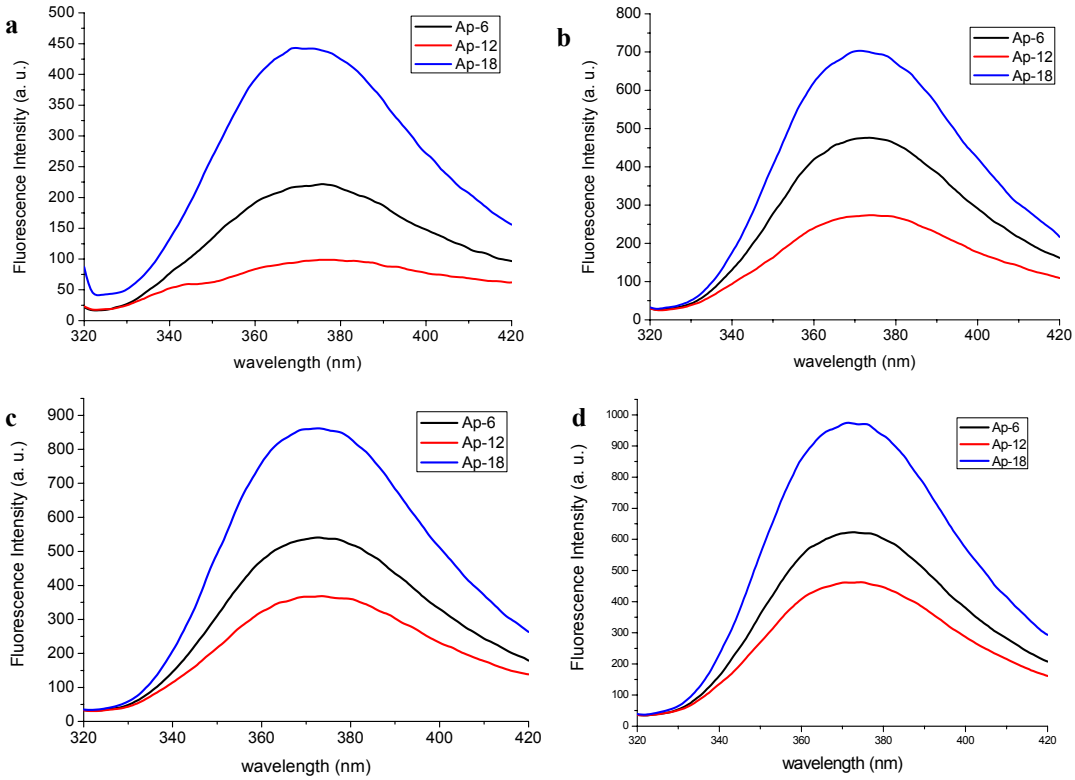
# Supplementary Information

**Materials.** 21G ($GGG(TTAGGG)_3$), and the dimeric sequence 45G ($GGG(TTAGGG)_7$) were all purchased from Invitrogen company. The labeled oligonucleotides, FAM-21G (5`-FAM-$GGG(TTAGGG)_3$-3`) with FAM: 6-carboxyfluorescein, FAM-45G (5`-FAM-$GGG(TTAGGG)_7$-3`), and the stochastic 45 mer oligonucleotide (5`-FAM-CCCTTCCCCTCTGATG -GACATCTCACCGACCGCAGGATCCTATAA-3`) were all purchased from TaKaRa Clontech. 21G substituted with 2-Ap (2-aminopurine) was purchased from TaKaRa Clontech. PEG 200 (polyethylene glycol, average mol wt 200) was purchased from Sigma-Aldrich. All the experiments were carried out in 150 mM $K^+$ solution if not stated otherwise

**Circular Dichroism Spectroscopy.** 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 0.1 cm path-length cuvette with a scanning speed of 200 nm/min, 3 nm bandwidth and 2 s response time. The concentration of all the oligonucleotides adopted in CD experiments was 10 μM in the samples including 10 mM pH=7.5 Tris-HCl buffer, 150 mM KCl, 1 mM EDTA and 40% (w/v) PEG 200. The initial states of samples were acquired according to the following procedure: samples were treated with heat in diluted $K^+$ solution and slowly cooled down before PEG was added, and then maintained at 37 °C for 10 minutes to make the influence of PEG be stable. This initial state was the same as the state of the sample in which the dried-up DNA sample or the DNA sample in pure water was added into the buffer in the presence of PEG and then maintained at 37 °C for 10 minutes. Besides, kinetic bahaviors of samples prepared according to different procedures had little difference shown in Figure S5.

