Regulation of DNA Strand Displacement Using G-quadruplex-mediated Toehold

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Electronic Supporting Information

General Methods

Materials and reagent. DNA sequences used in this work were listed in Table S1. All the fluorescence labeled oligonucleotides were purchased from TaKaRa Clontech. All the unlabeled sequences were purchased from Invitrogen Company. Sodium chloride, potassium chloride, and PEG 200 were purchased from Sinopharm Chemical Reagent Co. Ltd.

Formation and purification of substrate SC. Substrate SC were formed by strand S, strand M and strand T heating to 95 °C at 10 μM concentration in TE buffer with 20 mM $K^+$and 200 mM Na^+, then cooling to 4 °C rapidly. Then the complex were purified through native gel electrophoresis. The gel electrophoresis was run on 20% polyacrylamide gel at 4 °C, 12 V/cm. The target bands were cut from gels, and then eluted with 1×TEbuffer at 4 °C. The purified solutions were quantified by UV absorbance at 260 nm.

Fluorescence kinetic experiments. Fluorescence data were recorded using Perkin Elmer LS55 Fluorescence Spectrometer with the temperature controller set to 30°C. Excitation and emission wavelengths were 488 nm and 515 nm, respectively, with 10 nm bandwidths. The fluorescence data of 750 nM substrate SC in 200 μL solutions were collected for 5 minutes before addition of strand I. The runs were paused for about 30s to add 10 μL150 μM strand I and mix by gentle pipetting.The reaction contained25 mM HEPES-NH_3•H_2O (pH 8.0), 20 mM K^+, 200 mM Na^+, and different volume fraction of PEG.

CD analysis. Circular dichroism (CD) experiments were carried out with Chariscan circular dichroism photomultiplier (Applied Photophsics Limited. UK) equipped with a Quantum Nothwest TC125 temperature controller. All the CD spectra were measured from 220 nm to 320 nm in a 0.5 cm path-length cuvette with a scanning speed of 200 nm/min, 3 nm bandwidth and 2 s response time. The temperature was controlled at 30 °C. Thermal denaturation was performed by graduallyincreasingthe temperatures from 25 °C to 90 °C, and the ultraviolet absorbances at 265 nm were monitored to calculate the Tm values for each sample.

Native gel electrophoresis. 750 nM FAM-labeled substrate SC and 7.5 μM unlabeled strand I were mixed together in the solution containing 25 mM HEPES-NH_3•H_2O (pH 8.0), 20 mM K^+, 200 mM Na^+, and 20% PEG. The total volume of each sample was 10 μL. Each sample was incubated at 30 °C for 2 hours. Native gel electrophoresis was run on 20% polyacrylamide gel at 4 °C, 5 V cm^{-1} in 1×TBE buffer. Gels with FAM-labeled oligonucleotides were photographed under irradiation of UV light (Vilber Lourmat, Bio-Print, VL).

Table S1. Oligonucleotides used in this work.

	component	Sequences (5’ to 3’)
Universal strand	Strand M	GTTGTTCAGGTCGATAGTCA(3'-FAM)
	Strand S	TGACTATCGACCTGAACAACTCGCTAGGCTCTGAGACC(5'-DABCYL)
$2G_{3:1}$	Strand T	GGTCTCAGAGCCTAGCGTGGTTGGTTGGT
	Strand I	TGGTGTTGTTCAGGTCGATAGTCA
$3G_{3:1}$	Strand T	GGTCTCAGAGCCTAGCGTGGTTGGGTTGGGT
	Strand I	TGGGTGTTGTTCAGGTCGATAGTCA
$4G_{3:1}$	Strand T	GGTCTCAGAGCCTAGCGTGGGGTTGGGGTTGGGGT
	Strand I	TGGGGTGTTGTTCAGGTCGATAGTCA
$2G_{2:2}$	Strand T	GGTCTCAGAGCCTAGCGTGGTTGGT
	Strand I	TGGTTGGTGTTGTTCAGGTCGATAGTCA
$3G_{2:2}$	Strand T	GGTCTCAGAGCCTAGCGTGGGTTGGGT
	Strand I	TGGGTTGGGTGTTGTTCAGGTCGATAGTCA
$4G_{2:2}$	Strand T	GGTCTCAGAGCCTAGCGTGGGGTTGGGGT
	Strand I	TGGGGTTGGGGTGTTGTTCAGGTCGATAGTCA
CD spectrum	2G	TGGTTGGTTGGTTGGT
	3G	TGGGTTGGGTTGGGTTGGGT
	4G	TGGGGTTGGGGTTGGGGTTGGGGT
	Strand M-C	TGACTATCGACCTGAACAAC

