^{cp}G-containing oligonucleotides, (a) 5'- TAT TAA GAA AGA TGA TTA AAA AA ^{cp}G GTA GTG AAT AAA TTG AAA -3' for PyU-cpG1 (calculated mass: 13161.8) and (b) 5'- TAT TAA GAA AGA TAA TTA AAA AA ^{cp}G ATA GTG AAT AAA TTG AAA -3' for PyU-cpG2 (calculated mass: 13129.8)

(a)



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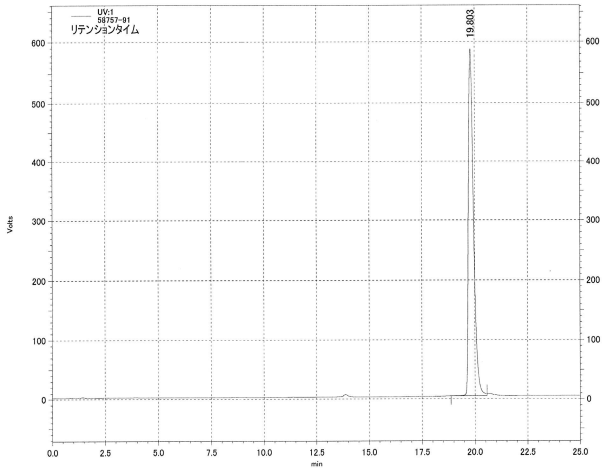
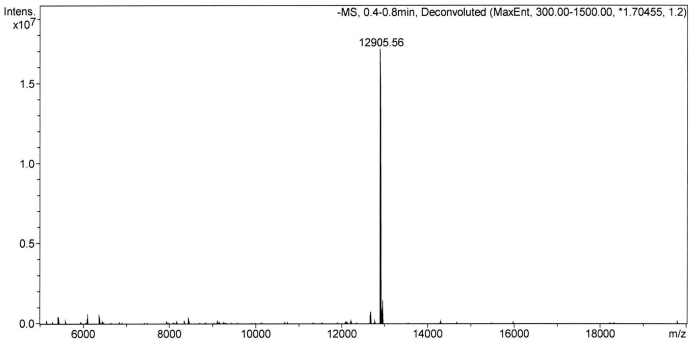


Fig. S4 HPLC profile of purified ^{Py}U-containing oligonucleotides, (a) 5'-TTT CAA TTT ATT CAC TAC CTT TTT ^{Py}UAA TCA TCT TTC TTA ATA -3’ for PyU-cpG1 and (b) 5’-TTT CAA TTT ATT CAC TAT CTT TTT ^{Py}UAA TTA TCT TTC TTA ATA-3’ for PyU-cpG2.

(a)



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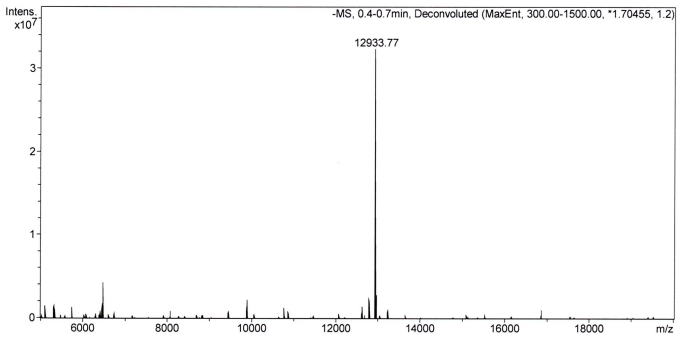


Fig. S5 Mass spectra of ^{Py}U-containing oligonucleotides, (a) 5'-TTT CAA TTT ATT CAC TAC CTT TTT ^{Py}UAA TCA TCT TTC TTA ATA -3’ for PyU-cpG1 (calculated mass: 12903.5) and (b) 5’-TTT CAA TTT ATT CAC TAT CTT TTT ^{Py}UAA TTA TCT TTC TTA ATA-3’ for PyU-cpG2 (calculated mass: 12933.6).

