Molecular crowding of the cosolutes induces an intramolecular i-motif structure of triplet repeat DNA oligomers at neutral pH†

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Experimental methods

Polyethylene glycols 200 and 8000 (PEG, average molecular weight 200 and 8000, respectively) were purchased from Wako Pure Chemical Co., Ltd. (Japan), and used without further purification. Oligonucleotides (custom synthesized and purified by HPLC) were purchased from Hokkaido System Science (Japan). Before any experiment, the samples were heated to 90°C and cooled down to 4°C at a rate of 2°C min^{-1} to remove any non-equilibrium intermolecular structures. All measurements were performed in 10 mM Na_2HPO_4 containing 1 mM Na_2EDTA.

The UV melting curves of the DNA samples (2 μM of total strand concentration) were recorded at 260 nm as a function of temperature using a Shimadzu UV-1700 instrument (Shimadzu Co., Ltd., Japan) with a 1 cm path length quartz cell. The samples were heated at a rate of 0.5°C min^{-1} to obtain the melting curves. The water condensation on the cuvette exterior at low temperature was avoided by flushing a constant stream of dry nitrogen. The experiments were repeated three times to estimate the uncertainty in the melting temperature.

The CD spectra of the DNA samples (2 μM of total strand concentration) were obtained using a J-820 spectropolarimeter (JASCO Co., Ltd., Japan) with a 1 cm path length quartz cell at 4°C. The cuvette-holding chamber was flushed with a constant steam of dry N_2 gas to avoid water condensation on the cuvette surface. Data were collected from 200 to 350 nm with a 4 s response time, 1 nm slit width and the scan speed of 100 nm min^{-1}. Each spectrum shown was the average of three individual scans and is buffer corrected.

The native gel electrophoresis was carried out with 15% polyacrylamide gel (acrylamide:bisacrylamide = 19:1) in a cold room at 4°C. Ice-cold loading buffer (1 μL, 40% glycerol and 0.2% blue dextran) was mixed with the DNA sample (1 μL, 4 μM) in the buffer solution containing PEG. The experiments were performed with the gels containing no cosolutes. Only the DNA solution containing cosolute was mixed with the loading buffer and loaded in the gel. After the electrophoresis, the nucleotides in the gel were stained by Cybergold (TaKaRa Bio., Inc., Japan) and the fluorescence emission of the stains was visualized using FLS-5100 film (Fuji Photo Film Co., Ltd., Japan).

