Structure and Stability of Higher-Order Human Telomeric Quadruplexes

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Figure S1. Sedimentation coefficient distributions as determined by the program Sedfit are shown for $(TTAGGG)_4$ (Panel A), $(TTAGGG)_4TT$ (Panel B), $(TTAGGG)_8$ (Panel C), $(TTAGGG)_8TT$ (Panel D), $(TTAGGG)_{12}$ (Panel E) and $(TTAGGG)_{12}TT$ (Panel F).

Figure S2. A) Structures of the Hybrid-1, Hybrid-2 and propeller quadruplexes; B) Schematic representation of the two trimer models simulated in this study.

Figure S3. CD melting and annealing curves for all the oligonucleotides used in this study.

Table 1S. Sedimentation coefficient values.

Figure S4. Singular values from SVD analysis of the CD vs temperature spectra for the six oligonucleotides.

Table 2S. Results from SVD analysis of the CD vs temperature spectra for the six oligonucleotides.

Figure S5. Deconvolution of $(TTAGGG)_n$ thermograms.

FIGURE S1

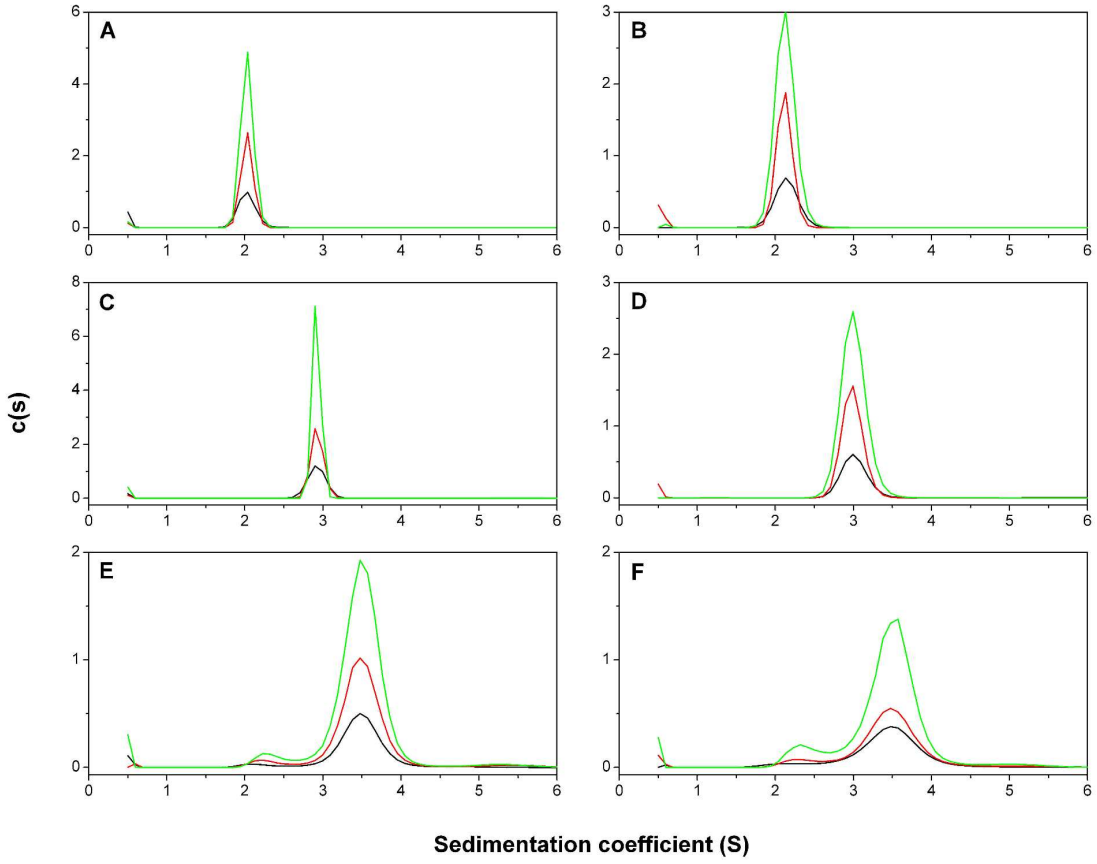
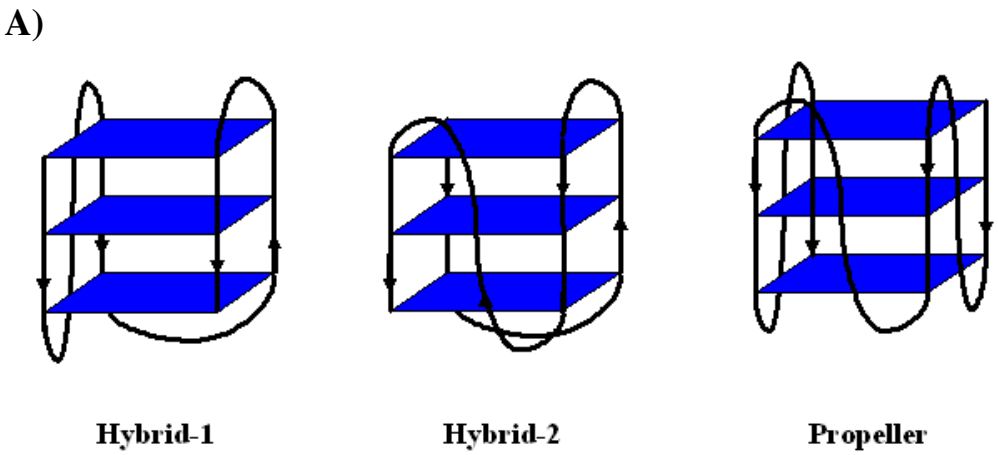


