Silver Cations Fold i-Motif DNA at Neutral pH

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SUPPORTING INFORMATION for *Chem. Commun.*

Contents

1. GENERAL EXPERIMENTAL

All the oligonucleotides and their fluorescent conjugates were purchased from Eurogentec and were HPLC purified. Solid DNA samples were initially dissolved as a stock solution in MilliQ water (100 µM for hTeloC$_{FRET}$, 1 mM for hTeloC and hairpinC) and further dilutions were carried out in the appropriate buffer. Sodium cacodylate buffer was chosen as the pH stays constant when varying the temperature; it has also been the buffer of choice for previous i-motif studies.[1, 2] Annealed samples were thermally annealed by heating in a heat block at 95°C for 5 minutes and were allowed to cool slowly to room temperature overnight. Non-annealed samples had the DNA diluted into the respective buffer and were used immediately.

2. FRET MELTING SCREEN

The ability of Ag^+ to stabilise an i-motif forming DNA sequence was investigated using a fluorescence resonance energy transfer (FRET) assay. The labelled oligonucleotide $hTeloC_{FRET}$ (5'-FAM-d[TAACCCTAACCCTAACCCTAACCC]-TAMRA-3'; donor fluorophore FAM is 6-carboxyfluorescein; acceptor fluorophore TAMRA is 6-carboxytetramethyl-rhodamine) was prepared as a 400 nM solution in a 10 mM sodium cacodylate buffer (pH 7.4 or 5.5) with 5 mM NaCl and then thermally annealed. $AgNO_3$ and $NaNO_3$ (Sigma) were dissolved in the same respective buffer. Strip-tubes (QIAgen) were prepared by aliquoting 10 µL of the annealed DNA, followed by 10 µL of the $AgNO_3$ solutions. Fluorescence melting curves were determined in a QIAgen Rotor-Gene Q-series PCR machine, using a total reaction volume of 20 µL. Measurements were made with excitation at 483 nm and detection at 533 nm. Final analysis of the data was carried out using QIAgen Rotor-Gene Q-series software and Origin.

The FRET melting experiments monitor the emission of the donor fluorophore (FAM) as the temperature of the sample is increased. When the DNA is folded, the fluorophores are close together and FAM is quenched; as the temperature increases, the DNA melts, FAM and TAMRA move further apart and FAM is no longer quenched. This results in an increase in the fluorescence of the donor fluorophore FAM; we can use these signals to indicate how much of the sample is folded and also monitor DNA melting.

FRET Melting at pH 7.4

At pH 7.4, $hTeloC_{FRET}$ is unfolded at room temperature, this is indicated in our FRET experiments by high fluorescence signal (ie low FRET as the fluorophores are far apart). Figure S1 shows representative melting curves for hTeloC at pH 7.4 in the presence of 0-100 µM Ag^+. At low concentration (0.5 µM) there is little effect on the stability of the DNA, all the DNA populations in solution are unfolded at room temperature (T_m < 20°C). At 1 µM Ag^+ concentration the initial fluorescence intensity at the start of the experiment is much lower compared to the DNA sample in the absence of Ag^+. This indicates the fluorophores are closer together (ie there is more FRET), at this concentration of Ag^+ the melting temperature of the DNA is observed to be ~60°C. As the temperature increases, a gradual increase in fluorescence is also observed, indicative of melting. At 5 µM the sample is quenched until

~70°C and then the fluorescence increases until upper limit of 95°C. This is indicative that 5 µM of Ag^+ stabilises the DNA structure enough for it not to fully melt within the experimental conditions (T_m > 95°C). 10-100 µM of Ag^+ is enough to quench FAM for the whole experiment, indicative that a concentration of 10 µM of Ag^+ stabilises the DNA structure enough for it not to melt in our buffer conditions. Further melting experiments using 1, 1.5, 2, 3 and 4 µM Ag^+ gave melting temperatures of 55, 60, 68, 74 and 80°C respectively (Figure S2).