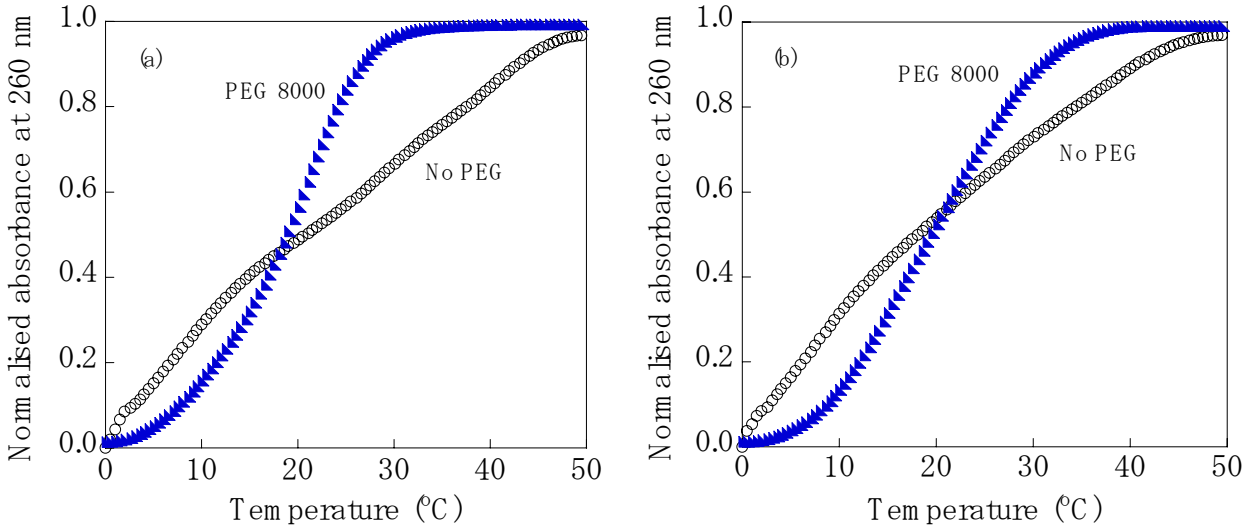

Fig. S1. The UV-melting curves of **(a) $(CCT)_8$ and (b) $(CCT)_{10}$** in the absence and presence of PEG 8000 at **pH 8.0**. [ss-DNA] = 2 μM; **$[Na^+]$ = 22 mM**.

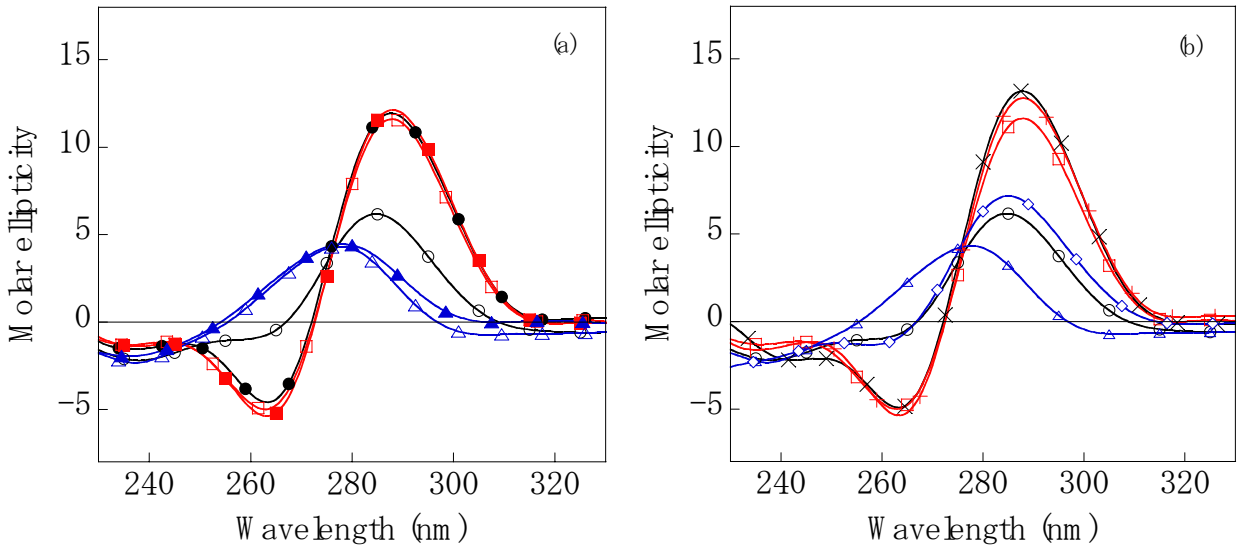

Fig. S2. CD spectra of **$(CCT)_8$** in the absence and presence of 20 wt% (a) PEG 200 and (b) PEG 8000. ○-without PEG at pH 7.0; ●-with PEG 200 at pH 7.0; Δ-without PEG at pH 8.0; ▲-with PEG 200 at pH 8.0; □-without PEG at pH 6.0; ■-with PEG 200 at pH 6.0; ×-with PEG 8000 at pH 7.0; ◊-with PEG 8000 at pH 8.0; +-with PEG 8000 at pH 6.0. Molar ellipticity in 10^5 deg cm^2 $dmol^{-1}$; [ss-DNA] = 2 μM; **[Na^+] = 22 mM**;