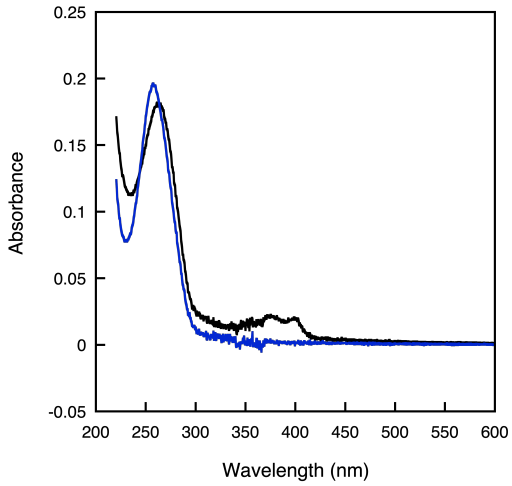


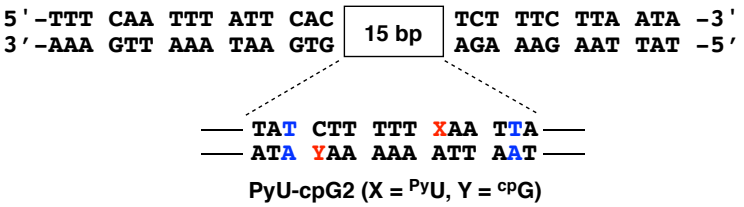
Fig. S6 Absorption spectra of 5'-TTT CAA TTT ATT CAC TAC CTT TTT ^{Py}UAA TCA TCT TTC TTA ATA -3' (black line) and 5'- TAT TAA GAA AGA TGA TTA AAA AA ^{cp}G GTA GTG AAT AAA TTG AAA -3' (blue line) for PyU-cpG1 in aqueous buffer solution. The conditions were as follows: [DNAs] = 4.0 μM in pH 7.4 Tris-HCl buffer (50 mM) and [NaCl] = 100 mM.

Table S1. Melting temperatures (T_m) for PyU-cpG1 in the absence and presence of PEG.

cosolute	T_m, ºC
none	62.9
10% (w/v) PEG	65.0
21% (w/v) PEG	64.3
30% (w/v) PEG	63.7
40% (w/v) PEG	58.8

Experimental conditions: [PyU-cpG1] = 4.0 μM in 50 mM Tris-HCl (pH7.4), [NaCl] = 100 mM. T_m is determined by monitoring the UV absorption at 260 nm. The error of T_m was less than 0.5 ºC.

(a)



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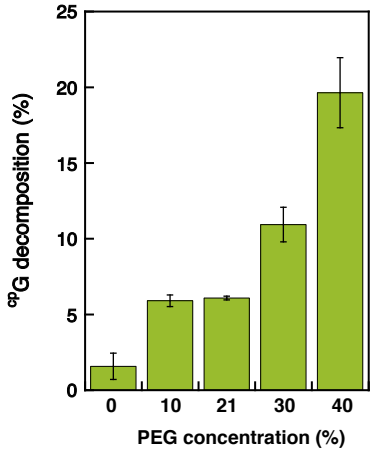

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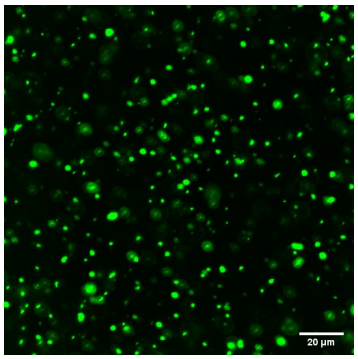
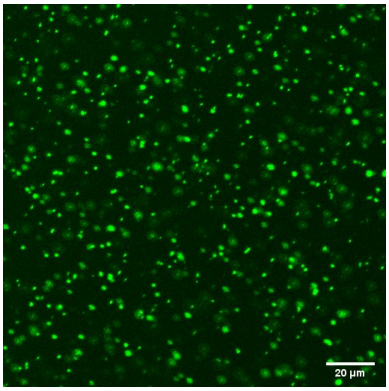


