Supporting information for:
**Pif1 helicase unfolding of G-quadruplex DNA is highly dependent on sequence and reaction conditions**
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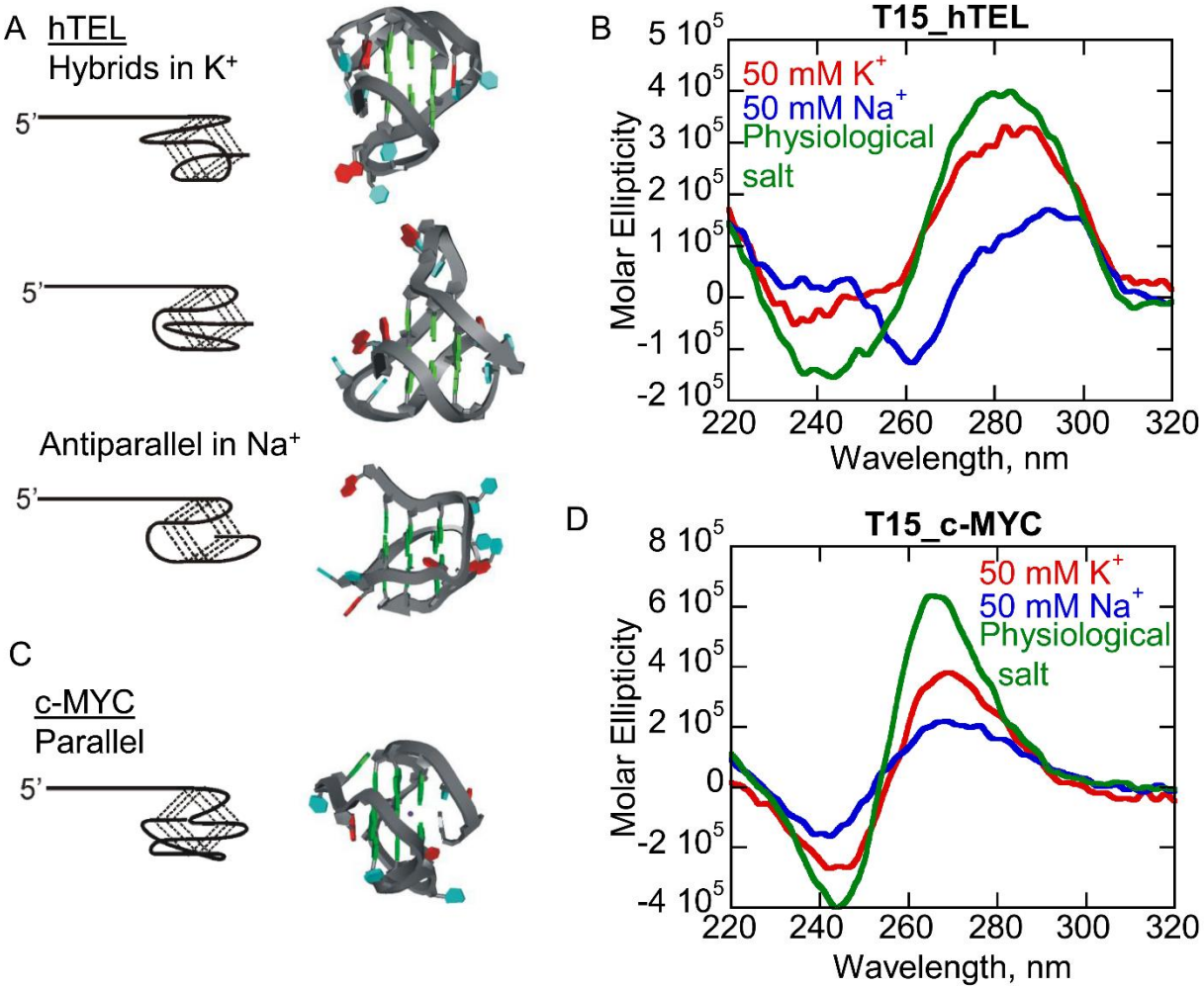
**Supplementary Table 1.** Sequences of oligonucleotides.