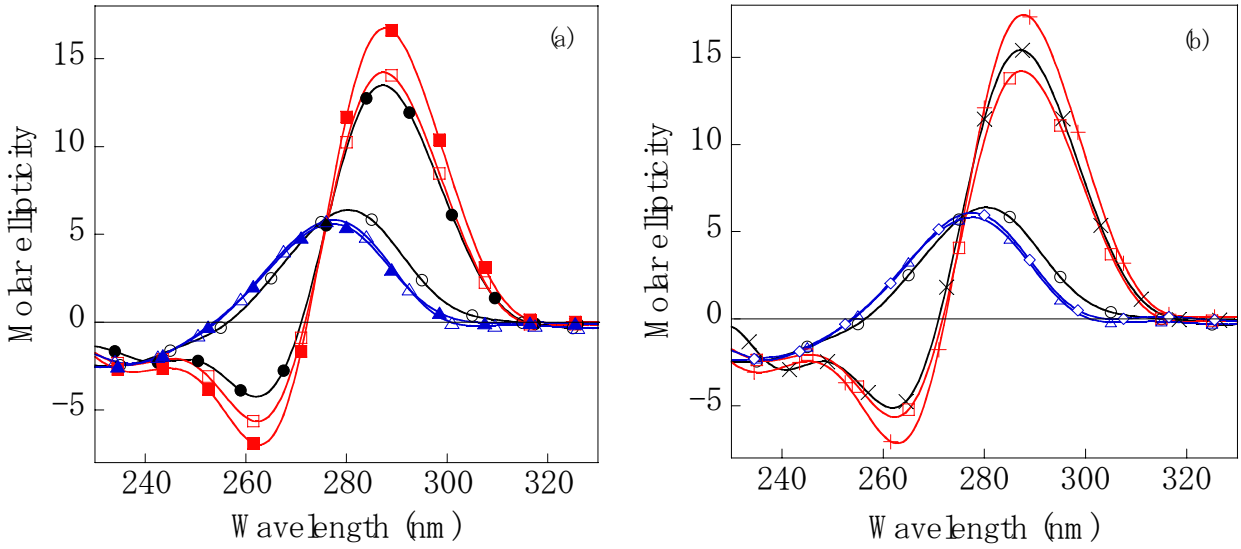

Fig. S3. CD spectra of **$(CCT)_{10}$** in the absence and presence of 20 wt% (a) PEG 200 and (b) PEG 8000. ○-without PEG at pH 7.0; ●-with PEG 200 at pH 7.0; Δ-without PEG at pH 8.0; ▲-with PEG 200 at pH 8.0; □-without PEG at pH 6.0; ■-with PEG 200 at pH 6.0; ×-with PEG 8000 at pH 7.0; ◊-with PEG 8000 at pH 8.0; +-with PEG 8000 at pH 6.0. Molar ellipticity in 10^5 deg cm^2 $dmol^{-1}$; [ss-DNA] = 2 μM; **[Na^+] = 122 mM;**

