Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

DNA methylation is regulated by both stability and topology of G-quadruplex

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Contents:

Experimental

Materials

All unmodified and fluorescently labelled oligodeoxynucleotides purified by high-performance liquid chromatography were purchased from Fasmac Co., Ltd. (Japan). Concentrations of single-stranded DNA oligonucleotides were calculated from the absorbance measured at 260 nm and 95 °C using single-strand extinction coefficients. The absorbance was measured using a Shimadzu 1800 spectrophotometer (Shimadzu, Japan) connected to a thermoprogrammer.

Thermodynamic analysis

UV absorbance was measured using a Shimadzu 1800 spectrophotometer (Shimadzu, Japan) equipped with a temperature controller. The UV melting curves of G4s were measured at 295 nm, at which G4s exhibited a hypochromic transition. All experiments were conducted in 10 mM Tris-HCl (pH 7.8), 30 mM NaCl, 10 mM KCl and 10 mM $MgCl_2$. The heating rates were 0.5 °C min^{-1} from 0 °C to 95 °C. To calculate thermodynamic parameters, including the melting temperature (T_m), enthalpy change (ΔH^o), entropy change (ΔS^o) and free energy at 37 °C (ΔG^o_{37}) for intramolecular G4 formation, the melting curves were obtained at least three times and were fit to the theoretical equation for an intramolecular association.[1] Before the measurement, the sample was heated to 95 °C, and then cooled at a rate of 1.0 ºC min^{-1}. Intramolecular G4 formation was confirmed by measuring UV melting at oligonucleotide concentrations of 2, 5, 10 and 20 μM.

CD measurements

CD spectra were recorded on a JASCO J-1500 spectropolarimeter (JASCO, Japan) equipped with a temperature controller, using a total DNA concentration of 0.5 or 5 μM in 10 mM Tris-HCl (pH 7.8), 30 mM NaCl, 10 mM KCl and 10 mM $MgCl_2$. The spectra were obtained at 37 °C by capturing at least three scans from 200 to 350 nm for a cuvette with a path length of 0.1 cm for 5 μM DNA or 1 cm for 0.5 μM DNA. The cuvette-holding chamber was flushed with a constant stream of dry N_2 gas to avoid the condensation of water on the cuvette exterior. Before the measurement, the sample was heated to 95 °C and then cooled at a rate of 1.0 °C min^{-1}.

DNA methylation assay

DNA methylation was performed using a CpG methylase (M. SssI) kit (Zymo Research, USA). Before the methylation reaction, substrate oligonucleotides (Final concentration is 0.5 μM in methylation reaction) were annealed from 95 °C to 20 °C at a cooling rate of 1.0 °C min^{-1} in

10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 10 mM KCl, 10 mM $MgCl_2$ and 1 mM DTT to fold DNA structures. After annealing, CpG methylase and *S*-adenosylmethionine (SAM) (final concentration of 600 μM) were added to the annealed samples and reacted at 37 °C for 1 h, heated to 65 °C and incubated for 20 min to inactivate methyltransferase. To prevent inhibition of restriction enzyme digestion by the formation of DNA structure, methylated products were annealed in the buffer of HhaI digestion, 1 × M (TAKARA, Japan) containing 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10 mM $MgCl_2$ and 1 mM DTT, in which all the oligonucleotides were confirmed to form duplexes. After annealing, HhaI was added and the mixture was allowed to react at 37 °C for 1 h. As methylated cytosine can inhibit the digestion of HhaI, only non-methylated DNA should be cleaved by HhaI. The product was analysed using 10 wt% polyacrylamide gels in 1 × TBE buffer. The gels were run at 250 V at 25 °C for 50 min and imaged using a fluorescent imager (FLA-5100, FUJIFILM).

Native gel electrophoresis

Native polyacrylamide gel electrophoresis (PAGE) was conducted on non-denaturing gels containing 10 or 15 wt% polyacrylamide gels in 1×TBE buffer containing 30 mM KCl or NaCl. DNA samples (2 μL of 2.5 μM) were loaded and analysed by electrophoresis. The gels were stained with SYBR® Gold (Thermo Fisher Scientific) and imaged using a fluorescent imager (FLA-5100, FUJIFILM). Before the measurement, the samples were heated to 80 °C and then cooled at a rate of 1.0 °C min^{-1}. The gels were run at 100 V and 25 °C for 140 min.

