Supporting Information to

Single-Molecule Investigation of G-Quadruplex Folds of the Human Telomere Sequence in a Protein Nanocavity

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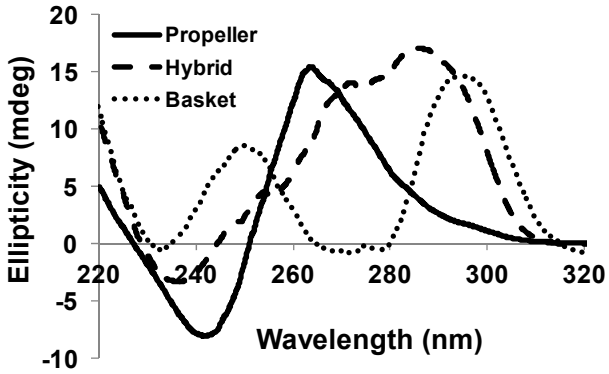
Fig. S1. Characterization of the G-quadruplexes by CD spectroscopy. Propeller (solid, 20 mM KCl and 5 M LiCl), hybrid (dashed, 50 mM KCl and 1 M LiCl), and basket folds (dotted, 1 M NaCl).

Human telomere sequence: 5'-TAGGG(TTAGGG)$_3$TT

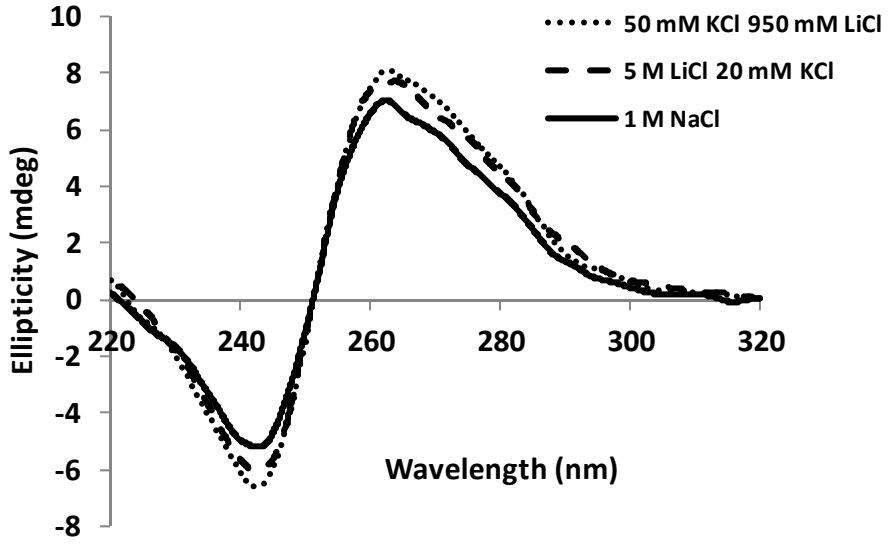


Strikingly, when the LiCl concentration was increased to 5 M with 20 mM KCl, the CD spectra dramatically changed, which is consistent with those reported for the propeller fold.(1, 2) We conclude that the 5 M LiCl solution shifts the folding topology toward the propeller fold, and away from the hybrid fold, due to the decreased water activity of the electrolyte solution (5 M LiCl, $a_w \sim 0.75$),(3) as previously described by Miller et al. with solutions containing 40% CH_3CN ($a_w \sim 0.81$).(3, 4)

Fig. S2. CD spectra for the control ODN and G-quadruplexes with tails.

Hybrid (50 mM/1 M LiCl), Basket (1 M NaCl) and Propeller (20 mM in 5 M LiCl).