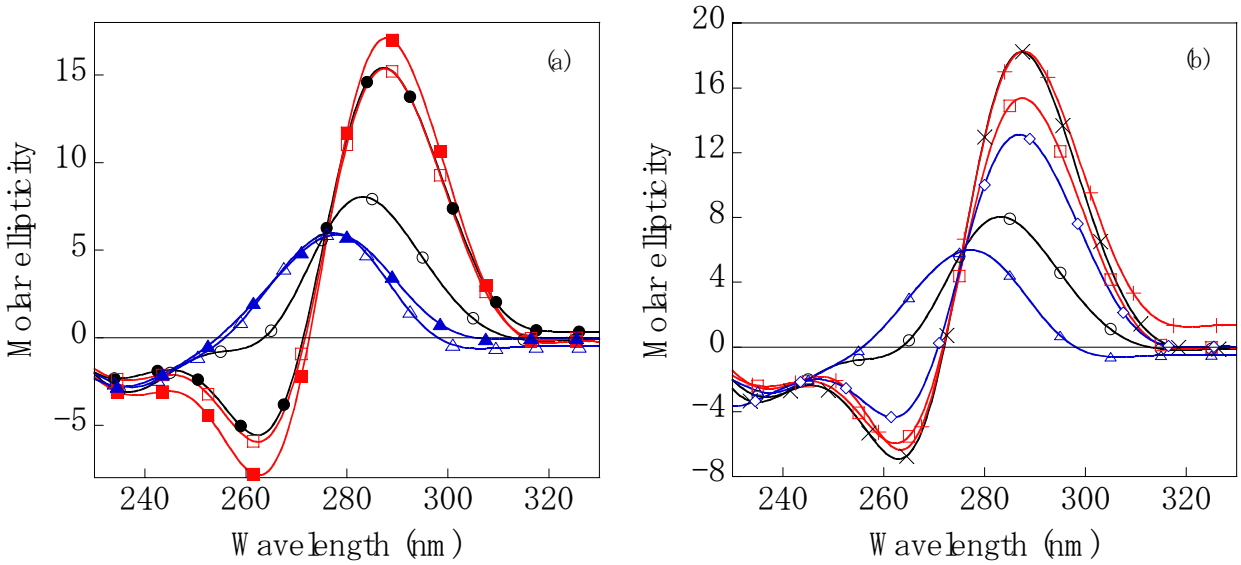

Fig. S4. CD spectra of **$(CCT)_{10}$** in the absence and presence of 20 wt% (a) PEG 200 and (b) PEG 8000. ○-without PEG at pH 7.0; ●-with PEG 200 at pH 7.0; Δ-without PEG at pH 8.0; ▲-with PEG 200 at pH 8.0; □-without PEG at pH 6.0; ■-with PEG 200 at pH 6.0; ×-with PEG 8000 at pH 7.0; ◊-with PEG 8000 at pH 8.0; +-with PEG 8000 at pH 6.0. Molar ellipticity in 10^5 deg cm^2 $dmol^{-1}$; [ss-DNA] = 2 μM; **[Na^+] = 22 mM;**