1. H. Tateishi-Karimata, S. Nakano and N. Sugimoto, *Curr. Protoc. Nucleic Acid Chem.*, 2013, **Chapter 7**, Unit7 19.

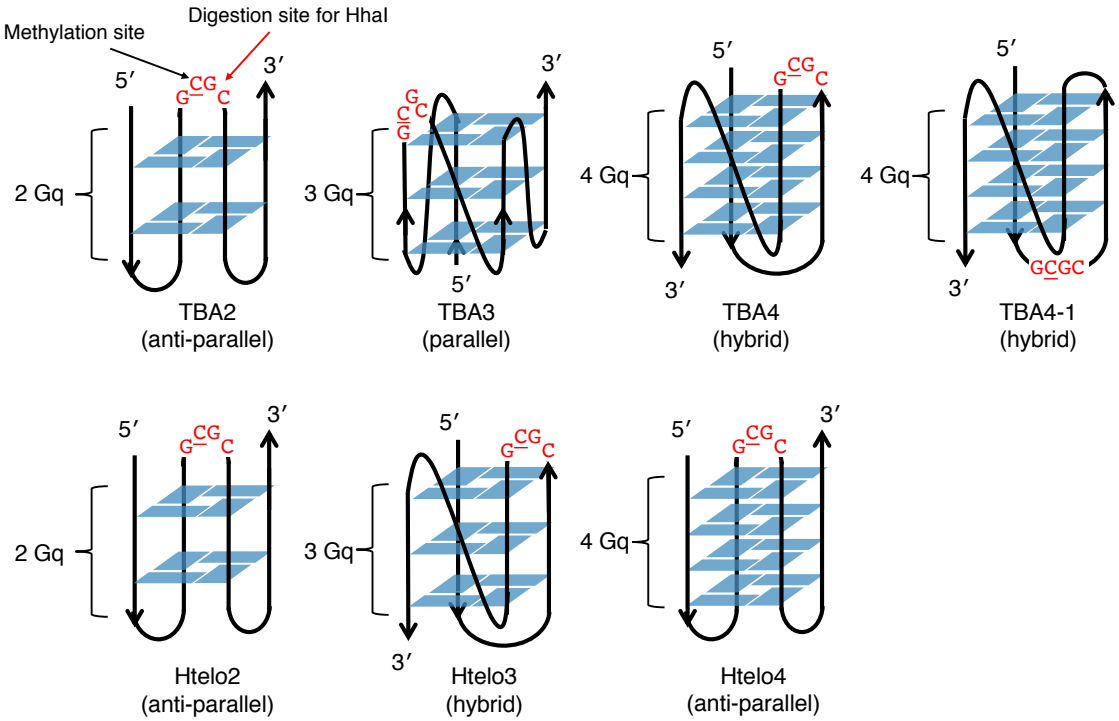

Fig. S1 Schematic illustrations of G4s with parallel, hybrid and anti-parallel topologies. The cytosines for the methylation site are underlined and the digestion sites for restriction enzyme HhaI, 5'-GCGC-3' are in red.

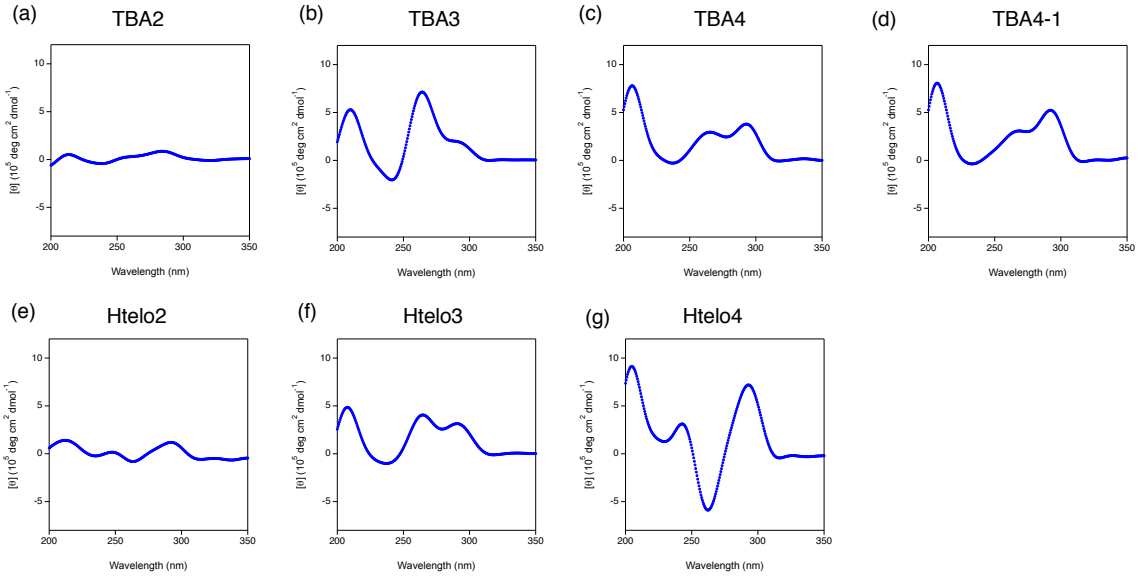

Fig. S2 CD spectra of (a) TBA2, (b) TBA3 (c) TBA4, (d) TBA4-1, (e) Htelo2, (f) Htelo3 and (g) Htelo4. All experiments were carried out at 37 ºC in 10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 10 mM KCl and 10 mM $MgCl_2$.

