Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Effect of DNA modifications on the transition between canonical and non-canonical DNA structures in CpG islands during senescence

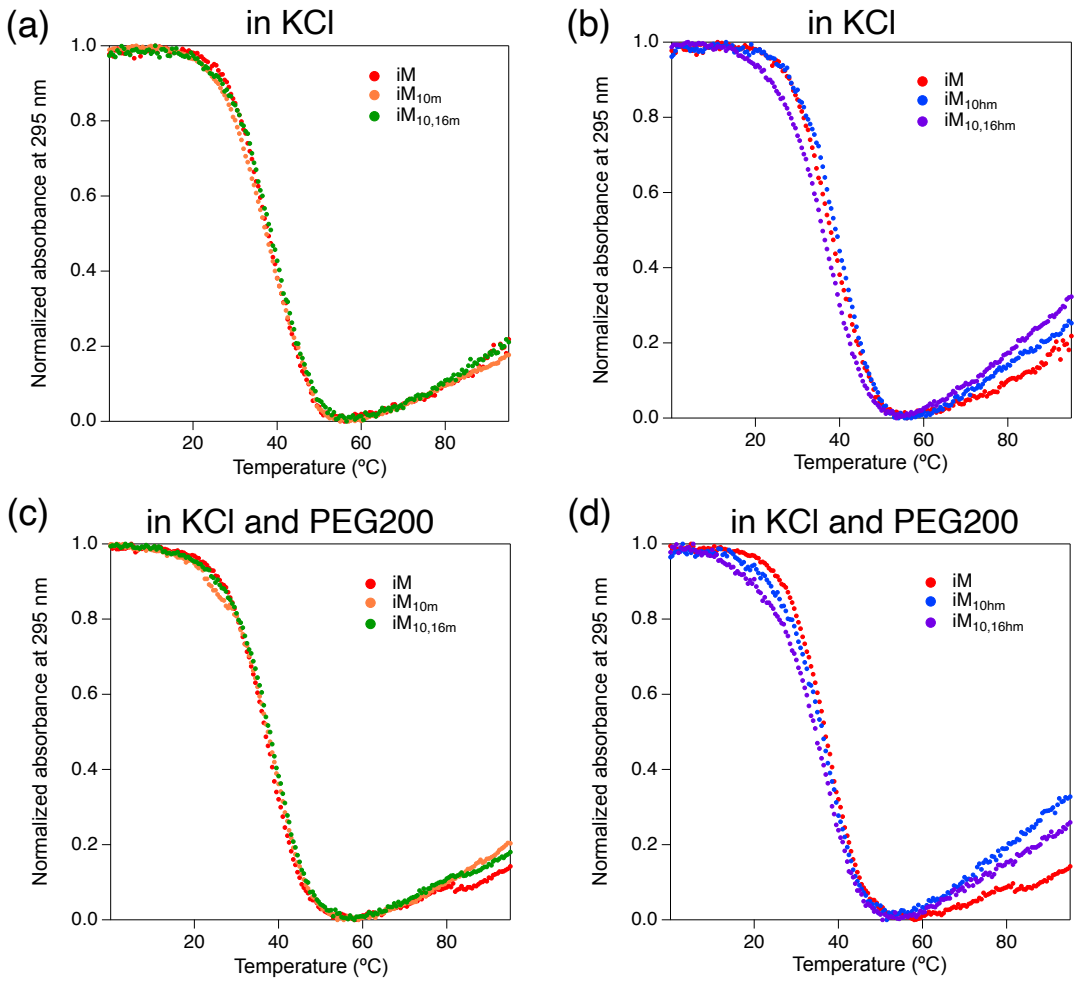
Saki Matsumoto[a], Hisae Tateishi-Karimata[a], Tatsuya Ohyama[a], Naoki Sugimoto[a, b, *]

[a] Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, 7-1-20 Minatojima-Minamimachi, Kobe 650-0047, Japan.

[b] Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, 7-1-20 Minatojima-Minamimachi, Kobe 650-0047, Japan.

*To whom correspondence should be addressed. Tel: (+81)78-303-1416; Fax: (+81)78-303-1495; Email: sugimoto@konan-u.ac.jp

Contents:


(a)
in KCl
Normalized absorbance at 295 nm
Temperature (°C)
iM
iM10m
iM10,16m
(b)
in KCl
Normalized absorbance at 295 nm
Temperature (°C)
iM
iM10hm
iM10,16hm
(c)
in KCl and PEG200
Normalized absorbance at 295 nm
Temperature (°C)
iM
iM10m
iM10,16m
(d)
in KCl and PEG200
Normalized absorbance at 295 nm
Temperature (°C)
iM
iM10hm
iM10,16hm

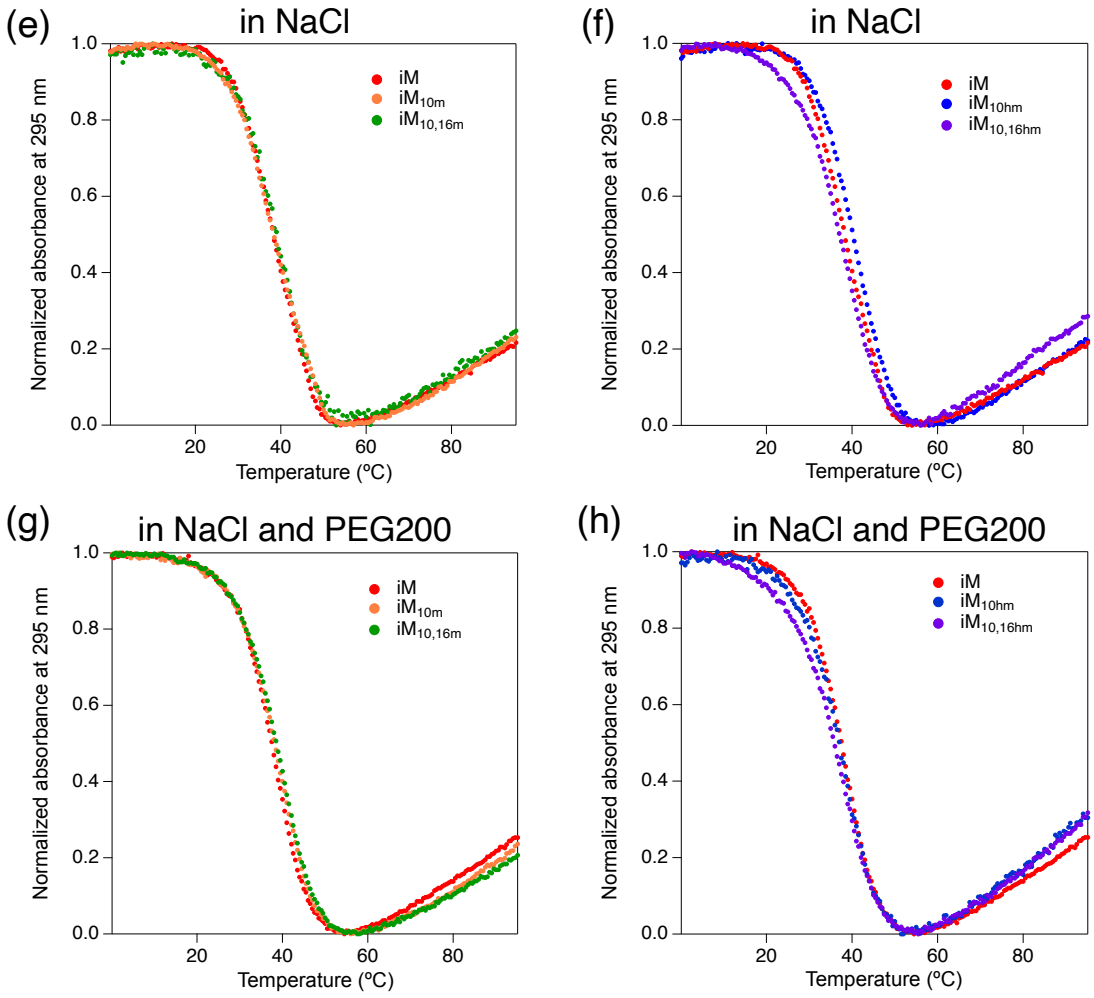


Figure S1. UV melting curves for 5 μM of (a) iM, iM_{10m}, and $iM_{10,16m}$ in the presence of K^+, (b) iM, iM_{10hm}, and $iM_{10,16hm}$ in the presence of K^+, (c) iM, iM_{10m}, and $iM_{10,16m}$ in the presence of K^+ and 40 wt% PEG200, (d) iM, iM_{10hm}, and $iM_{10,16hm}$ in the presence of K^+ and 40 wt% PEG200, (e) iM, iM_{10m}, and $iM_{10,16m}$ in the presence of Na^+, (f) iM, iM_{10hm}, and $iM_{10,16hm}$ in the presence of Na^+, (g) iM, iM_{10m}, and $iM_{10,16m}$ in the presence of Na^+ and 40 wt% PEG200, and (h) iM, iM_{10hm}, and $iM_{10,16hm}$ in the presence of Na^+ and 40 wt% PEG200.

