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Supporting Information

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for

Plasmodium Telomeric Sequences: Structure, Stability and Quadruplex Targeting by Small Compounds

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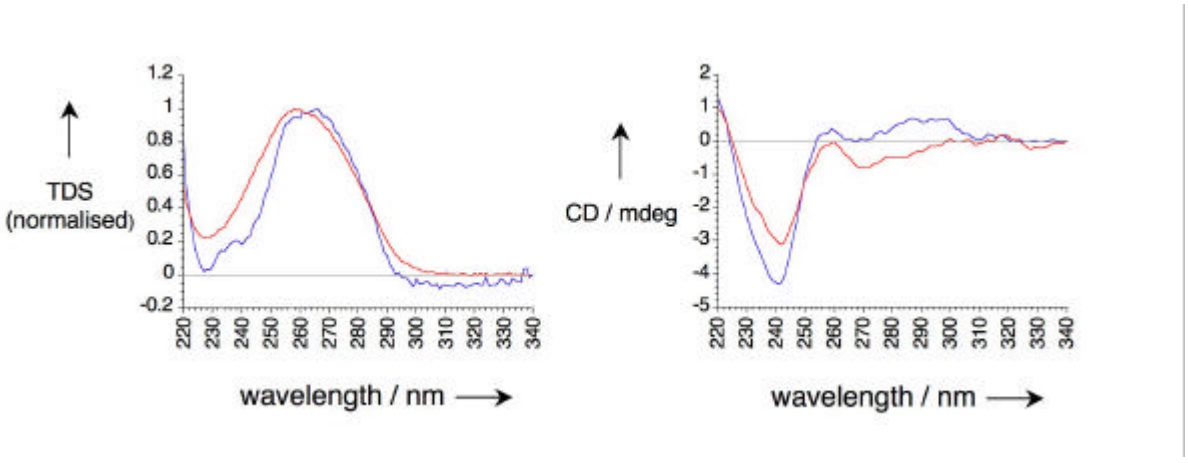

Figure S1: To rule out the possibility of G-probe intermolecular assembly, the G-probe (3 µM) was incubated overnight under the same ionic and temperature conditions of the hybridisation experiments presented in Figure 3 (NaCl 50 mM and $MgCl_2$ 10 mM, 37 °C or 50°C). TDS and CD spectra after overnight incubation (red spectra: 50°C, blue spectra: 37°C) showed that the G-probe did not assembly into G-quadruplex structures, even at a concentration 100-fold greater (3 µM) than the one used in the hybridisation experiments presented in Figure 3 (30 nM).

