Supporting Information

Label-Free Single-Molecule Identification of Telomere G-quadruplexes With a Solid-State Nanopore Sensor

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Materials

Nanochips used in this work (SiN_x membrane grown on both sides of silicon substrate and free-standing on top of the window) were purchased from Nanopore Solution, Portugal, with a membrane thickness of 20 nm and a window size of 20 μm × 20 μm. KCl, NaCl, LiCl, CsCl, NH_4Cl, Tris(hydroxymethyl)aminomethane (Tris) and Ethylenediaminetetraacetic acid (EDTA) were ordered from Aldrich, Shanghai; HPLC purified telomeric DNA sequences were purchased from Sangon, Shanghai. The incubation and translocation buffers were composed of 1

mM EDTA, 10 mM Tris, and 1 M KCl or NaCl or LiCl or CsCl or NH_4Cl with a pH 7.4. All the aforementioned solutions were prepared using HPLC-grade water from a water purification system (Molecular 1850D).

Telomeric sequences used in this work:

DNA1: 5'-$(TTAGGG)_4$ -3'; DNA2: 5'-$(AGGGTT)_4$ -3';

DNA3: 5'-$(TTAGGG)_2$ -3'; DNA4: 5'-$(AGGGTT)_2$ -3';

DNA5: 5'-$(AGGGTT)_8$ -3'; DNA6: 5'-$(AGGGTT)_{12}$ -3'.

Apparatus and data analysis

Circular dichroism spectra (CD) were recorded with a JASCO J-810 spectropolarimeter equipped with a temperature controlled water bath, CD spectra were collected with 10 μM of DNA in folding buffer and scanned from 310 to 210 nm in 1 nm increments with an average time of 2 second, three scans were accumulated and automatically averaged. The physical size of the nanopore was measured via a transmission electron microscope (Tecnai F20, FEI). Electric pulse breakdown was performed using Keithley 2450 that was controlled with a Labview program to measure the conductivity of the SiN_x membrane through the current-voltage (*I-V*) curves. *I-V* characteristics and ion current blockade of the analytes were measured on a patch-clamp amplifier (Axopatch 200B) with a 25 kHz sampling rate and a 5 kHz low-pass Bessel filter, which was housed inside a Faraday cage. Data analysis was performed on Clampfit using lowpass Gaussian filter at 5 KHz, all the event spikes were collected at the level of 10% of open pore current which is around four times larger than the noise; all the dwell time longer than 50 ms and normalized current blockage lower than 0.05 were excluded in the data analysis; all the histograms of normalized current blockage was divided into 30 bins with a gaussian fitting and dwell time with an equal bin size of 0.2 ms fitting with exponential decay except special indication.

Nanopore Fabrication and sensing principal

SiN_x nanochips were rinsed sequentially with ethanol and ultrapure water to get rid of any contaminants on the membrane surface before nanopore generation. The pore size was finely adjusted by precisely controlling the applied voltages across the membrane and the step length, as the time of breakdown is closely related to the quality and lifespan of resulting nanopores. Nanopore sensing of G4 were performed on a SiN_x chip with a nano-scaled pore which sandwiched into flow cells chambers where the buffers were filled and Ag/AgCl electrodes were inserted; the pre-folded telomeric sequences in 1 M K^+ or Na^+ electrolyte was loaded in the *cis* chamber and a positive transmembrane potential was applied to drive the analytes to thread through the orifice during which the produced ion current drop by the physical occupation and the blockage duration represent the size and surface properties of the analytes.

Illustration of the optimized nanopore sensing conditions for the monitoring of G4 formation:

We have started with a physiological concentration of Na+ as folding buffer and tended to monitor the gradual formation of G4 via recording the current traces produced by analytes perforation. Unfortunately, with low electrolyte concentration, debye radius is longer and is comparable with the size of nanopore, the baseline of ion current measured in nanopore is quite unstable and the capture rate for analytes is extremely low arised from the nanopore surface charge modulation. Cs^+ and Li^+ are reported to have weak ability of stabilizing G4 skeletons compared with K^+ and Na^+ due to the size of metal ions. Hence, we utilized mixed cations and kept high concentration of Cs^+ or Li^+ and gradually increased the ratio of K^+ or Na^+ while fix the total concentration of cations to 1 M accordingly. Again the expected phenomenon is not observed in both Cs^+ and Li^+ buffers, as in both cases the distribution of current blockage is extremely wide and is hard to declare if any migration of the relative ratio of current distribution, eventually the assays were performed in 1 M K^+ or Na^+ buffer solution.

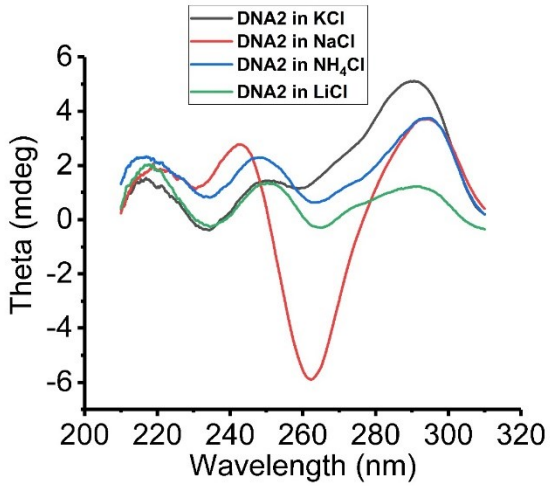

Figure S1. CD spectra of 10 μM DNA2 prepared in 1 M of the four indicated cation buffer solutions composed of 10 mM Tris, 1 mM EDTA with a pH 7.4 at 22 ℃.

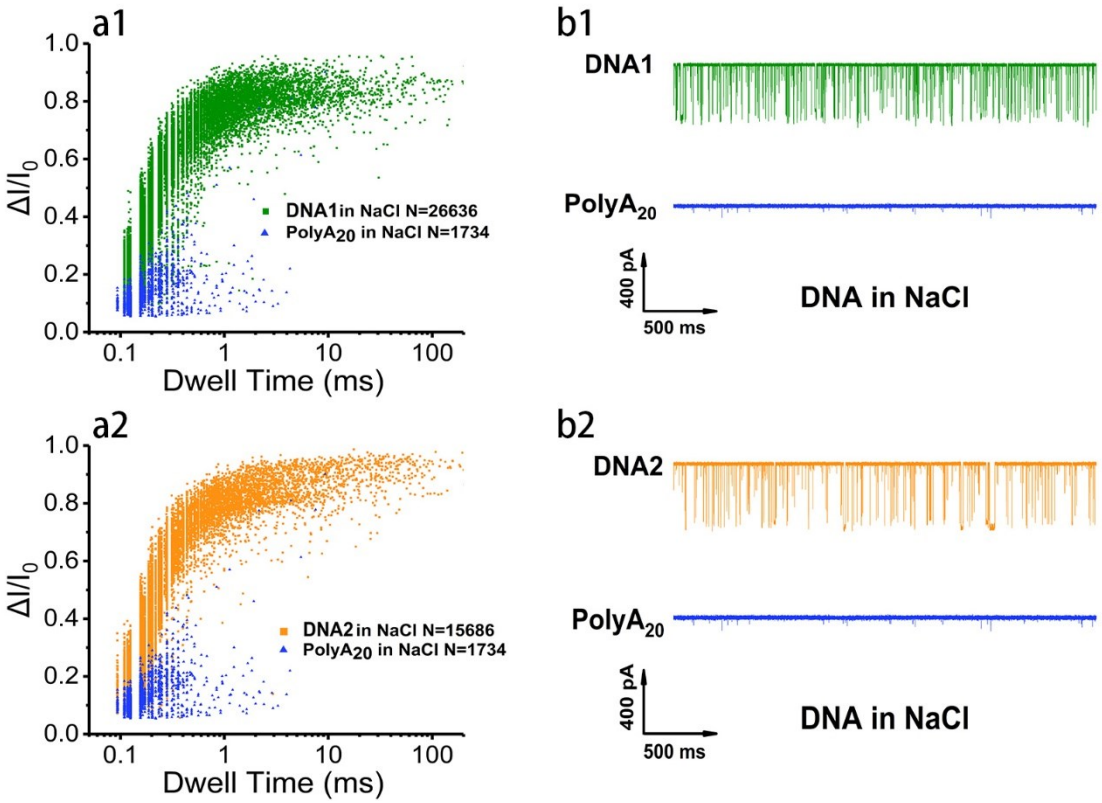

Figure S2. Discriminative analysis of G4 translocation properties. (a1-a2) Scatter plots showing events distribution of G4 folded with 100 nM of DNA1, DNA2 and $PolyA_{20}$ in 1 M NaCl, 10 mM Tris, 1 mM EDTA at pH 7.4 with a ~3.2 nm nanopores under 200 mV external voltage; (b1-b2) Representative translocation current traces of indicated DNA during 2 s.