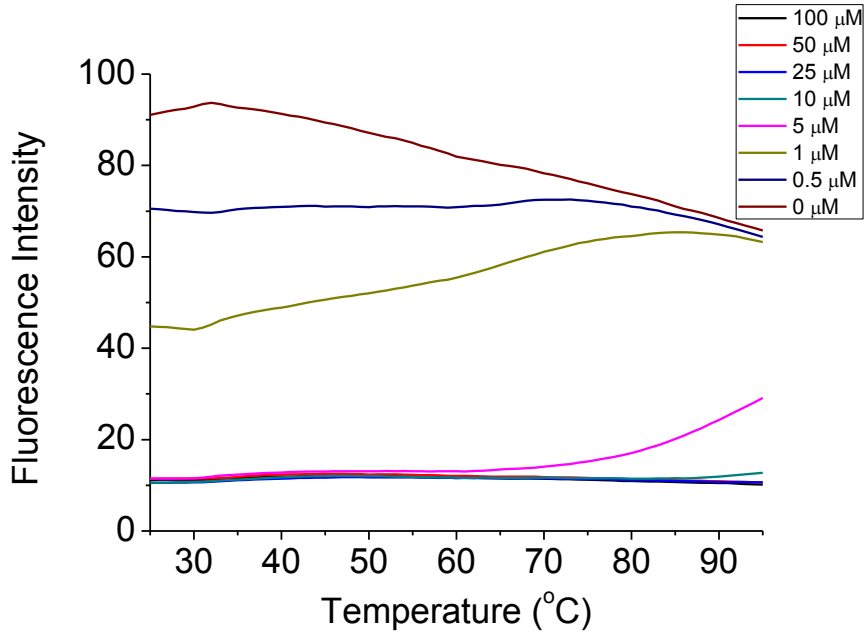

Figure S1: Representative FRET melting experiments of 0.2 µM hTeloC$_{FRET}$ in 10 mM sodium cacodylate buffer, pH 7.4 supplemented with 5 mM of NaCl and between 0 and 100 µM of $AgNO_3$

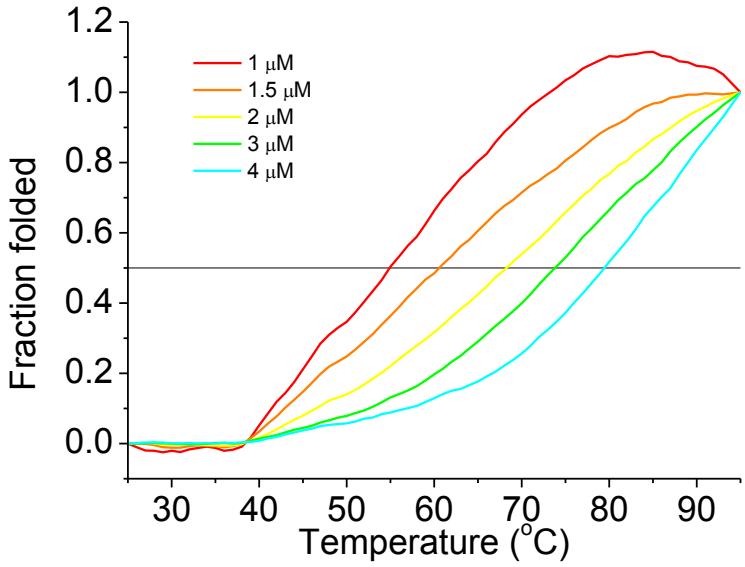

Figure S2: Representative FRET melting experiments of 0.2 μM hTeloC$_{FRET}$ in 10 mM sodium cacodylate buffer, pH 7.4 supplemented with 5 mM of NaCl and between 0 and 4 μM of $AgNO_3$

FRET Melting at pH 5.5

At pH 5.5, hTeloC$_{FRET}$ is folded at room temperature, this is indicated in our FRET experiment by low fluorescence signal (ie FAM is quenched). Figure S3 shows representative melting curves for i-motif DNA at pH 5.5 in the presence of 0-100 μM $AgNO_3$. At low concentrations (0-1 μM Ag^+) there is little effect on the stability of the i-motif DNA, the melting temperature (T_m) is 46°C. At concentrations of 5 μM Ag^+ and above there are two melting transitions, one at 46°C and another at a higher temperature, indicating two different populations which have different stability. This is consistent with an acid-stabilised population and an additional population stabilised by Ag^+.