**Fluorescence Experiments.** The fluorescence spectra were collected with Perkin Elmer LS55 Fluorescence Spectrometer. Three oligonucleotides with 2-aminopurine (Ap) substitutions at different adenine residues in 21G were shown in Figure S2. Samples containing 1 μM oligonucleotide, 10 mM Tris-HCl buffer, 150 mM KCl and 40% (w/v) PEG 200 were excited at 305 nm, and emission spectra were collected from 320–420 nm.

Ap-6: GGGTTApGGGTTAGGGTTAGGG
Ap-12: GGGTTAGGGTTApGGGTTAGGG
Ap-18: GGGTTAGGGTTAGGGTTApGGG



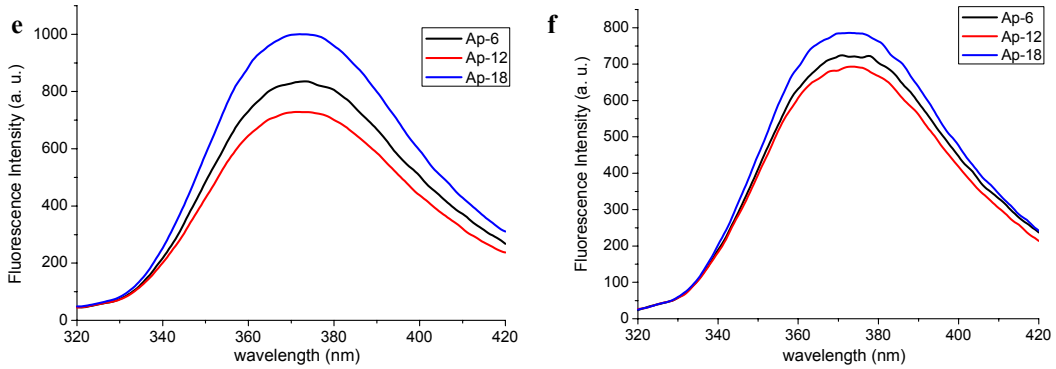



**Figure S1**. Fluorescence of Ap at different positions along with time. a-e depicted the spectra of samples which were measured after bathed for 0, 1, 3, 6 and 24h at 37 °C respectively. f showed the spectra of the samples treated with heat denaturation and renaturation before measured. Figure S1a-S1e showed the changing process along with time at 37. The large differences between the spectra of the Aps at different positions reflected the heterogeneity of the TTA looping conformations, and vice versa. We clearly observed the closer process between the different Aps in Fluorescence spectra, implying it is a slow dynamic course from unsymmetrical to symmetrical structures for G-quadruplex.

**Native Gel Electrophoresis.** Native gel electrophoresis was run on 15% polyacrylamide gel containing 40% (w/v) PEG 200 and 150 mM KCl at 4 °C, 8 V/cm in 1×TBE buffer containing 150 mM KCl. The oligonucleotids were all labeled with FAM and imaged under irradiation of UV light. The samples were all treated with heat in diluted $K^+$ solution and then slowly cooled down to 4 °C. PEG was then added into the samples at different situations.

**CD Kinetic Experiments.** Kinetic experiments were carried out with CD to detect the signal at 265 nm along with time. The concentration of all the oligonucleotides adopted in kinetic experiments was 10 μM in the samples including 10 mM Tris-HCl buffer, 150 mM KCl and 40% (w/v) PEG 200. The first-order rate constant $k_1$ was calculated according to the slope of the linear ship between the logarithms of reactant mol fraction and time, while the second-order rate constant $k_2$ was obtained from the slope of the linear relationship between the reciprocals of reactant mol fraction and time. Herein, the mole fraction $\chi$ of the untransformed structure can be calculated according to the Equation 1.