Control ODN 5'-TGAGTGTGAGTGTGAGTGTGAGTGT



G-Quadruplexes with a tail 5'-A_{25}-TAGGG(TTAGGG)$_3$TT

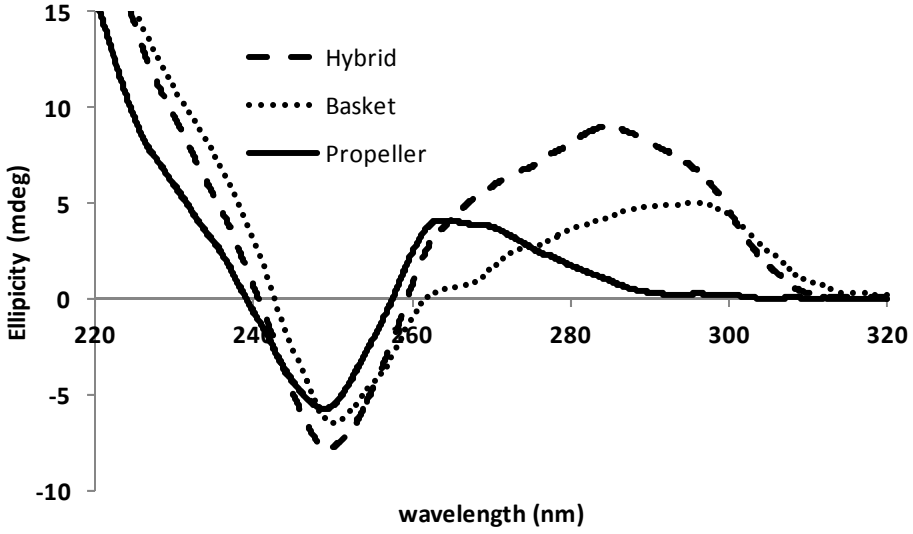
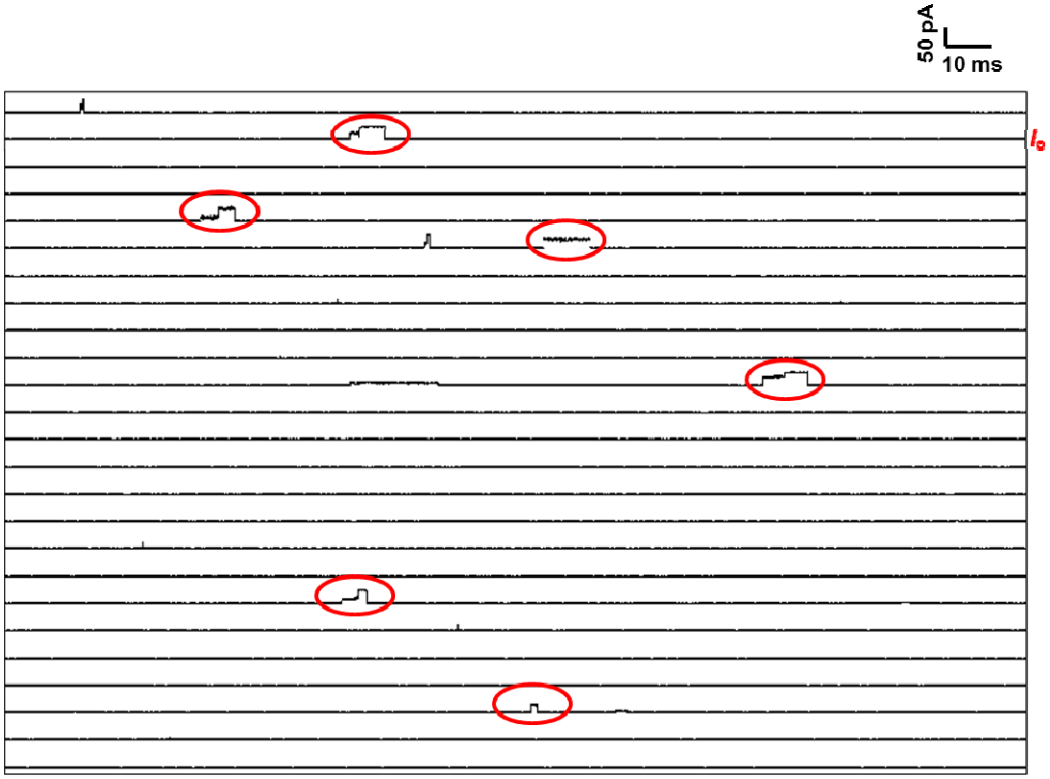
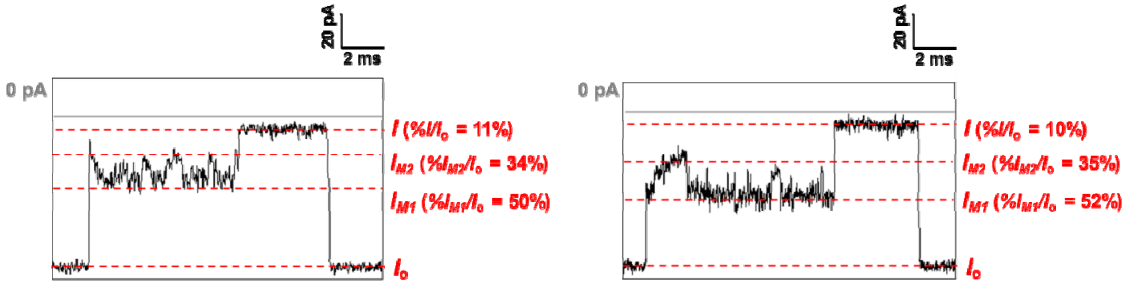