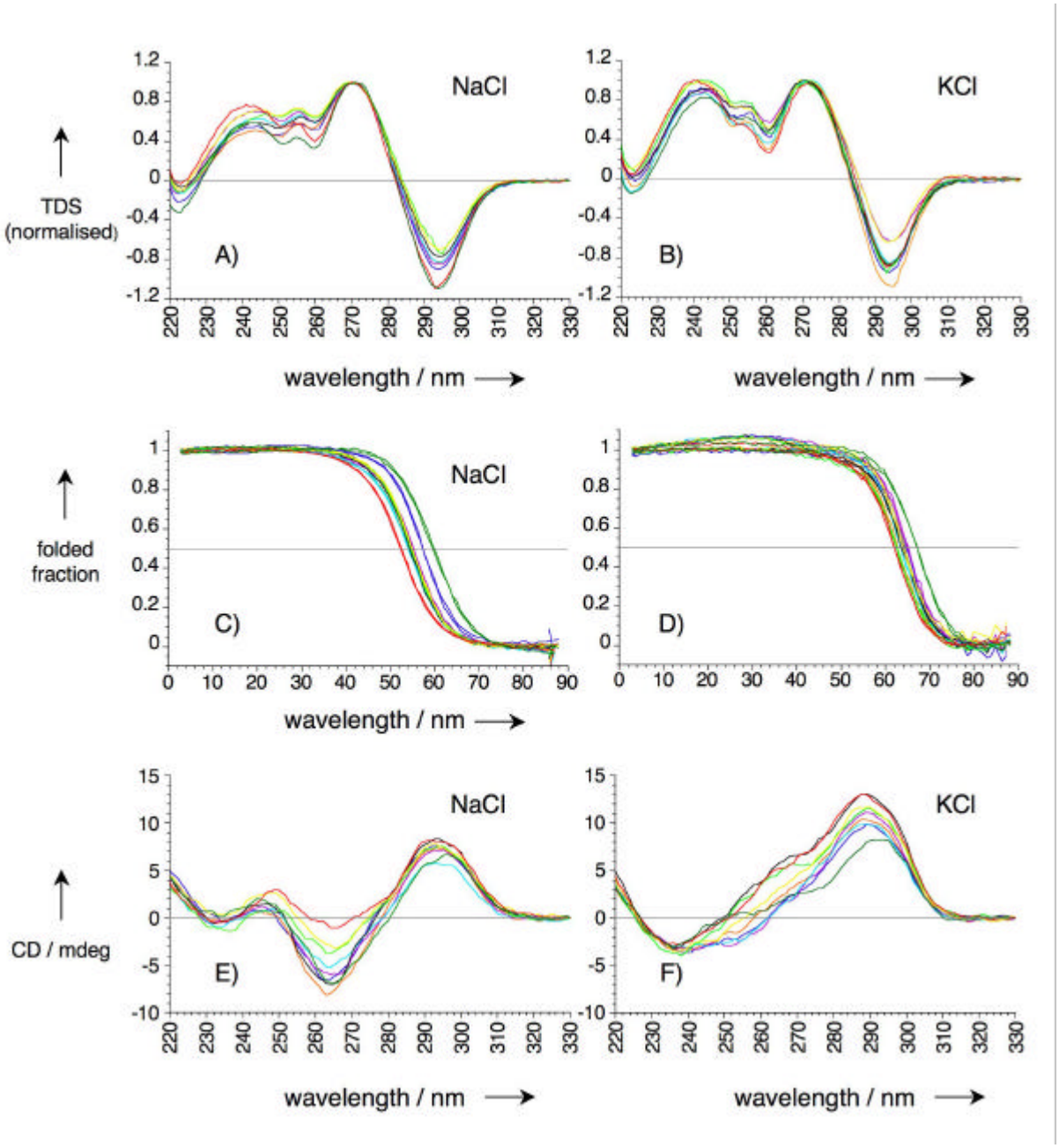

Figure S2: A, B) Normalised thermal difference spectra; C, D) oligonucleotide folded fractions as a function of temperature deduced from melting curves recorded at 295 nm assuming low- and high-temperature linear baseline corrections; E, F) circular dichroism spectra at 20°C of the eight *Plasmodium* telomeric sequences (Pf1, Pf2, Pf3, Pf4, Pf5, Pf6, Pf7, Pf8) and of the human telomeric sequence (21G). All the measurements were carried out at 3 µM oligonucleotide concentration in a cacodylic acid buffer (10 mM) pH 7.2 (adjusted with LiOH) containing NaCl or KCl (100 mM).

