Supplementary Material
ILPR G-quadruplexes Demonstrate High Mechanical Stabilities

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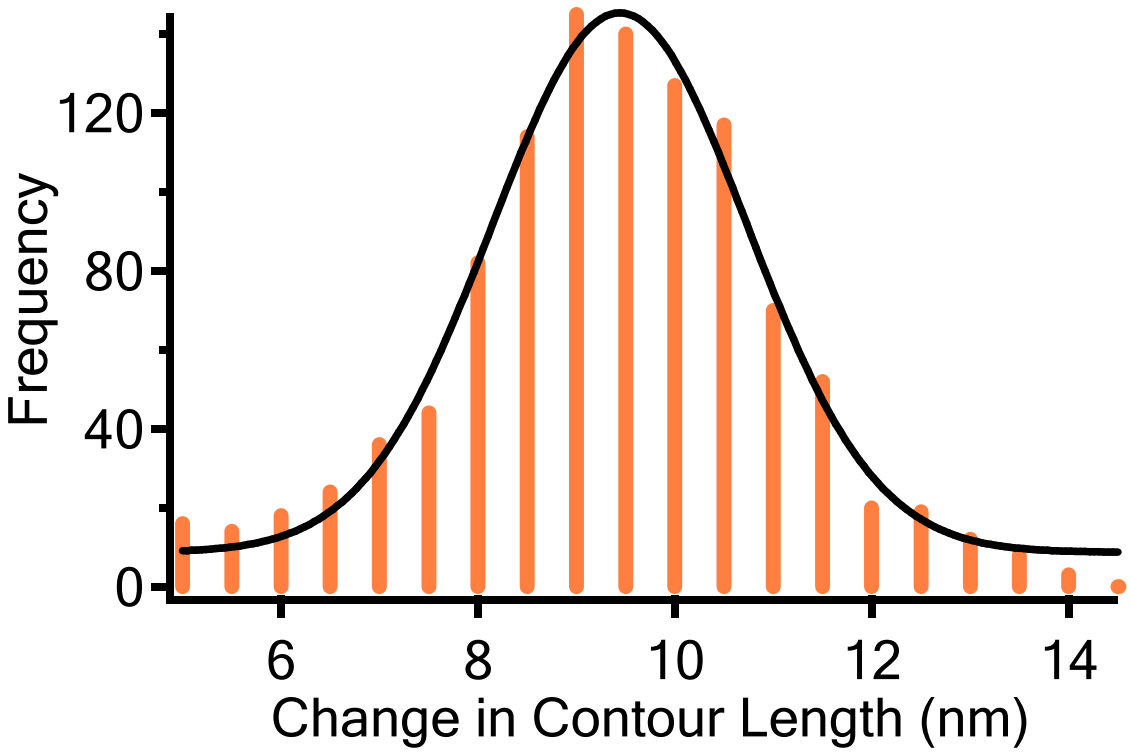

Figure S1. Histogram of change in contour length for the 31-mer ILPR sequence (see manuscript for detailed sequence) in 100 mM KCl, 2 mM EDTA, 10 mM Tris buffer, pH 8.0 (n=1069). The total population is fit by a Gaussian distribution (solid line).