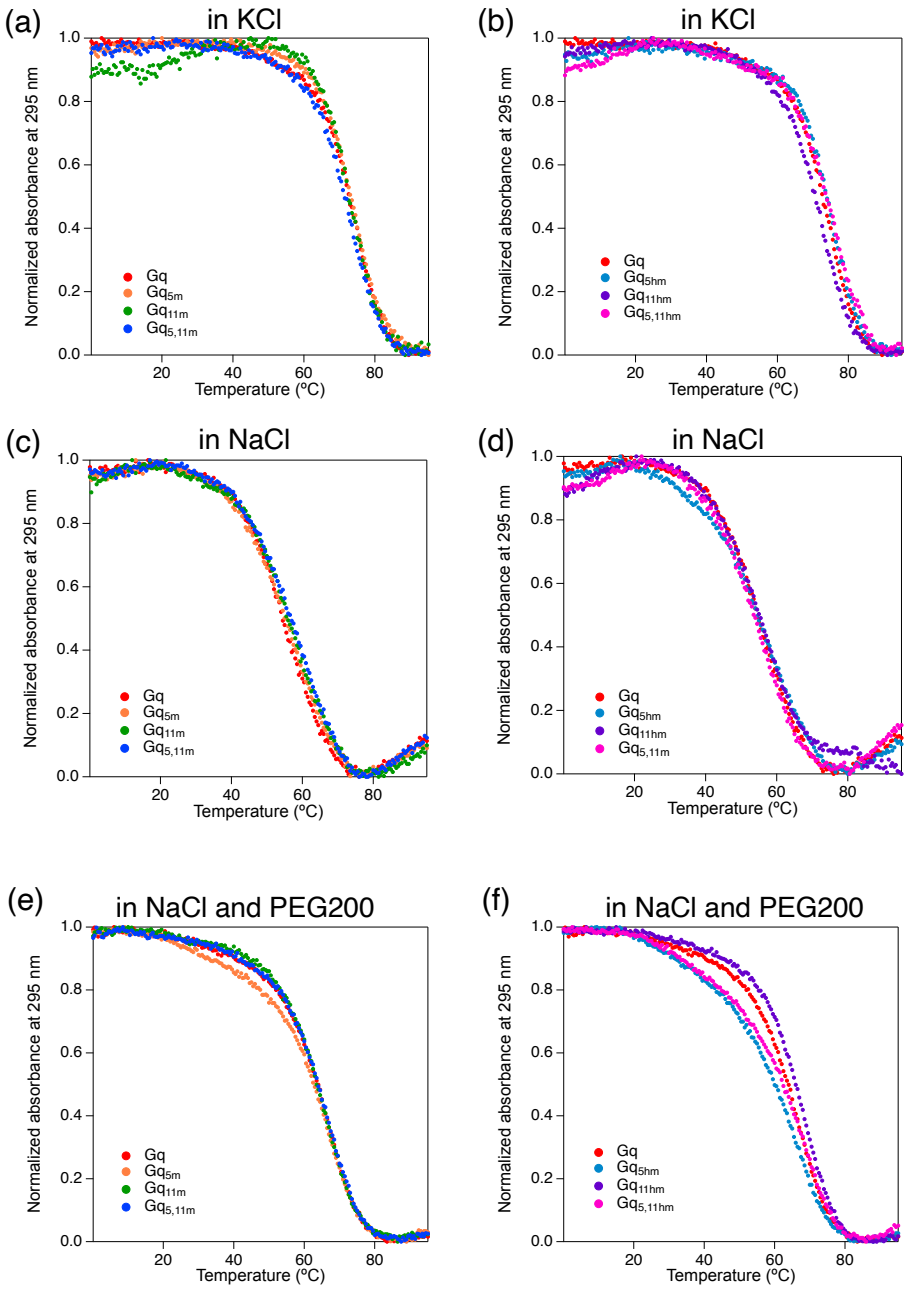


Figure S2. UV melting curves for 5 μM of (a) Gq, Gq_{5m}, Gq_{11m}, and $Gq_{5,11m}$ in the presence of K^+, (b) Gq, Gq_{5hm}, Gq_{11hm}, and $Gq_{5,11hm}$ in the presence of K^+, (c) Gq, Gq_{5m}, Gq_{11m}, and $Gq_{5,11m}$ in the presence of Na^+, (d) Gq, Gq_{5hm}, Gq_{11hm}, and $Gq_{5,11hm}$ in the presence of Na^+, (e) Gq, Gq_{5m}, Gq_{11m}, and $Gq_{5,11m}$ in the presence of Na^+ and 40 wt% PEG200, and (f) Gq, Gq_{5hm}, Gq_{11hm}, and $Gq_{5,11hm}$ in the presence of Na^+ and 40 wt% PEG200.

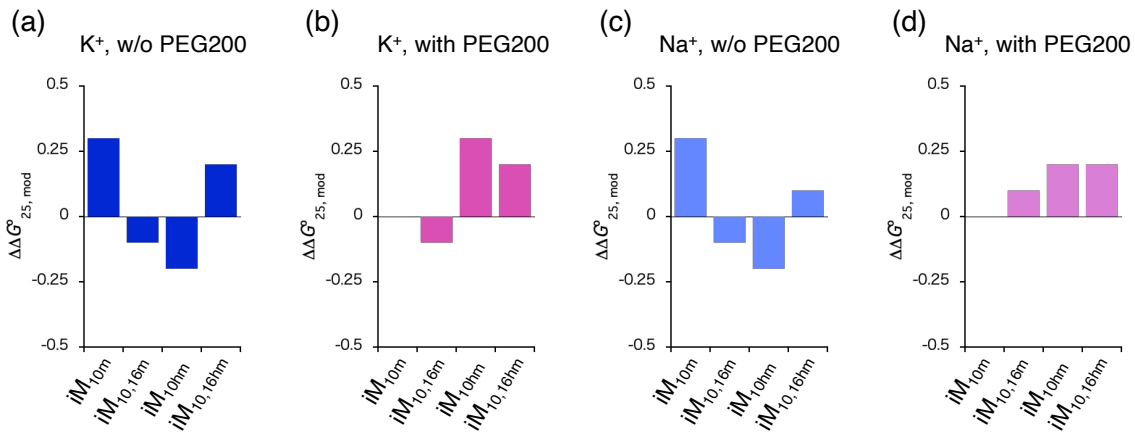


Figure S3. The effect of modifications on the formation of i-motifs in 100 mM KCl or NaCl in 0 wt% or 40 wt% PEG200. The $\Delta\Delta G^{\circ}_{25,\mathrm{mod}}$ values in (a) 100 mM KCl with 0 wt% PEG200, (b) 100 mM KCl with 40 wt% PEG200, (c) 100 mM NaCl with 0 wt% PEG200, and (d) 100 mM NaCl with 40 wt% PEG200.

