Supplementary Data

Small circular DNAs encoding the human telomere repeat: Varied sizes, structures, and telomere-encoding activities

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Synthesis of DNA nanocircles

The N6-dimethylacetamidine-dA (Dma-dA) phosphoramidite was prepared according to the method described by McBride et al. (McBride, L. J., Kierzek, R., Beaucage, S. L. and Caruthers, M. H.: *J. Am. Chem. Soc.* **1986**, *108*, 2040-2048). Spectroscopic data were in accordance with published data. DNA oligonucleotides were synthesized as described previously (U. M. Lindstrom, E. T. Kool, *Nucleic Acids Res*. **2002**, *30*, e101) on an Applied Biosystems 394 synthesizer using β-cyanoethylphosphoramidite chemistry. Ultra-mild deprotection phosphoramidites were purchased from Glen Research. No changes to the standard protocol were needed for the couplings of the Dma-dA phosphoramidite. 5′-Phosphorylation was carried out with chemical phosphorylation reagent (Glen Research). Deprotection was done with 0.05 M K_2CO_3/MeOH for 12 h at room temperature for the ultra-mild deprotection bases. Neutralization of the carbonate solution was accomplished by adding an equal volume of 2 M tetraethylammonium acetate. For the Dma-dA base, cleavage was achieved with ammonia/methylamine (AMA) reagent [NH_4OH/$MeNH_2$ (40% in water) 1:1] for at least 8 h at room temperature. Oligomers were purified by preparative 15% denaturing polyacrylamide gel electrophoresis (PAGE) and quantified by absorbance at 260 nm.

Circularization of the linear precursor oligonucleotides was carried out using T4 DNA ligase (New England Biolabs). The corresponding precursor (1 μM) was combined with a 18mer ligation splint (GTTAGGGTTAGGGTTAGG, 1.5 μM) in 50 mM Tris, pH 7.5, 10 mM $MgCl_2$. After denaturation for 5 minutes at 75°C, each solution was allowed to slowly reach room temperature. ATP, DTT, and bovine serum albumin (BSA) were added to final concentrations of 100 μM, 10 mM, and 25 μg/ml, respectively. The reaction was initiated by adding T4 DNA ligase to a final concentration of 800 U/ml. The reaction was incubated for 12 h at room temperature, followed by heat-inactivation of the ligase and dialysis against water. The reaction mixture was concentrated, followed by purification of circular oligonucleotides by 15% PAGE. Confirmation of circularity was provided by nicking with S1 endonuclease; initial cleavage of circle produces a single band with the mobility of the linear precursor, see Figure 2 (main paper).

Thermal denaturation experiments:

Solutions for thermal denaturation studies were prepared as 1 mL samples at 1 µM concentration. For pH 5.0, 50 mM NaOAc and for pH 7.0, 10 mM Na•PIPES buffer was used. Solutions were heated to 90°C for 5 minutes and annealed by slowly cooling to room temperature and then to 0°C. The melting studies were carried out in quartz cells with 1 cm pathlength on a Varian Cary 1 UV-Vis spectrophotometer equipped with a Peltier temperature controller. Absorbance was monitored at 260 nm while temperature was raised from 15 to 80°C at a rate of 0.5°C/minute. Melting temperatures (T_m) were determined by fitting the first derivative of absorbance with respect to 1/T.

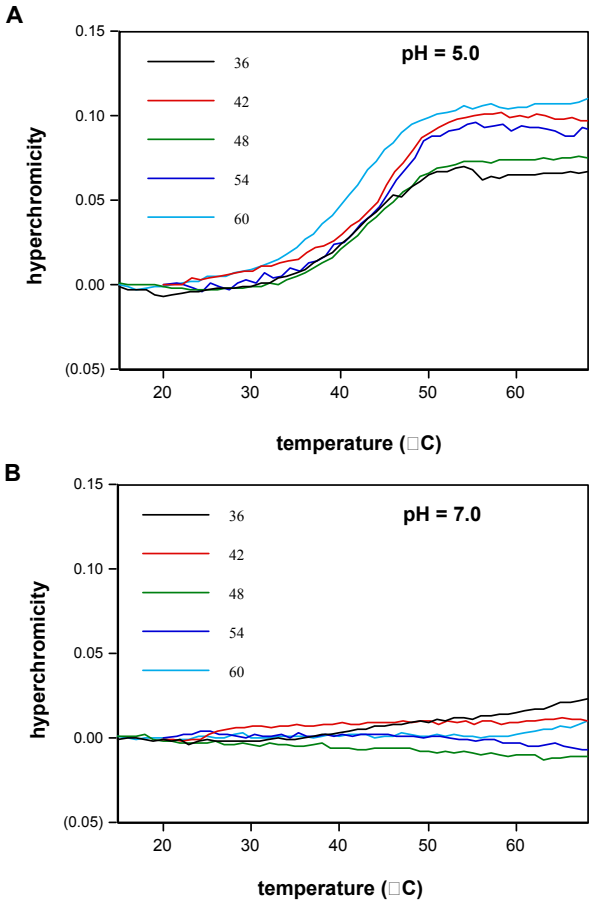

Figure S1. UV-monitored thermal denaturation plots for the five circles in this study at pH=5.0 (A) and 7.0 (B).

Circular dichroism:

Samples for circular dichroism measurements were prepared as described for thermal denaturation studies. CD-spectra were recorded from 320 to 220 nm on an Aviv spectrometer at 25°C. Data was collected for 2 seconds in 2 nm intervals in a 1 cm pathlength quartz cell; raw data was corrected for pathlength and concentration of the sample to yield molar ellipticity. Baseline correction was carried out by subtracting buffer signals.