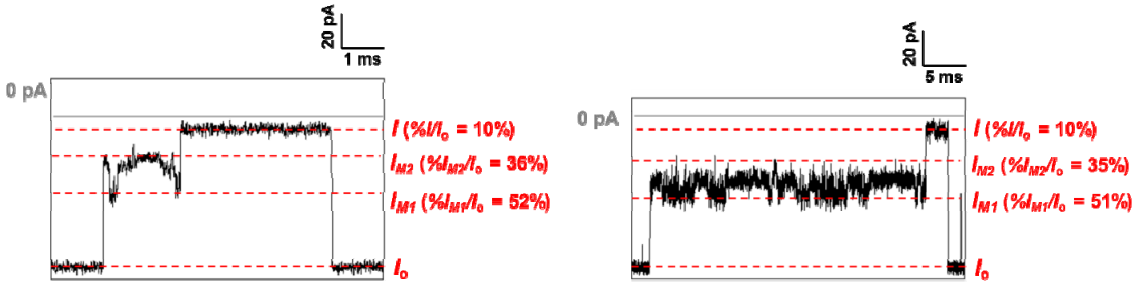
Fig. S3 Sample *i-t* traces representing translocation events of the basket fold (no tail) in 1 M NaCl under 120 mV.



The red circles indicate events that were interpreted as the interaction between the basket fold and the protein channel. See below for enlarged *i-t* traces.

Type 1:




20 pA
1 ms
0 pA
I (%I/I$_o$ = 10%)
I$_{M2}$ (%I$_{M2}$/I$_o$ = 36%)
I$_{M1}$ (%I$_{M1}$/I$_o$ = 52%)
I$_o$
20 pA
5 ms
0 pA
I (%I/I$_o$ = 10%)
I$_{M2}$ (%I$_{M2}$/I$_o$ = 35%)
I$_{M1}$ (%I$_{M1}$/I$_o$ = 51%)
I$_o$

Type 2

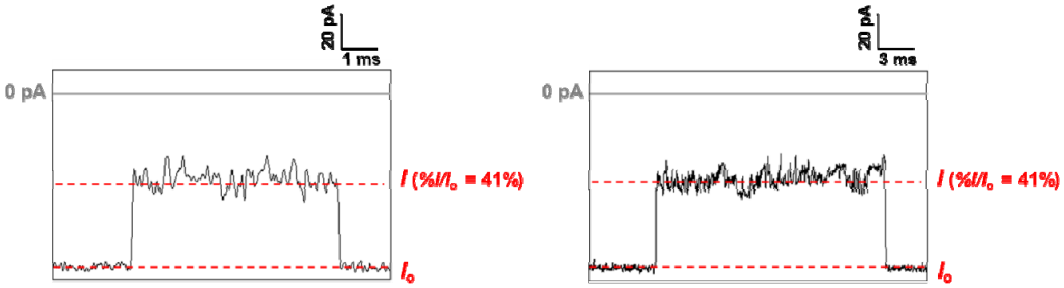

20 pA
1 ms
0 pA
I (%I/I$_o$ = 41%)
I$_o$
20 pA
3 ms
0 pA
I (%I/I$_o$ = 41%)
I$_o$

Fig. S4. Sample *i-t* trace representing interactions between the propeller fold (no tail) and the α-HL ion channel in 20 mM KCl and 5 M LiCl under 120 mV.