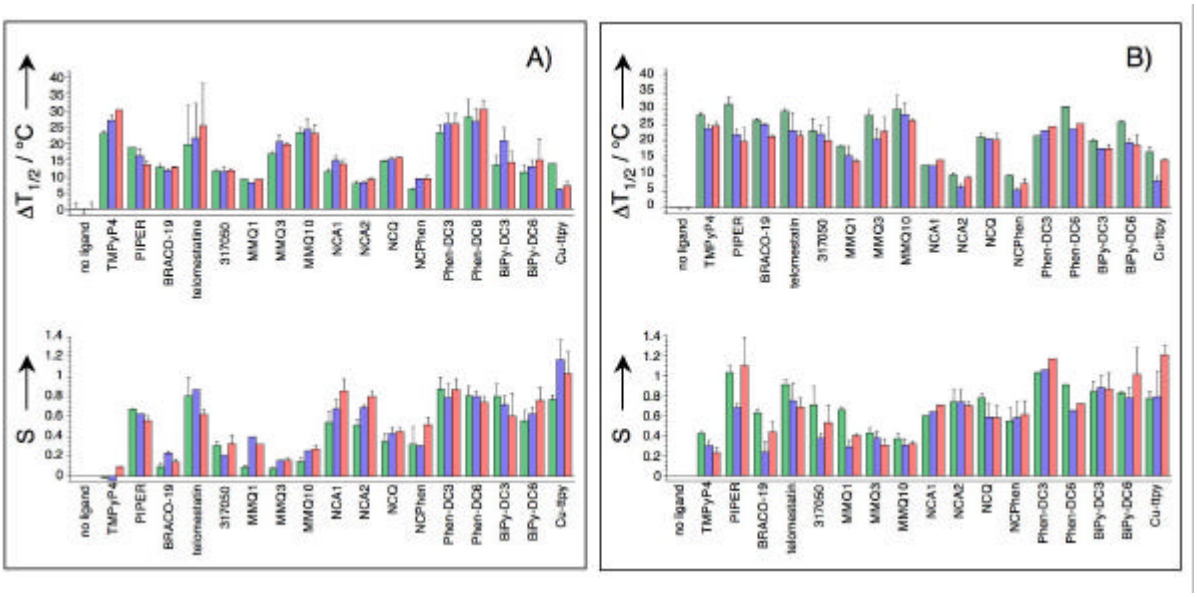

Figure S3: Thermal stabilisation ($\Delta T_{1/2}$) induced by compound (1 µM) on telomeric quadruplexes (0.2 µM) and selectiviy (*S*) in the presence of ds26 (10 µM) , in a cacocylic acid buffer (10 mM) pH 7.2 (adjusted with LiOH) and NaCl (100 mM) (A) or KCl (10 mM)+LiCl (90 mM) (B) (green column: F21GT; blue column: FPf1T; red column: FPf8T).

In NaCl (100 mM) the $T_{1/2}$ of FPf1T, FPf8T and F21GT alone (with no ligand and no competitor) were 43.8°C, 44.7°C and 49.7°C (±1°C), respectively. In KCl (10 mM) + LiCl (90 mM) the $T_{1/2}$ of FPf1T, FPf8T and F21GT were 48.5°C, 48.5°C and 52.7.°C (±1°C), respectively, that is. near the $T_{1/2}$ values in 100 mM NaCl. LiCl was added in order to keep the same ionic strength as in the FRET-melting experiments carried out in 100 mM NaCl. The presence of the duplex competitor ds26 did not affect the melting curves of the three telomeric sequences, neither in NaCl nor in KCl.

The error bars are the standard deviations obtained from several experiments. For telomestatin in NaCl, two different stock solutions were used. These two stocks solution did not induce the same $\Delta T_{1/2}$: a $\Delta T_{1/2}$ of about 58°C were obtained with the first one and of about 78°C with the second one. Despite this strong difference, probably due to telomestatin solubility/stability problems, each stock displayed a strong quadruplex vs. duplex selectivity for the three sequences.

Compared to NaCl, in KCl most of the ligands induced a higher quadruplex stabilisation ($\Delta T_{1/2}$) and displayed a stronger quadruplex vs. duplex selectivity (*S)*. Nevertheless, in KCl as well as in NaCl, all the tested compounds displayed, in the limit of the experimental error, roughly the same stabilisation and selectivity profiles towards the three telomeric sequences.

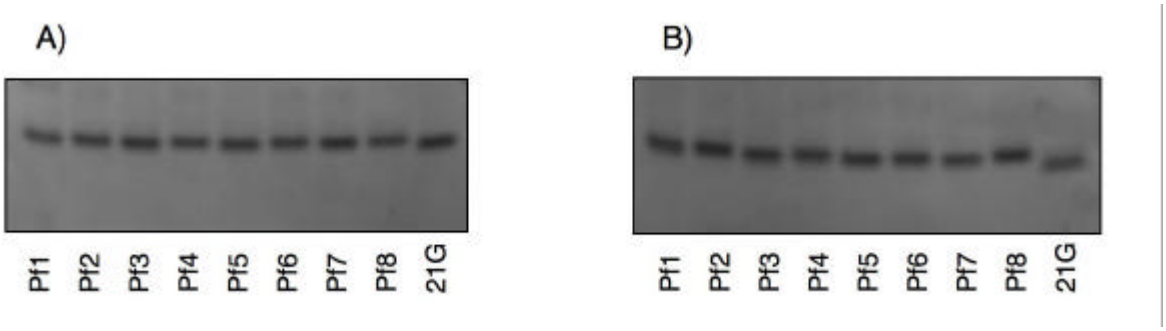

Figure S4: Nondenaturing PAGE of the eight *Plasmodium* telomeric sequences (Pf1 to Pf8) and of the human one (21G) in sodium (A) and in potassium (B). Oligonucleotides (30 µM) were annealed from 92°C to 2°C at a rate of 1°C min^{-1} in a cacodylic acid buffer (10 mM) pH 7.2 (adjusted with LiOH) containing NaCl or KCl (100 mM). Samples (20 µL, 10% sucrose) were loaded on a polyacrylamide gel (acrylamide:bisacrylamide 19:1 12% in TBE 1x and NaCl or KCl 20 mM); after 3 h migration at 4°C in a TBE (1x) buffer containing NaCl or KCl (20 mM), gels were imaged by UV shadow at 254 nm with a G:BOX (Syngene).