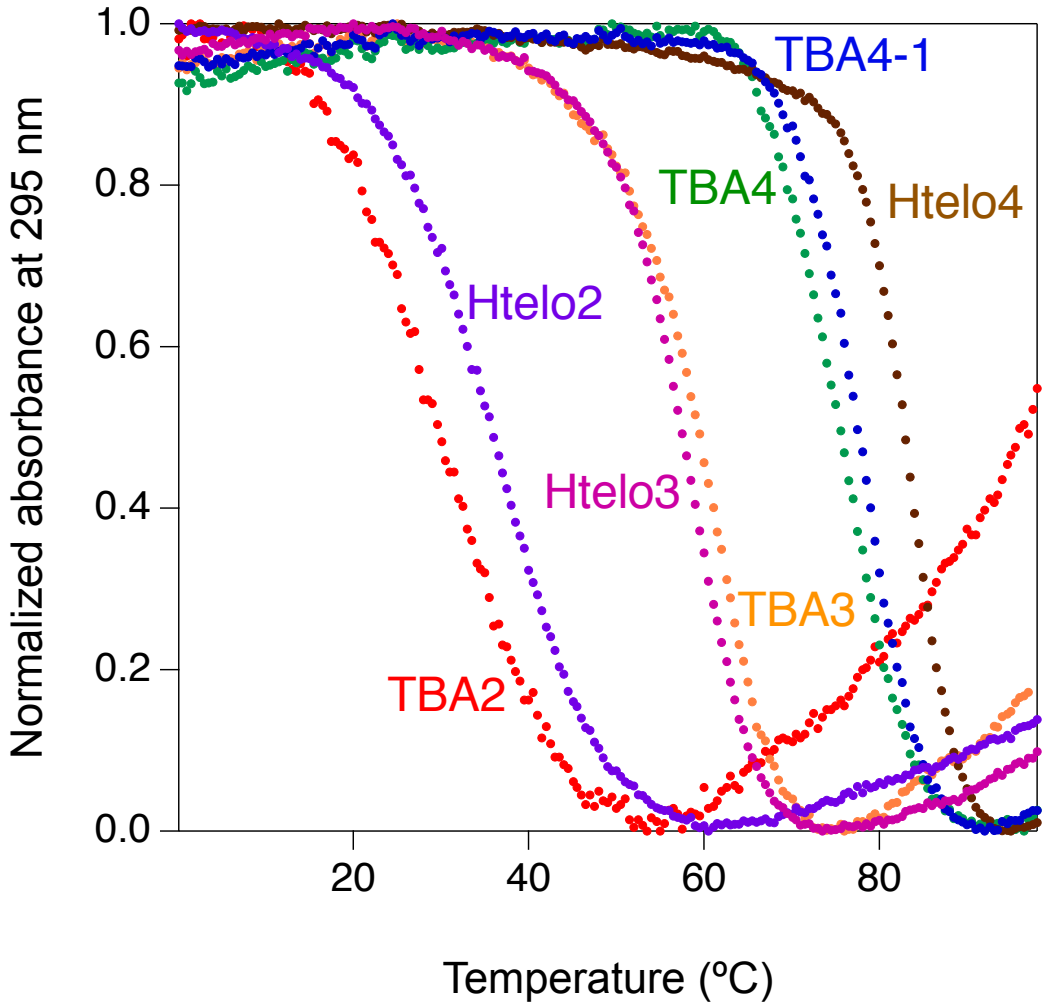

Fig. S3 Normalized UV melting curves of TBA2 (red), TBA3 (orange), TBA4 (green), TBA4-1 (blue), Htelo2 (purple), Htelo3 (pink) and Htelo4 (brown). All experiments were carried out in 10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 10 mM KCl and 10 mM $MgCl_2$.

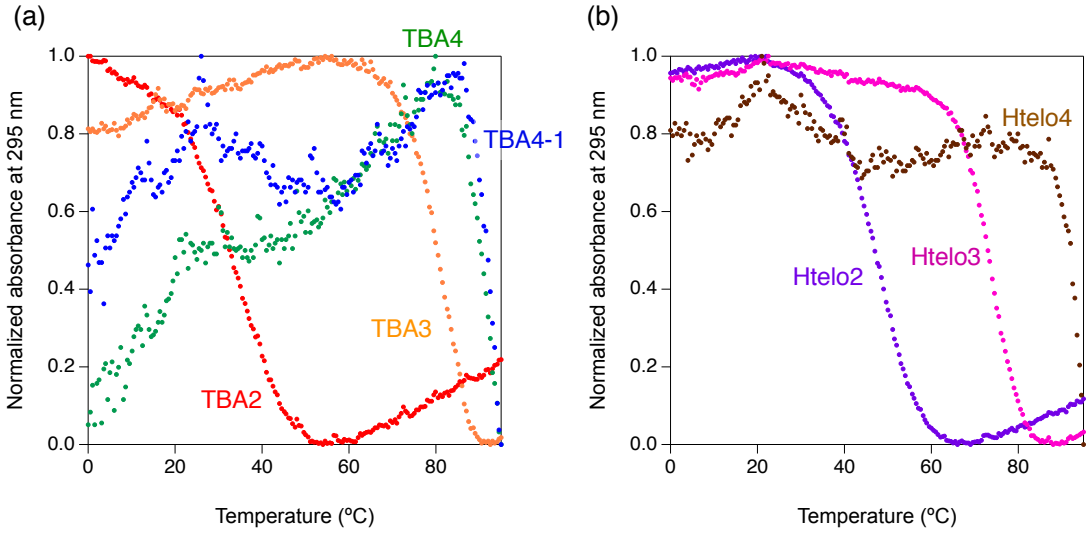

Fig. S4 Normalized UV melting curves of TBA2 (red), TBA3 (orange), TBA4 (green), TBA4-1 (blue), Htelo2 (purple), Htelo3 (pink) and Htelo4 (brown). All experiments were carried out in 10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 140 mM KCl and 10 mM $MgCl_2$.