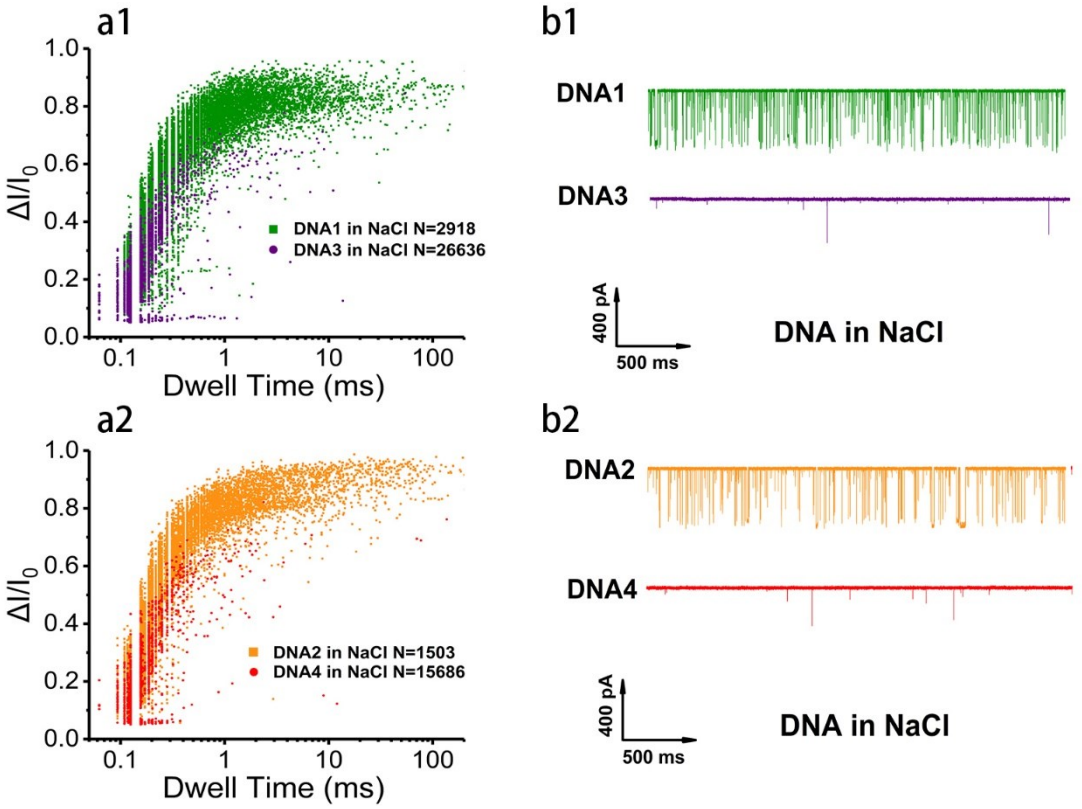

Figure S3. Discriminative analysis of G4 translocation properties. (a1-a2) Scatter plots showing events distribution of G4 folded with 100 nM of DNA1-4 in 1 M NaCl, 10 mM Tris, 1 mM EDTA at pH 7.4 with a ~3.2 nm nanopores under 200 mV external voltage; (b1-b2) Representative translocation current traces of indicated DNA during 2 s.

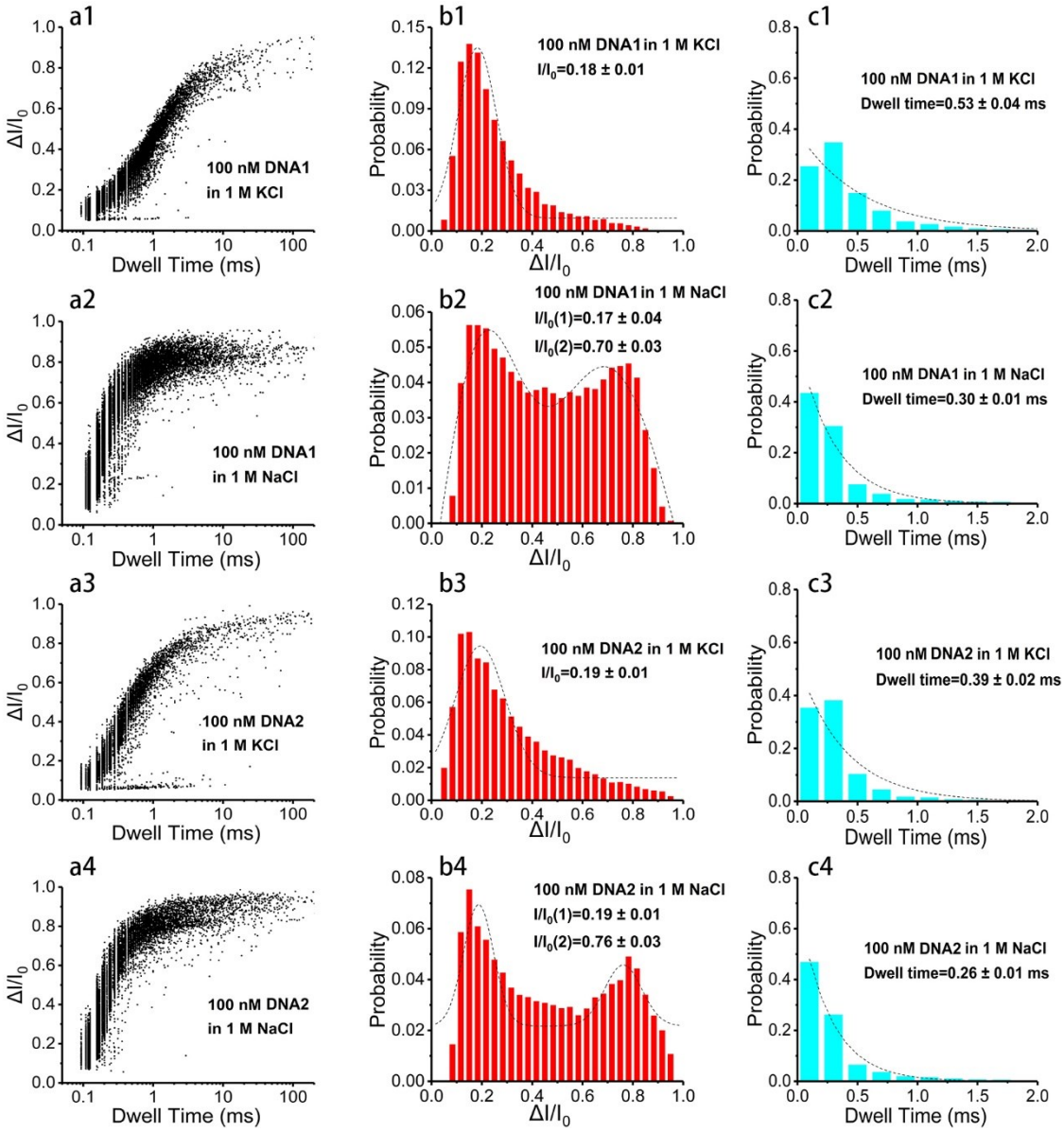

Figure S4. Representation of nanopore translocation properties of the G-quadruplexes formed with 100 nM DNA1 and DNA2, performed in 1 M KCl and 1 M NaCl, 10 mM Tris, 1 mM EDTA at pH 7.4 under 200 mV bias voltage in a ~3.2 nm nanopore. (a1-a4) Scatter plots showing event distribution of DNA1 and DNA2 in the two indicated ion buffers; (b1-b4) Histograms of the normalized current amplitude with Gaussian fitting curves, all the data of $\Delta I/I_0$ were divided into 30 bins from 0.05 to 1; (c1-c4) Histograms of dwell time with exponential decay fitting curves, all the data were treated with a same bin size of 0.2 ms.

