Supplementary Material

The parallel G-quadruplex structure of vertebrate telomeric repeat sequences is not the preferred folding topology under physiological conditions

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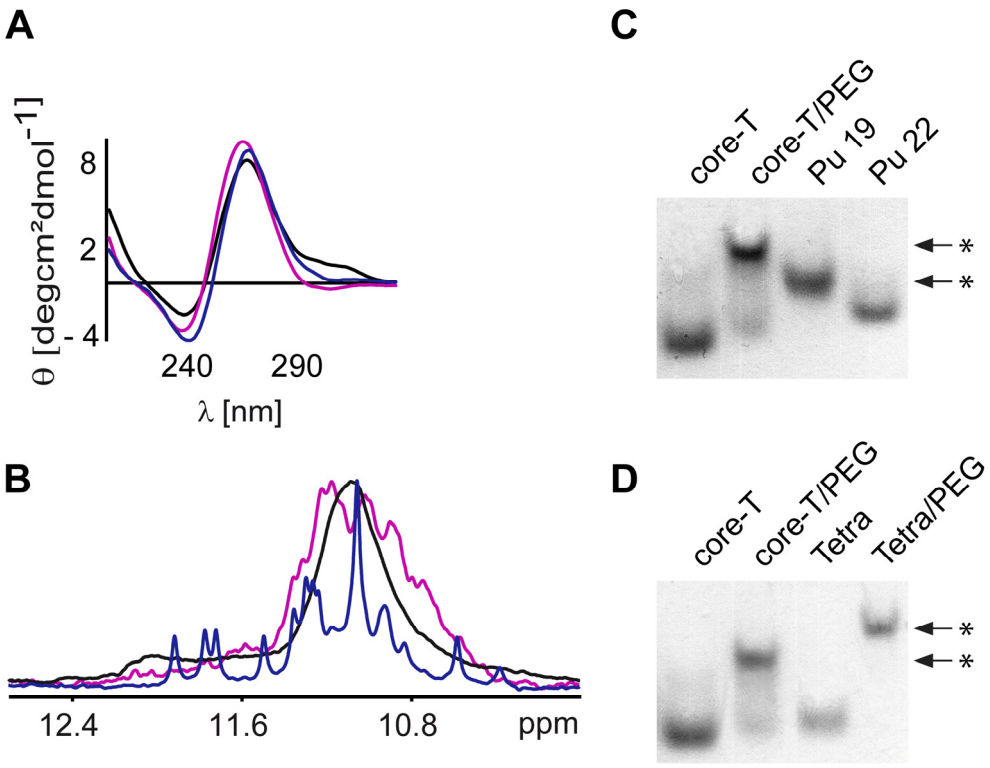

Figure S1. Overlays of CD (**A**) and NMR (**B**) spectra of [core-T] in the presence of PEG (black line), Pu19 d($AG_3TG_4AG_3TG_4A$) and Pu22mut 14/23 d($TGAG_3TG_3TAG_3TG_3TAA$) constructs in intraoocyte buffer (magenta and blue lines, respectively). The Pu19 and Pu22mut 14/23 constructs originate from the c-Myc nuclease hypersensitivity element III_1, which exclusively forms a parallel conformation (27). (**C**) Native PAGE for [core-T] the absence and presence of 40% PEG 200 and for Pu19 and Pu22mut constructs in intraoocyte buffer. (**D**) Comparison of the native PAGE behavior of [core-T] and the *Tetrahymena thermophila* (Tetra) telomeric sequence d($G_4T_4G_4T_4G_4T_4G_4$) in the absence and presence of 40% PEG 200. The *T. thermophila* telomeric repeat was shown to form high-order structures in the presence of PEG (28). The CD spectrum of the Pu19 and the Pu22mut 14/23 constructs are characterized by a dominant band at 260 nm, typical for the parallel G-quadruplex topology. While the 1D ^{1}H 11-echo NMR spectrum for Pu22mut 14/23 shows well-resolved imino resonances, the 1D ^{1}H 11-echo NMR spectrum for Pu19 shows unresolved, broad imino signals. Slower electrophoretic mobility of Pu19 compared to Pu22mut 14/23 on a native 10% PAGE indicates that the line broadening observed in the NMR spectrum of Pu19 results from high-order parallel G-quadruplex formation (*), while a well-resolved spectrum for Pu22mut 14/23 corresponds to a monomeric form. Analogous response of electrophoretic mobilities for [core-T] and Tetra constructs in the presence of 40% PEG 200 suggest that [core-T], similarly to the *T. thermophila* telomeric repeat, forms high-order structures (*).