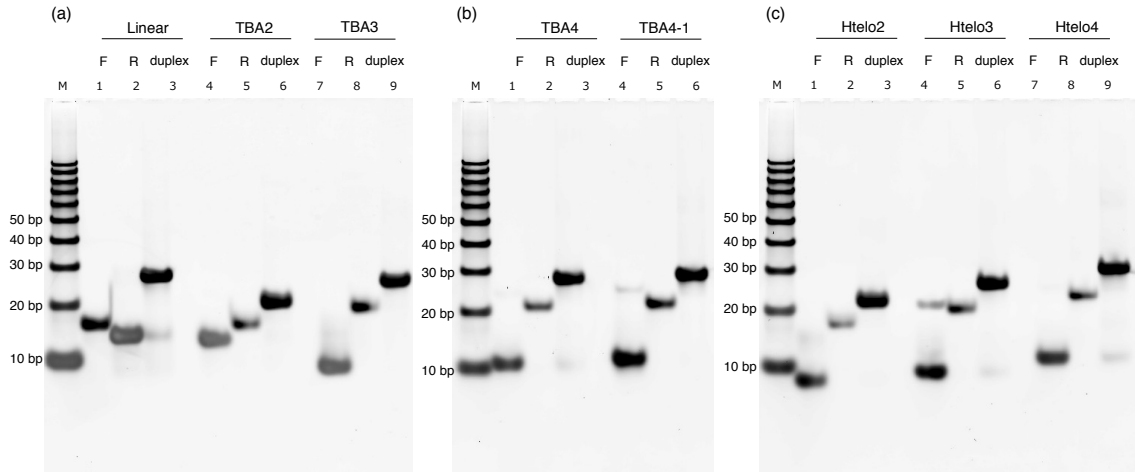

Fig. S5 Nondenaturing gel electrophoresis of forward strand (F), reverse strand (R), and mixture of forward and reverse strands (duplex) of (a) Linear, TBA2, and TBA3, (b) TBA4 and TBA4-1 and (c) Htelo2, Htelo3, and Htelo4 in the buffer for HhaI: 10 mM Tris-HCl (pH 7.5) containing 100 mM NaCl and 10 mM $MgCl_2$. The gels were run at 100 V and at 25 ºC for 140 min and stained by SYBR Gold. M represents the 10 bp DNA step ladder.

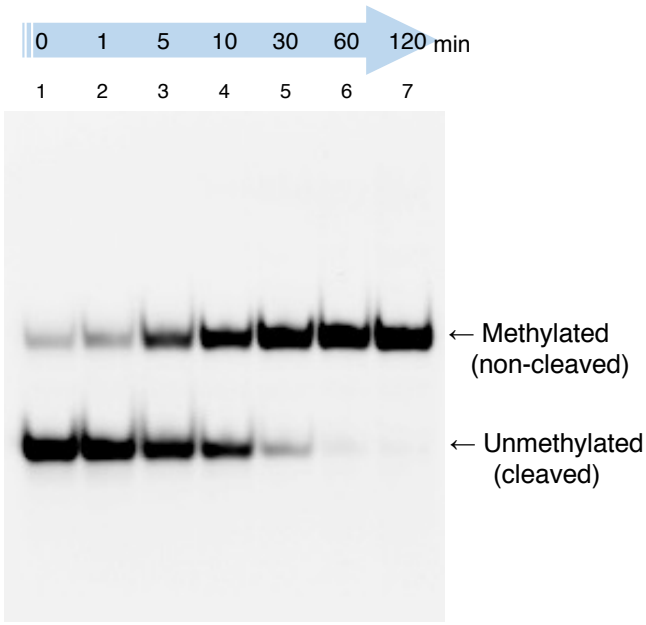

Fig. S6 Time course of methylation reaction of Linear for 0, 1, 5, 10, 30, 60 and 120 min at 37 ºC. Methylation reaction was conducted in the buffer of 10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 10 mM KCl, 10 mM $MgCl_2$ and 1 mM DTT.

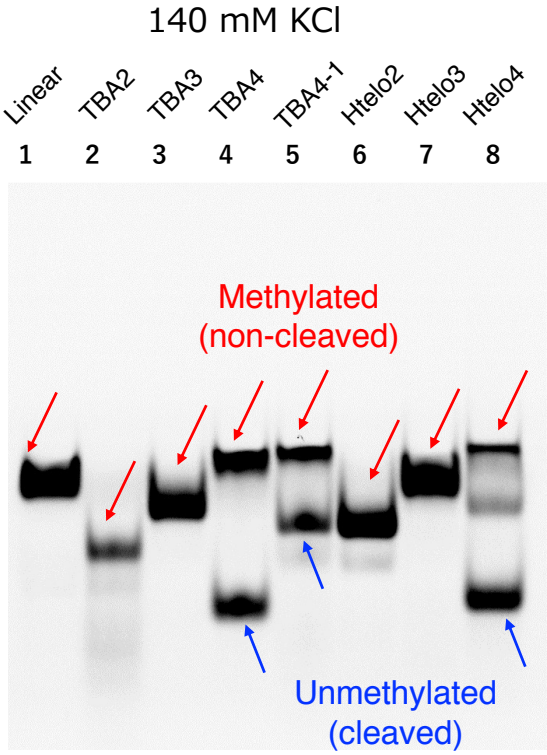

Fig. S7 Effect of KCl concentration on methylation efficiency. DNA oligonucleotide was methylated in 10 mM Tris-HCl (pH 7.8) buffer containing 30 mM NaCl, 140 mM KCl, 10 mM $MgCl_2$ and 1 mM DTT for 60 min at 37 °C, followed by the digestion reaction by HhaI.