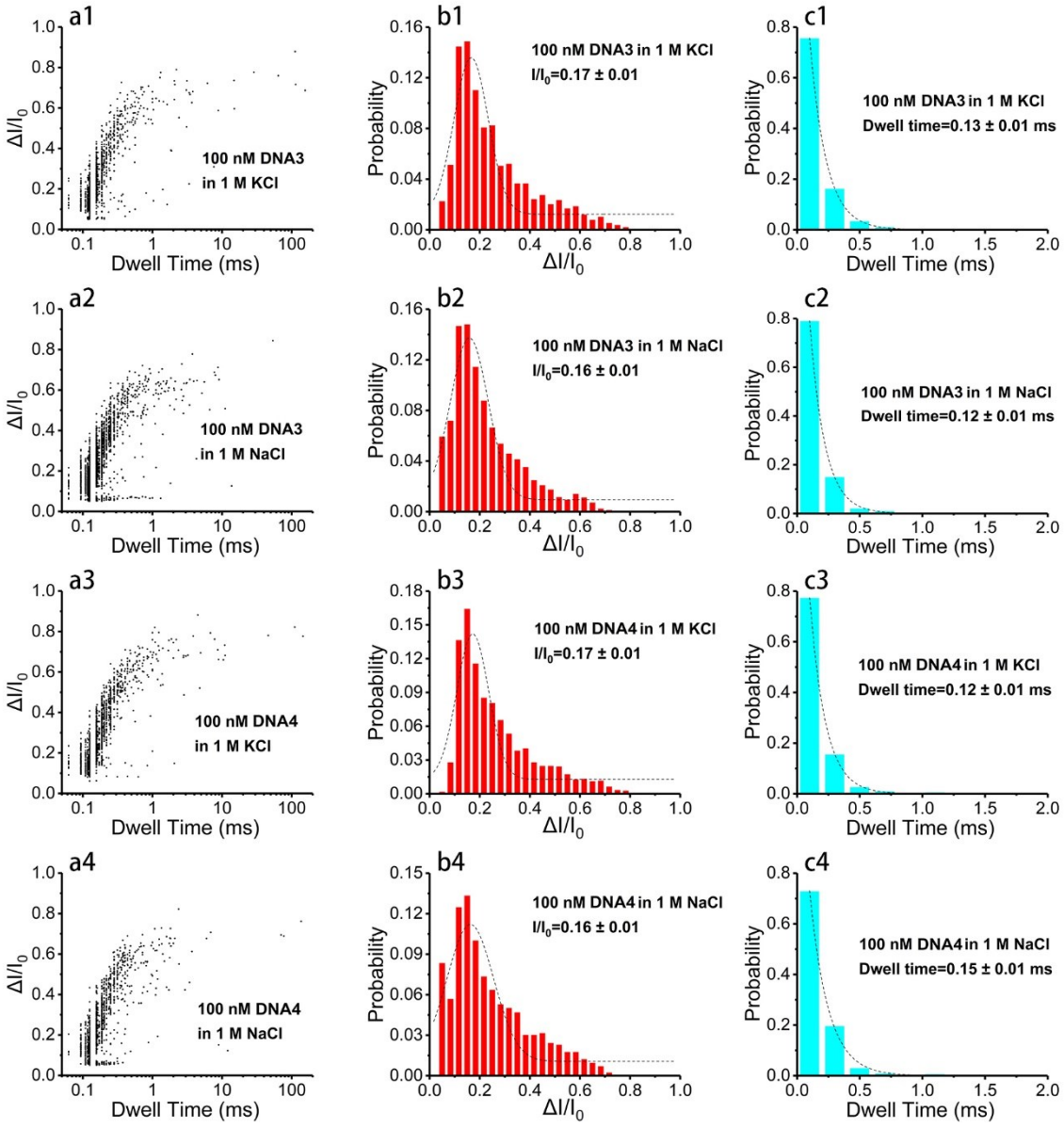

Figure S5. Representation of nanopore translocation properties of the G-quadruplexes formed with 100 nM DNA3 and DNA4, performed in 1 M KCl and 1 M NaCl, 10 mM Tris, 1 mM EDTA at pH 7.4 under 200 mV bias voltage in a ~3.2 nm nanopore. (a1-a4) Scatter plots showing event distribution of DNA3 and DNA4 in the two indicated ion buffers; (b1-b4) Histograms of the normalized current amplitude with Gaussian fitting curves, all the data of $\Delta I/I_0$ were divided into 30 bins from 0.05 to 1; (c1-c4) Histograms of dwell time with exponential decay fitting curves, all the data were treated with a same bin size of 0.2 ms.

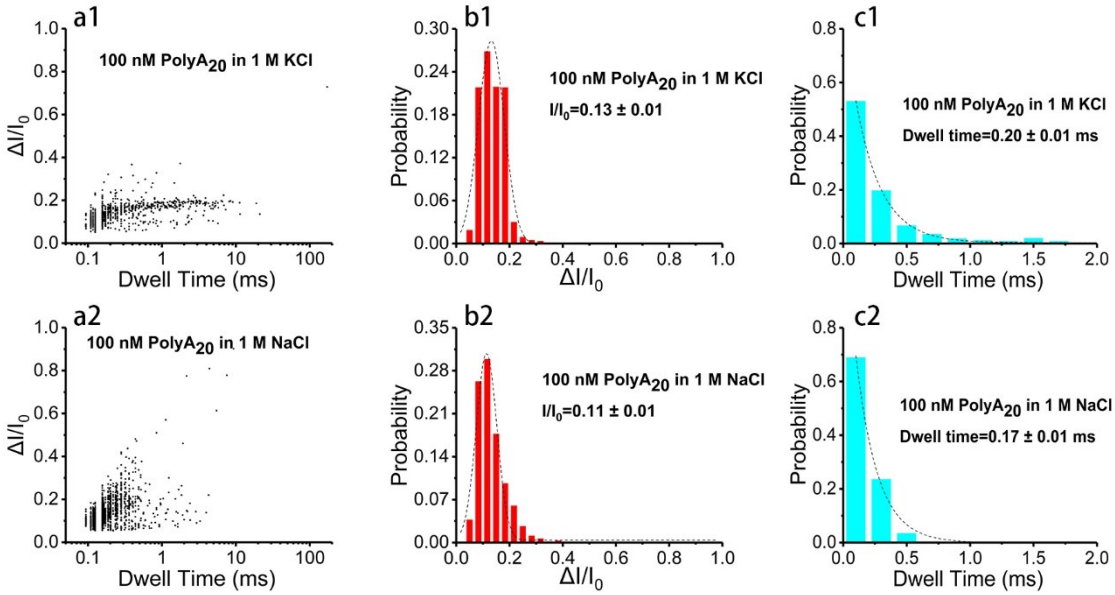

Figure S6. Representation of nanopore translocation properties of the G-quadruplexes folded with 100 nM $PolyA_{20}$, performed in 1 M KCl and 1 M NaCl, 10 mM Tris, 1 mM EDTA at pH 7.4 under 200 mV bias voltage in a ~3.2 nm nanopore. (a1-a2) Scatter plots showing event distribution of $PolyA_{20}$ in the two indicated ion buffers; (b1-b2) Histograms of the normalized current amplitude with Gaussian fitting curves, all the data of $\Delta I/I_0$ were divided into 30 bins from 0.05 to 1; (c1-c2) Histograms of dwell time with exponential decay fitting curves, all the data were treated with a same bin size of 0.2 ms.