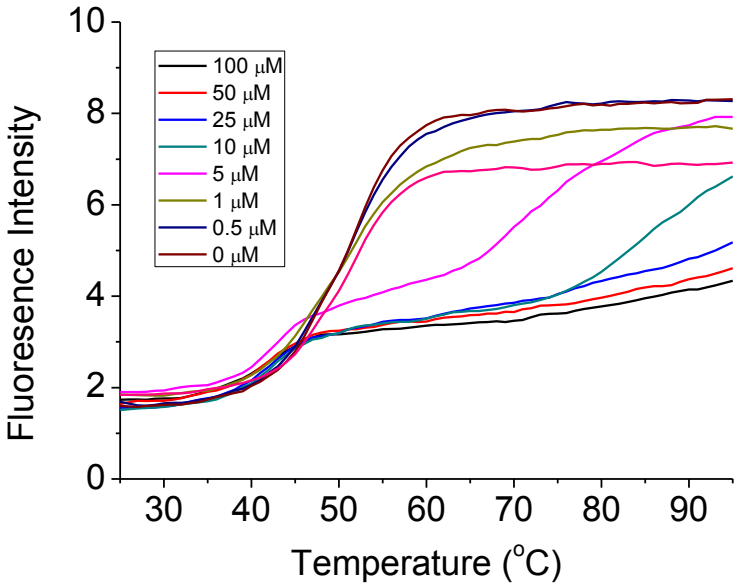

Figure S3: Representative FRET melting experiments of 0.2 μM hTeloC$_{FRET}$ in 10 mM sodium cacodylate buffer, pH 5.5 supplemented with 5 mM of NaCl and between 0 and 100 μM of AgNO$_3$

Control FRET Experiments

To rule out the contribution of the nitrate anions we performed all the experiments using $NaNO_3$ and found no significant effect on the DNA stability (Figure S4 and S5). To rule out any potential quenching effects by silver cations with the fluorophores, we also performed a melting experiment with a DNA sequence capable of forming a duplex[3] (ds$_{FRET}$, 5'-FAM-TATAGCTATA-HEG-TATAGCTATA-TAMRA-3') in the presence and absence of 100 μM Ag^+. There were no significant differences between the samples (Figure S6), indicating that Ag^+ do not interfere with the fluorphores.

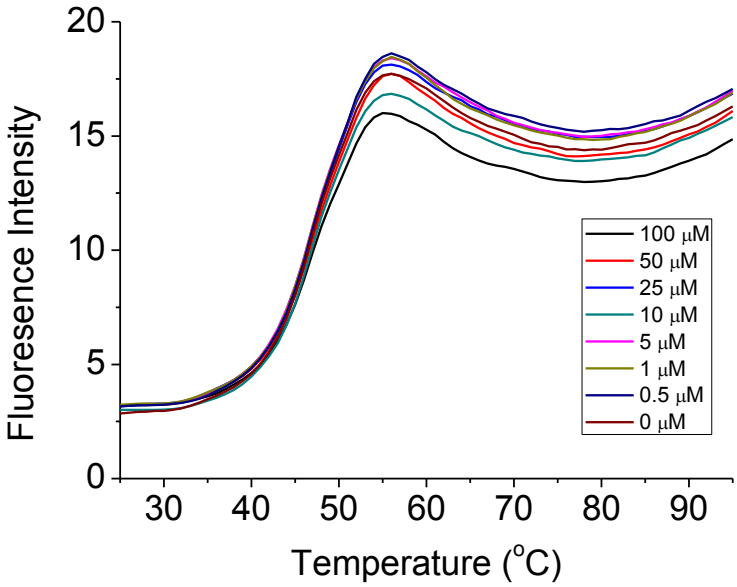

Figure S4: Representative FRET melting experiments of 0.2 µM hTeloC$_{FRET}$ in 10 mM sodium cacodylate buffer, pH 5.5 supplemented with 5 mM of NaCl and between 0 and 100 µM of NaNO$_3$