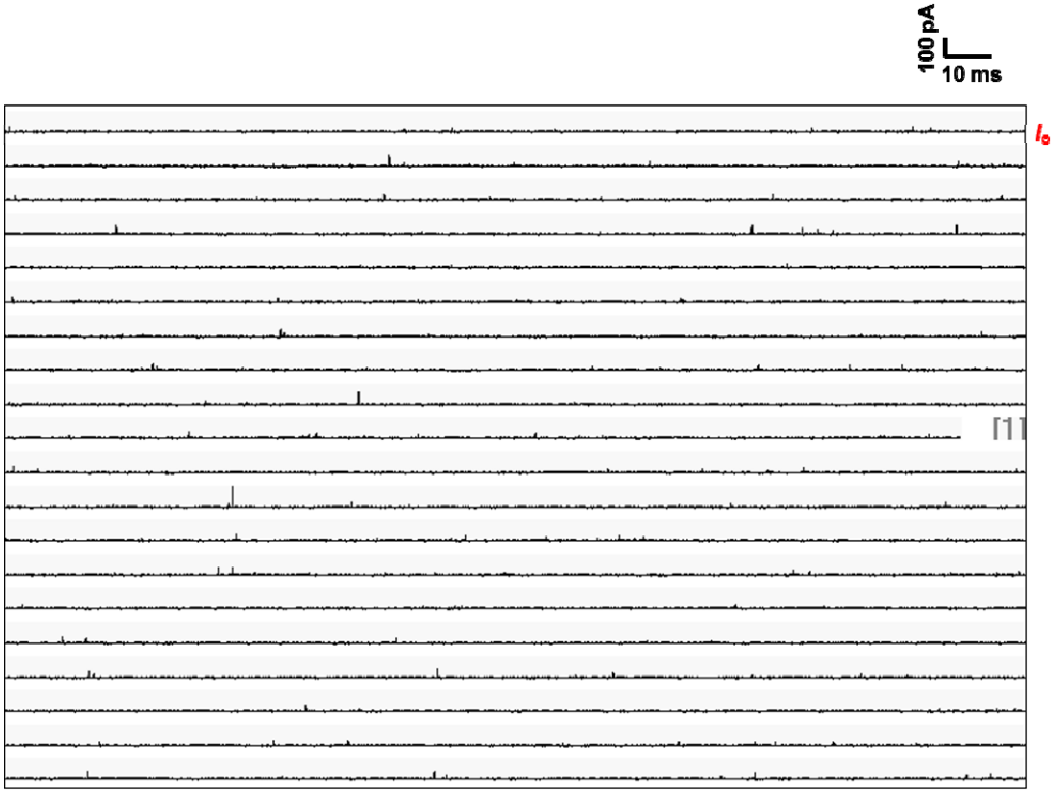
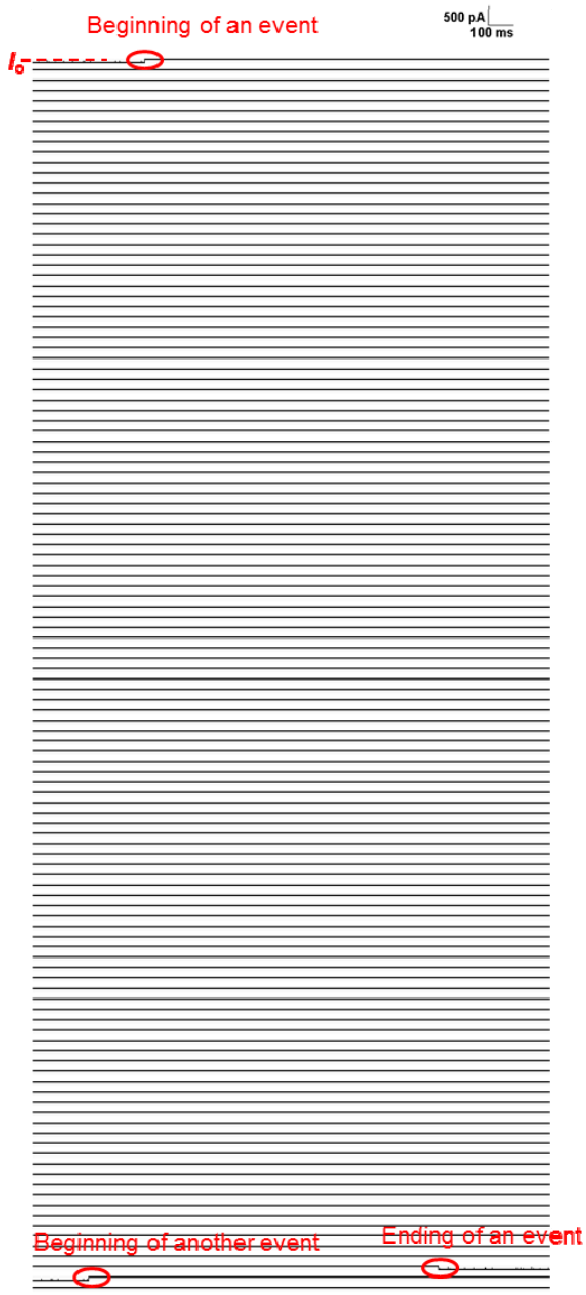


Fig. S5. Sample *i-t* traces representing translocation events of 5'-A_{25}-hybrid DNA (50 mM KCl and 950 mM LiCl) under 120 mV.