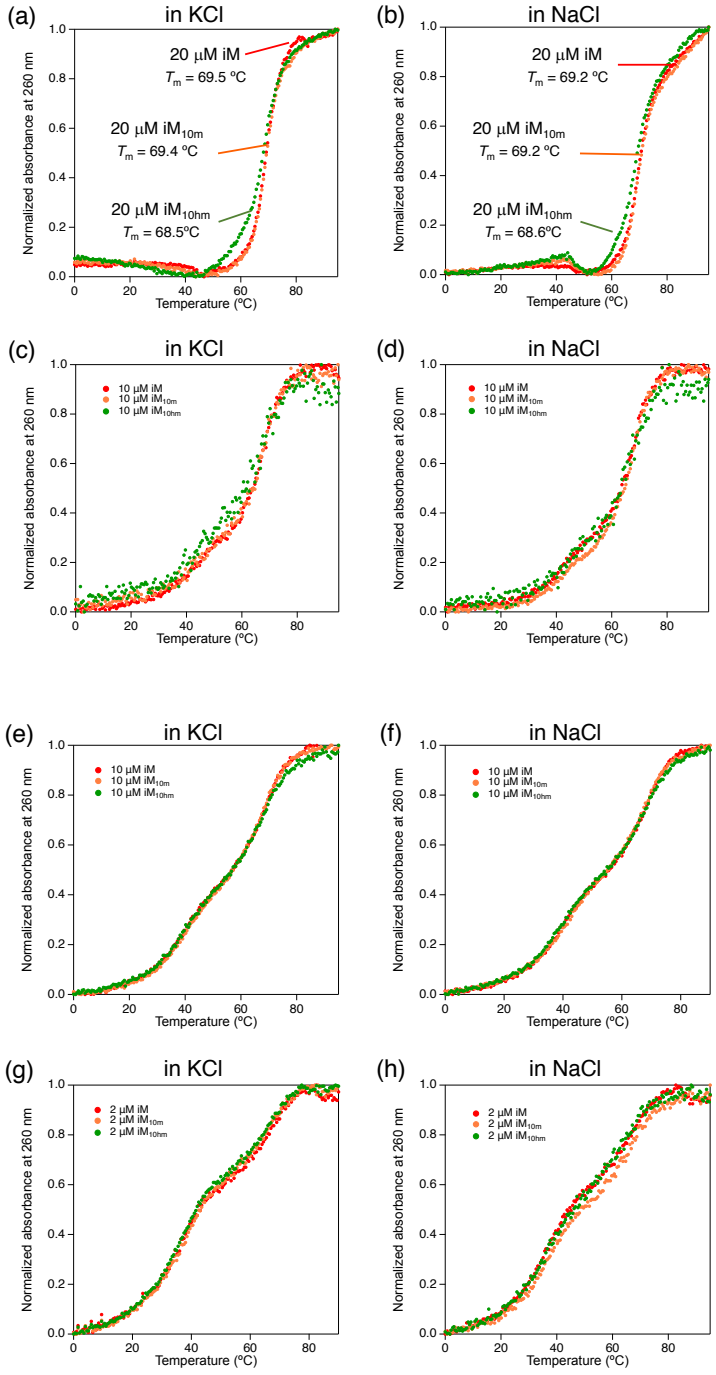


Figure S4. UV melting curves at pH 5.0 for (a) 20 μM of iM, iM$_{10m}$, and iM$_{10hm}$ in the presence of K^+, (b) 20 μM of iM, iM$_{10m}$, and iM$_{10hm}$ in the presence of Na^+, (c) 10 μM of iM, iM$_{10m}$, and iM$_{10hm}$ in the presence of K^+, (d) 10 μM of iM, iM$_{10m}$, and iM$_{10hm}$ in the presence of Na^+, (e) 5 μM of iM, iM$_{10m}$, and iM$_{10hm}$ in the presence of K^+, (f) 5 μM of iM, iM$_{10m}$, and iM$_{10hm}$ in the presence of Na^+, (g) 2 μM of iM, iM$_{10m}$, and iM$_{10hm}$ in the presence of K^+, and (h) 2 μM of iM, iM$_{10m}$, and iM$_{10hm}$ in the presence of Na^+.

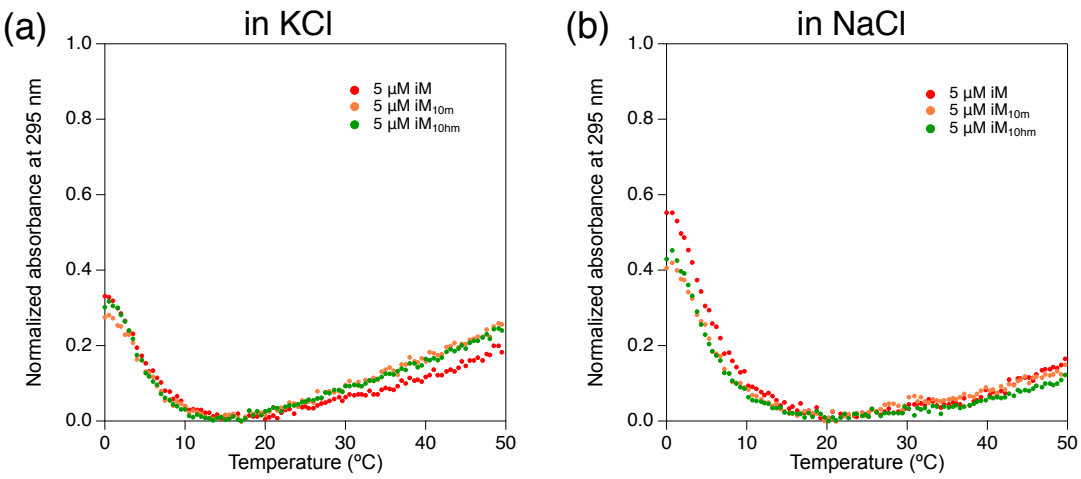


Figure S5. UV melting curves at pH 7.0 for (a) 5 μM of iM, iM_{10m}, and iM_{10hm} in the presence of K^+ and (b) 5 μM of iM, iM_{10m}, and iM_{10hm} in the presence of Na^+.

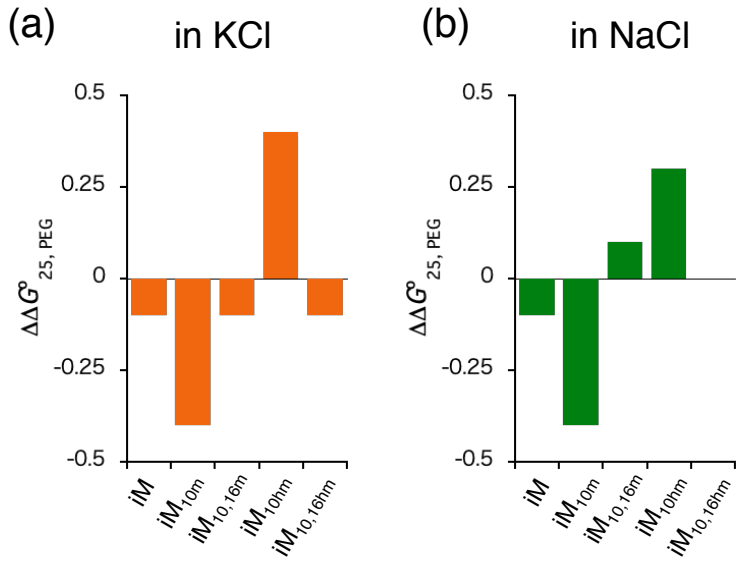


Figure S6. The effect of PEG200 on the formation of i-motifs. The $\Delta\Delta G^{o}_{25,PEG}$ values in (a) 100 mM KCl and (b) 100 mM NaCl

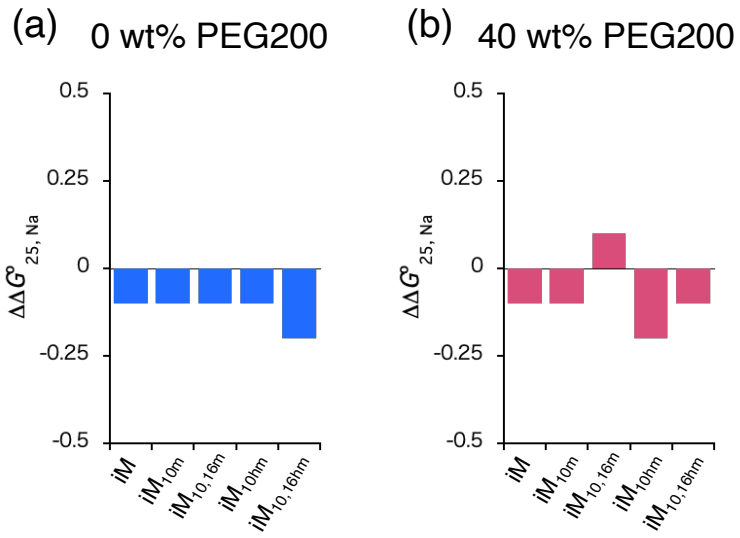


Figure S7. The effect of cations on the formation of i-motifs. The $\Delta\Delta G^{\circ}_{25,Na}$ values in (a) 0 wt% PEG200 and (b) 40 wt% PEG200

