Supporting Information for

Folding Kinetics of Single Human Telomeric G-quadruplex Affected by Cisplatin

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1. Details of DNA Constructions

Table S1. DNA oligonucleotides used for central DNA complex in magnetic tweezers experiments

Name	ssDNA sequence
Upper dsDNA handle primer 1	5'-Biotin-CGGGAGGGCTTACCAT-3'
Upper dsDNA handle primer 2	5'-TGCTCTAGATTGCTCACCCAGAAACG-3'
Lower dsDNA handle primer 1	5'-CGGGGTACCTGGCTGGTTTATTGCTGAT-3'
Lower dsDNA handle primer 2	5'-Digoxigenin -GCCGCCCTATACCTTGT-3'

The above three oligonucleotides were annealed in 1:1:1 ratio to a central dsDNA-ssDNA-dsDNA complex structure with two sticky ends (heated to 95 °C for 10 minutes and cooled slowly to room temperature).

Table S2. DNA oligonucleotides used for dsDNA handles PCR in magnetic tweezers experiments

Name	ssDNA sequence
G-quadruplex ssDNA sequence	5'-phosphate-CTAGCGGTGTGAAATACCGACACA-GGGTTAGGGTTAGGGTTA GGGTTAACAGCCA-GCAAGACGTAGCCAGCT-3'
Left complementary flanking ssDNA segments	5'-TGTGTCGGTATTTCACACCG-3'
Right complementary flanking ssDNA segments	5'-phosphate-GGCTACGTCTTGCTGGCTGT-3'

The dsDNA handles were prepared by PCR amplification with pBR322 templets following a standard NEB protocol. The primers were designed using Primer5.0. The PCR products were purified by QIAquick PCR Purification Kit (QIAGEN), and then digested by restriction endonucleases XbaI and KpnI respectively to obtain sticky ends which were just complementary to these of the above central DNA complex. The digested products were further purified by QIAquick PCR Purification Kit. Finally, the two dsDNA handles and the central DNA complex were ligated by ligase T4 following standard NEB protocol.

Name	ssDNA sequence

G-quadruplex ssDNA sequence	5'- -GACACAGGGTTAGGGTTAGGGTTAGGGTTAACAGCC -3'
Left complementary flanking ssDNA segments	5'-TGTGTC-3'
Right complementary flanking ssDNA segments	5'-GGCTAC-3'

Table S3. DNA oligonucleotides used for the G-quadruplex sequence with 6 bp dsDNA handles in CD measurements

The above three oligonucleotides were annealed in 1:1:1 ratio to the G-quadruplex sequence with 6 bp dsDNA handle at each side (heated to 95 °C for 10 minutes and cooled slowly to room temperature).

2. Force Calibration

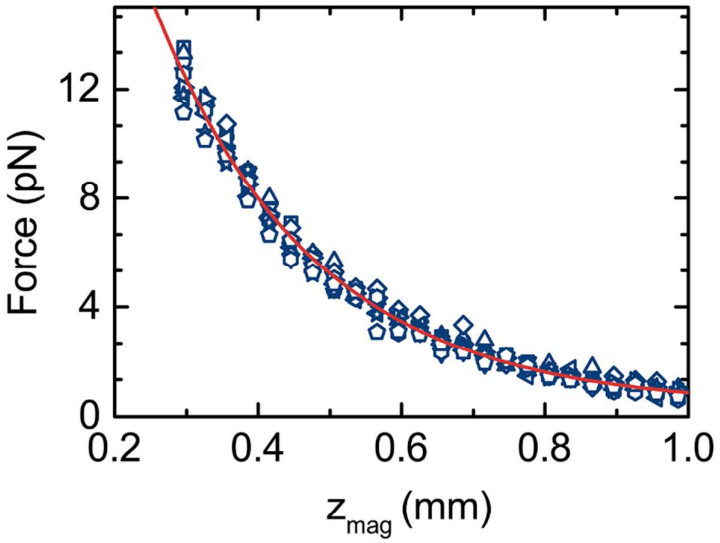

Figure S1. Force versus z_{mag} data curve obtained with 10 individual λ-DNA molecules. Each data point was calculated by a horizontal bead trajectory of 4096 frames. The red line is a single-exponential fitting.