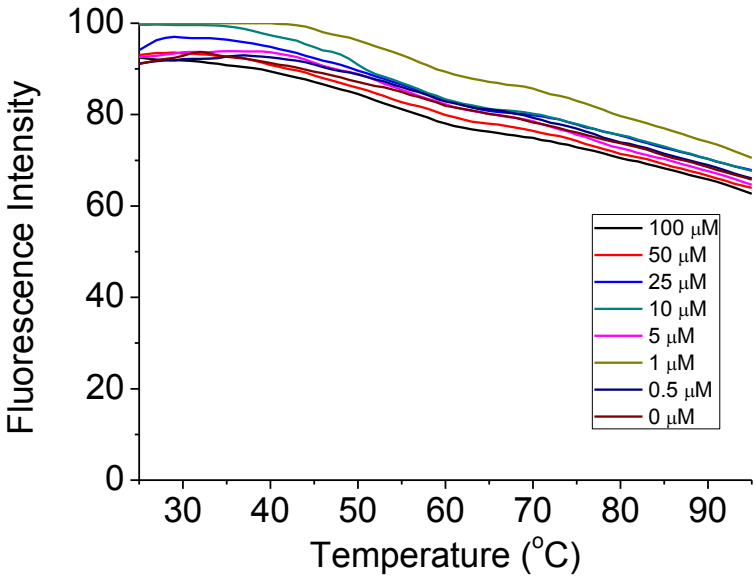

Figure S5: Representative FRET melting experiments of 0.2 µM hTeloC$_{FRET}$ in 10 mM sodium cacodylate buffer, pH 7.4 supplemented with 5 mM of NaCl and between 0 and 100 µM of NaNO$_3$

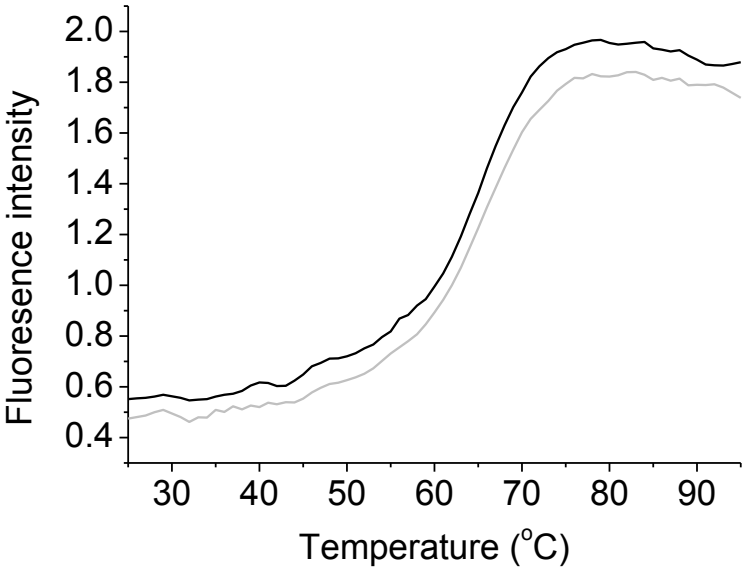

Figure S6: Representative FRET melting experiments of 0.2 µM ds_{FRET} in 10 mM sodium cacodylate buffer, pH 7.4 supplemented with 100 mM of NaCl (black) and 100 µM of $AgNO_3$ (grey).