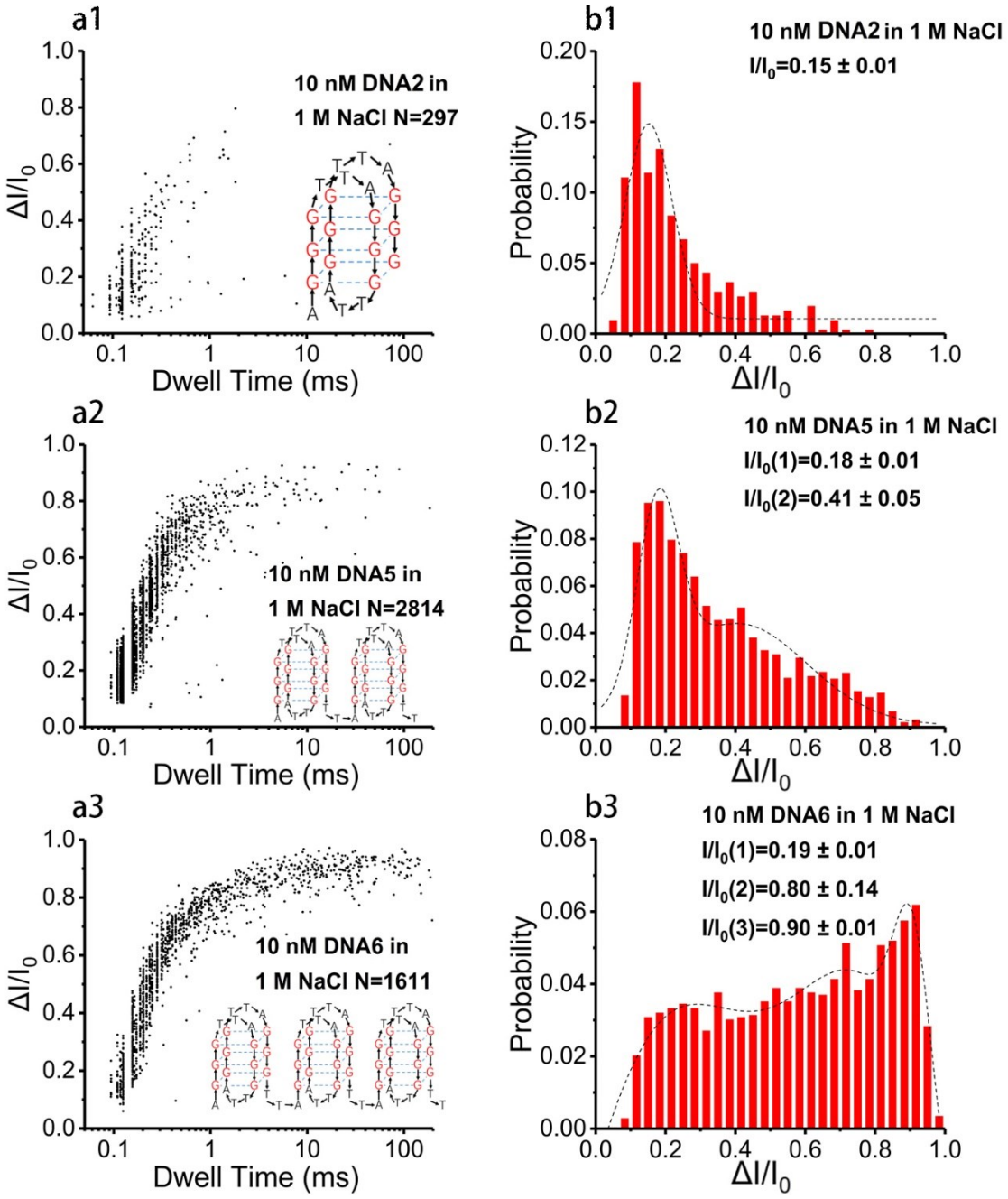

Figure S7. Representation of nanopore translocation properties of the G-quadruplexes formed with 10 nM DNA2, DNA5 and DNA6 performed in 1 M NaCl, 10 mM Tris, 1 mM EDTA at pH 7.4 under 200 mV in a single nanopore of 4.2 nm in diameter. (a1-a3) Scatter plots showing event distribution of DNA2, DNA5 and DNA6 in 1 M NaCl buffers, the insert is the G4 skeleton folded with the corresponding DNA; (b1-b3) Histograms of the normalized current amplitude with Gaussian fitting curves, all the data of $\Delta I/I_0$ were divided into 30 bins from 0.05 to 1.

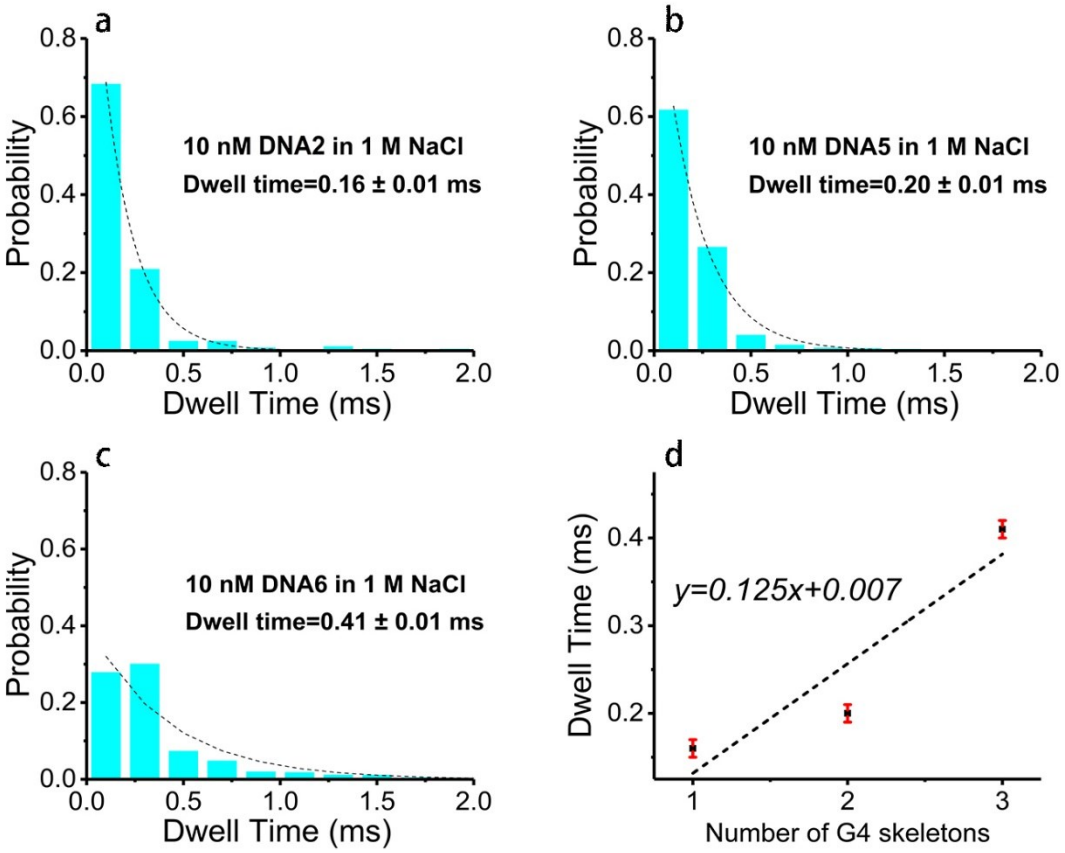

Figure S8. Nanopore translocation duration of the G-quadruplexes recorded with 100 nM DNA2, DNA5 and DNA6, performed in 1 M NaCl, 10 mM Tris, 1 mM EDTA at pH 7.4 under 200 mV bias voltage in a ~4.2 nm nanopore. (a-c) Histograms of dwell time with exponential decay fitting curves, all the data were treated with a same bin size of 0.2 ms; (d) Line fitting graph of dwell time as a function of the number of G4 skeletons.