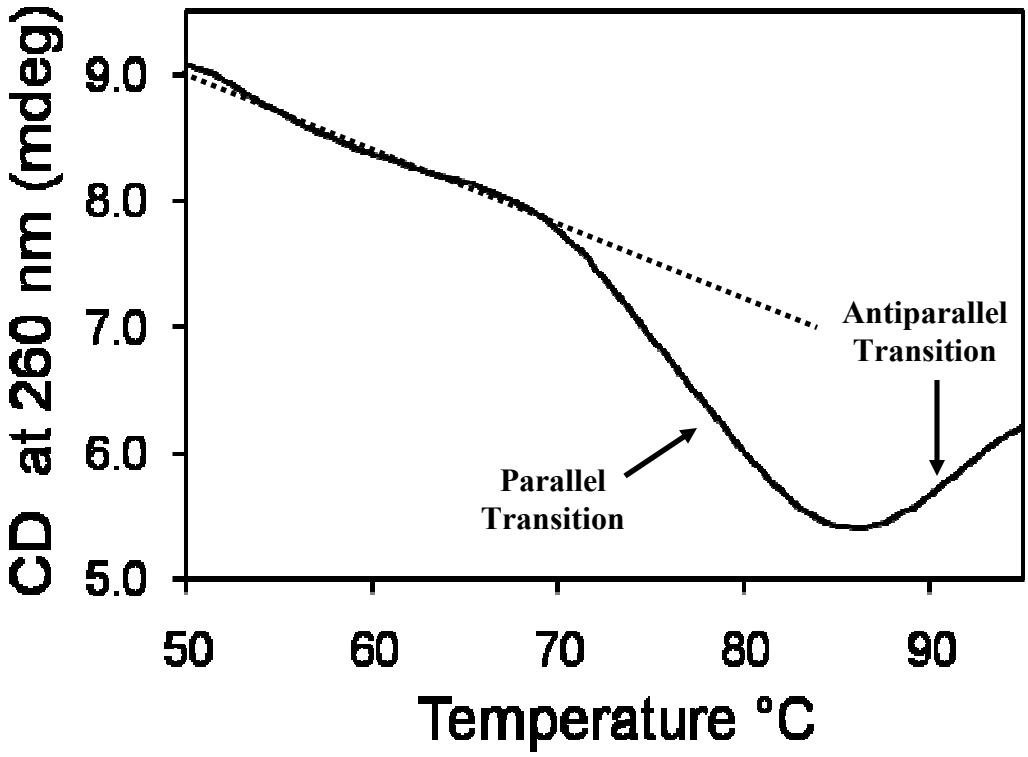

Figure S2. CD melting for a mixture of ILPR variants 5′-$(ACAGGGGTGTGGGG)_2$ (overnight reannealing to ensure all antiparallel structure, melting temperature ~93 °C) and 5′-$(ACAGGGGTCTGGGG)_2$ (parallel structure, melting temperature ~83 °C) in 10 mM Tris-HCl pH 8.0 with 100 mM KCl. Each variant has a concentration of 5μM. The dotted line is a guide for the base line.

Calculations of Free Energy Change ($\Delta G_{23\ ^\circ C}$) from CD Melting Experiments.

CD melting experiments were performed according to Fig 3b in the manuscript. Free energy changes for the observed transitions of the melting curves (Fig 3b) were calculated[1] by treating each transition separately. Briefly, the sloping baseline of the melting curve was first corrected[2] according to respective temperature ranges (73 °C-83 °C for the first transition; and 83°C-93°C for the second transition). The fraction of DNA in the folded state at the melting temperature, α, was then calculated separately for the two transitions. These values were then used to derive $\Delta G_{23\ ^\circ C}$ for the two transitions separately[1]. This calculation yielded $\Delta G_{23\ ^\circ C}$ (for melting) of 14.5 ± 0.8 kcal/mol and 28 ± 1 kcal/mol for parallel and antiparallel G quadruplex transitions, respectively. Compared to the Jarzynski calculation (see manuscript), the first value is identical whereas the second value is larger. The discrepancy of ΔG for the second transition calculated from these two methods could come from the systematic error in the CD melting analysis. Notice that α is dependent on the melting range, which is difficult to determine accurately, especially when there are two neighboring transitions as shown in Fig 3b.

Calculation of Unfolding Kinetics of G Quadruplexes.

The unfolding kinetics of G quadruplexes was calculated by the following equation: $\ln K_{eq} = \ln \frac{k_{unfold}}{k_{fold}} = -\frac{\Delta G_{unfold}}{k_B T}$, where K_{eq} is the unfolding equilibrium constant at 23 °C, k_{fold} and k_{unfold} are folding and unfolding rate constants at zero force, respectively, and ΔG_{unfold} is the unfolding free energy change of G quadruplexes calculated by the Jarzynski equation (see manuscript for details). This calculation yielded k_{unfold} of 1.5×10^{-11} s^{-1} for parallel and 9.4×10^{-18} s^{-1} for antiparallel G quadruplexes. The exceptionally slow unfolding kinetics qualitatively agrees with that of an *Oxytricha* telomeric repeat at 37 °C in 50 mM K^+.[3]

References

1. Marky, L.A., and Breslauer, K.J., Calculating thermodynamic data for transitions of any molecularity from equilibrium melting curves. *Biopolymers*, **1987**, 26, 1601-1620;
2. Mergny J.L. and Lacroix, L., Analysis of thermal curves. *Oligonucleotides*, **2003**, 13, 515-537.
3. Brown, N. M.; Rachwal, P. A.; T., B.; Fox, K. R., Exceptionally slow kinetics of the intramolecular quadruplex formed by the Oxytricha telomeric repeat. *Org. Biomol. Chem.* **2005,** 3, 4153-4157.