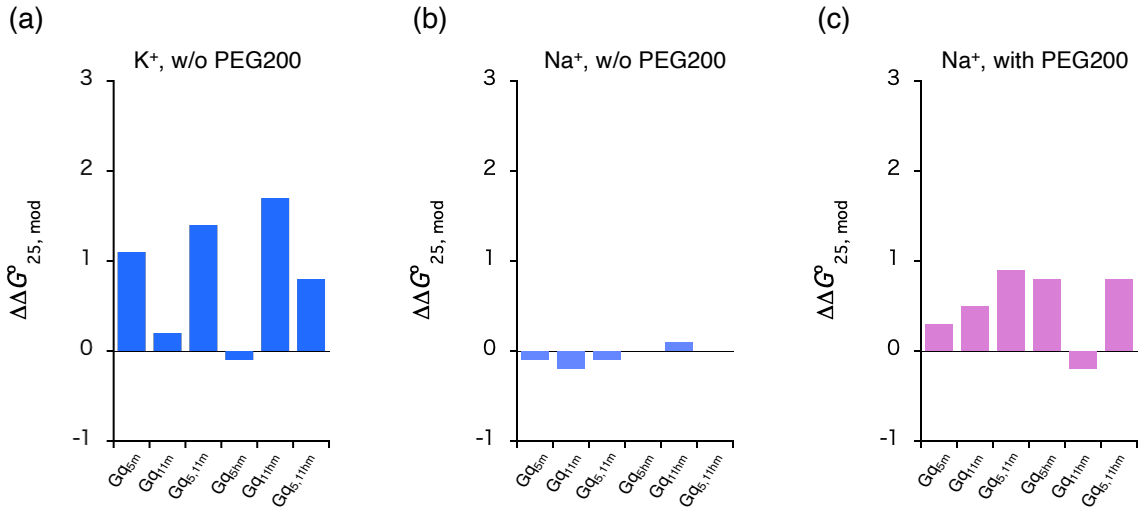


Figure S8. The effect of the modifications on the formation of G-quadruplexes. The $\Delta\Delta G^{\circ}_{25,\mathrm{mod}}$ values in (a) 100 mM KCl with 0 wt% PEG200, (b) 100 mM NaCl with 0 wt% PEG200, (c) 100 mM NaCl with 40 wt% PEG200.

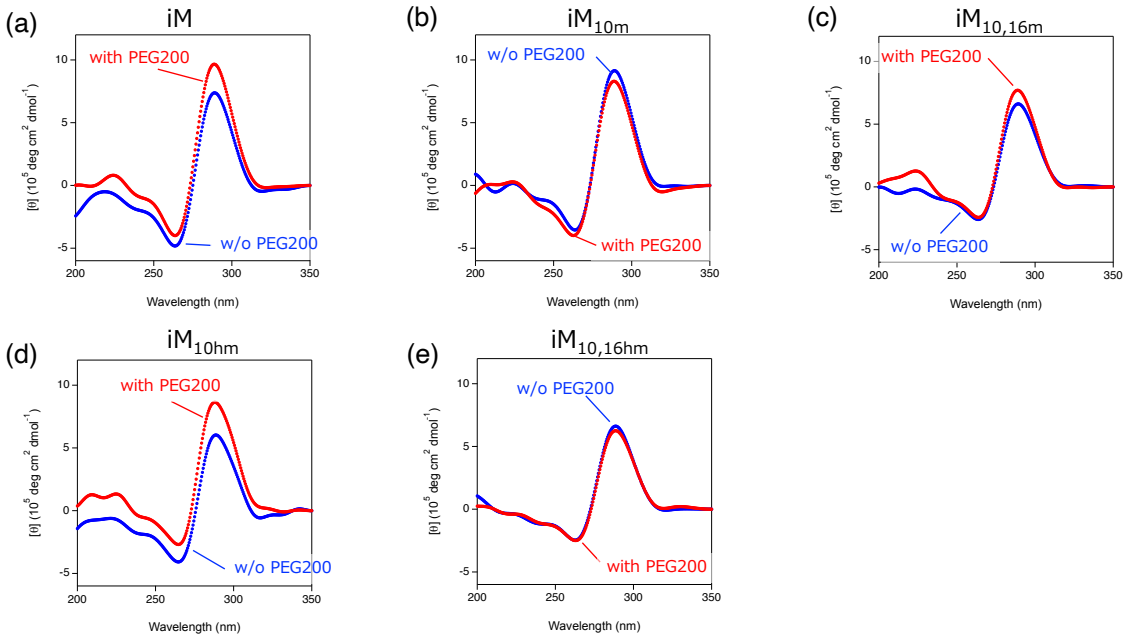


Figure S9. CD spectra of (a) iM, (b) iM_{10m} (c) $iM_{10,16m}$, (d) iM_{10hm}, and (e) $iM_{10,16hm}$ in the presence of K^+. All experiments were carried out at 4 ºC in 50 mM MES-LiOH (pH 6.0) containing 100 mM KCl containing 0 wt% (blue) or 40 wt% PEG200 (red).

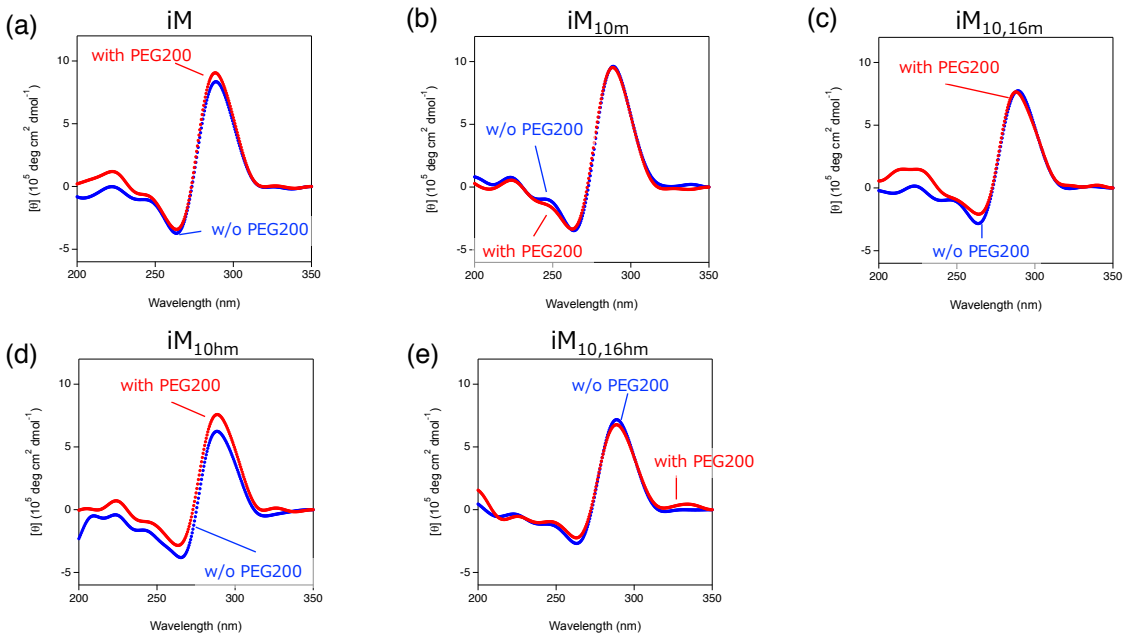


Figure S10. CD spectra of (a) iM, (b) iM_{10m} (c) $iM_{10,16m}$, (d) iM_{10hm}, and (e) $iM_{10,16hm}$ in the presence of Na^+. All experiments were carried out at 4 ℃ in 50 mM MES-LiOH (pH 6.0) containing 100 mM NaCl containing 0 wt% (blue) or 40 wt% PEG200 (red).