Figure S1. Sedimentation coefficient distributions as determined by the program Sedfit are shown for $(TTAGGG)_4$ (Panel A), $(TTAGGG)_4TT$ (Panel B), $(TTAGGG)_8$ (Panel C), $(TTAGGG)_8TT$ (Panel D), $(TTAGGG)_{12}$ (Panel E) and $(TTAGGG)_{12}TT$ (Panel F). Data for three different loading concentrations of A_{260}(1.2 cm) ~ 0.25, 0.5 and 1 are shown by the black, red and green lines, respectively.

FIGURE S2



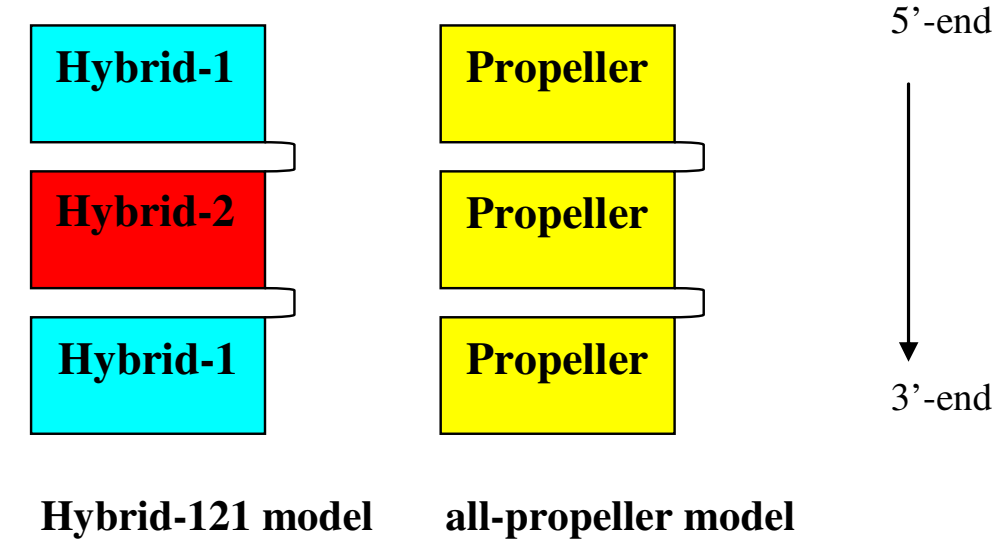


Figure S2. A) Structures of the Hybrid-1, Hybrid-2 and propeller quadruplexes; B) Schematic representation of the two trimer models simulated in this study, the 5'-3' orientation is indicated by the black arrow on the left.

