Supporting Information For

Incorporation of O^6-methylguanine restricts conformational conversion of human telomere G-quadruplex under molecular crowding

Experimental Section

Materials and Methods

DNA oligomers were purchased from Sangon (Shanghai, China) and used without further purification. Concentrations of these oligomers were determined by measuring the absorbance at 260 nm after melting. Extinction coefficients were estimated by the nearest neighbor method by using mononucleotide and dinucleotide values. All the experiments were carried out in 10 mM Tris buffer containing 100 mM NaCl or KCl with corresponding PEG or dehydration regeants. PEG200, PEG1000 and PEG6000 were purchased from Sigma-Aldrich. Acetonitrile was obtained from Beijing Chemical reagent Corporation.

UV melting experiment: melting experiments were performed on a Cary 300 UV/Vis spectrophotometer equipped with a Peltier temperature control accessory. All UV/Vis spectra were measured in 1.0-cm path-length cell. Absorbance changes at 295 nm versus temperature were collected at a heating rate of 0.5 °C·min^{-1}. Before measurement, the DNA samples were heated at 95 °C for 5 min, and gently cooled from 95 °C to room temperature.

CD Spectroscopy：CD spectra were recorded on a JASCO J-810 spectropolarimeter. CD spectra were recorded from 320 to 220 nm in 1 nm increment with an average time of 2 seconds and three scans were accumulated and automatically averaged. The correspondingly buffer was scanned as a control and subtracted from the spectra of DNA to eliminate its influence on DNA CD signal.

Gel electrophoresis: Native gel electrophoresis was carried out on acrylamide gel (12 %) containing different content of PEG200 and run at 4 ºC, 1×TB buffer containing 10 mM KCl/NaCl and was silver stained. DNA was heated at 95 ºC for 5 min in 10 mM Tris buffer containing corresponding cation (K^+/Na^+) and PEG200, and gently cooled from 95 ºC to room temperature, following by incubation at 4 ºC overnight.

Table. S1 Oligonucleotide sequences used in this work.

Sequene (5'-3')	Abbreviation
d[AGGGTTAGGGTTAGGGTTAGGG]	AG_3
d[A^{m}**G**GGTTAGGGTTAGGGTTAGGG]	mG1
d[AG^{m}**G**GTTAGGGTTAGGGTTAGGG]	mG2
d[AGG^{m}**G**TTAGGGTTAGGGTTAGGG]	mG3

The ^{m}G represents the position of 6mG.

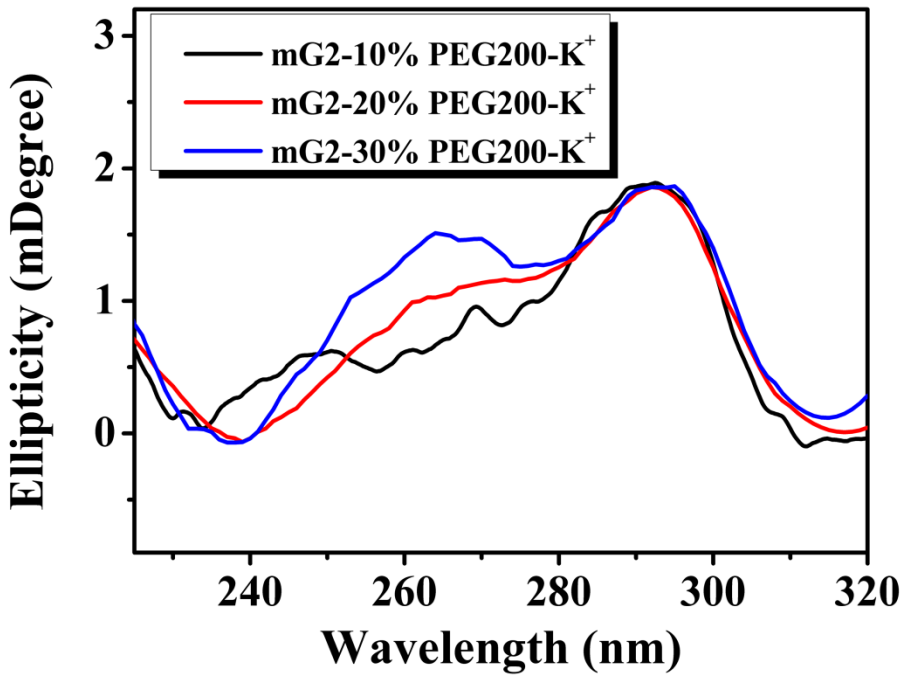

Fig. S1 CD spectra of mG2 measured in 10 mM Tris buffer (pH 7.2) containing 100 mM KCl and different content of PEG 200 at 25 ºC.