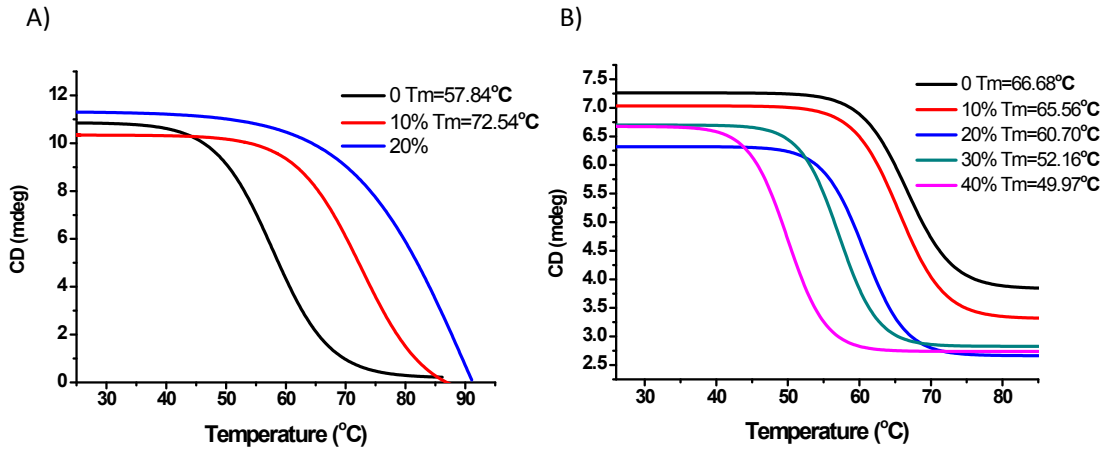

Figure S1. A) The Tm values of the G-quadruplex with different volume fraction of PEG. The experiments were carried out at 10 μM G-quadruplex of 3G (TGGGTTGGGTTGGGTTGGGT). Tm value increased as the volume fraction of PEG increased. When PEG reached 20%, the G-quadruplex was too stable to calculate the value of Tm. B) The Tm values of the double stranded DNA with different volume fraction of PEG. The experiments were performed at 10 μM dsDNA of strand M. The values of Tm were positive correlated with the PEG concentration. The system contained25 mM HEPES-NH_3•H_2O (pH 8.0), 200 mMNaCl, 20 mM KCl. The temperatures were increased gradually from 25 °C to 90 °C, and the ultraviolet absorbances at 265 nm were monitored to calculate the Tm values for each sample.

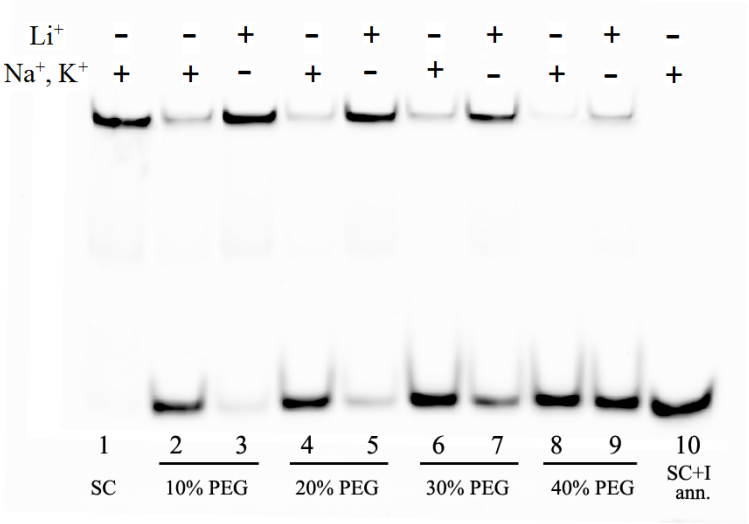

Figure S2. 20% native PAGE analysis of the G-quadruplex toehold-mediated strand displacement at 30°C for 2h. The reactions (lane 2 to lane 9) were carried out with 200 mM NaCl, 20 mM KCl or only 150 mM LiClwith different concentration of PEG 200. [SC]= 750 nM, [I]= 7.5 μM, "ann." denoted the sample was annealed.