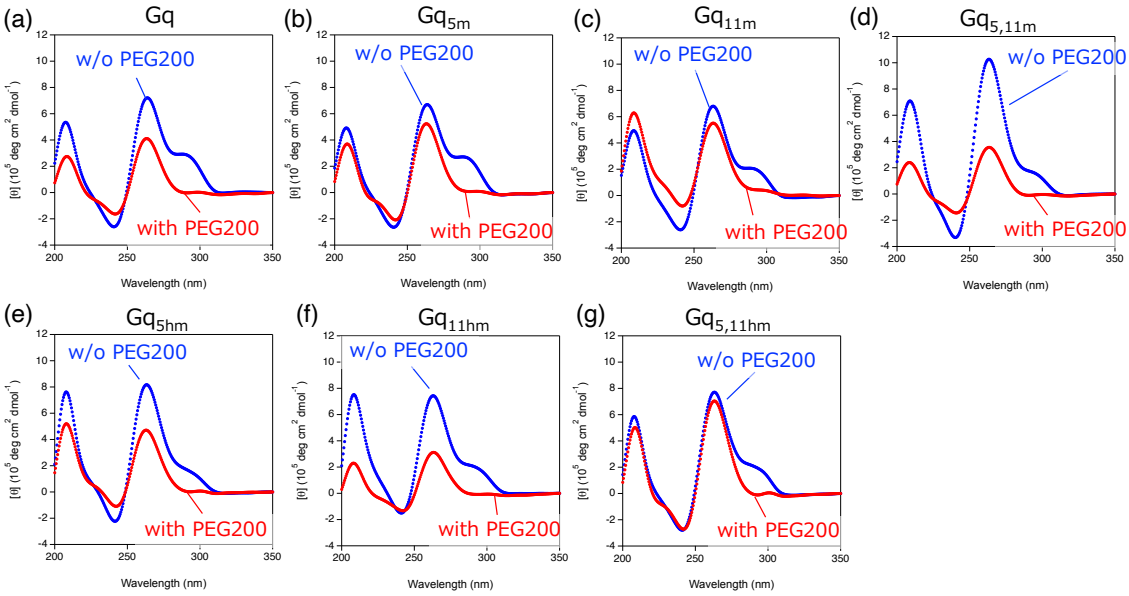


Figure S11. CD spectra of (a) Gq, (b) Gq_{5m} (c) Gq_{11m}, (d) $Gq_{5,11m}$, (e) Gq_{5hm}, (f) Gq_{11hm}, and (g) $Gq_{5,11hm}$ in the presence of K^+. All experiments were carried out at 4 ºC in 50 mM MES-LiOH (pH 6.0) with 100 mM KCl containing 0 wt% (blue) or 40 wt% PEG200 (red).

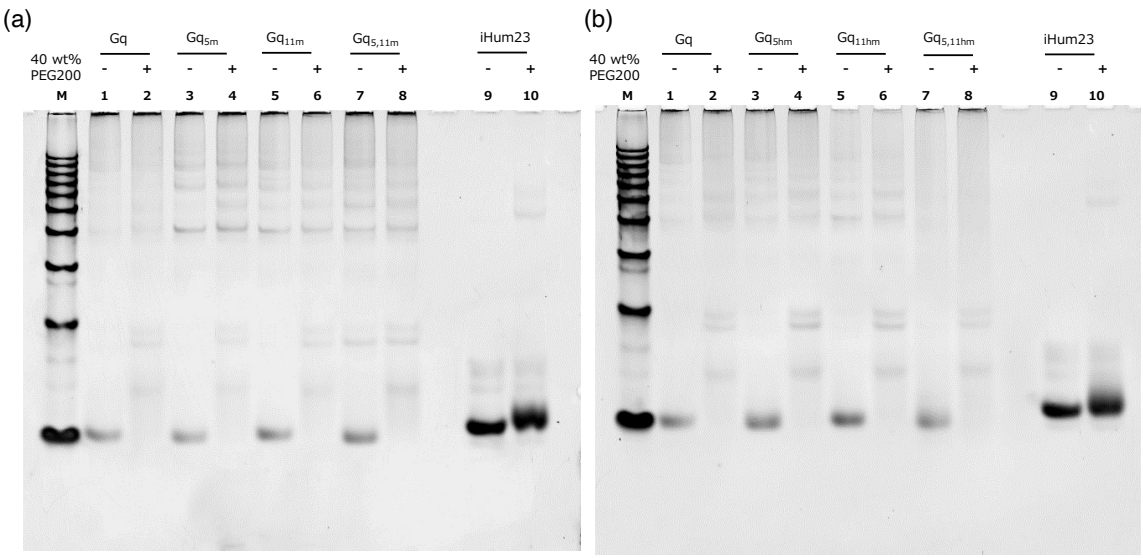


Figure S12. Nondenaturing gel electrophoresis of (a) Gq, Gq_{5m}, Gq_{11m}, and $Gq_{5,11m}$ and (b) Gq, Gq_{5hm}, Gq_{11hm}, and $Gq_{5,11hm}$ at 25 ºC in 50 mM MES-LiOH (pH 6.0) containing 100 mM KCl without or with 40 wt% of PEG200. Lane M: 10 bp DNA standard; Lanes 1, 3, 5, 7 and 9; without PEG200: lanes 2, 4, 6, 8, and 10; with 40 wt% PEG200. iHum23 indicates telomeric G-quadruplex, $TA(G_3TTA)_3G_3$ as the marker of monomer G-quadruplex with antiparallel.

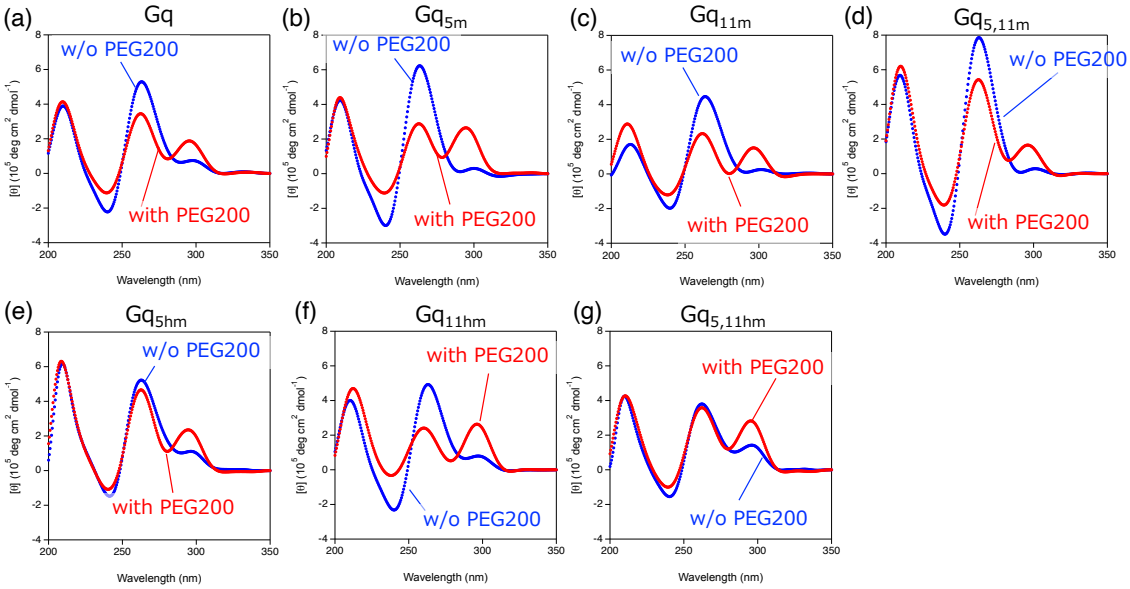


Figure S13. CD spectra of (a) Gq, (b) Gq_{5m} (c) Gq_{11m}, (d) $Gq_{5,11m}$, (e) Gq_{5hm}, (f) Gq_{11hm}, and (g) $Gq_{5,11hm}$ in the presence of Na^+. All experiments were carried out at 4 ºC in 50 mM MES-LiOH (pH 6.0) with 100 mM NaCl containing 0 wt% (blue) or 40 wt% PEG200 (red).