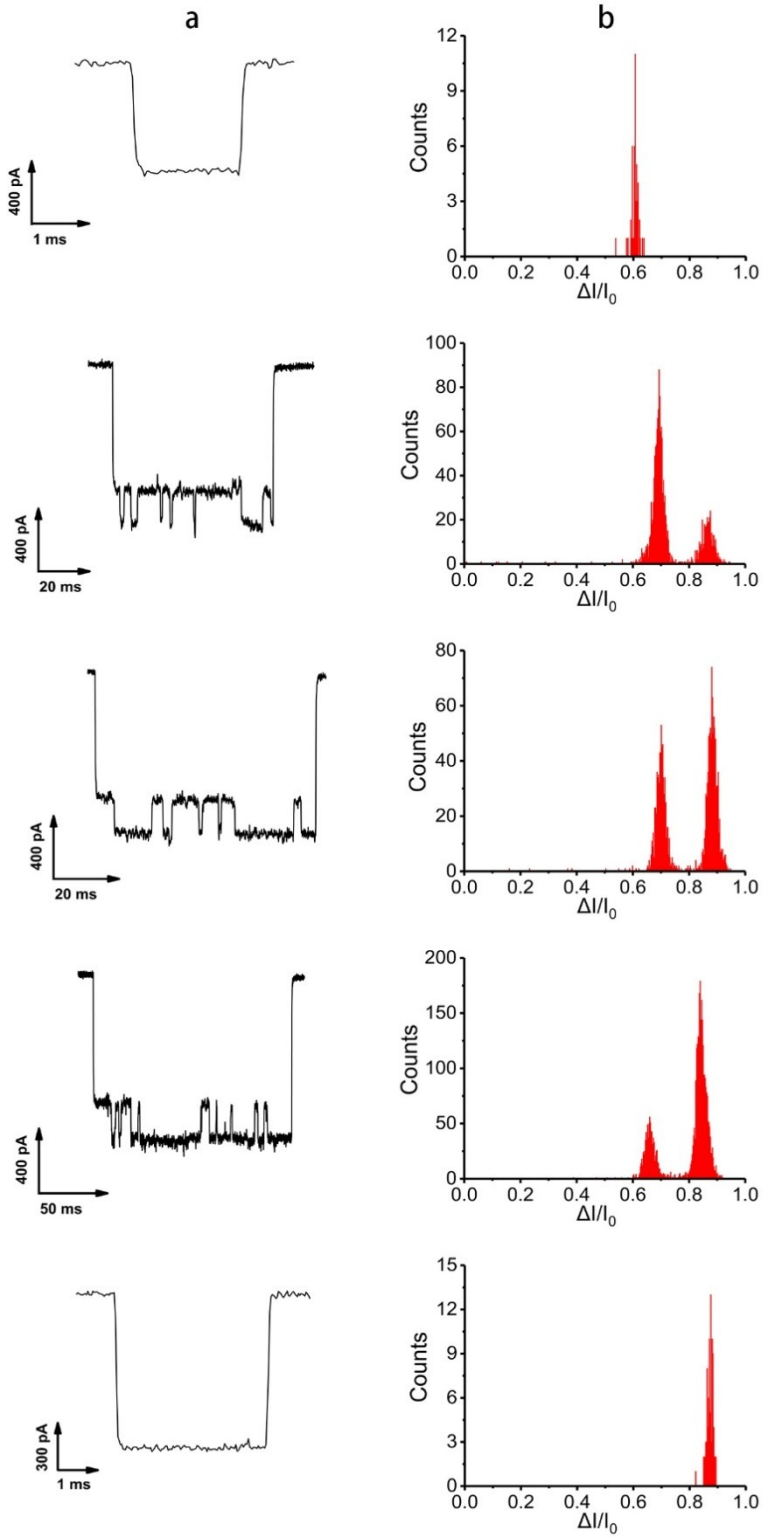

Figure S9. Representative events of G4 folded with 10 nM DNA2 in 1 M KCl, 10 mM Tris, 1 mM EDTA with a pH 7.4 under 200 mV bias voltage, recorded during the kinetic process in a nanopore of 3.5 nm in diameter. (a) Original translocation signals of single-molecule DNA; (b) All points histograms showing the distribution profiles of normalized current blockage $\Delta I/I_0$, signifying the real-time conformation change of single-molecule G4 during translocation process.

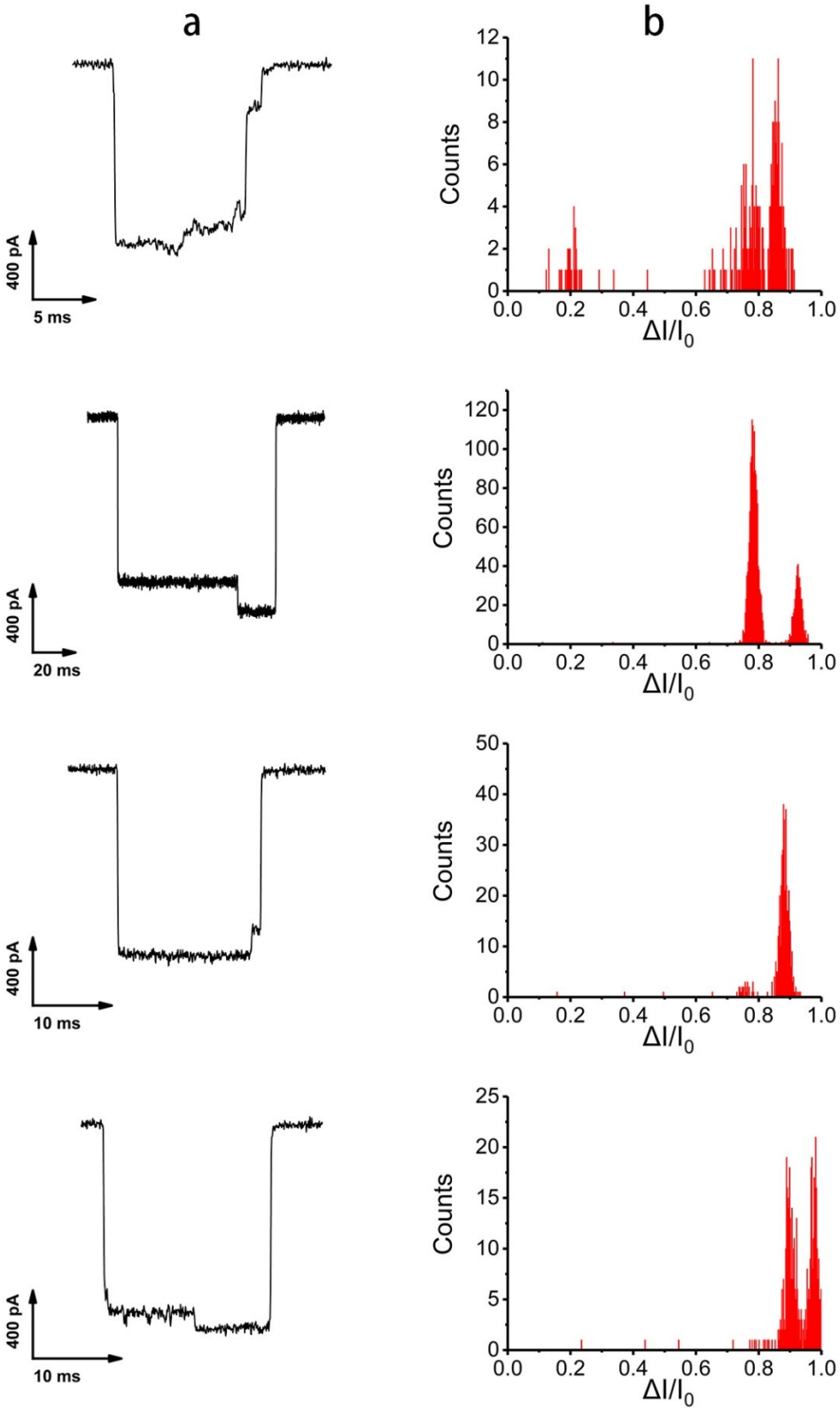

Figure S10. Representative events of G4 folded with 10 nM DNA6 in 1 M KCl, 10 mM Tris, 1 mM EDTA with a pH 7.4 under 200 mV bias voltage, recorded during the kinetic process with a 4.2 nm nanopore. (a) Original translocation signals of single-molecule G4; (b) All points histograms showing the distribution profiles of normalized current blockage $\Delta I/I_0$.