| Name | Sequence |
|---|---|
| $T_{15}$_hTEL | 5′-$T_{15}$A GGGTTAGGGTTAGGGTTAGGG-3′ |
| hTEL trap | 5′-CCCTAACCCTAACCCTAACCC-3′ |
| $T_{15}$_c-MYC | 5′-$T_{15}$GAGGGTGGGTAGGGTGGGTAA-3′ |
| c-MYC trap | 5′-CCCACCCTACCCACCC-3′ |
| 14T/14T-16bp fork | 5′- $T_{14}$CGCTGATGTCGCCTGG-3′ |
| | 5′-CCAGGCGACATCAGCG$T_{14}$-3′ |
| 16Trap | 5′-CGCTGATGTCGCCTGG-3′ |
| 14TiCy3/14T-16bp fork | 5′- $T_{13}$/iCy3/TCGCTGATGTCGCCTGG-3′ |
| | 5′-CCAGGCGACATCAGCG$T_{14}$-3′ |
| 14TAmCy3/14T-16bp fork[1] | 5′- $T_{13}$/iAmMC6T/TCGCTGATGTCGCCTGG-3′ |
| | 5′-CCAGGCGACATCAGCG$T_{14}$-3′ |
| $T_{15}$_hTEL reporter | 5′-$T_{15}$AGGGTTAGGGTTAGGGTTAGGGTTACGCTGATGTCGC-3' |
| $T_{15}$ reporter | 5′-$T_{15}$ATTACGCTGATGTCGC-3′ |
| $T_{15}$_c-MYC reporter | 5′-$T_{15}$GAGGGTGGGTAGGGTGGGTAACGCTGATGTCGC-3′ |
| $T_{24}$_c-MYC reporter | 5′-$T_{24}$GAGGGTGGGTAGGGTGGGTAACGCTGATGTCGC-3′ |
| $T_{32}$_c-MYC reporter | 5′-$T_{32}$GAGGGTGGGTAGGGTGGGTAACGCTGATGTCGC-3′ |
| $T_{40}$_c-MYC reporter | 5′-$T_{40}$GAGGGTGGGTAGGGTGGGTAACGCTGATGTCGC-3′ |
| Reporter displaced strand | 5′-GCGACATCAGCG$T_7$-3′ |
| Reporter trap | 5′-GCGACATCAGCG-3′ |

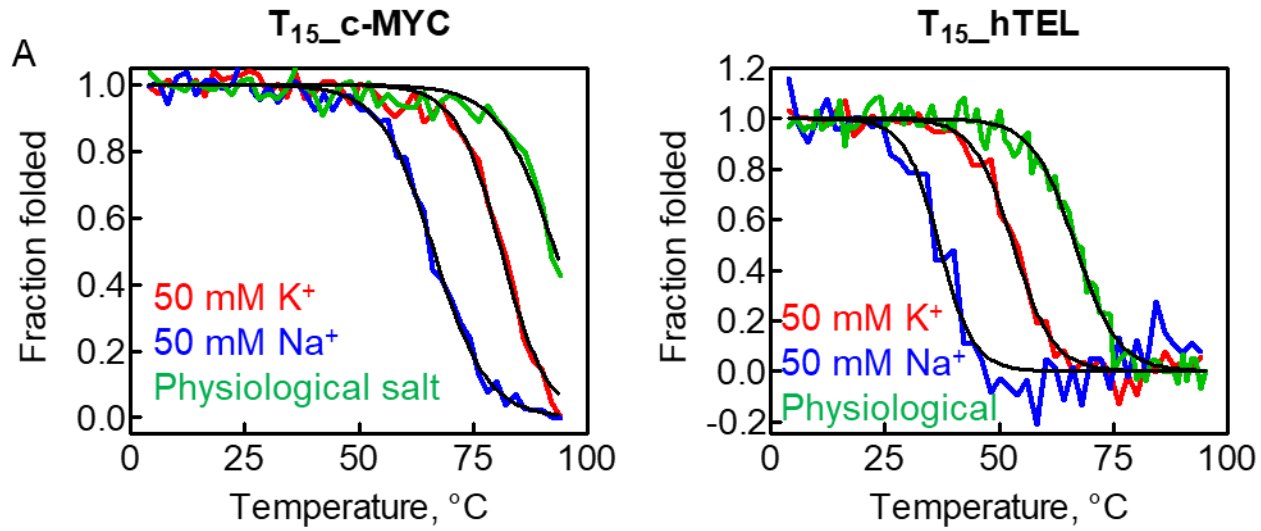
[1]Oligonucleotide was reacted with Cy3 NHS ester and gel purified to obtain Cy3 labeled species.
Guanines involved in tetrad formation are underlined.
Duplex forming regions of substrates are colored.