FIGURE S3

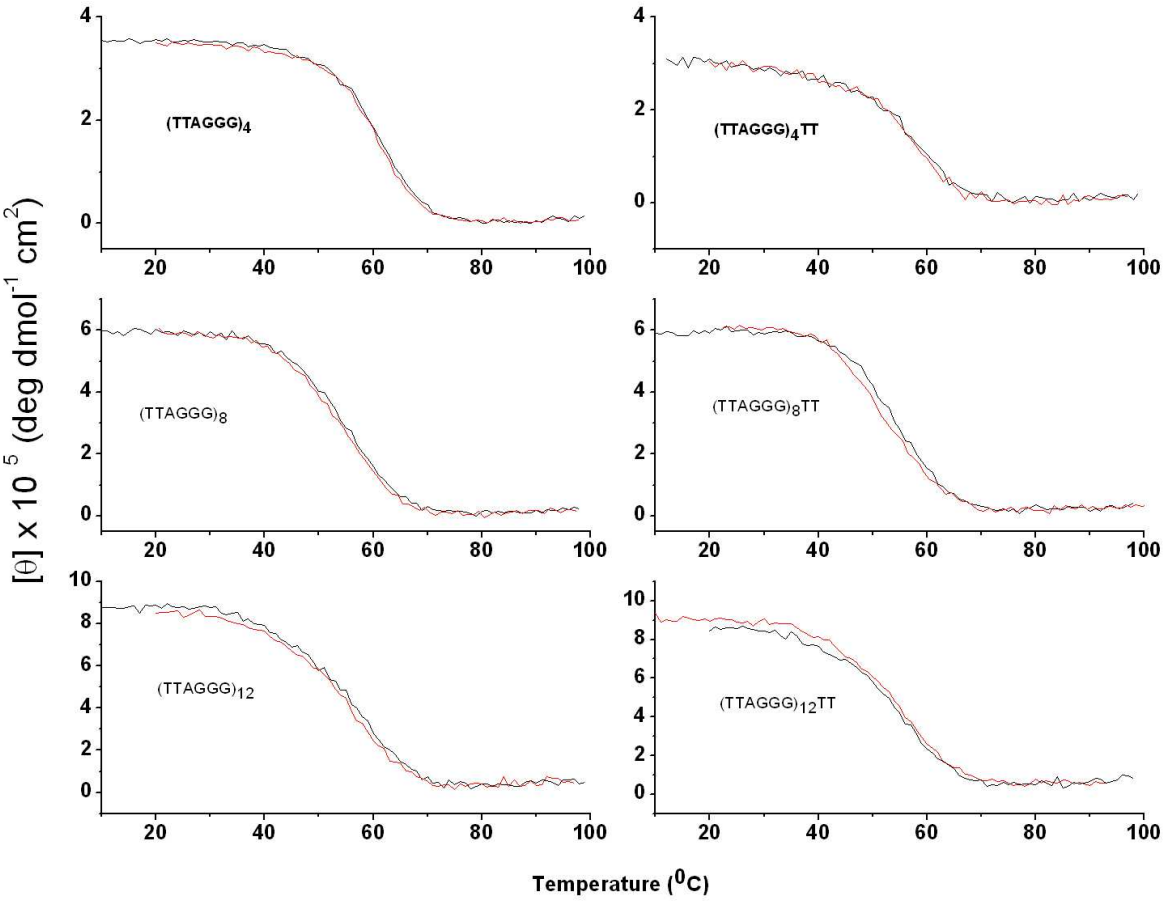


Figure S3. CD melting (black) and cooling (red) curves for all the oligonucleotides used in this study.

Table 1S

Table 1S: Sedimentation coefficients values

DNA sequence	S(20,w)[a]	S(20,buffer)	Calculated in buffer[b]
$(TTAGGG)_4$	2.05 ± 0.01	2.03 ± 0.01	
$(TTAGGG)_4TT$	2.15 ± 0.01	2.13 ± 0.01	
$(TTAGGG)_8$	2.95 ± 0.01	2.92 ± 0.01	
$(TTAGGG)_8TT$	3.02 ± 0.01	2.99 ± 0.01	
$(TTAGGG)_{12}$	3.51 ± 0.01 (first peak) 2.34 ± 0.03 (second peak)	3.49 ± 0.01 2.32 ± 0.03	3.40 ± 0.06 (hybrid-121) 3.87 ± 0.02 (all-propeller) 2.45 ± 0.08 (dt12)
$(TTAGGG)_{12}TT$	3.52 ± 0.01 (first peak) 2.40 ± 0.04 (second peak)	3.49 ± 0.01 2.38 ± 0.04	

[a] Corrected to standard conditions of water, 20°C. [b]Calculated from the MD trajectories with the program HYDROPRO5 in the same buffer conditions used in the experiments. All values are expressed in Svedberg units (S).