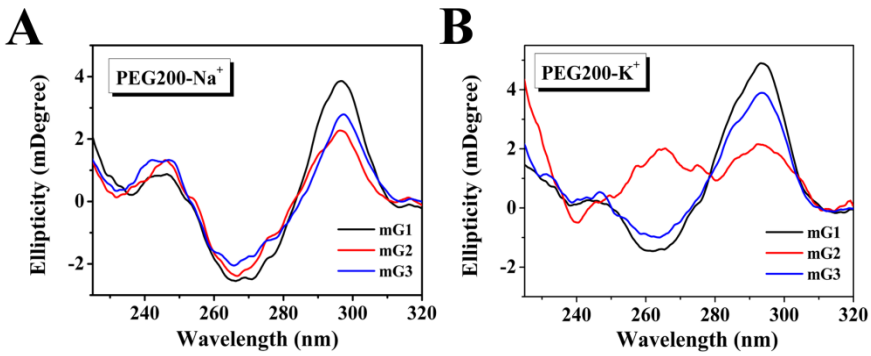

Fig. S2 CD spectra of AG_3, mG1, mG2 and mG3 measured in different buffer conditions at 37 ºC. (A) 10 mM Tris buffer (pH 7.2) containing 100mM NaCl and 40% PEG200. (B) 10 mM Tris buffer (pH 7.2) containing 100 mM KCl and 40% PEG200.

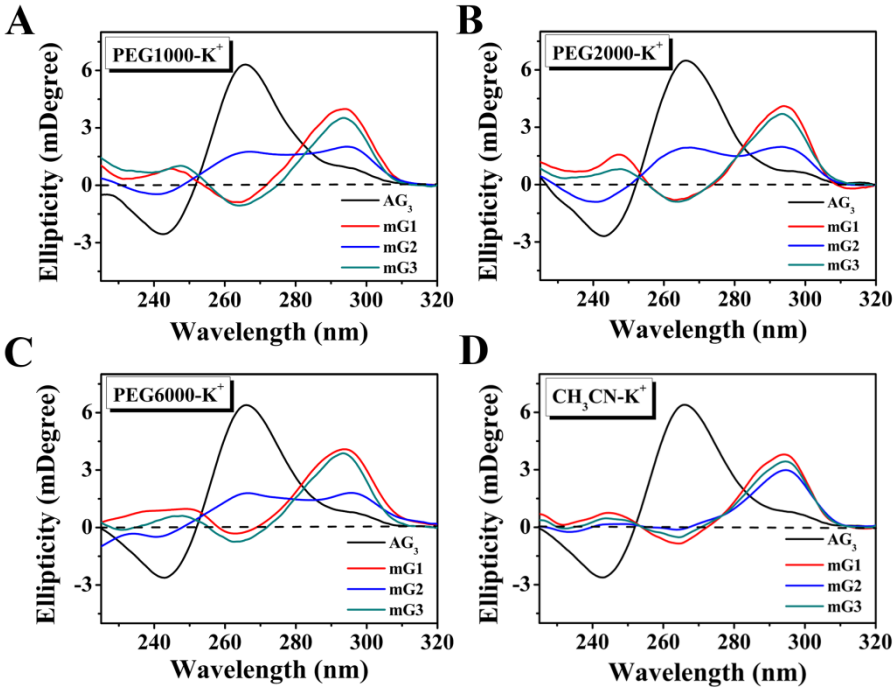

Fig. S3 CD spectra of AG_3, mG1, mG2 and mG3 measured in different buffer conditions at 25 ºC. (A) 10 mM Tris buffer (pH 7.2) containing 100 mM KCl and 40% PEG1000. (B) 10 mM Tris buffer (pH 7.2) containing containing 100 mM KCl and 40% PEG2000. (C) 10 mM Tris buffer (pH 7.2) containing 100 mM KCl and 40% PEG6000. (D) 10 mM Tris buffer (pH 7.2) containing 100 mM KCl and 40% CH_3CN.