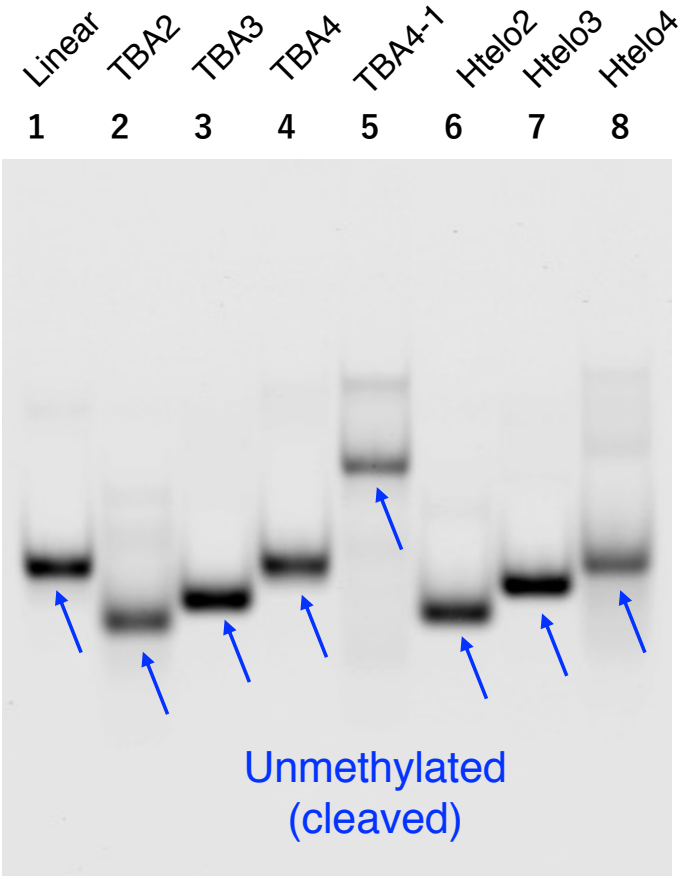

Fig. S8 Negative control of methylation reaction without methyltransferase. DNA oligonucleotide was incubated in the same buffer with methylation reaction (10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 10 mM KCl, 10 mM $MgCl_2$ and 1 mM DTT) for 60 min at 37 ℃, followed by the digestion reaction by HhaI.

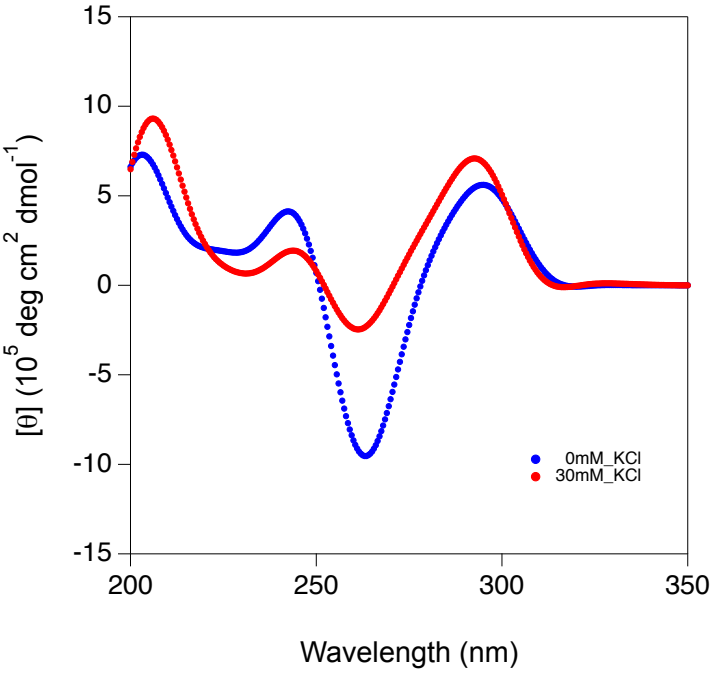

Fig. S9 CD spectra of Htelo4 in the presence of 0 and 30 mM of KCl. All experiments were carried out at 37 ºC in 10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl and 10 mM $MgCl_2$

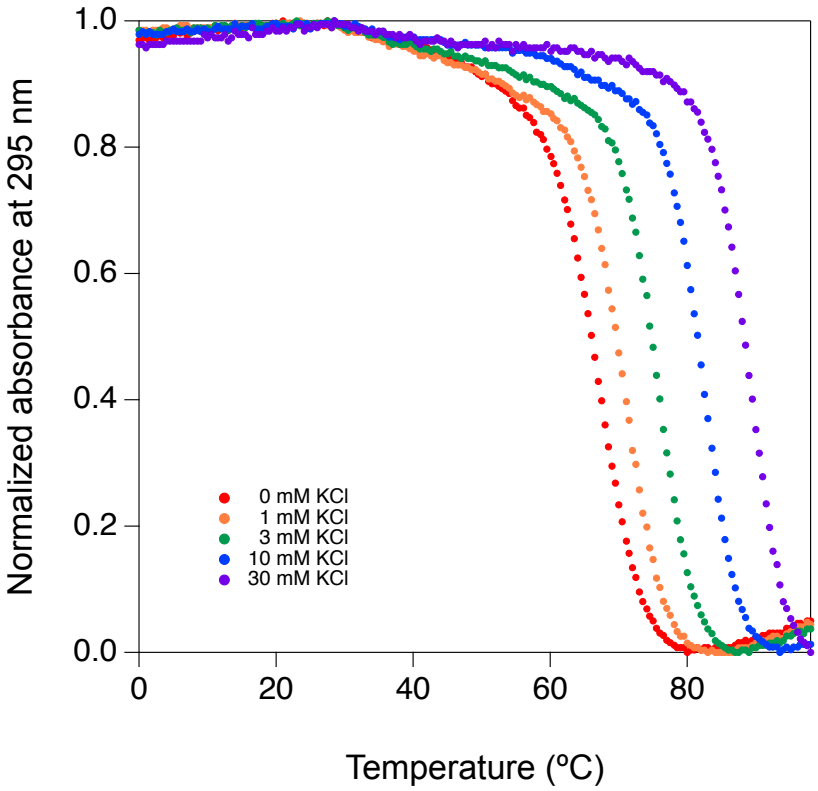

Fig. S10 UV melting curves of Htelo4 in the presence of various concentrations of KCl. The measurements of UV melting curves were carried out in 10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 10 mM $MgCl_2$ and 0 mM (red), 1 mM (orange), 3 mM (green), 10 mM (blue) and 30 mM (purple) KCl.