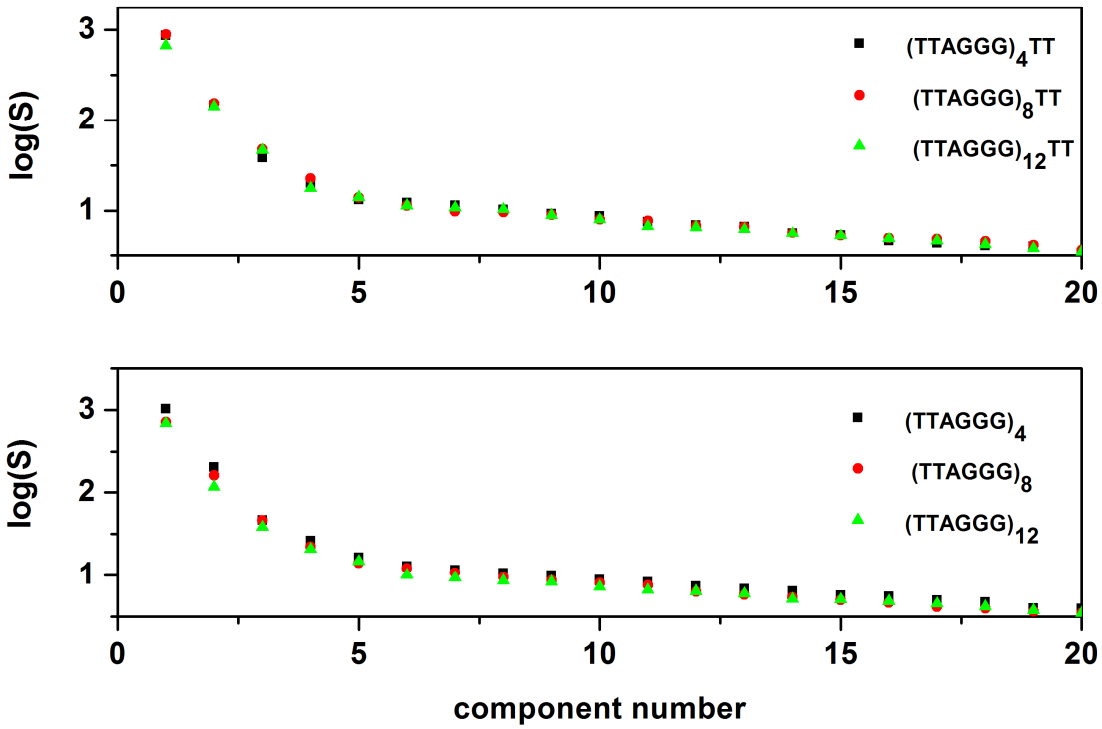


Figure S4. Plots of the logarithm of the first 20 singular values values resulting for the SVD anlaysis of the six sequences of this study.

Table 2S

Table 2S Results from Singular Values Decomposition analysis of the CD spectra series on changing the temperature.*