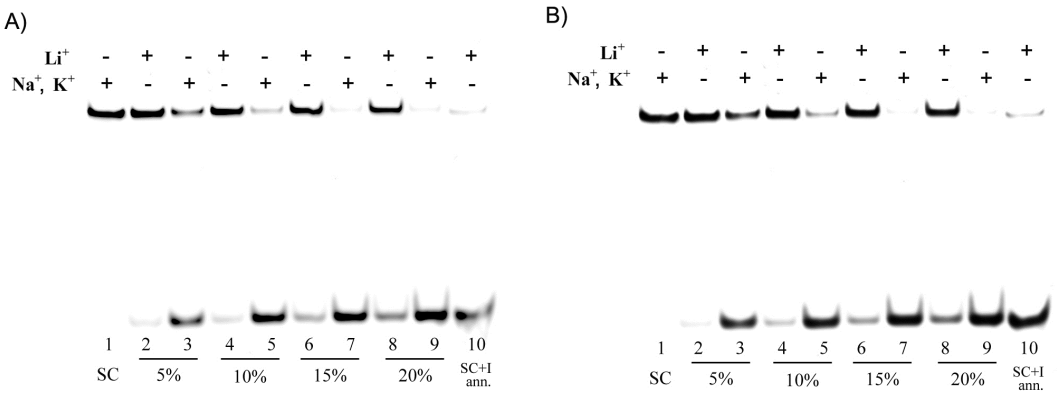

Figure S3. 20% native PAGE analysis of the G-quadruplex toehold-mediated strand displacement at 30°C for 2h. The reactions (lane 2 to lane 9) were carried out with 200 mM NaCl, 20 mM KCl or only 150 mM LiCl. [SC]= 750 nM, [I]= 7.5 μM, "ann." denoted the sample was annealed. A) different concentration of PEG 400. B) different concentration of PEG 600