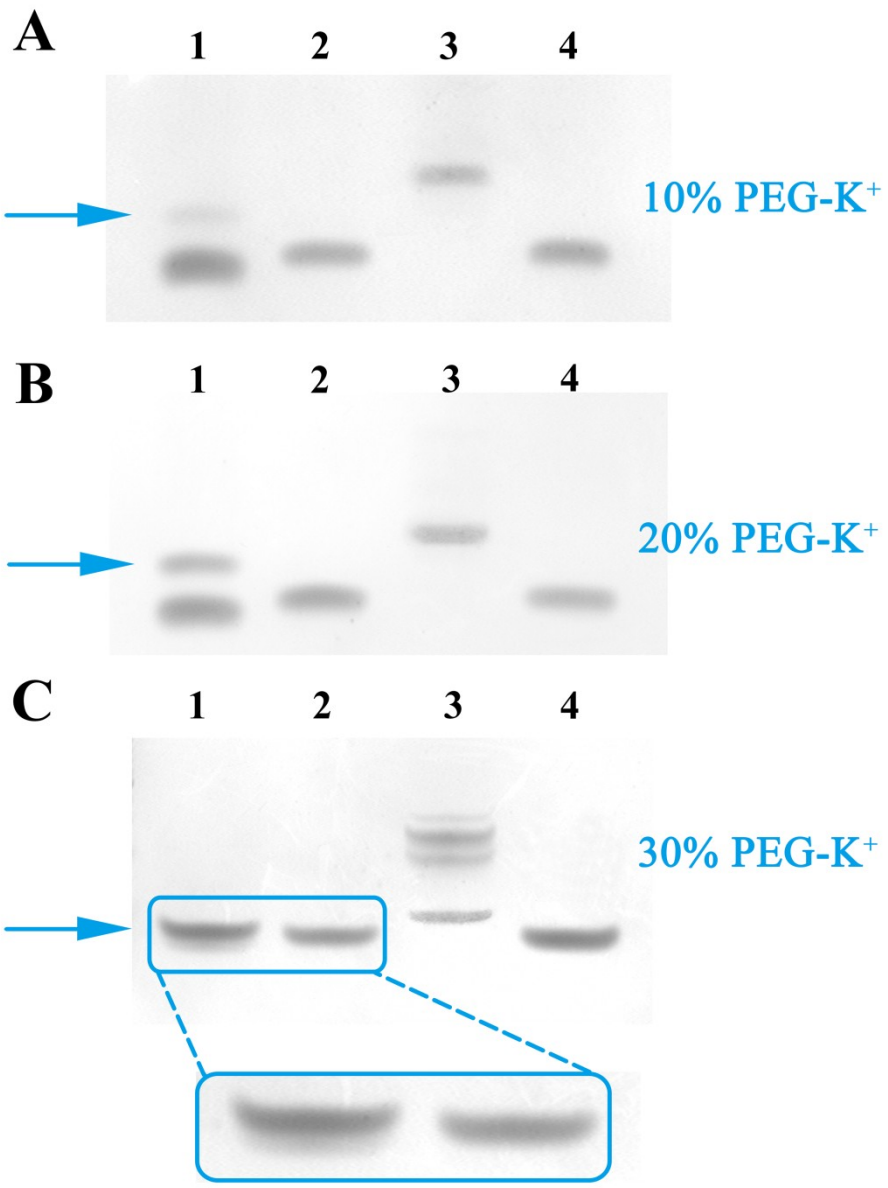

Fig. S4 Native 12% PAGE images of AG_3 (Lane 1), mG1 (Lane 2), mG2 (Lane 3) and mG3 (Lane 4) in K^+ buffer containing 10% PEG200 (A), 20% PEG200 (B) and 30% PEG200 (C), respectively.