$\chi = 1\text{-}[CD]/([CD]_f\text{-}[CD]_i)$ (Equation 1)

Herein, [CD] is observed CD intensity during the kinetic process; $[CD]_f$ is the peak value at 265 nm after total transformation, and this value can be obtained by heat denaturation and renaturation; $[CD]_i$ is the initial peak value at 265 nm when the PEG was added.

Activation energy parameters were calculated by plotting the rate constants as a function of 1/T. Activation energies were used to calculate the "quasi-thermodynamic" transition state enthalpies ($\Delta H^{\circ\ddagger}$); Gibbs free energies ($\Delta G^{\circ\ddagger}$), and entropies ($\Delta S^{\circ\ddagger}$) for the transitions were also calculated via equations[1]:

$E_A = \Delta H^{\circ\ddagger} + RT$ (Equation 2)

$k = (k_B T/h)K^{\ddagger}K$ (Equation 3)

$\Delta G^{\circ\ddagger} = -RT \ln K^{\ddagger}$ (Equation 4)

$\Delta G^{\circ\ddagger} = \Delta H^{\circ\ddagger} - T\Delta S^{\circ\ddagger}$ (Equation 5)

where $k_B$ is Boltzmann's constant, $h$ is Planck's constant, $K^{\ddagger}$ is the equilibrium constant for formation of the activated complex, and K is the "transmission coefficient", which describes the number of complexes that pass over the activation energy barrier without returning (assumed to be equal to 1) [2].

Thus, the thermodynamic parameters for the single quadruplex unit at 37 °C could be determined: $\Delta H^{\circ\ddagger}$=145.7 kJ/mol, $\Delta G^{\circ\ddagger}$ = 100.0 kJ/mol and $\Delta S^{\circ\ddagger}$ = 147.2 J/(mol•K).

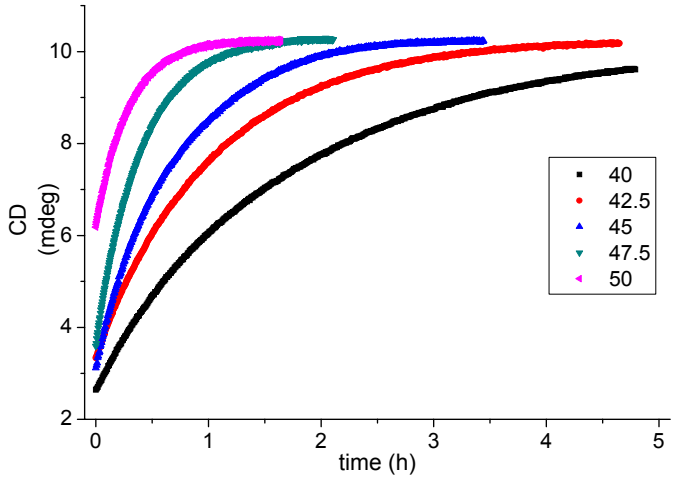



**Figure S2**. Real time recorded CD data of 21G at 265 nm at 40, 42.5, 35, 47.5, and 50 °C respectively.

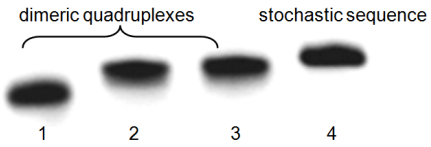



**Figure S3.** 15% native gel electrophoretic analysis of intramolecular folding of the long sequence containing two quadruplex units under molecular crowding condition. The sample of lane 1 was treated with PEG and then immediately loaded onto gel; the sample of lane 2 was loaded after bathing at 37 °C for 6h in the presence of PEG; the sample of lane 3 and 4 was treated with heat denaturation and renaturation under the PEG condition.