Type 1 (~ 4 min)



Type 2 (~ 25 s)

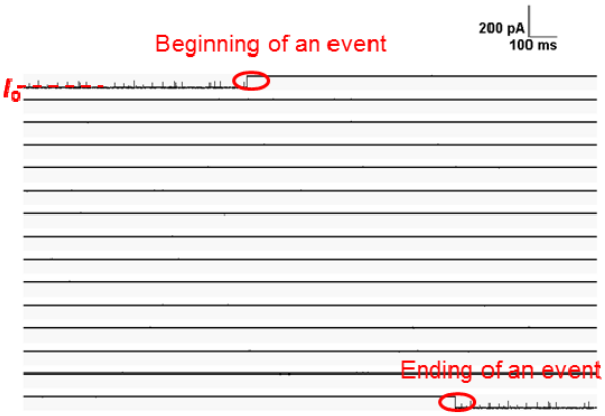


Fig. S6. Sample *i-t* traces representing translocation events of 5'-A_{25}-basket DNA (1 M NaCl) under 120 mV.

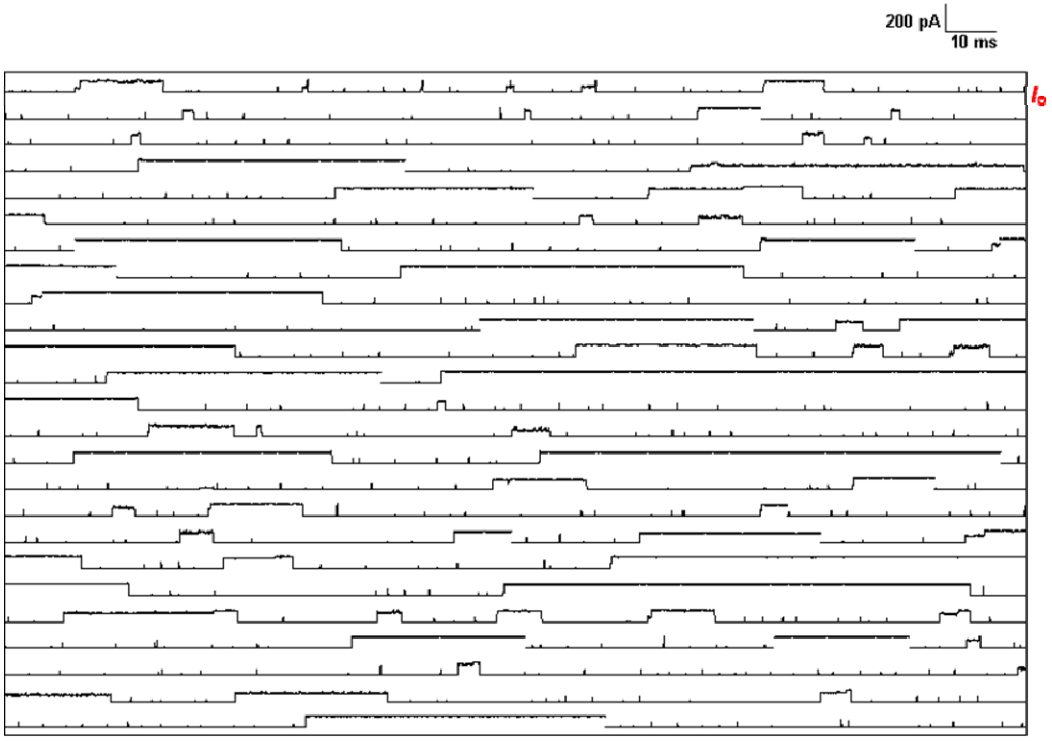
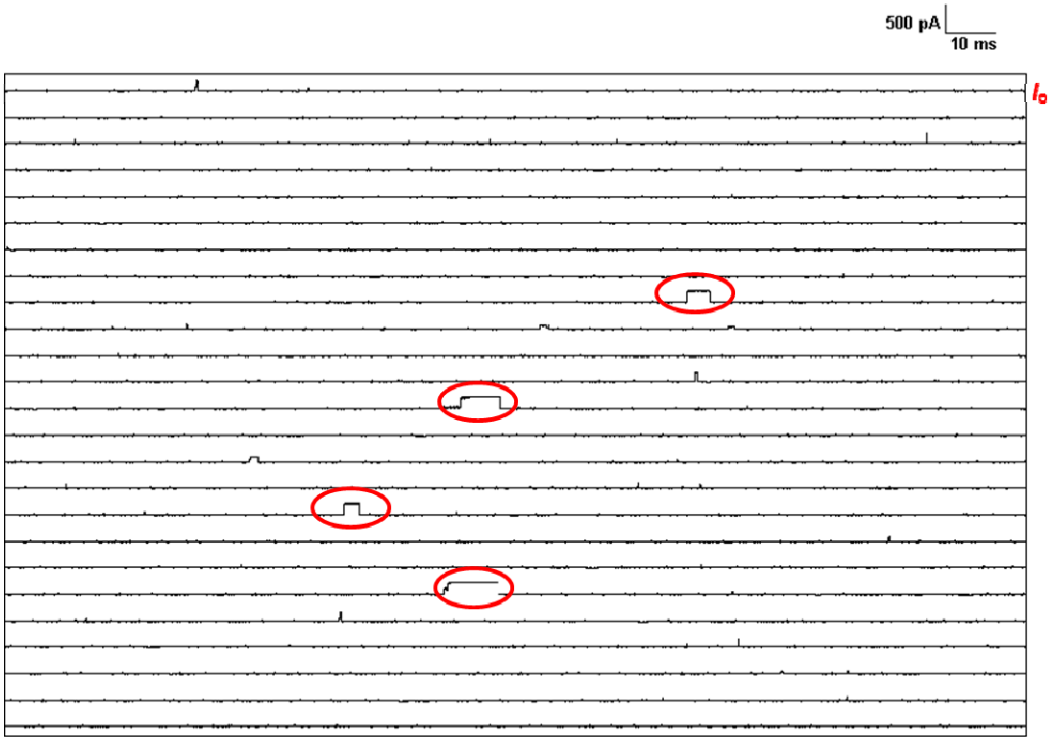