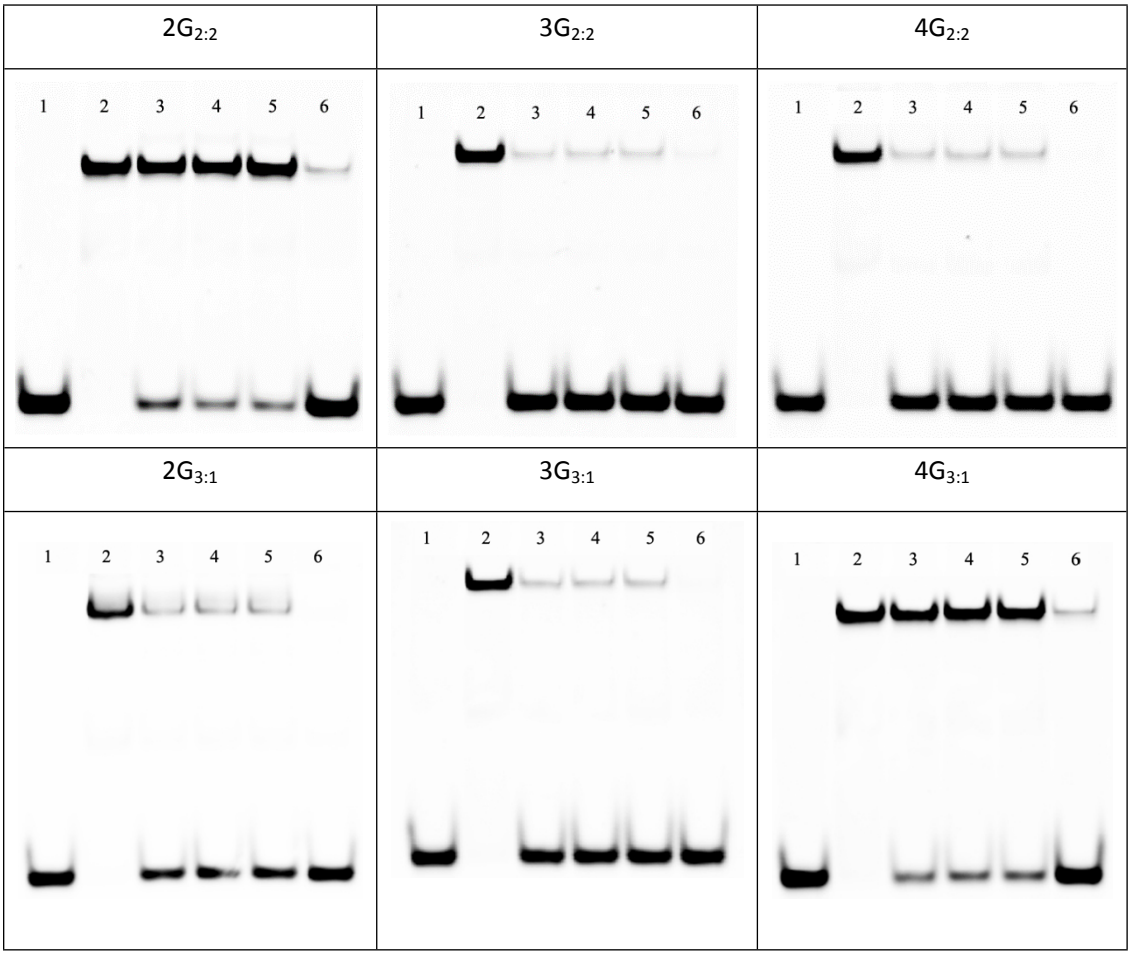

Figure S4. 20% native PAGE analysis of the G-quadruplex toehold-mediated strand displacement of $2G_{2:2}$, $3G_{2:2}$, $4G_{2:2}$and $2G_{3:1}$, $3G_{3:1}$, $4G_{3:1}$. Lane 1: 750 nM strand M labeled by FAM, lane 2: 750 nM substrate SC, lane 3-5: 750 nM SC and 7.5 μM I in 20% PEG, lane 6: 750 nM SC and 7.5 μM I after annealed. The reactions all contained 25

mM HEPES-NH_3•H_2O (pH 8.0), 20 mM K^+, 200 mM Na^+, the temperature was controlled at 30°C for 2h..

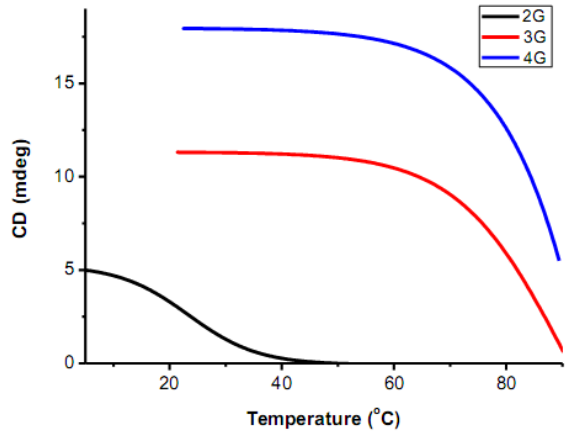

Figure S5. The Tm values of different number of G-quartet with 20% PEG. The experiments were carried out at 10 μM G-quadruplex for each sample. Tm value increased as the number of G-quartet increased. The system contained 25 mM HEPES-NH_3•H_2O (pH 8.0), 200 mM NaCl, 20 mM KCl. The temperatures were increased gradually from 25 °C to 90 °C for 3G and 4G, and 5°C to 50°C for 2G. The ultraviolet absorbances at 265 nm were monitored to calculate the Tm values for 3G and 4G, 295 nm for 2G.