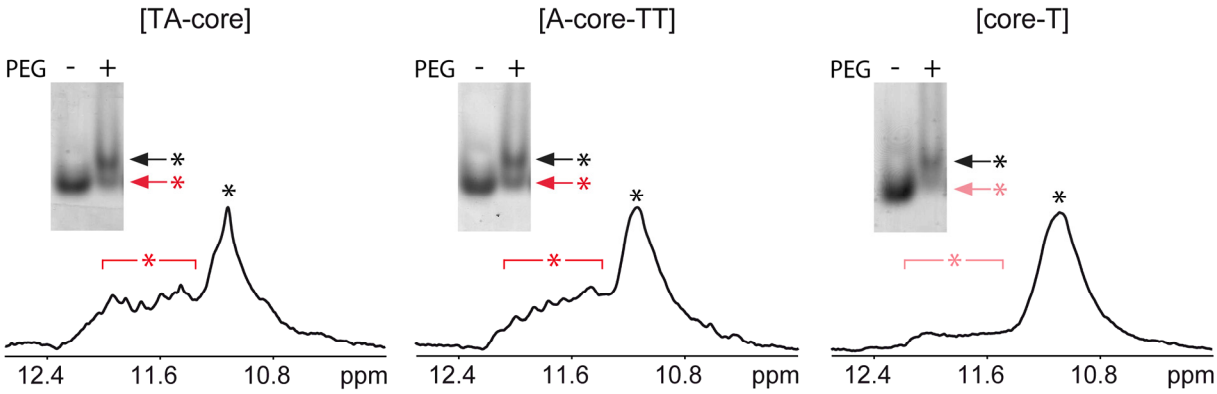

Figure S2. NMR spectra and native PAGE analysis of [TA-core], [A-core-TT], and [core-T] constructs in intraoocyte buffer supplemented with 40% PEG. The presence of several small peaks between 11.5 and 12.4 ppm in the NMR spectra and the presence of two resolved bands on native 8% PAGE gels suggest that the dominant high-order parallel structure (*) coexists with low-level (*) populated alternative monomeric conformation(s).