Supplementary Figure 1.




**Supplementary Figure 1.** c-MYC and hTEL G4DNA form different structures. (A) Schematic illustrations and solution NMR structures (hybrids PDB IDs: 2JSM and 2JSQ; (80); anti-parallel PDB ID: 2MCC; (81)) of the hybrid G4DNA structures formed by the hTEL sequence in $K^+$ and the anti-parallel structure formed in $Na^+$. Guanines are shown in green, adenines in red, and thymidines in blue. (B) Circular dichroism spectra of 10 µM $T_{15}$_hTEL in 50 mM $K^+$ (red), 50 mM $Na^+$ (blue), and physiological salt (green) indicate formation of hybrid G4DNA structures with maxima around 285 nm and minima around 240 nm in $K^+$ and physiological salt and formation of an anti-parallel G4DNA structure in $Na^+$ with a maximum around 290 nm and a minimum around 260 nm. (C) Schematic illustration and solution NMR structure (PDB ID: 1XAV; (51)) of the parallel G4DNA structure formed by a sequence modified from that in the c-MYC promoter which forms only the predominant G4DNA structure formed from the wild-type 27mer sequence. Coloring is the same as in A. (D) Circular dichroism spectra of 10 µM $T_{15}$_c-MYC oligonucleotide in 50 mM $K^+$ (red), 50 mM $Na^+$ (blue), and physiological salt (green) indicate formation of a parallel G4DNA structures with maxima around 265 nm and minima around 245 nm. Duplicate experiments produced similar results.