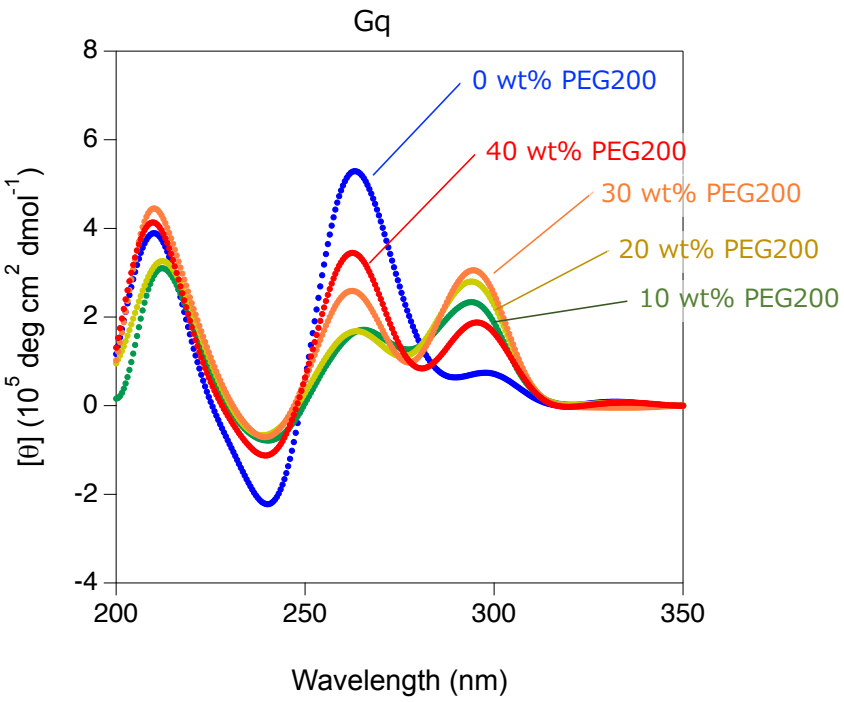


Figure S14. CD spectra of Gq in the presence of Na^+. All experiments were carried out at 4 ºC in 50 mM MES-LiOH (pH 6.0) with 100 mM NaCl containing 0 wt% (blue), 10 wt% (green), 20 wt% (yellow), 30 wt% (orange), or 40 wt% PEG200 (red).

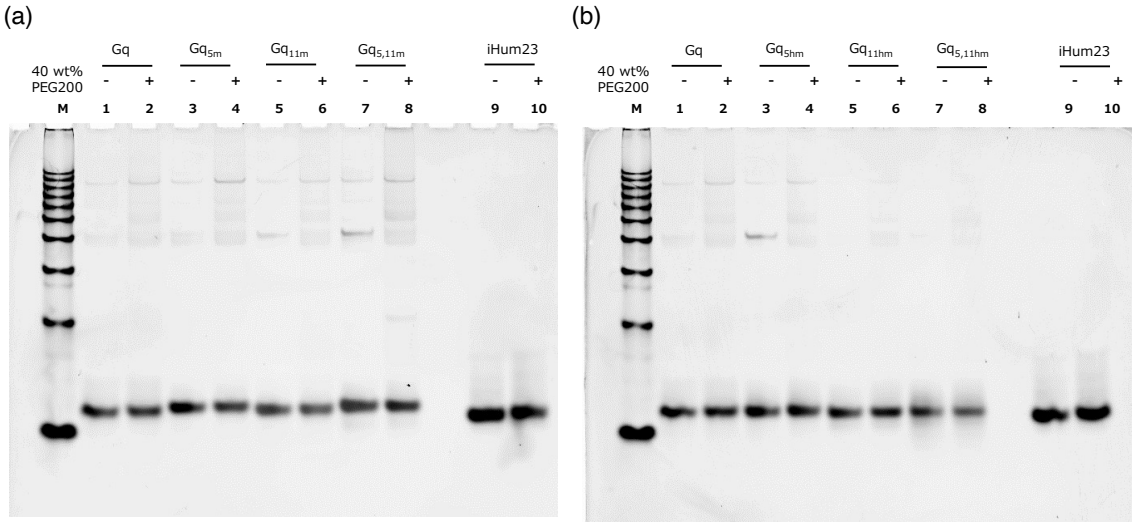


Figure S15. Nondenaturing gel electrophoresis of (a) Gq, Gq_{5m}, Gq_{11m}, and $Gq_{5,11m}$ and (b) Gq, Gq_{5hm}, Gq_{11hm}, and $Gq_{5,11hm}$ at 25 ºC in 50 mM MES-LiOH (pH 6.0) containing 100 mM NaCl without or with 40 wt% of PEG200. Lane M: 10 bp DNA standard; Lanes 1, 3, 5, 7 and 9; without PEG200: lanes 2, 4, 6, 8, and 10; with 40 wt% PEG200. iHum23 indicates telomeric G-quadruplex, $TA(G_3TTA)_3G_3$ as the marker of monomer G-quadruplex with antiparallel.

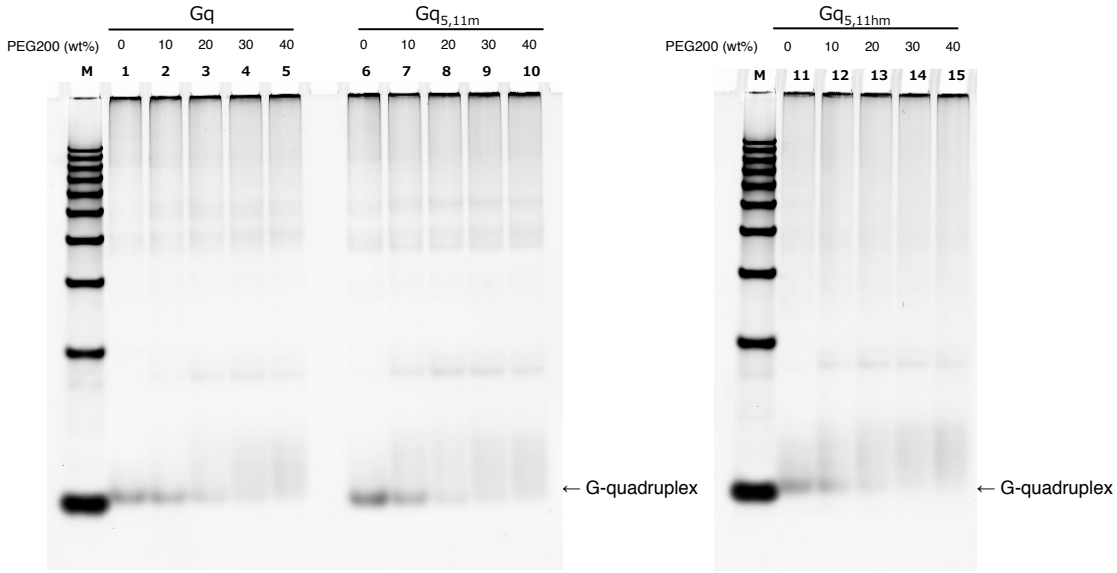


Figure S16. Nondenaturing gel electrophoresis of Gq, $Gq_{5,11m}$, and $Gq_{5,11hm}$, at 25 ºC in 50 mM MES-LiOH (pH 6.0) containing 100 mM KCl with 0, 10, 20, 30, and 40 wt% of PEG200. Lane M: 10 bp DNA standard; lanes 1, 6, and 11; 0 wt % PEG200: lanes 2, 7, and 12; with 10 wt% PEG200: lanes 3, 8, and 13; with 20 wt% PEG200: lanes 4, 9, and 14; with 30 wt% PEG200: lanes 5, 10, and 15; with 40 wt% PEG200.

Table S1. i-motif and G-quadruplex forming sequences in CpG island of *ELOVL2*

No.	sense sequences	T_m (ºC) [a]	antisense sequences	T_m (ºC) [a]	N_{CpG} [b]
1	GGGCAG**CG**GGTGGGTATTCCTGGGG	N.D.[c]	CCCCAGGAATACCCACC**CG**CTGCCC	34.8	1
2	GGGGG**CG**GGGAGG**CGCG**GG**CG**GG	60.6	CC**CG**CC**CGCG**CCTCCC**CG**CCCCC	36.8	4
3	GGG**CG**TGGGTGTGGGTGGGGG	58.7	CCCCCACCCACACCCA**CG**CCC	34.3	1
4	GGG**CG**GGAAAGGGC**CG**AG**CG**GG	N.D. [c]	CC**CG**CT**CG**GCCCTTTCC**CG**CCC	27.8	3
5	GGGGC**CG**GGTC**CGCGCG**GGCCTGGGGAG**CG**GG	72	CC**CG**CTCCCCAGGCC**CGCGCG**GACC**CG**GCCCC	34.4	5
6	CCCACCCTC**CG**ACCCTC**CG**GACCCCC	34.5	GGGGGTC**CG**GAGGGT**CG**GAGGGTGGG	45.2	2
7	CCCCTCTCCC**CG**GTCC**CG**CCCC	45.4	GGGG**CG**GGAC**CG**GGGAGAGGGG	56.0	2
8	CCCTG**CG**CCCCTCCCC**CGCGCG**CCC	24.5	GGG**CGCGCG**GGGGAGGGG**CG**CAGGG	60.5	4
9	CCCCCAGAGAAACCCACCCAACCCCC	26.3	GGGGGTTGGGTGGGTTTCTCTGGGGG	N.D. [c]	0
10	CCCAC**CG**CACCCCCAGAGAAACCCACCC	36.0	GGGTGGGTTTCTCTGGGGGTG**CG**GTGGG	43.2	1

[a]The value of melting temperature (T_m) was determined by differentiation of UV melting curve of each DNA (5 μM) in 50 mM MES-LiOH (pH 6.0) and 100 mM NaCl. [b]N_{CpG} represents the number of CpG sites within structure forming sequences and CpG sites are marked in bold.
[c]"N.D." indicates that values could not be obtained from the melting curves.