$(TTAGGG)_4$			$(TTAGGG)_4TT$		
S	**U autocorr**	**V autocorr**	**S**	**U autocorr**	**V autocorr**
1030.6	0.9986	0.9887	864.4	0.9987	0.9890
201.3	0.9971	0.9813	147.1	0.9969	0.9750
46.2	0.9986	0.8947	38.6	0.9971	0.8165
26.1	0.9850	0.7232	20.1	0.9938	0.5480
16.4	0.9986	0.1805	13.2	0.9974	-0.0236
12.9	0.9967	-0.0362	12.2	0.9932	0.0871
11.6	0.9917	0.0123	11.5	0.9972	-0.2503
10.7	0.9939	-0.1105	10.3	0.9968	-0.1763
$(TTAGGG)_8$			$(TTAGGG)_8TT$		
S	**U autocorr**	**V autocorr**	**S**	**U autocorr**	**V autocorr**
713.4	0.9984	0.9870	888.6	0.9986	0.9899
159.8	0.9968	0.9782	153.5	0.9961	0.9811
46.1	0.9996	0.9016	47.8	0.9996	0.8678
22.1	0.9984	0.7508	22.6	0.9858	0.5883
14.1	0.9968	-0.1066	14.0	0.99778	0.1109
12.2	0.9948	0.0359	11.4	0.9949	0.0309
10.7	0.9975	-0.1167	9.9	0.9848	-0.0015
9.5	0.9964	-0.1527	9.6	0.9962	-0.1273
$(TTAGGG)_{12}$			$(TTAGGG)_{12}TT$		
S	**U autocorr**	**V autocorr**	**S**	**U autocorr**	**V autocorr**
802.8	0.9987	0.9876	670.4	0.9985	0.9868
142.9	0.9972	0.9815	142.6	0.9965	0.9747
40.9	0.9993	0.9074	46.5	0.9997	0.8943
19.5	0.9825	0.4477	17.7	0.9858	0.4993
11.7	0.9984	0.1752	14.0	0.9986	0.050
10.9	0.9964	0.0291	11.4	0.9955	-0.0980
9.9	0.9972	0.1834	10.8	0.9966	0.0629
8.2	0.9886	-0.0668	10.4	0.9958	-0.0332

* S is the singular value, Uautocorr and Vautocorr represent the autocorrelations factors of the column of the matrix of the basis spectra (U matrix) and the matrix of the coefficient vectors (V matrix).

Comments on the analysis of $(TTAGGG)_n$ and $(TTAGGG)_nTT$ DSC Transitions

Below is a summary of how each of the data sets was analyzed with the best resolved numbers for enthalpies, entropies and transition temperatures for the resolved components. Table 2 in the main text summarizes the results. Species plots and fitted thermograms are shown in Figure 5 (main text) and Figure S5 (below).

1. $(TTAGGG)_4$
   a. Recursion treatment of this transition showed almost two-state behavior with a small amount of an intermediate.
   Resolved thermodynamic data:
   Δh_i = 68.7 kJ/mole; Δs_i = 202 J/K-mole;
   Δh_n = 172.3 kJ/mole; Δs_n = 520 J/K-mole;
   b. The intermediate did not appear to be a sequential transition and has low enthalpy, so to analyze the data the small cumulative enthalpy of the intermediate was subtracted from the experimental cumulative enthalpy and the resulting curve was treated as two-state to yield:
   ΔH = 228 kJ/mole; ΔS = 681 J/K-mole; T_m = 63.09
   This procedure may overcorrect at low temperature, but the correction is small so doesn't make too much difference.

2. $(TTAGGG)_4TT$
   a. This transition is clearly a two-state melt, so was analyzed as such.
   Result: ΔH = 213 kJ/mole; ΔS = 639 J/K-mole; T_m = 59.84

3. $(TTAGGG)_8$
   a. This one was the hardest to analyze. There is a rather large seemly non-sequential intermediate from the recursion analysis. Using a 2-intermediate model and the recursion results the best fit gave the following:

   Δh_1 = 138.6 kJ/mole; Δs_1 = 432 J/K-mole; T_1 = 47.61
   Δh_2 = 171.2 " Δs_2 = 524 " T_2 = 53.53
   Δh_n = 203.4 " Δs_n = 605 " T_n = 63.08

   b. Assuming the first component is not part of the sequential transition and subtracting its contribution to the total cumulative enthalpy yields the results for a single intermediate:

   Δh_i = 263 kJ/mole; Δs_i = 806 J/K-mole; T_i = 53.44
   Δh_n = 249 " Δs_n = 739 " T_n = 63.33

4. $(TTAGGG)_8TT$
   a. Recursion of this one showed a small low temperature component with small enthalpy, the results of the two intermediate analysis being:
   Δh_1 = 32.4 kJ/mole; Δs_1 = 83 J/K-mole T_1 = 43.97

Δh_2 = 191.9 " Δs_2 = 607 " T_2 = 42.93
Δh_n = 216.8 " Δs_n = 647 " T_n = 61.87

b. Again the first component was subtracted from the overall cumulative enthalpy and treated as though there is a single intermediate.