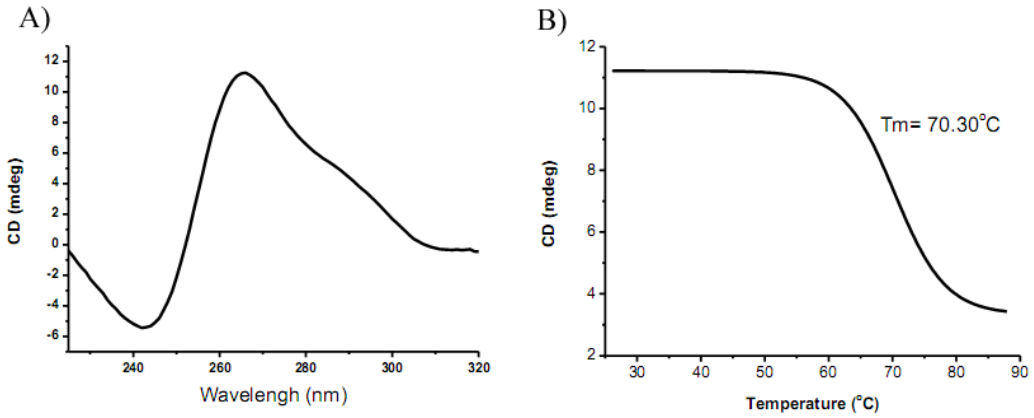

Figure S6. CD spectroscopy analysis of toehold domain in $4G_{3:1}$. [strand T] = 10 μM, 25 mM HEPES-NH_3•H_2O (pH 8.0), 20 mM K^+, 200 mM Na^+, and 20% PEG. The system was heated to 95 °C for 5 min, then cooling to 4 °C rapidly. A) CD spectrum was measured at 30°Cfrom 220 nm to 320 nm. B) The temperatures were increased gradually from 25 °C to 90 °C. The ultraviolet absorbance at 265 nm was monitored to calculate the Tm values.

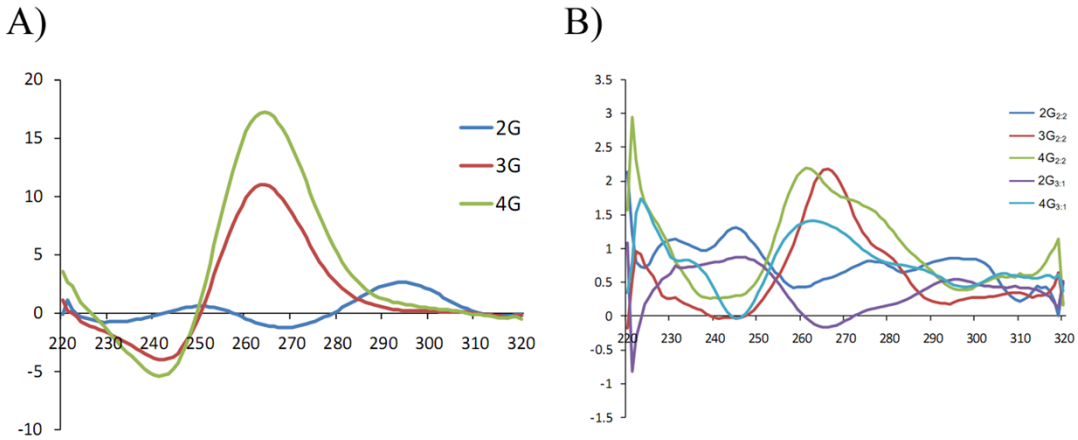

Figure S7. A) CD spectroscopy analysis of 2G, 3G and 4G. [strand] = 10 μM, 25 mM HEPES-NH_3•H_2O (pH 8.0), 20 mM K^+, 200 mM Na^+, and 20% PEG. The system was heated to 95 °C for 5 min, then cooling to 4 °C rapidly. CD spectrum was measured at 30°C from 220 nm to 320 nm. B) CD spectroscopy analysis of G-quadruplex structure formation in the displacement reaction of $2G_{3:1}$, $4G_{3:1}$, $2G_{2:2}$, $3G_{2:2}$, $4G_{2:2}$. The results were treated by subtracting the spectra of SC and strand I from the reaction spectrum. Initial concentrations: [substrate SC] = 2 μM, [strand I] = 20 μM, 25 mM HEPES-NH_3•H_2O (pH 8.0), 20 mM K^+, 200 mM Na^+, and 20% PEG. The experiment was carried out after 2h incubation at 30°C.