Table S2. The thermodynamic parameters of i-motif forming DNA in the presence of K^+ [a]

	without PEG200				40 wt% PEG200			
	ΔH^o ($\Delta\Delta H^o_{mod}$)[b] [kcal mol^{-1}]	$T\Delta S^o$ [$\Delta(T\Delta S^o_{mod})$][b] [kcal mol^{-1}]	ΔG^o_{25} ($\Delta\Delta G^o_{25,mod}$)[b] [kcal mol^{-1}]	T_m ($\Delta T_{m,mod}$)[b] [°C]	ΔH^o ($\Delta\Delta H^o_{mod}$)[b] [kcal mol^{-1}]	$T\Delta S^o$ [$\Delta(T\Delta S^o_{mod})$][b] [kcal mol^{-1}]	ΔG^o_{25} ($\Delta\Delta G^o_{25,mod}$)[b] [kcal mol^{-1}]	T_m ($\Delta T_{m,mod}$)[b] [°C]
iM	-37.1 ± 0.7	-35.5 ± 0.8	-1.7 ± 0.0	39.1 ± 0.5	-41.2 ± 0.7	-39.4 ± 0.7	-1.8 ± 0.0	38.6 ± 0.4
iM_{10m}	-32.6 ± 0.8 (+4.5)	-31.1 ± 0.9 (+4.4)	-1.4 ± 0.1 (+0.3)	38.8 ± 1.0 (- 0.3)	-38.7 ± 4.4 (+2.5)	-37.0 ± 4.2 (+2.4)	-1.8 ± 0.3 (0.0)	39.5 ± 0.5 (+ 0.9)
$iM_{10,16m}$	-35.9 ± 1.0 (+1.2)	-34.2 ± 1.0 (+1.3)	-1.8 ± 0.1 (-0.1)	40.4 ± 0.7 (+ 1.3)	-38.1 ± 1.8 (+3.1)	-36.1 ± 1.8 (+3.3)	-1.9 ± 0.1 (-0.1)	40.9 ± 0.7 (+ 2.3)
iM_{10hm}	-36.9 ± 0.5 (+0.2)	-35.0 ± 0.5 (+0.5)	-1.9 ± 0.1 (-0.2)	41.2 ± 0.7 (+ 2.1)	-35.2 ± 2.4 (+6.0)	-33.7 ± 2.3 (+5.7)	-1.5 ± 0.1 (+0.3)	38.4 ± 0.1 (- 0.2)
$iM_{10,16hm}$	-34.9 ± 0.5 (+2.2)	-33.4 ± 0.5 (+2.1)	-1.5 ± 0.0 (+0.2)	38.8 ± 0.1 (- 0.3)	-36.6 ± 1.1 (+4.6)	-35.1 ± 1.1 (+4.3)	-1.6 ± 0.0 (+0.2)	38.2 ± 0.5 (- 0.4)

[a]The values were determined in 50 mM MES-LiOH (pH 6.0) and 100 mM KCl with or without 40 wt% PEG200. Each value is the average of three determinations and the standard deviation is shown with the average. Melting temperatures were measured at 5 μM of DNA. [b]The amount of change resulting from the modifications was calculated by using ΔX = [X(without modification) – X (modification)], in which X is ΔH^o, $T\Delta S^o$, ΔG^o_{25}, or T_m and included in parentheses.

Table S3. The thermodynamic parameters of i-motif forming DNA in the presence of Na^+ [a]

	without PEG200				40 wt% PEG200			
	ΔH^o ($\Delta\Delta H^o_{mod}$)[b] [kcal mol^{-1}]	$T\Delta S^o$ [$\Delta(T\Delta S^o_{mod})$][b] [kcal mol^{-1}]	ΔG^o_{25} ($\Delta\Delta G^o_{25,mod}$)[b] [kcal mol^{-1}]	T_m ($\Delta T_{m,mod}$)[b] [ºC]	ΔH^o ($\Delta\Delta H^o_{mod}$)[b] [kcal mol^{-1}]	$T\Delta S^o$ [$\Delta(T\Delta S^o_{mod})$][b] [kcal mol^{-1}]	ΔG^o_{25} ($\Delta\Delta G^o_{25,mod}$)[b] [kcal mol^{-1}]	T_m ($\Delta T_{m,mod}$)[b] [ºC]
iM	-36.8 ± 1.1	-35.0 ± 1.0	-1.8 ± 0.0	40.1 ± 0.4	-41.6 ± 2.8	-39.7 ± 2.7	-1.9 ± 0.1	39.3 ± 0.4
iM_{10m}	-31.4 ± 1.0 (+5.4)	-29.9 ± 1.0 (+5.1)	-1.5 ± 0.1 (+0.3)	40.2 ± 0.8 (+ 0.1)	-40.0 ± 2.8 (+1.6)	-38.1 ± 2.6 (+1.6)	-1.9 ± 0.2 (0.0)	39.7 ± 0.2 (+ 0.4)
$iM_{10,16m}$	-37.5 ± 0.6 (-0.7)	-35.7 ± 0.6 (-0.7)	-1.9 ± 0.0 (-0.1)	40.7 ± 0.1 (+ 0.6)	-40.2 ± 2.0 (+1.4)	-38.5 ± 2.0 (+1.2)	-1.8 ± 0.1 (+0.1)	38.6 ± 1.0 (-0.7)
iM_{10hm}	-38.6 ± 1.0 (-1.8)	-36.5 ± 1.0 (-1.5)	-2.0 ± 0.1 (-0.2)	41.7 ± 0.4 (+ 1.6)	-37.8 ± 2.5 (+3.8)	-36.1 ± 2.3 (+3.6)	-1.7 ± 0.2 (+0.2)	38.8 ± 0.8 (- 0.5)
$iM_{10,16hm}$	-35.5 ± 2.8 (+1.3)	-33.8 ± 2.7 (+1.2)	-1.7 ± 0.1 (+0.1)	40.0 ± 0.7 (- 0.1)	-37.0 ± 2.2 (+4.6)	-35.3 ± 2.1 (+4.4)	-1.7 ± 0.0 (+0.2)	39.1 ± 0.6 (- 0.2)

[a]The values were determined in 50 mM MES-LiOH (pH 6.0) and 100 mM NaCl with or without 40 wt% PEG200. Each value is the average of three determinations and the standard deviation is shown with the average. Melting temperatures were measured at 5 μM of DNA. [b]The amount of change resulting from the modifications was calculated by using ΔX = [X(without modification) – X (modification)], in which X is ΔH^o, $T\Delta S^o$, ΔG^o_{25}, or T_m and included in parentheses.