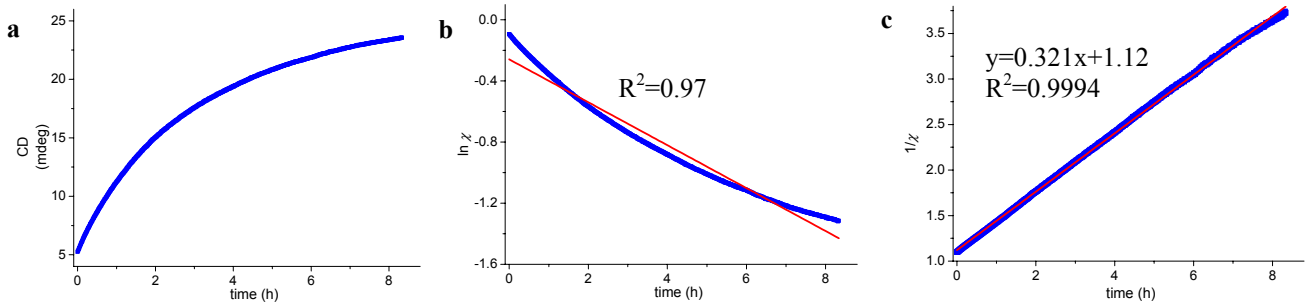
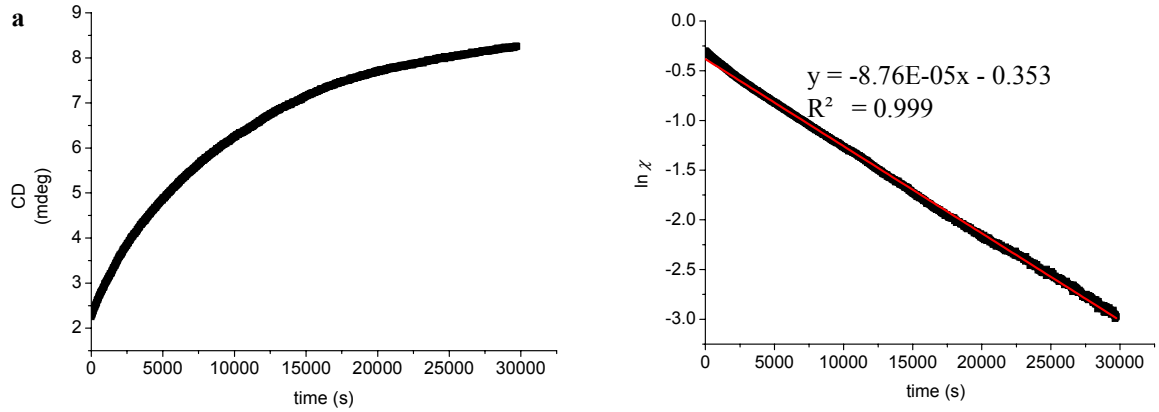



**Figure S4**. Kinetic data of the long sequence containing dimeric quadruplex units. a, real time recorded CD data at 265 nm at 37 °C. b, The logarithms of untransformed structure mol fraction plotted with time at 37 °C. c, The reciprocals of untransformed structure mol fraction plotted with time at 37 °C.