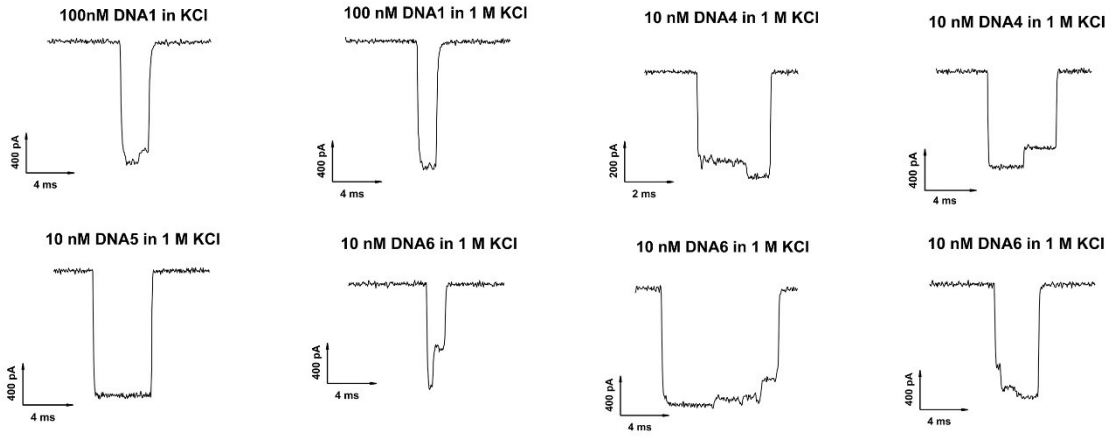

Figure S11. Representative events recorded while monitoring the single-molecule G4 translocation process.

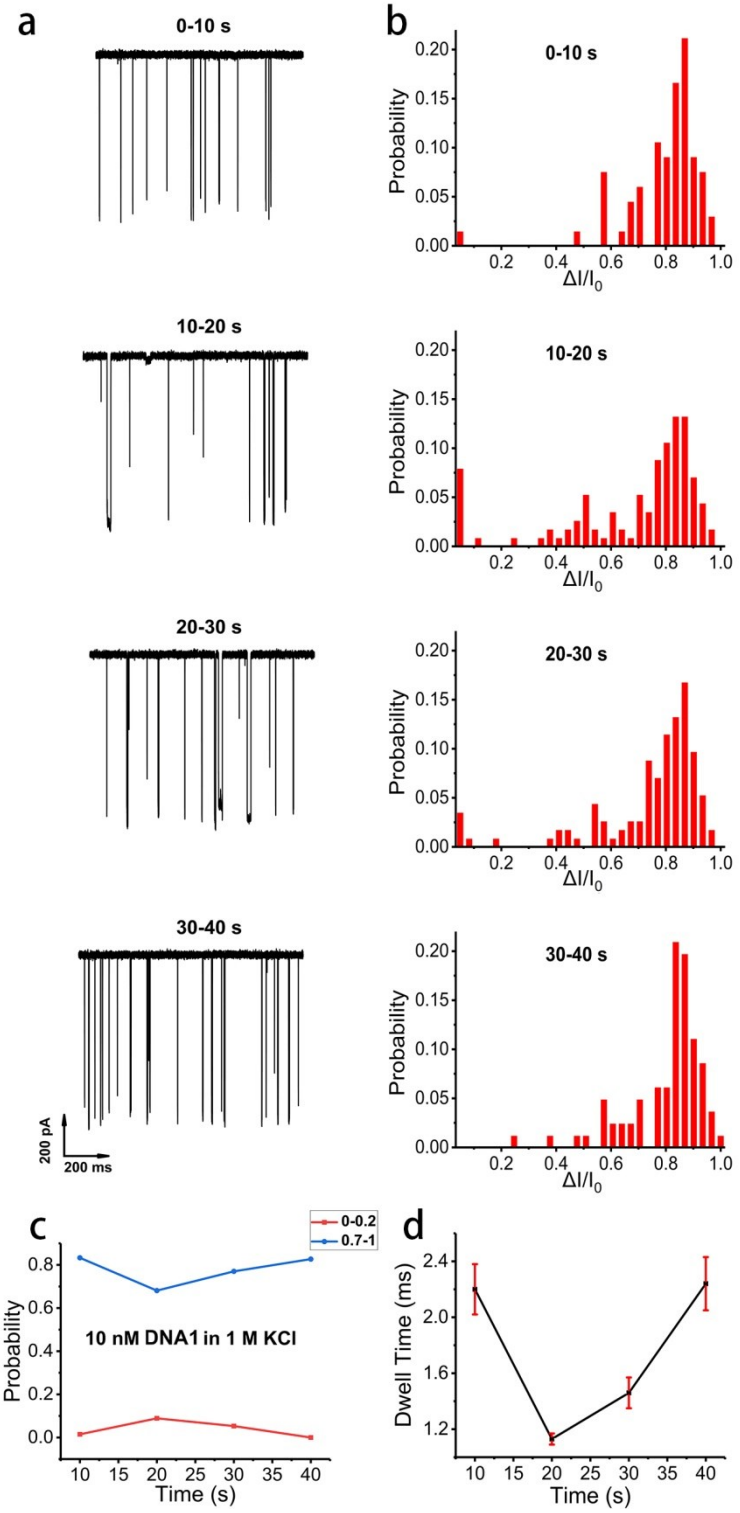

Figure S12. Real-time monitoring the formation of G4 folded with 10 nM of DNA1 in 1 M KCl, 10 mM Tris, 1 mM EDTA with a pH 7.4 under 200 mV bias voltage in a ~3.5 nm nanopore. (a) A part of the total indicated 10 seconds representative current traces of G4 translocation during the first 40 seconds; (b) Histograms of the normalized current amplitude during the indicated time period; (c) Line graphs indicating the ratio of $\Delta I/I_0$ (0.7-1) increase along with time while the ratio of $\Delta I/I_0$ (0-0.2) decrease along with time during the first 40 seconds; (d) Line graphs displaying that nanopore blockage duration fluctuated with time.