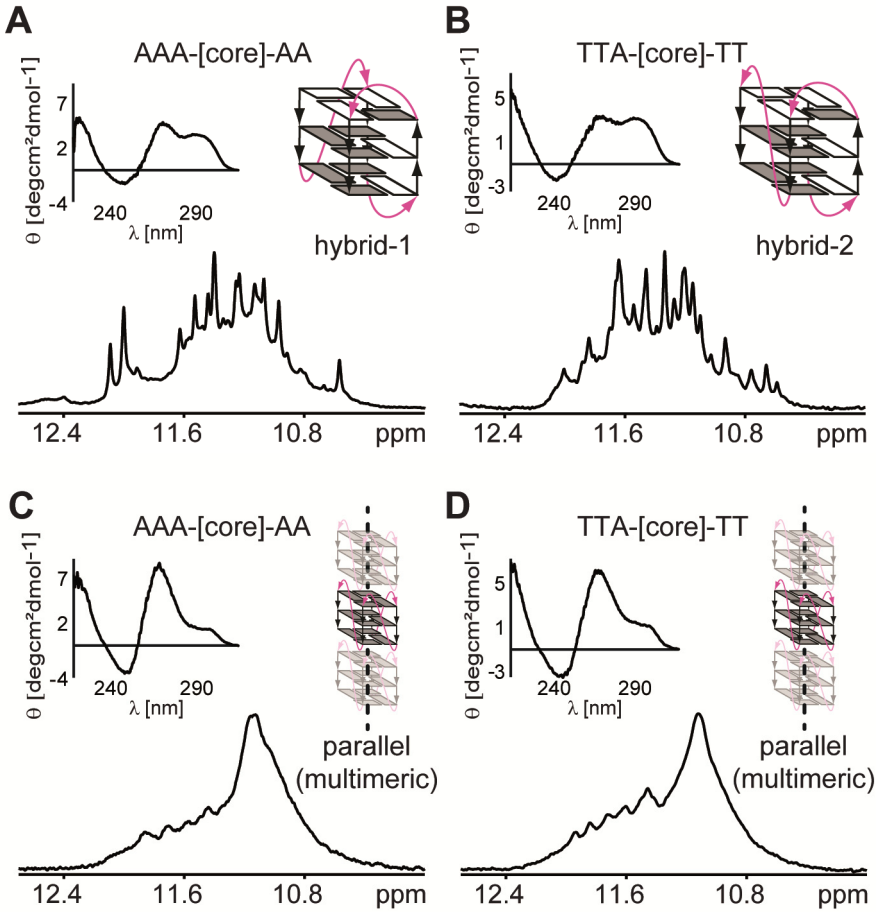

Figure S3. CD spectra and imino regions of 1D ^{1}H 11-echo NMR spectra for [AAA-core-AA] and [TTA-core-TT] constructs in intraoocyte buffer (**A-B**), and in intraoocyte buffer supplemented with 40% PEG 200 (**C-D**).

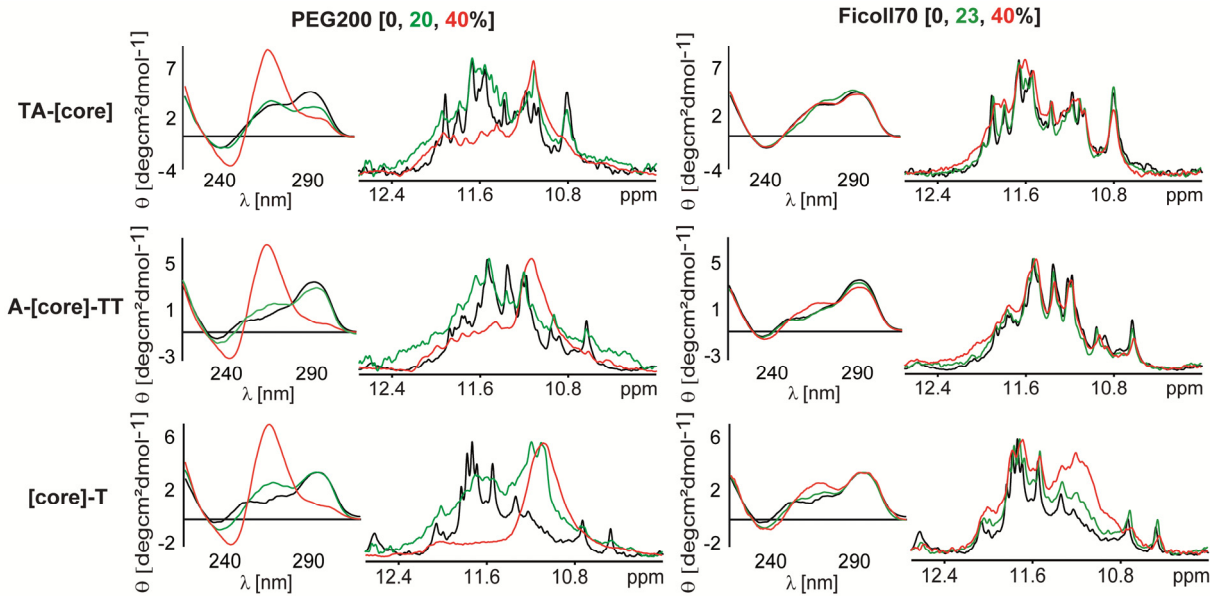

Figure S4. Overlays of CD spectra and imino regions of 1D ^{1}H 11-echo NMR spectra for [TA-core], [A-core-TT], and [core-T] constructs (rows) in intraoocyte buffer, and in intraoocyte buffer supplemented either with PEG 200 (20%, 40%) or Ficoll 70 (23%, 40%). Note that a 23% (w/v) Ficoll 70 solution simulates equivalent volume exclusion as a 40% (v/v) PEG 200 solution.

Excluded volumes of PEG 200 and Ficoll 70 were calculated based on a simple geometric model analogous to that employed by Spink and Chaires (37). In this model, the PEG 200 and Ficoll molecules were approximated as spheres with radii r_{PEG} = 0.57 nm and r_{Ficoll}= 5.5 nm, respectively.