Results: Δh_i = 219 kJ/mole; Δs_i = 676 J/K-mole; T_i = 51.96
Δh_n = 222 " Δs_n = 662 " T_n = 61.82

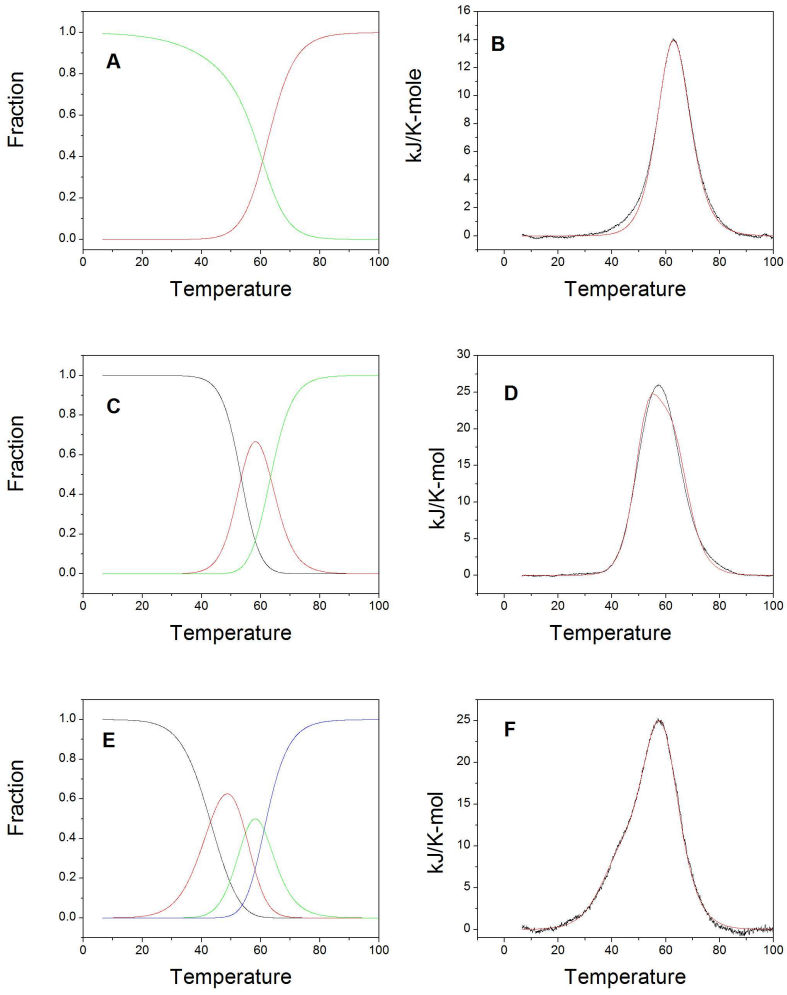
These numbers seem reasonable in light of above monomers.

5. $(TTAGGG)_{12}$
   a. Recursion in this case was straight-forward 2-intermediate states, so was analyzed accordingly.

Results: Δh_1 = 159.1 kJ/mole; Δs_1 = 503 J/K-mole; T_1 = 43.05
Δh_2 = 203.4 " Δs_2 = 620 " T_2 = 54.87
Δh_n = 216.0 " Δs_n = 646 " T_n = 61.15

6. $(TTAGGG)_{12}TT$
   a. Same as trimer above.

Results: Δh_1 = 176.1 kJ/mole; Δs_1 = 553 J/K-mole; T_1 = 45.37
Δh_2 = 221.4 " Δs_2 = 672 " T_2 = 56.30
Δh_3 = 203.5 " Δs_3 = 606 " T_3 = 62.59


A
Fraction
Temperature
B
kJ/K-mole
Temperature
C
Fraction
Temperature
D
kJ/K-mol
Temperature
E
Fraction
Temperature
F
kJ/K-mol
Temperature