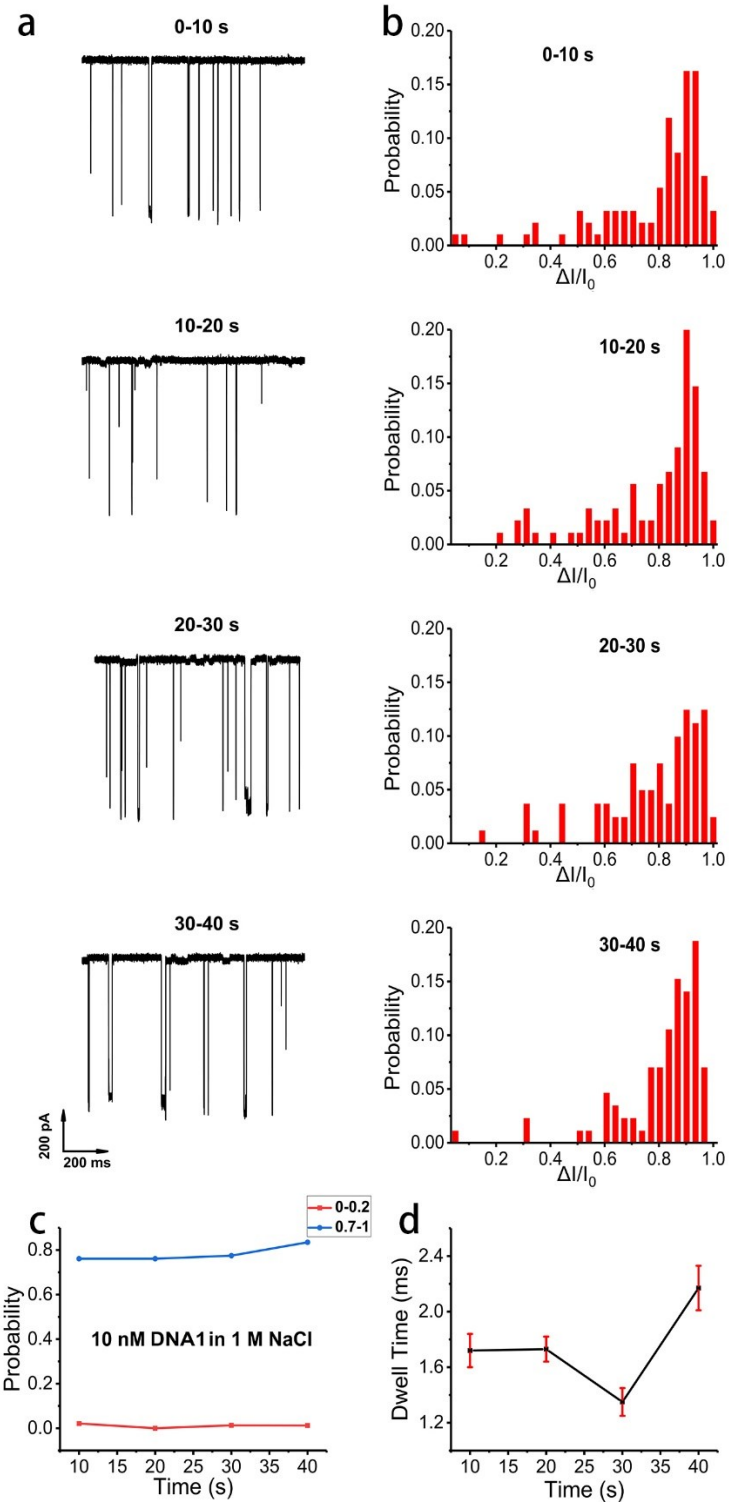

Figure S13. Real-time monitoring the formation of G4 folded with 10 nM of DNA1 in 1 M NaCl, 10 mM Tris, 1 mM EDTA with a pH 7.4 under 200 mV bias voltage in a ~3.5 nm nanopore. (a) A part of the total indicated 10 seconds representative current traces of G4 perforation during the first 40 seconds; (b) Histograms of the normalized current amplitude during the indicated time period; (c) Line graphs indicating the ratio of $\Delta I/I_0$ (0.7-1) increase along with time while the ratio of $\Delta I/I_0$ (0-0.2) decrease along with time during the first 40 seconds; (d) Line graphs displaying that nanopore blockage duration fluctuated with time.

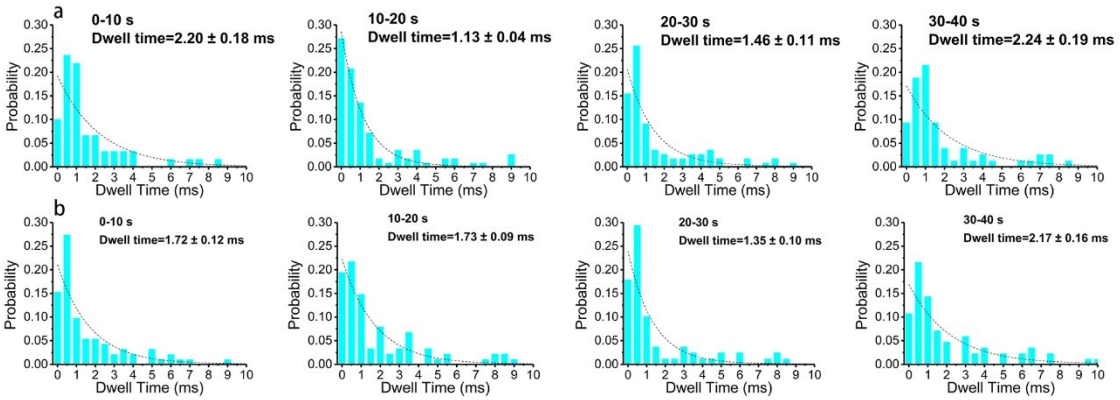

Figure S14. Real-time monitoring the formation of G4 folded with 10 nM of DNA1 in 1 M KCl (a) and 1 M NaCl (b), 10 mM Tris, 1 mM EDTA with a pH 7.4 under 200 mV bias voltage in a ~3.5 nm nanopore. Histograms of dwell time with exponential decay fitting curves, all the data were treated with a same bin size of 0.5 ms.

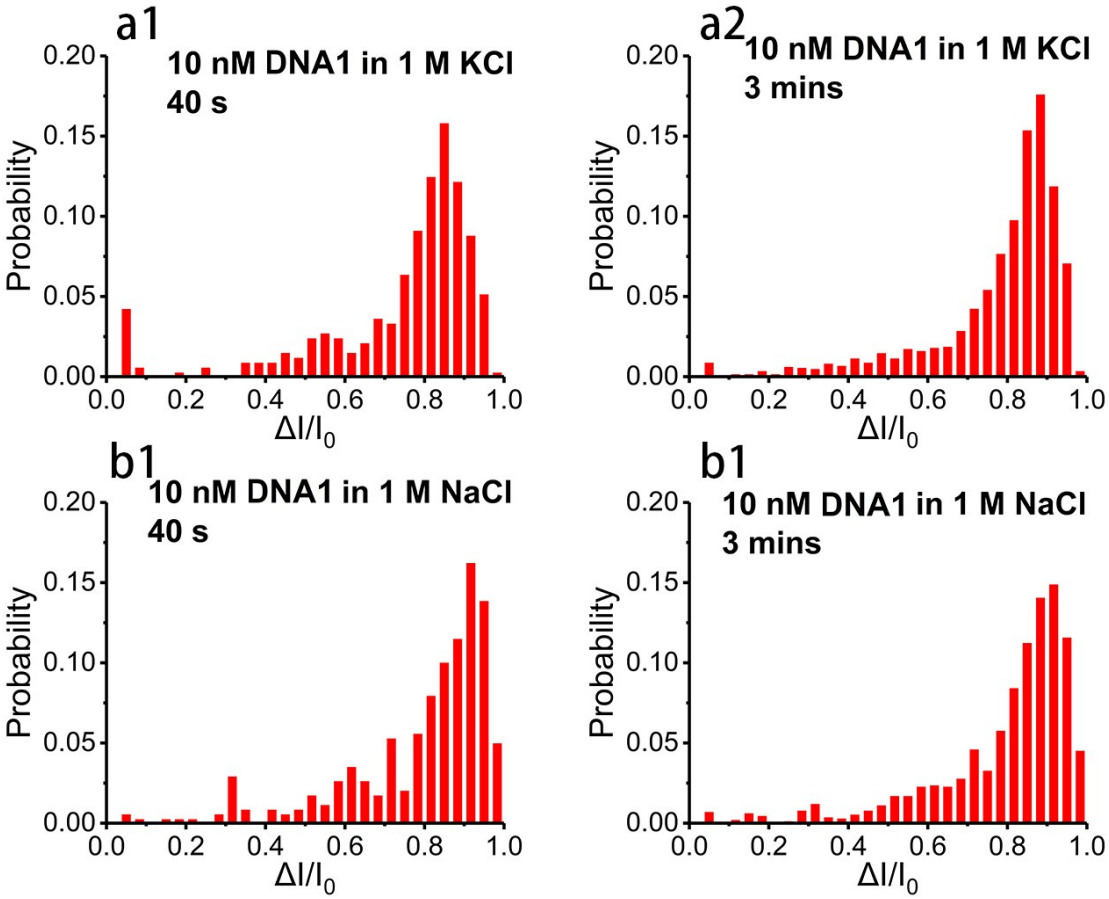

Figure S15. Real-time monitoring the formation of G4 folded with 10 nM of DNA1 in 1 M KCl (a1-a2) and 1 M NaCl (b1-b2), 10 mM Tris, 1 mM EDTA with a pH 7.4 under 200 mV bias voltage in a ~3.5 nm nanopore. Histograms of the normalized current amplitude with Gaussian fitting curves, all the data of $\Delta I/I_0$ were divided into 30 bins from 0.05 to 1. (a1) DNA1 in KCl in the first 40 seconds; (a2) DNA1 in KCl in the first 3 minutes; (c1) DNA1 in NaCl in the first 40 seconds; (b2) DNA1 in NaCl in the first 3 minutes in monitoring the translocation process.