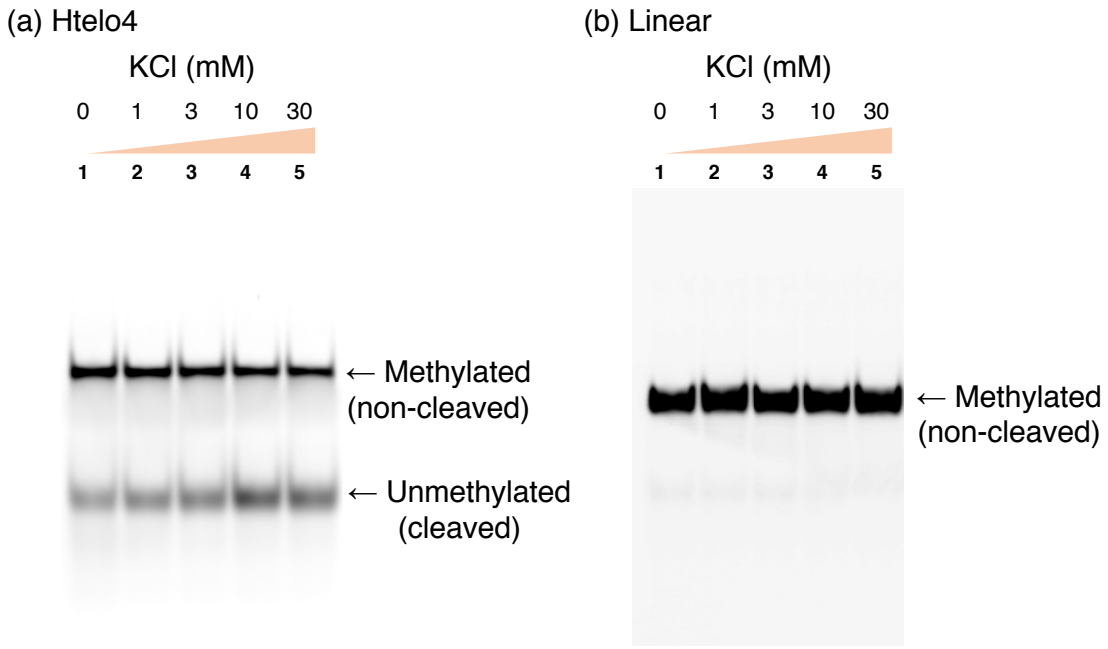

Fig. S11 The effect of the concentration of KCl on methylation of (a) Htelo 4 and (b) linear. Methylation was conducted in 10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 10 mM $MgCl_2$ and 0 to 30 mM KCl. After methylation, the product was annealed and digested by the HhaI and analyzed by gel electrophoresis. The gels were run at 100 V and 25 °C for 140 min. The bands of Htelo4 in the presence of various KCl concentrations were quantified and used for the calculation of methylation efficiencies, which are plotted in Fig. 2.