Table S4. The thermodynamic parameters of G-quadruplex forming DNA in the presence of K^+ [a]

	without PEG200				40 wt% PEG200			
	ΔH° ($\Delta\Delta H^\circ_{mod}$)[b] [kcal mol^{-1}]	$T\Delta S^\circ$ [$\Delta(T\Delta S^\circ)_{mod}$][b] [kcal mol^{-1}]	ΔG°_{25} ($\Delta\Delta G^\circ_{25,mod}$) [kcal mol^{-1}][b]	T_m (ΔT_m) [°C][b]	ΔH° [kcal mol^{-1}]	$T\Delta S^\circ$ [kcal mol^{-1}]	ΔG°_{25} [kcal mol^{-1}]	T_m [°C]
Gq	-53.1 ± 1.1	-45.7 ± 0.9	-7.4 ± 0.2	73.6 ± 0.3	N.D.[c]	N.D.[c]	N.D.[c]	N.D.[c]
Gq_{5m}	-45.4 ± 2.0 (+7.7)	-39.1 ± 1.8 (+6.6)	-6.3 ± 0.2 (+1.1)	73.0 ± 0.5 (-0.6)	N.D.[c]	N.D.[c]	N.D.[c]	N.D.[c]
Gq_{11m}	-52.5 ± 0.5 (+0.6)	-45.2 ± 0.4 (+0.5)	-7.2 ± 0.1 (+0.2)	72.5 ± 0.1 (-1.1)	N.D.[c]	N.D.[c]	N.D.[c]	N.D.[c]
$Gq_{5,11m}$	-44.1 ± 3.4 (+9.0)	-38.2 ± 3.0 (+7.5)	-6.0 ± 0.4 (+1.4)	71.8 ± 0.5 (-1.8)	N.D.[c]	N.D.[c]	N.D.[c]	N.D.[c]
Gq_{5hm}	-52.6 ± 2.0 (+0.5)	-45.2 ± 1.7 (+0.5)	-7.5 ± 0.3 (-0.1)	74.4 ± 0.1 (+0.8)	N.D.[c]	N.D.[c]	N.D.[c]	N.D.[c]
Gq_{11hm}	-42.1 ± 3.9 (+11.0)	-36.4 ± 3.3 (+9.3)	-5.7 ± 0.6 (+1.7)	71.3 ± 1.1 (-2.3)	N.D.[c]	N.D.[c]	N.D.[c]	N.D.[c]
$Gq_{5,11hm}$	-47.1 ± 1.6 (+6.0)	-40.5 ± 1.3 (+5.2)	-6.6 ± 0.4 (+0.8)	73.6 ± 1.2 (0.0)	N.D.[c]	N.D.[c]	N.D.[c]	N.D.[c]

[a]The values were determined in 50 mM MES-LiOH (pH 6.0) and 100 mM KCl with or without 40 wt% PEG200. Each value is the average of three determinations and the standard deviation is shown with the average. Melting temperatures were measured at 5 μM of DNA. [b] The amount of change resulting from the modifications was calculated by using ΔX = [X(without modification) – X (modification)], in which X is ΔH°, $T\Delta S^\circ$, ΔG°_{25}, or T_m and included in parentheses. [c] "N.D." indicates that values could not be obtained from the melting curves.

Table S5. The thermodynamic parameters of G-quadruplex forming DNA in the presence of Na^{+} [a]

	without PEG200				40 wt% PEG200			
	ΔH^{o} ($\Delta\Delta H^{o}_{mod}$)[b] [kcal mol^{-1}]	$T\Delta S^{o}$ [$\Delta(T\Delta S^{o})_{mod}$][b] [kcal mol^{-1}]	ΔG^{o}_{25} ($\Delta\Delta G^{o}_{25,mod}$) [kcal mol^{-1}][b]	T_m (ΔT_m) [°C][b]	ΔH^{o} ($\Delta\Delta H^{o}_{mod}$)[b] [kcal mol^{-1}]	$T\Delta S^{o}$ [$\Delta(T\Delta S^{o})_{mod}$][b] [kcal mol^{-1}]	ΔG^{o}_{25} ($\Delta\Delta G^{o}_{25,mod}$) [kcal mol^{-1}][b]	T_m (ΔT_m) [°C][b]
Gq	-31.9 ± 0.9	-29.1 ± 0.8	-2.8 ± 0.1	53.7 ± 0.6	-42.2 ± 0.5	-37.3 ± 0.4	-4.9 ± 0.0	64.0 ± 0.3
Gq_{5m}	-31.6 ± 0.3 (+0.3)	-28.7 ± 0.2 (+0.4)	-2.9 ± 0.0 (-0.1)	54.9 ± 0.2 (+1.2)	-39.8 ± 0.0 (+2.4)	-35.2 ± 0.0 (+2.1)	-4.6 ± 0.0 (+0.3)	63.7 ± 0.3 (-0.3)
Gq_{11m}	-31.8 ± 0.6 (+0.1)	-28.8 ± 0.5 (+0.3)	-3.0 ± 0.1 (-0.2)	55.7 ± 0.3 (+2.0)	-38.6 ± 0.2 (+3.6)	-34.2 ± 0.2 (+3.1)	-4.4 ± 0.1 (+0.5)	63.0 ± 0.5 (-1.0)
$Gq_{5,11m}$	-30.5 ± 0.5 (+1.4)	-27.6 ± 0.5 (+1.5)	-2.9 ± 0.1 (-0.1)	55.8 ± 0.4 (+2.1)	-35.4 ± 0.4 (+6.8)	-31.4 ± 0.4 (+5.9)	-4.0 ± 0.0 (+0.9)	63.0 ± 0.2 (-1.0)
Gq_{5hm}	-30.4 ± 0.2 (+1.5)	-27.6 ± 0.2 (+1.5)	-2.8 ± 0.0 (0.0)	55.3 ± 0.2 (+1.6)	-37.3 ± 0.8 (+4.9)	-33.1 ± 0.7 (+4.2)	-4.1 ± 0.1 (+0.8)	62.2 ± 0.1 (-1.8)
Gq_{11hm}	-30.2 ± 0.5 (+1.7)	-27.5 ± 0.5 (+1.6)	-2.7 ± 0.0 (+0.1)	54.3 ± 0.1 (+0.6)	-42.0 ± 1.9 (+0.2)	-37.0 ± 1.6 (+0.3)	-5.1 ± 0.2 (-0.2)	66.0 ± 0.2 (+2.0)
$Gq_{5,11hm}$	-31.9 ± 0.9 (0.0)	-29.2 ± 0.8 (-0.1)	-2.8 ± 0.1 (0.0)	53.2 ± 0.8 (-0.5)	-35.0 ± 1.3 (+7.2)	-30.9 ± 1.2 (+6.4)	-4.1 ± 0.2 (+0.8)	64.2 ± 0.4 (+0.2)

[a]The values were determined in 50 mM MES-LiOH (pH 6.0) and 100 mM NaCl with or without 40 wt% PEG200. Each value is the average of three determinations and the standard deviation is shown with the average. Melting temperatures were measured at 5 μM of DNA. [b] The amount of change resulting from the modifications was calculated by using ΔX = [X(without modification) – X (modification)], in which X is ΔH^{o}, $T\Delta S^{o}$, ΔG^{o}_{25}, or T_m and included in parentheses.

Table S6. Melting temperatures of i-motif forming DNA in the presence of complementary G-quadruplex forming DNA and K^+ [a]

	T_m (ºC)	
	100 mM KCl	100 mM KCl 40 wt% PEG200
iM and Gq	39.3 ± 1.1	39.5 ± 0.4
$iM_{10,16m}$ and $Gq_{5,11m}$	N.D. [b]	41.1 ± 0.3
$iM_{10,16hm}$ and $Gq_{5,11hm}$	N.D. [b]	37.5 ± 0.3

[a]The values were determined by UV melting curves at 295 nm in 50 mM MES-LiOH (pH 6.0) and 100 mM KCl with or without 40 wt% PEG200. Each value is the average of three determinations and the standard deviation is shown with the average. Melting temperatures were measured at 5 μM of DNA. [b] "N.D." indicates that values could not be obtained from the melting curves.

Table S7. Melting temperatures of mixture of i-motif forming DNA and G-quadruplex forming DNA in the presence of Na^+ [a]

	T_m (ºC)	
	100 mM NaCl	100 mM NaCl 40 wt% PEG200
iM and Gq	78.5 ± 1.1	61.4 ± 1.1
$iM_{10,16m}$ and $Gq_{5,11m}$	79.8 ± 0.7	62.4 ± 1.7
$iM_{10,16hm}$ and $Gq_{5,11hm}$	77.6 ± 0.8	60.7 ± 1.7

[a]The values were determined by UV melting curves at 260 nm in 50 mM MES-LiOH (pH 6.0) and 100 mM NaCl with or without 40 wt% PEG200. Each value is the average of three determinations and the standard deviation is shown with the average. Melting temperatures were measured at 5 μM of DNA.