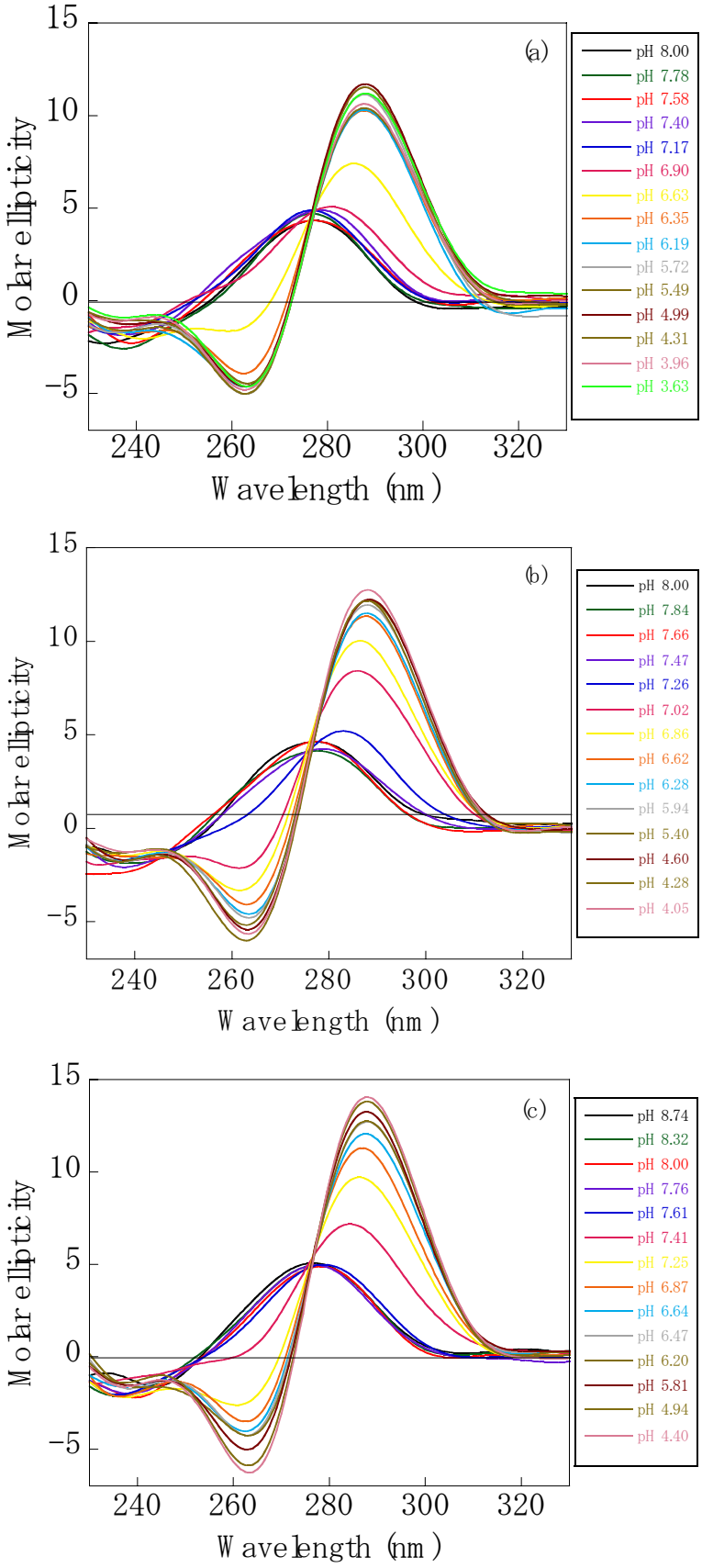

Fig. S5. The pH dependent CD spectra of **$(CCT)_8$** in (a) the absence and presence of 20 wt% of (b) PEG 200 and (c) PEG 8000. Molar ellipticity in 10^5 deg cm^2 $dmol^{-1}$; [ss-DNA] = 2 μM; **[Na^+] = 122 mM**;

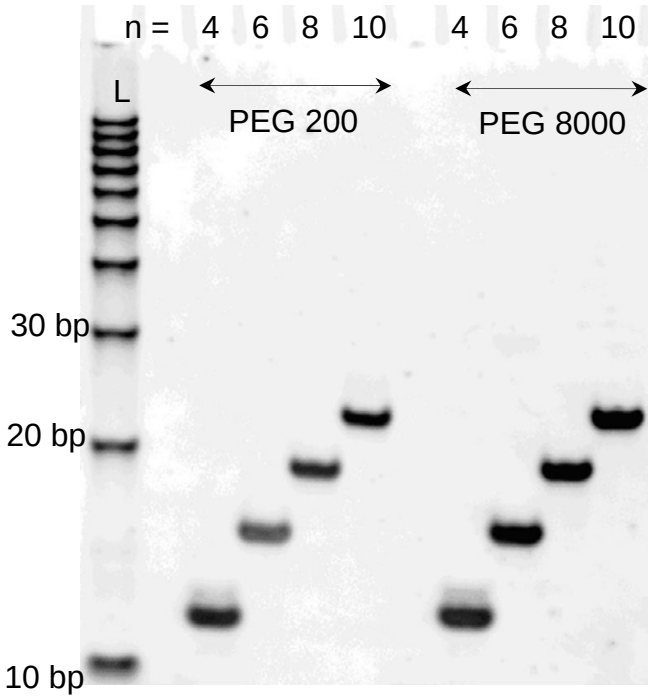

Fig. S6. Native gel electrophoretogram of the CCT triplet repeat sequences in the presence of 20 wt% PEG 200 and PEG 8000. **The ionic strength of Na^+ is 122 mM**. [Na_2EDTA] = 1 mM; [Na_2HPO_4] = 10 mM; **pH = 7.0**; L: 10 bp DNA step ladder; n = triplet repeat number.