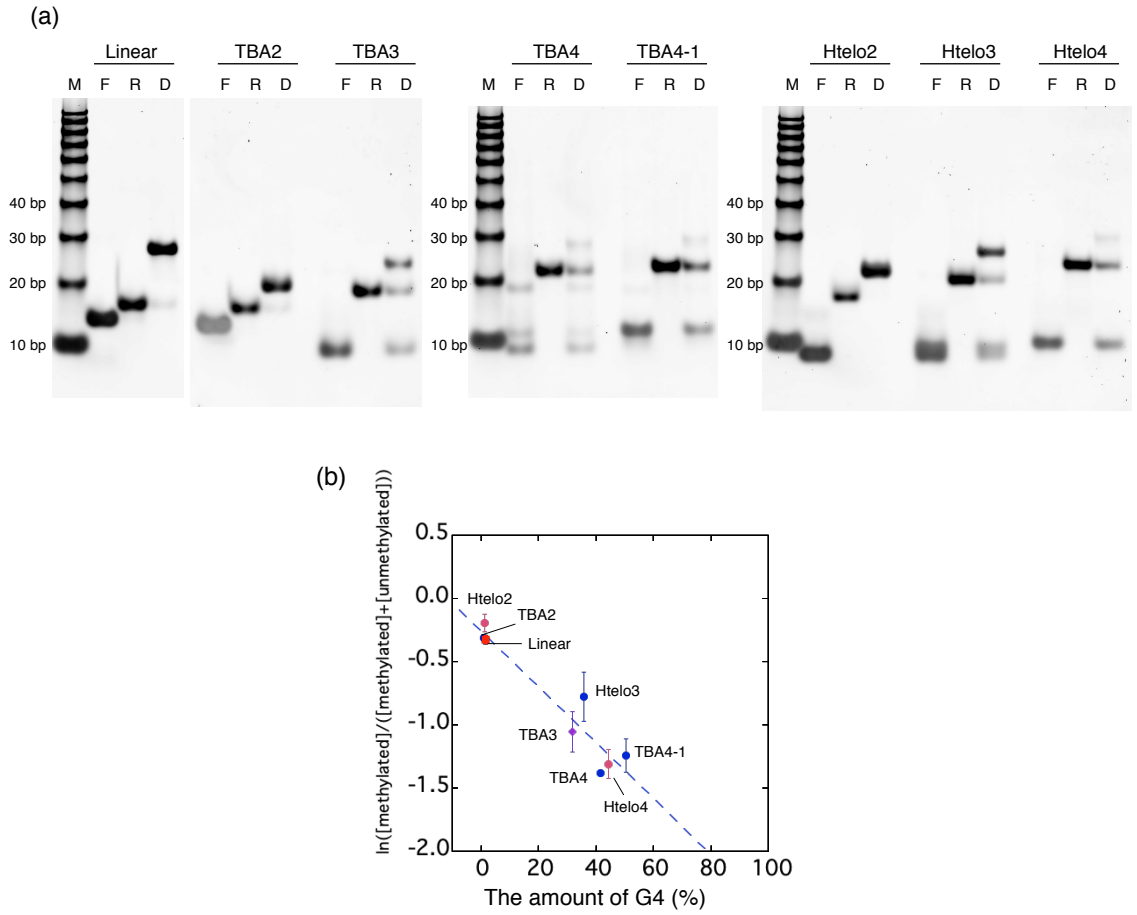

Fig. S12 Effect of equilibrium between duplexes and quadruplexes before methylation reaction on methylation efficiency. (a) Nondenaturing gel electrophoresis of forward strand (F), reverse strand (R) and mixture of forward and reverse strands (D) of Linear, TBA2, and TBA3, TBA4, TBA4-1, Htelo2, Htelo3 and Htelo4 in the buffer for methylation: 10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 10 mM KCl and 10 mM $MgCl_2$. The gels were run at 100 V and at 25 °C for 140 min and stained by SYBR Gold. M represents the 10 bp DNA step ladder. (b) Plot of the amount of G4 (%) vs. ln([methylated] / ([methylated] + [unmethylated])) as the methylation efficiency. The red square indicates linear, the blue dots indicate hybrid G4, the purple rhombus indicates parallel G4, and the pink dots indicate anti-parallel G4. The dots show the average of three experiments, and the error bars indicate the standard deviation although some of the error values are too small to be indicated.

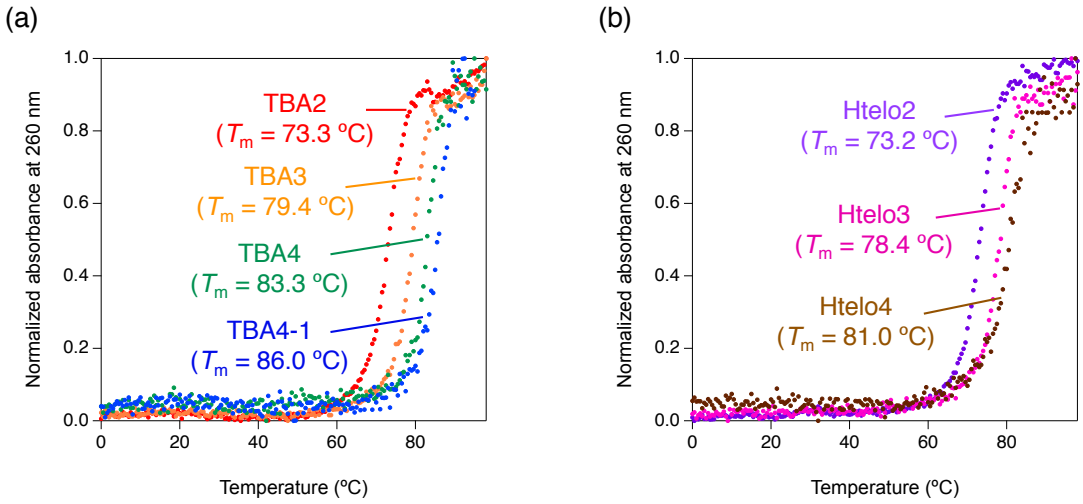

Fig. S13 Normalized UV melting curves of duplexes for TBA2 (red), TBA3 (orange), TBA4 (green), TBA4-1 (blue), Htelo2 (purple), Htelo3 (pink) and Htelo4 (brown). All experiments were carried out in 10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 10 mM KCl, and 10 mM $MgCl_2$. The melting temperatures (T_ms) were described in the figure.