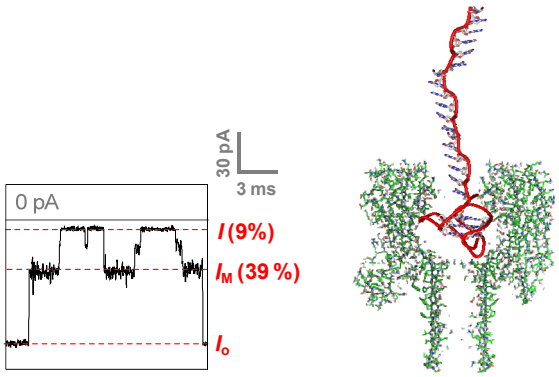
Fig. S7. Sample *i-t* traces representing translocation events of 5'-A_{25}-propeller DNA (20 mM KCl and 5 M LiCl) under 120 mV.



The red circles indicate events that were interpreted as the translocation of propeller folded strands through α-HL ion channel.

Fig. S8. Sample *i-t* traces representing non-translocation interactions between the α-HL and the hybrid folds (~5% of the total events) or basket fold (~15% of the total events).

Hybrid folds:



Basket fold:

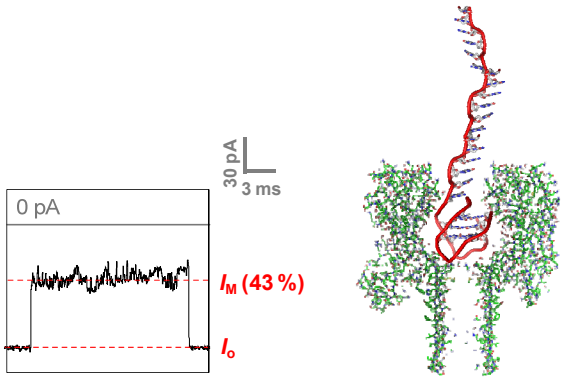
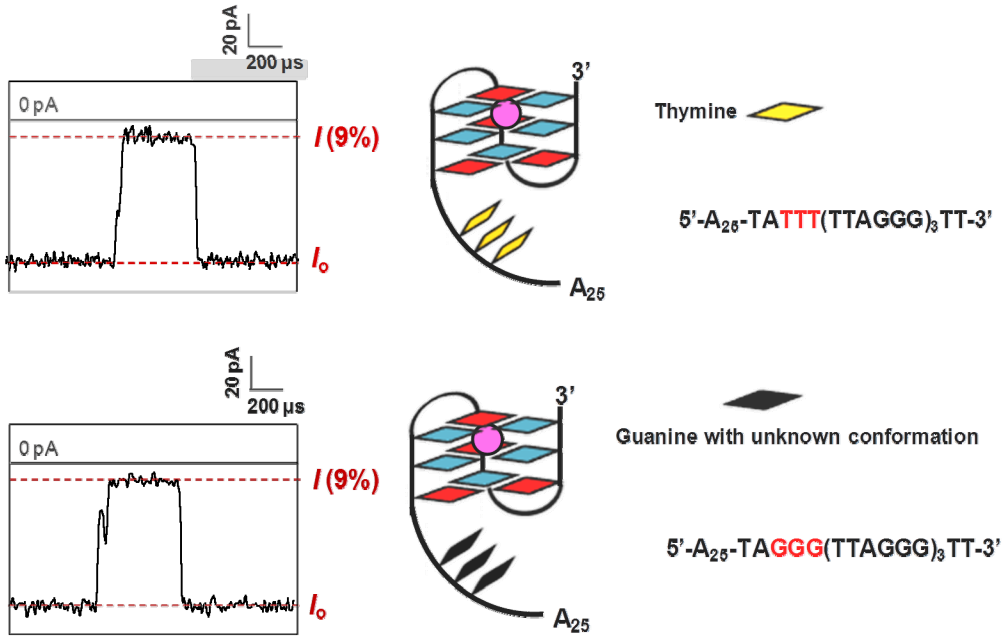
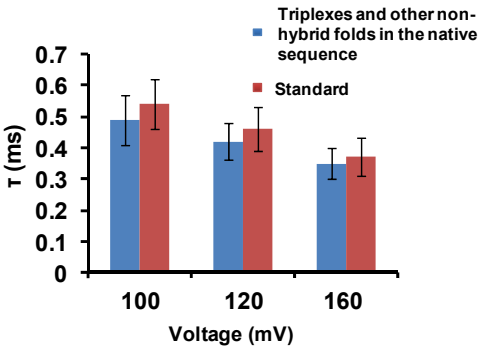


Fig. S9. The presence of triplexes in the KCl solution (50 mM KCl, 950 mM LiCl). The sequence with the 5'-GGG replaced by 5'-TTT was forced to form a triplex and used as a standard under these conditions. **a,** Sample i-t traces of the control triplex and triplex present in the native sequence. **b,** Plot of their duration decay constants τ as a function of applied voltage. The decay constants τ were obtained by fitting their duration histograms into a single-exponential decay model.

a,