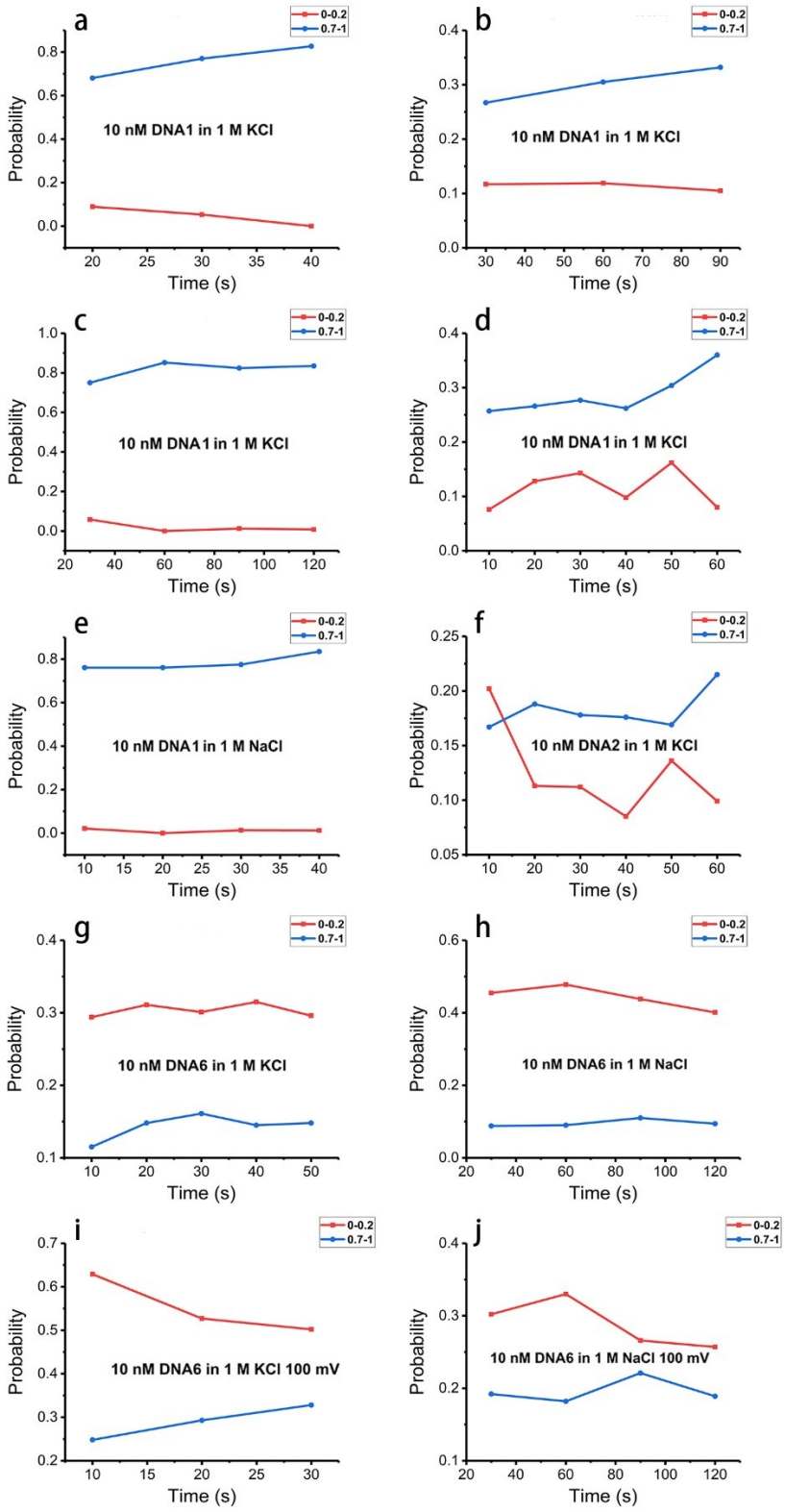

Figure S16. Real-time monitoring the formation of G4 folded with 10 nM DNA in 1 M KCl and 1 M NaCl, 10 mM Tris, 1 mM EDTA with a pH 7.4 under 200 mV (a-h) or 100 mV (i-j) bias voltage with nanopore of 3.5 nm (for DNA1 and DNA2) and 4.2 nm (for DNA6) in diameter. Line graphs showing a ratio increase of normalized current blockage $\Delta I/I_0$ (0.7-1) while a ratio of $\Delta I/I_0$ (0-0.2) decrease along with time during the first few seconds or minutes, recorded with (a-d) DNA1 in KCl; (e) DNA1 in NaCl; (f) DNA2 in KCl; (g) DNA6 in KCl; (h) DNA6 in NaCl; (i) DNA6 in KCl under 100 mV; (j) DNA6 in NaCl under 100 mV.

Table 1. Translocation behaviors of G4 folded with the indicated sequences in this study.

	DNA1		DNA2		DNA3		DNA4		$PolyA_{20}$	
Electrolyte	1 M NaCl	1 M KCl	1 M NaCl	1 M KCl	1 M NaCl	1 M KCl	1 M NaCl	1 M KCl	1 M NaCl	1 M KCl
$\Delta I/I_0$	0.17±0.04 0.70±0.03	0.18±0.01	0.19±0.01 0.76±0.03	0.19±0.01	0.16±0.01	0.17±0.01	0.16±0.01	0.17±0.01	0.11±0.01	0.13±0.01
Dwell Time (ms)	0.30±0.01	0.53±0.04	0.26±0.01	0.39±0.02	0.12±0.01	0.13±0.01	0.15±0.01	0.12±0.01	0.17±0.01	0.20±0.01

Statistical data of normalized current blockages and dwell time recorded with 100 nM of the four telomeric G-quadruplexes and $PolyA_{20}$ in 1 M KCl and 1 M NaCl, 10 mM Tris, 1 mM EDTA at pH 7.4 under 200 mV with nanopores of ~3.2 nm in diameter. All the data of $\Delta I/I_0$ were divided into 30 bins from 0.05 to 1 for Gaussian fitting and all the data of dwell time were treated with a same bin size of 0.2 ms with fitting mode of exponential decay. The setups were repeated for three times and the data were not all shown but could be provided on requests.

Table 2. Translocation behaviors of G4 folded with the indicated sequences in this study.

	DNA2		DNA5		DNA6	
Electrolyte	1 M NaCl	1 M KCl	1 M NaCl	1 M KCl	1 M NaCl	1 M KCl
$\Delta I/I_0$	0.15±0.01	0.18±0.01	0.18±0.01 0.41±0.05	0.25±0.02 0.97±0.11	0.19±0.01 0.80±0.14 0.90±0.01	0.52±0.05 0.74±0.01 0.89±0.01
Dwell Time (ms)	0.16±0.01	0.16±0.01	0.20±0.01	0.28±0.01	0.41±0.01	0.54±0.02

Statistic data of normalized current blockages, dwell time recorded with 10 nM of the three telomeric G-quadruplexes in 1 M KCl or 1 M NaCl, 10 mM Tris, 1 mM EDTA at pH 7.4 under 200 mV with nanopores of 4.2 nm in diameter. All the data of $\Delta I/I_0$ were divided into 30 bins from 0.05 to 1 for Gaussian fitting and all the data of dwell time were treated with a same bin size of 0.2 ms with fitting mode of exponential decay. The setups were repeated for three times and the data were not all shown but could be provided on requests.