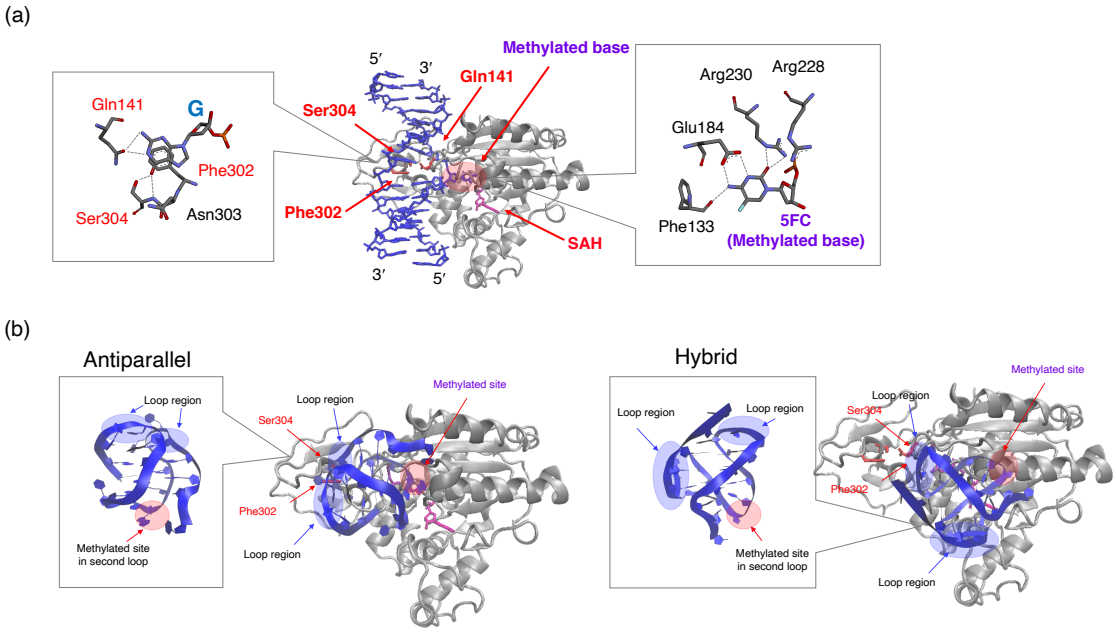

Fig. S14 (a) Structure of M.MpeI-DNA complexes (PDB codes 4DKJ). The DNA and DNA intercalating residues (Gln141, Phe302, and Ser304 in M.MpeI) are shown in stick representation. Gln141, Phe302, and Ser304 are indicated in red, 5FC as methylated base as the substrate is indicated in purple, and SAH is indicated in magenta. M.Mpel MTase interacts with the strand containing substrate cytosine, which is flipped out and recognised by Phe133, Glu184, Arg228 and Arg230 by hydrogen bonding. The Gln141 of MTase intercalates in bases where the cytosine substrate was originally located. The opposite strand of the cytosine substrate is also recognised by several hydrogen bonds of Ser304 and Phe302. (b) Structural comparison of the interaction between MTase and G-quadruplex with antiparallel and hybrid. Second loop with methylation sites is shown in red, other loops in blue.

Table S1. DNA sequences of X in Fig. 1, the complementary sequences of X, and length in Fig. 1

Abbreviation [a]	Sequences in X (5′ to 3′) [b]	Complementary sequences of X (5′ to 3′) [b]	Length (nt)
Linear	CGGGTCCGTCAGCGCCCGCTCAACA	TGTTGAGCGGGCGCTGACGGACCCG	25
TBA2	GGTTGGTGCGCTGGTTGG	CCAACCAGCGCACCAACC	18
TBA3	GGGTTGGGTGCGCTGGGTTGGG	CCCAACCCAGCGCACCCAACCC	22
TBA4	GGGGTTGGGGTGCGCTGGGGTTGGGG	CCCCAACCCCAGCGCACCCCAACCCC	26
TBA4-1	GGGGTGCGCTGGGGTGTGGGGTTGGGG	CCCCAACCCCACACCCCAGCGCACCCC	27
Htelo2	GGTTAGGTGCGCTGGTTAGG	CCTAACCAGCGCACCTAACC	20
Htelo3	GGGTTAGGGTGCGCTGGGTTAGGG	CCCTAACCCAGCGCACCCTAACCC	24
Htelo4	GGGGTTAGGGGTGCGCTGGGGTTAGGGG	CCCCTAACCCCAGCGCACCCCTAACCCC	28

[a] Linear cannot fold into any significant structures while TBA2, TBA3, TBA4 and TBA4-1 can fold G4 based on TBA, and Htelo2, Htelo3 and Htelo4 can fold G4 based on Htelo. [b] The loop regions are underlined and the sequence for the digestion enzyme recognition site is indicated in red.

Table S2. Thermodynamic parameters for G-quadruplex formation[a]

	$-\Delta H$ (kcal mol^{-1})	$-T\Delta S$ (kcal mol^{-1})
TBA2	32.2 ± 4.7	33.0 ± 4.7
TBA3	47.1 ± 4.6	43.5 ± 4.1
TBA4	53.3 ± 1.8	47.4 ± 1.6
TBA4-1	62.6 ± 3.0	54.8 ± 2.3
Htelo2	30.0 ± 0.7	29.9 ± 0.7
Htelo3	51.9 ± 0.4	48.4 ± 0.3
Htelo4	96.4 ± 2.8	84.0 ± 2.4

[a]All experiments were carried out in a buffer of 10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 10 mM KCl and 10 mM $MgCl_2$. Thermodynamic parameters were evaluated using the average values obtained from curve fitting at the different DNA concentrations.

Table S3. The melting temperatures and thermodynamic parameters for G-quadruplex formation of Htelo4[a] at various concentrations of KCl

Conc. of KCl	$-\Delta H$ (kcal mol^{-1})	$-T\Delta S$ (kcal mol^{-1})	$-\Delta G^{o}_{37}$ (kcal mol^{-1})	T_m (ºC)
0 mM	66.2 ± 0.4	60.3 ± 0.4	6.0 ± 0.1	67.6 ± 0.2
1 mM	76.3 ± 0.8	68.7 ± 0.8	7.6 ± 0.1	71.4 ± 0.2
3 mM	88.1 ± 2.0	78.2 ± 1.8	9.9 ± 0.3	76.4 ± 0.3
10 mM	96.4 ± 2.8	84.0 ± 2.4	12.4 ± 0.4	82.9 ± 0.3
30 mM	N.D.[b]	N.D.[b]	N.D.[b]	> 95 ºC

[a]All experiments were carried out in a buffer of 10 mM Tris-HCl (pH 7.8) containing 30 mM NaCl, 10 mM $MgCl_2$ and various concentrations of KCl. Thermodynamic parameters were evaluated using the average values obtained from curve fitting at the different DNA concentrations. [b]"N.D." indicates that values could not be obtained from the melting curves due to too high melting temperatures.