b,



Note: We also observed much less dominant events (< 20%) with longer durations (τ = 18.6, 9.3, 4.4 ms at 100, 120, 160 mV) in the standard experiment, which correlated to the 3' side of the triplex entering the protein instead of the tail.

Since the triplexes were not the dominant species in the solution under our experimental conditions, the tail entering events of the standard were used for comparison.

References

1. Heddi B & Phan AT (2011) Structure of human telomeric DNA in crowded solution. . *J. Am. Chem. Soc.* 133:9824-9833.
2. Karsisiotis AI*, et al.* (2011) Topological Characterization of Nucleic Acid G-Quadruplexes by UV Absorption and Circular Dichroism. *Angew. Chem., Int. Ed.* 50(45):10645-10648.
3. Blandamer MJ, Engberts JBFN, Gleeson PT, & Reis JCR (2005) Activity of water in aqueous systems; A frequently neglected property. *Chem. Soc. Rev.* 34(5):440-458.
4. Miller MC, Buscaglia R, Chaires JB, Lane AN, & Trent JO (2010) Hydration Is a Major Determinant of the G-Quadruplex Stability and Conformation of the Human Telomere 3'-Sequence of d($AG_3(TTAG_3)_3$). *J. Am. Chem. Soc.* 132(48):17105-17107.