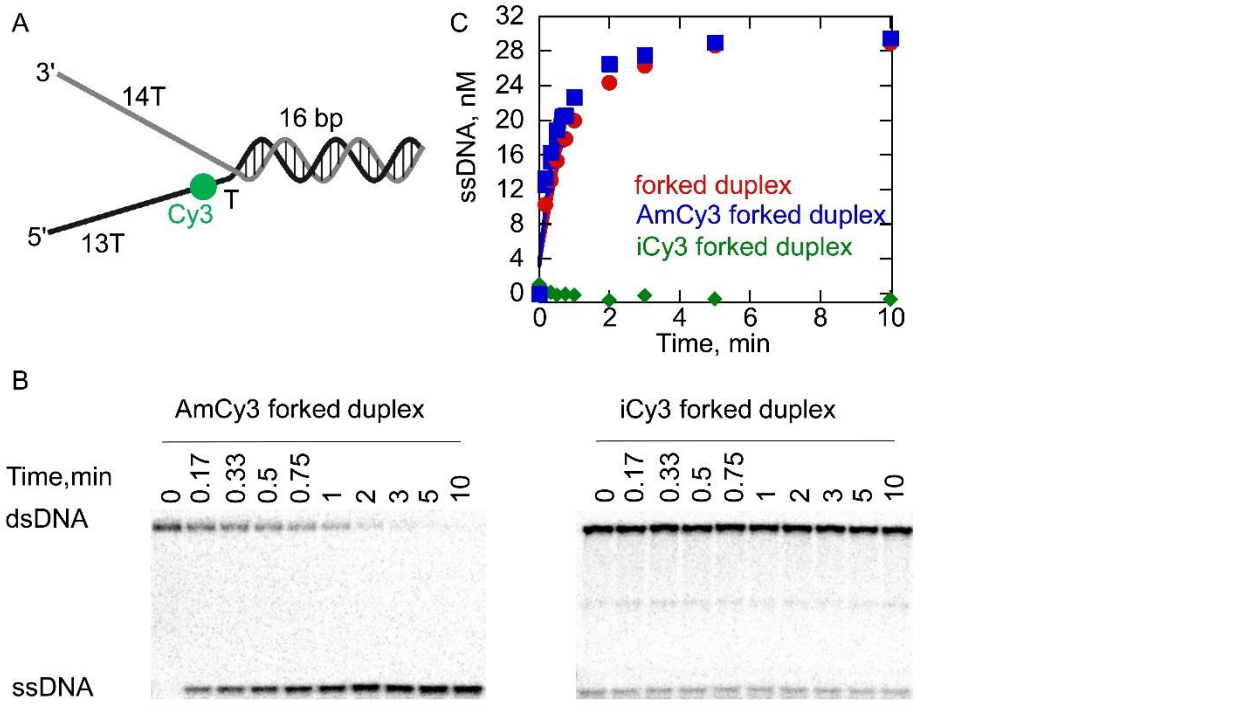
Supplementary Figure 2




**Supplementary Figure 2.** Thermal stability is affected by both the sequence and the monovalent cation. Thermal melting curves were measured by circular dichroism for $T_{15}$_c-MYC at 268 nm (A) and for $T_{15}$_hTEL at 290 nm (B) and the data were fit using GraphPad Prism using a non-linear regression from 1.0 down to 0.0 with a variable slope to obtain the midpoint of the melting curve. The melting temperatures ($T_m$) for $T_{15}$_c-MYC in 50 mM $K^+$ (red), 50 mM $Na^+$ (blue), and physiological salt (green) were 81 ± 1 °C, 66 ± 1 °C, and 93 ± 1 °C, respectively. The melting temperature of $T_{15}$_cMYC in physiological salt should be considered to be an estimate since the structure did not melt completely when the measurements were stopped at 95 °C. The melting temperatures ($T_m$) for $T_{15}$_hTEL in 50 mM $K^+$ (red), 50 mM $Na^+$ (blue), and physiological salt (green) were 53 ± 1 °C, 36 ± 1 °C, and 66 ± 1 °C, respectively. Errors errors represent the standard error of the fit of a single experiment.

**Cy3 inserted into the backbone prevents Pif1 from unwinding**

Some of the studies of the effect of G4DNA on Pif1 helicase activity have relied on single molecule Förster Resonance Energy Transfer (smFRET) with fluorescently labeled oligonucleotides. In some cases, the Cy3 in the translocase strand was attached through amine modified thymidine linkages (45). In other cases, internal Cy3 modifications were used (46,50). Here we tested the ability of Pif1 to translocate past both amine-modified-dT-Cy3 and internal Cy3 modifications by measuring unwinding of a well characterized 16-bp forked duplex (44). Cy3 modifications were placed 1-nt before the ssDNA-dsDNA junction on the translocase strand (Supplementary Fig. 3A). Unwinding was measured under conditions in which the DNA substrate was in excess to the enzyme in order to mimic smFRET experimental conditions. Pif1 was readily able to unwind the substrate with the Cy3 attached through the amine-modified-dT linkage, but was unable to unwind the substrate with the internal Cy3 modification (Supplementary Fig. 3B-C), indicating that any fluorescent modifications in the translocase strand must be tested for their effect on helicase activity. Some of the differences observed that were thought to be due to Pif1 G4DNA unfolding activity may in fact be affected by the inability of Pif1 to translocate past some fluorescent modifications.




**Supplementary Figure 3.** Pif1 unwinding with Cy3 modifications in the translocase strand. (A) Substrates were an unmodified 16 bp forked duplex (14T/14T-16bp fork), and two forked duplexes with different Cy3 modifications one nucleotide from the ssDNA-dsDNA junction on the translocase strand. One contained Cy3 attached through an amine modified thymidine (14TAmCy3/14T-16bp fork), and the other contained an internal Cy3 modification attached through the backbone (14TiCy3/14T-16bp fork). (B) Radiolabeled duplex (30 nM) was incubated with 10 nM Pif1 in reaction buffer with KCl. Reactions were initiated with the addition of 5 mM ATP, 10 mM MgCl2, and 100 nM 16trap. Timepoints were quenched at various times with 400 mM EDTA, 5 µM 16trap, and 2 µM $T_{50}$ to sequester the protein and separated by electrophoresis. (C) The initial linear portions of the curves were fit with a line to obtain steady-state unwinding rates of 22 ± 6 nM $s^{-1}$ for the 14T/14T-16bp unmodified fork and 29 ± 7 nM $s^{-1}$ for the amine linked Cy3 fork (14TAmCy3/14T-16bp fork). No unwinding was observed with the internal Cy3 modification.