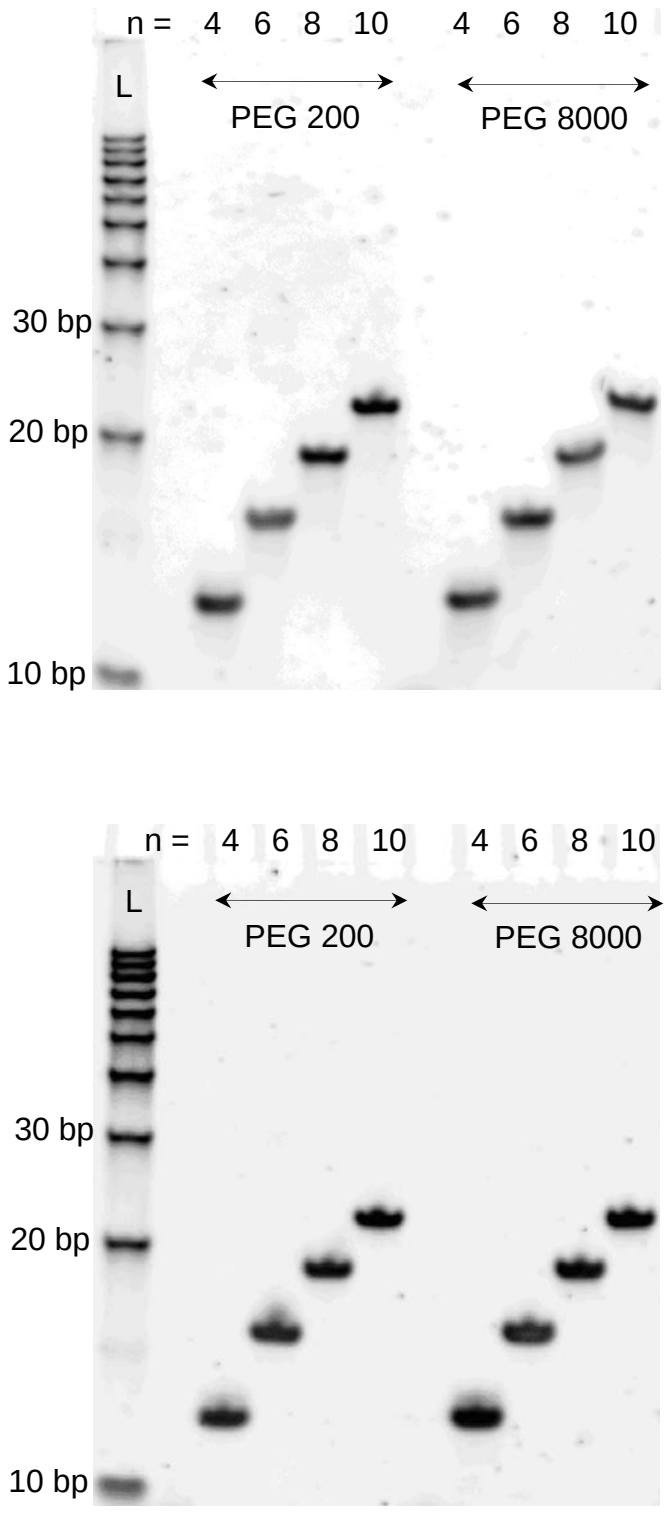

Fig. S7. Native gel electrophoretograms of the CCT triplet repeat sequences in the presence of 20 wt% PEG 200 and PEG 8000. **The ionic strength of Na^+ is 122 mM (top) and 22 mM (bottom)**. [Na_2EDTA] = 1 mM; [Na_2HPO_4] = 10 mM; **pH = 6.0**; L: 10 bp DNA step ladder; n = triplet repeat number.

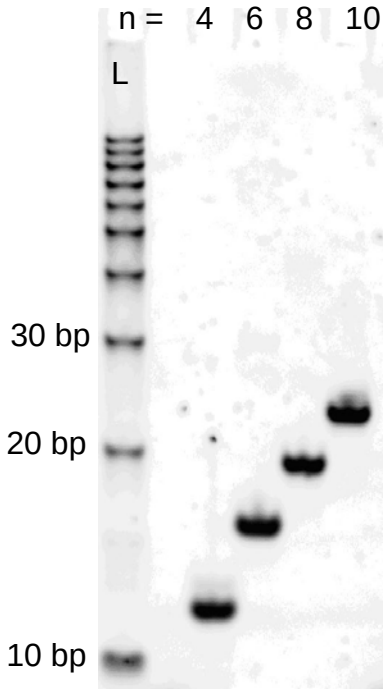

Fig. S8. Native gel electrophoretograms of the CCT triplet repeat sequences in the **absence of cosolute**. **The ionic strength of Na^+ is 122 mM** . [Na_2EDTA] = 1 mM; [Na_2HPO_4] = 10 mM; **pH = 8.0**; L: 10 bp DNA step ladder; n = triplet repeat number.