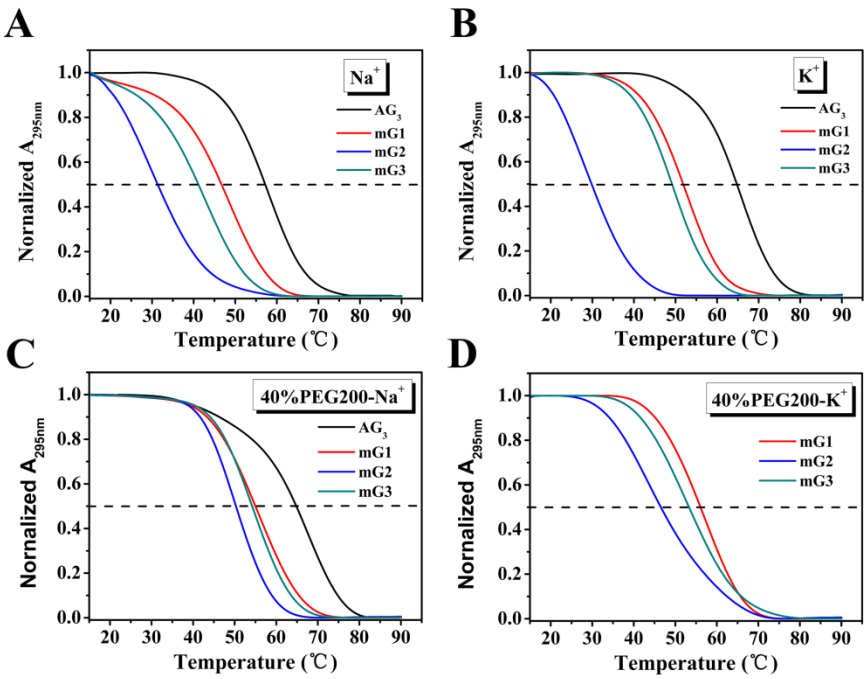

Fig. S5 The UV melting profiles of AG_3, mG1, mG2 and mG3 measured at 295 nm in different buffer conditions. (A) 10 mM Tris buffer (pH 7.2) containing 100 mM NaCl. (B) 10 mM Tris buffer (pH 7.2) containing 100 mM KCl. (C) 10 mM Tris buffer (pH 7.2) containing 100 mM NaCl and 40% PEG200. (D) 10 mM Tris buffer (pH 7.2) containing 100 mM KCl and 40% PEG200 (The melting profile of AG_3 was not given under this condition because its Tm was out of range).

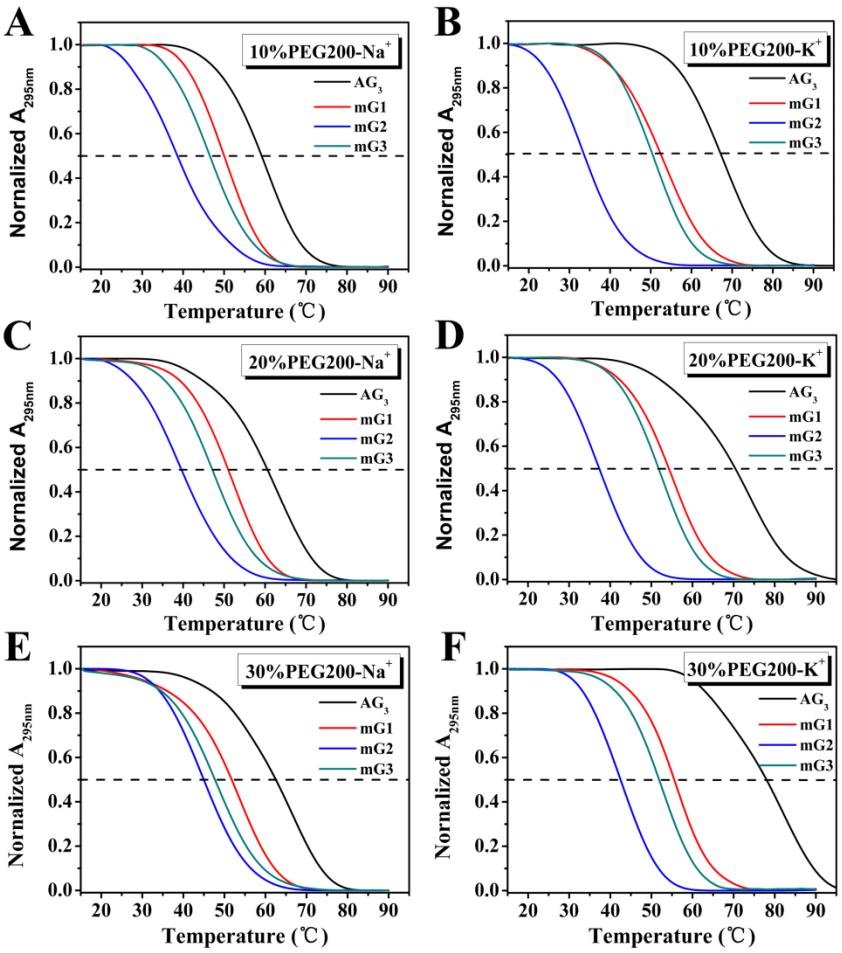

Fig. S6 The UV melting profiles of AG_3, mG1, mG2 and mG3 measured at 295 nm in different buffer conditions. (A) 10 mM Tris buffer (pH 7.2) containing 100 mM NaCl and 10% PEG200. (B) 10 mM Tris buffer (pH 7.2) containing 100 mM KCl and 10% PEG200. (C) 10 mM Tris buffer (pH 7.2) containing 100 mM NaCl and 20% PEG200. (D) 10 mM Tris buffer (pH 7.2) containing 100 mM KCl and 20% PEG200. (E) 10 mM Tris buffer (pH 7.2) containing 100 mM NaCl and 30% PEG200. (F) 10 mM Tris buffer (pH 7.2) containing 100 mM KCl and 30% PEG200.