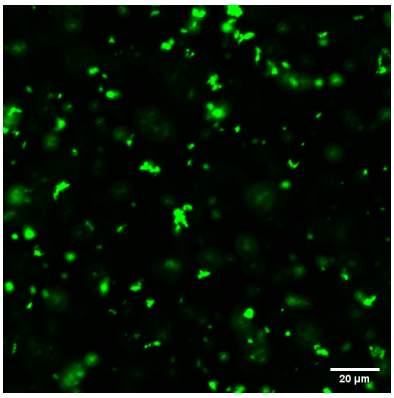
Fig. S7 (a) DNA sequence of PyU-cpG2. (b) ^{cp}G decomposition percentages of PyU-cpG2 obtained from photoirradiation (10 sec) in PEG mixed solutions. (c) Fluorescence images of condensed PyU-cpG2 (scale bar 20 μm). Images were collected with a confocal laser scanning microscope. The conditions were as follows: [PyU-cpG2] = 4.0 μM and [NaCl] = 100 mM in pH 7.4 Tris-HCl buffer (50 mM) containing 40 % (w/v) PEG.

(a)



PyU-G1

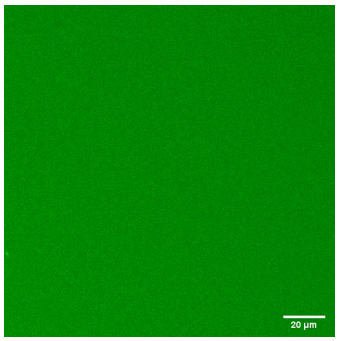
(b)



T-^{cp}G1

Fig. S8 (c) Fluorescence images of condensed (a) PyU-G1 and (b) T-cpG1 (scale bar 20 μm). Images were collected with a confocal laser scanning microscope. The conditions were as follows: [DNA] = 4.0 μM and [NaCl] = 100 mM in pH 7.4 Tris-HCl buffer (50 mM) containing 40 % (w/v) PEG.

(a)



PyU-G1 + T-cpG1

(b)

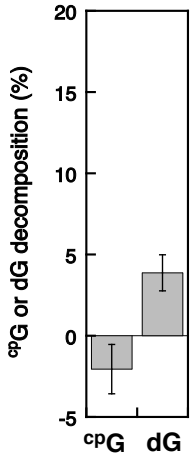


Fig. S9 (a) Fluorescence image of 30% (w/v) PEG solution containing PyU-G1 and T-cpG1. No DNA condensate was observed by confocal fluorescence microscopy. (scale bar 20 μm). (b) The amount of ^{cp}G decomposition and the total dG decomposition of DNAs ([pyU-G1] = 4.0 μM, [T-cpG1] = 4.0 μM) in 30% (w/v) PEG solution after 10 sec photoirradiation (λ_{ex}> 350 nm).

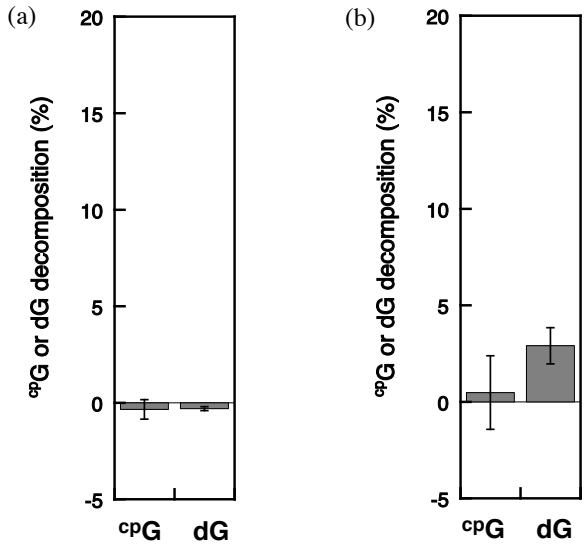


Fig. S10 The amount of ^{cp}G and dG decomposition percentages of T-cpG1 (a) in the absence and (b) presence of 40 % (w/v) PEG after 10 sec photoirradiation (λ_{ex}> 350 nm). The conditions were as follows: [T-cpG1] = 4.0 μM and [NaCl] = 100 mM in pH 7.4 Tris-HCl buffer (50 mM).