Discussion on the Gel experiments:

The unstructured and i-motif DNA migrate at the same speed and the bands produced by them can not be distinguished each other. For example, the migration of the bands in the gel carried out at pH 8.0 (in the absence of cosolute, ESI Fig. S8) resembles same as that carried out at pH 7.0 (with cosolute, Fig. 3) and even at pH 6.0 (ESI Fig S7). However, the T_m and CD experiments evidenced the formation of i-motif at pH 7.0 and pH 6.0 and unstructured DNA at pH 8.0. So we have concluded that the bands in the gel correspond to the i-motif at lower pH and unstructured DNA at higher pH. The positive charge in the i-motif may lead to the slow migration and hence the unstructured and i-motif may migrate at the same speed. Though the unstructured and i-motif DNAs can not be distinguished by the gel experiments, the distinction between the intra and intermolecular i-motif structures were possible. Moreover, the intramolecular i-motif structures were also confirmed by the concentration dependent T_m experiments in the absence of cosolute and in the presence of 20 wt% PEG 200 (ESI, Table S1).

Table S1. Melting temperature (T_m, °C) of the CCT triplet repeat sequences with 20 times higher concentration of the DNA (40 μM) in the absence and presence of 20 wt% PEG 200 and PEG 8000.

Conditions	Melting temperature (T_m, °C)			
	$(CCT)_4$	$(CCT)_6$	$(CCT)_8$	$(CCT)_{10}$
		pH 7.0 & 22 mM Na^+		
PEG 200		16.5 ± 0.7 (15.4 ± 0.8)	32.6 ± 0.3) (31.8 ± 0.4)	35.1 ± 0.7) (35.1 ± 0.5)
PEG 8000		30 ± 0.2 (19.7 ± 0.4)		47.5 ± 0.9 (39.3 ± 0.7)
		pH 6.0 & 122 mM Na^+		
No cosolute		18.2 ± 0.9 (17.0 ± 0.5)	35.1 ± 0.2 (34.0 ± 0.1)	
PEG 200			43.6 ± 0.3 (42.0 ± 0.5)	47.2 ± 0.6 (46.5 ± 0.1)
PEG 8000	42.6 ± 0.5 (37.0 ± 0.5)	49.0 ± 2.0 (33.6 ± 1.0)		59.1 ± 0.3 (52.0 ± 0.1)
		pH 6.0 & 22 mM Na^+		
No cosolute	34.0 ± 1.0 (34.6 ± 0.9)	26.1 ± 0.3 (26.1 ± 0.2)		46.6 ± 0.2 (46.1 ± 0.1)
PEG 200	40.1 ± 0.4 (39.0 ± 0.9)	37.0 ± 0.5) (35.5 ± 0.5)		54.3 ± 0.3 (53.1 ± 0.1)
PEG 8000		53.5 ± 1.5 (44.5 ± 0.1)		69.0 ± 0.5 (63.0 ± 0.1)

[ss-DNA] = 40 μM; [Na_2HPO_4] = 10 mM; [Na_2EDTA] = 1 mM; **In paranthesis the T_m values for 20 times lower concentration of the DNA (2 μM, this data is same as listed in Table 1).**

Discussion on the concentration dependent T_m experiment:

To confirm whether the i-motif is intramolecular or intermolecular, we have performed the melting temperature experiments with 20 times higher concentration of DNA (40 μM) and the T_m values are given in Table S1. The T_m remains either unaltered or increased only about 1.6 °C in the absence of cosolute and in the presence of 20% PEG 200. This concentration independence of T_m explains that the intramolecular i-motif structure is formed under this condition. However, the T_m is increased about 5.6 to 15.4 °C depending on the Na^+ concentration and pH in the presence of PEG 8000. This indicates that at higher concentration of DNA the PEG 8000 may induces the formation of intermolecular i-motif structure.