



Supplementary Figure S6 - Thermal stability of the G4 structures formed by G4#1 and its mutants

(A) Melting profiles recorded by UV-absorption and by fluorescence were reversible and were not dependent on oligonucleotide strand concentration (2-20 μM for UV-absorption, 0.2-2 μM for fluorescence). The UV-absorption melting profiles of m14 at strand concentrations of 2 μM and 20 μM in KCl (10 mM) are shown as an example. **(B)** CD spectra and analyses of melting transition amplitudes revealed that a large proportion of the G4 structures formed at strand concentrations of 2 μM and 20 μM did not melt, even at 95°C. The CD spectra of m14 (2 μM) in KCl (10 mM) at 20 and 95°C are shown as an example. Overall, these features (reversible, with strand concentration-independent melting transitions) suggest that the observed transitions originate mostly from intramolecular G4, the intermolecular structures being too stable to melt. **(C)** Temperatures of half-dissociation ($T_{1/2}$) determined by fluorescence on double-dye-labeled oligonucleotides (0.2-2 μM) in KCl (10 mM) were consistent with the melting temperatures (T_m) determined by absorbance at 295 nm for unlabeled oligonucleotides (2-20 μM), and were dependent on sequence. Thermal stability is partially correlated with origin activity, except for m6. However, this result does not contradict data obtained by hRPA binding (reported in Figure 7), because thermal stabilities are not necessarily correlated with thermodynamic stabilities at a given temperature. EMSA with hRPA was used to compare the stability of the structures formed at a given temperature and at a lower oligonucleotide strand concentration, at which the fraction of intermolecular G4 was much lower.