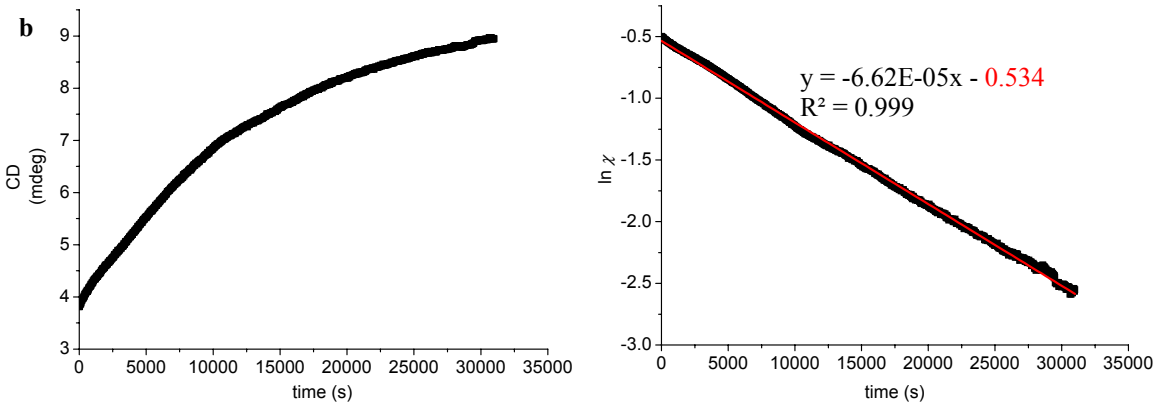



**Figure S5.** Kinetic behaviors of samples prepared according to different procedures had similar phenomina. a, the sample in which the dried-up DNA sample (21G) was added into the buffer in the presence of PEG. The left figure was the real time recorded CD data at 265 nm at 37 °C; the right figure was the logarithms of untransformed structure mol fraction plotted with time at 37 °C. b, the sample in which the DNA sample (21G) in pure water was added into the buffer in the presence of PEG. The left figure was the real time recorded CD data at 265 nm at 37 °C; the right figure was the logarithms of untransformed structure mol fraction plotted with time at 37 °C. From there results, we could observe that the kinetic behaviors of samples prepared according to different procedures were similar ($8.76\times10^{-5}$ $s^{-1}$ and $6.62\times10^{-5}$ $s^{-1}$ VS. $9.56\times10^{-5}$ $s^{-1}$).

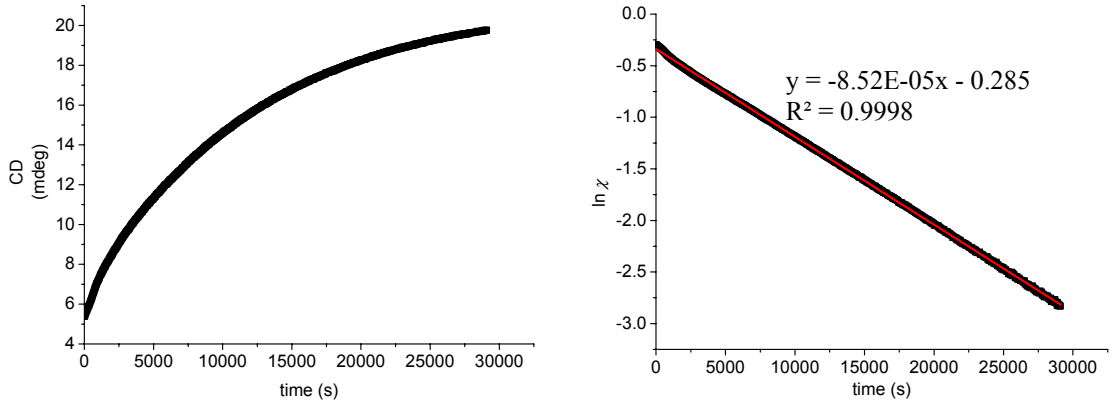



**Figure S6.** DNA concentrations had little influence on the kinetic behaviour. The left figure was the real time recorded CD data at 265 nm at 37 °C; the right figure was the logarithms of untransformed structure mol fraction plotted with time at 37 °C. The concentration of 21G was 20 μM. The rate constant at 37 °C at the DNA concentration of 20 μM was similar as the constant at 10 μM ($8.52\times10^{-5}$ $s^{-1}$ VS. $9.56\times10^{-5}$ $s^{-1}$).

**Ref:**

1 C. C. Hardin, A. G. Perry, and K. White, *Biopolymers* 56, **2001**, 147-194.

2 D. Eisenberg and D. M. Crothers, Physical Chemistry with Application to the Life Sciences. Benjamin-Cummings, Menlo Park, CA, 1979.