3. Unfolding Event Detection

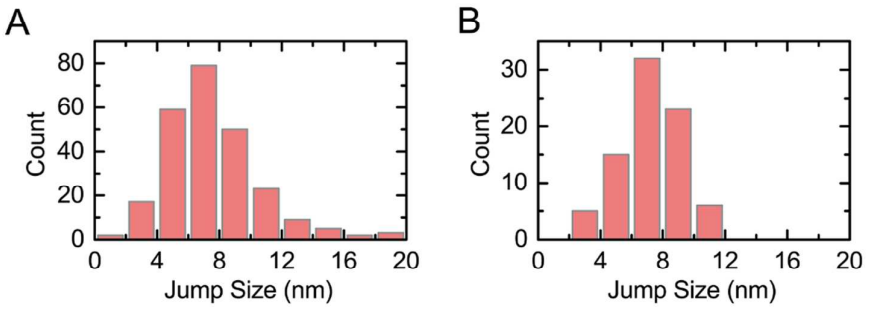

Figure S2. The jump size histograms for 0 μM (A) and 10 μM (B) cisplatin in force-jump experiments with an unfolding force of 13 pN. Each G-quadruplex unfolding event resulted in a discontinuous jump in the extension trajectory curve. A classic χ-square arithmetic was employed to detect the discontinuous position.[1,2] The extension trajectories were smoothed by a window of 20 frame and the analyzed by a MATLAB program. The size of jumps was also calculated as a criterion to reject abnormal jumps. Both the histograms show a peak around 7 nm. Once a G-quadruplex is unfolded to an ssDNA segment, the extension under force can be estimated as

$$x_{ss} = nh\left(\frac{a_1 \ln f/f_2}{1+a_3 e^{-f/f_2}} - a_2 - f/f_3\right) \quad (1)$$

where the force f is in pN, n is the number of nucleotide, h=0.34 nm, a_1 = 0.21, a_2 = 0.34, f_1 = -0.0037 pN, f_2 = 2.9 pN, f_3 = 8000 pN, a_3 = 2.1ln(M/0.0025) / ln(0.15/0.0025) - 0.1 and M is the concentration of monovalent cation in mol/L.[3] In 100 K^+ buffer a G-quadruplex sequence is a 21 nt ssDNA segment, whose extension is estimated to be 9.45 nm under 13 pN. In consideration of the size of G-quadruplex ~1.5 nm, the measured jump sizes agreed well with the theoretical predications. In our experiments, unfolding events with jump sizes larger than 16 nm were rejected as abnormal events.

4. Two-state Model Fitting

For the two-state model, $\mathrm{ssDNA} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{G4}$, we have

$$\begin{aligned} P_{\mathrm{ssDNA}} &= -k_1 \frac{\mathrm{d}P_{\mathrm{ssDNA}}}{\mathrm{d}t} + k_2 \frac{\mathrm{d}P_{\mathrm{G4}}}{\mathrm{d}t} \\ P_{\mathrm{G4}} &= k_1 \frac{\mathrm{d}P_{\mathrm{ssDNA}}}{\mathrm{d}t} - k_2 \frac{\mathrm{d}P_{\mathrm{G4}}}{\mathrm{d}t} \end{aligned} \tag{2}$$

Considering the initial conditions, the refolding probability has an obvious single exponential analytical expression

$$P_{\mathrm{G4}} = -\frac{k_1}{k_1 + k_2} \mathrm{e}^{-(k_1 + k_2)t} + \frac{k_1}{k_1 + k_2} \tag{3}$$

OriginPro 2015 was used to fit the refolding probability with the above equation.

5. Sequential Transition Model Fitting

The two states of free G-quadruplex and cisplatin-bound G-quadruplex could be regarded as a single equivalent state due to their degeneracy in force-jump experiments. The refolding probability observed was a sum of two states. The ratio of the two states may be obtained by a fitting of the corresponding histogram of unfolding force from force-ramp experiments, using $p = ap_1(f)+(1-a)p_2(f)$, where $p_1(f)$ and $p_2(f)$ correspond to the distributions of cisplatin-bound and cisplatin-free states, respectively..

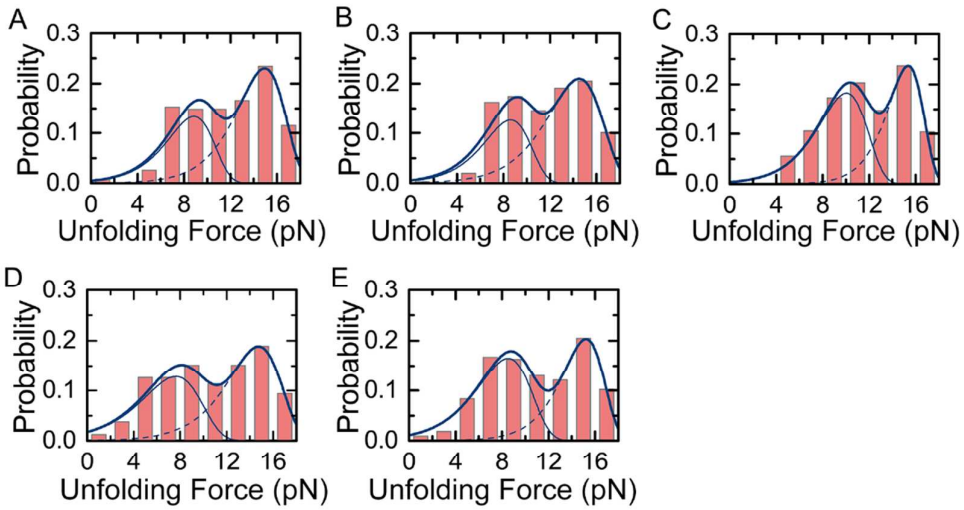

Figure S3. Histograms of unfolding fore for holding times of 10 s (A), 20 s (B), 30 s (C), 60 s (D) and 120 s (E). N = 235, 357, 434, 159 and 215, respectively. The lines represent the theoretical fits using $p = ap_1(f)+(1-a)p_2(f)$.

The histograms at 10 μM cisplatin and under holding force 2 pN for different holding times are shown as Figure S3. From fittings of these histograms, the ratios of cisplatin-bound state were obtained as a = 0.65, 0.66, 0.49, 0.58 and 0.61 for holding times of 10, 20, 30, 60 and 120 s, respectively. As the ratio a remains almost constant from 10 to 120 s, it may be induced that the process of cisplatin binding to refolded G-quadruplex was fast (i.e., taking less than 10 s) compared with the G-quadruplex refolding process. In addition, the refolding probability of cisplatin-bound state should depend linearly on that of cisplatin-free state as

$$P_{\text{G4-Cisplatin}} = AP_{\text{G4}} \tag{4}$$

Where $A = a/(1-a)$. Thus the sequential transition model, $\text{ssDNA} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \text{G4} \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} \text{G4-Cisplatin-Complex}$, was simplified to a two-state model and the master equation can be written as

$$
\begin{aligned}
P_{\mathrm{ssDNA}} &= -k_{12}\frac{\mathrm{d}P_{\mathrm{ssDNA}}}{\mathrm{d}t} + k_{21}\frac{\mathrm{d}P_{\mathrm{G4}}}{\mathrm{d}t} \\
(1+A)P_{\mathrm{G4}} &= k_{12}\frac{\mathrm{d}P_{\mathrm{ssDNA}}}{\mathrm{d}t} - k_{21}\frac{\mathrm{d}P_{\mathrm{G4}}}{\mathrm{d}t}
\end{aligned}
\tag{5}
$$

The total refolding probability of two states, which was obtained in the experiments, can be written as

$$
(1+A)P_{\mathrm{G4}} = -\frac{k_{12}(1+A)}{k_{12}(1+A)+k_{21}}\mathrm{e}^{-\left(\frac{k_{12}(1+A)+k_{21}}{1+A}\right)t} + \frac{k_{12}(1+A)}{k_{12}(1+A)+k_{21}} \tag{6}
$$

which was used to fit the refolding probability at different holding times to obtain the two rate constants k_{12} and k_{21}. According to the result in Figure S3, we used a = 0.6 in the fittings. For the two rates k_{23} and k_{32}, we could only obtain $k_{23}/k_{32} = a/(1\text{-}a)$ and the determination of their concrete values need experiments with higher temporal resolution

References

(1) Kerssemakers, J. W. J.; Munteanu, E. L.; Laan, L.; Noetzel, T. L.; Janson, M. E.; Dogterom, M. *Nature* **2006,** 442, 709-712.

(2) Aggarwal, T.; Materassi, D.; Davison, R.; Hays, T.; Salapaka, *M. Cell. Mol. Bioeng*. **2012**, 5, 14-31.

(3) Cocco, S.; Yan, J.; Leger, J. F.; Chatenay, D.; Marko, J. F. *Phys. Rev. E* **2004**, 70, 011910.