3. CIRCULAR DICHROISM EXPERIMENTS

Circular dichroism (CD) spectra were recorded on a Jasco J-810 spectropolarimeter using a 1 mm path length quartz cuvette. Human telomeric i-motif forming DNA sequence (hTeloC, 5'-[TAACCCTAACCCTAACCCTAACCC]-3') was diluted in a buffer containing sodium cacodylate (10 mM, pH 7.4 or 5.5) and NaCl (5 mM) to achieve a total volume of 200 µL. The scans were performed at 20°C over a wavelength range of 220-320 nm with a scanning speed of 200 nm/min, a response time of 1 s, 0.5 nm pitch and 2 nm bandwidth. A blank sample containing only buffer was treated in the same manner and subtracted from the collected data. Solutions of $AgNO_3$ and L-cysteine were added in small aliquots to the desired equivalent proportions using a Hamilton syringe. The CD spectra represent an average of three scans and are zero corrected at 320 nm. Final analysis and manipulation of the data was carried out using Origin.

CD at pH 5.5

The CD spectrum of hTeloC in pH 5.5 buffer shows a negative signal at 255 nm and a positive signal at 288 nm, indicative of folded i-motif structure. hTeloC in pH 5.5 buffer with 100 µM

$AgNO_3$ (either annealed in the presence or added directly) shows slightly reduced ellipticity at 288 nm (Figure S7), indicative that the population of acid-stabilised i-motif is slightly reduced. No further significant changes were observed, indicating that Ag^+ has little effect on the structure of acid-stabilised i-motif.

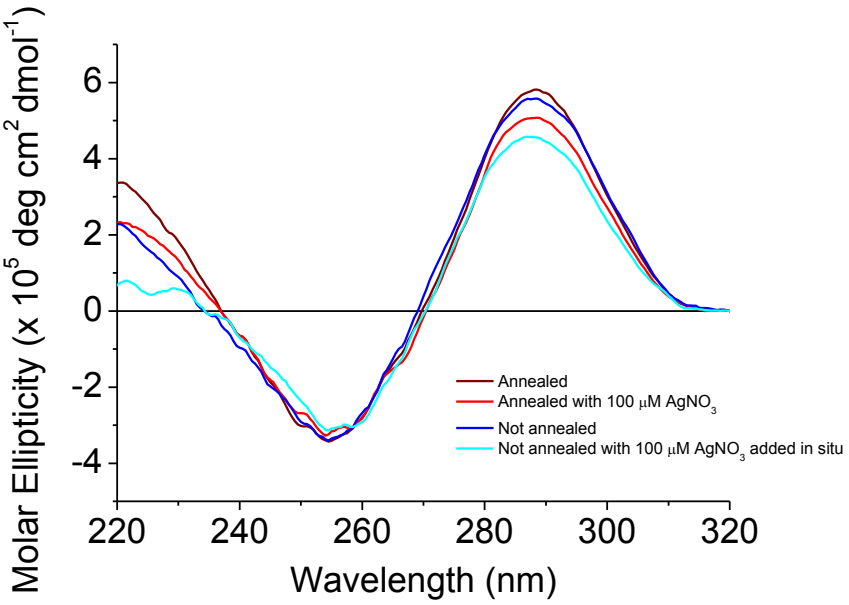

Figure S7: CD spectra of 10 µM hTeloC in 10 mM sodium cacodylate buffer, pH 5.5 supplemented with 5 mM of NaCl and between 0 and 100 µM of $AgNO_3$ as detailed

CD at pH 7.4

The CD spectrum of hTeloC annealed in pH 7.4 buffer shows a negative signal at 250 nm and a positive signal at 275 nm (Figure S8), indicative of unfolded DNA in a random coil structure. hTeloC in pH 7.4 buffer with 100 µM $AgNO_3$ (either annealed in the presence or added directly) shows a completely different spectrum (Figure S8). The signals at -260 nm and +288 nm are consistent with formation of a folded secondary structure which has characteristics similar to i-motif. The spectrum also displays similarity with that of a zinc-specific anti-parallel homoduplex,[4] the structure of which is yet unknown.

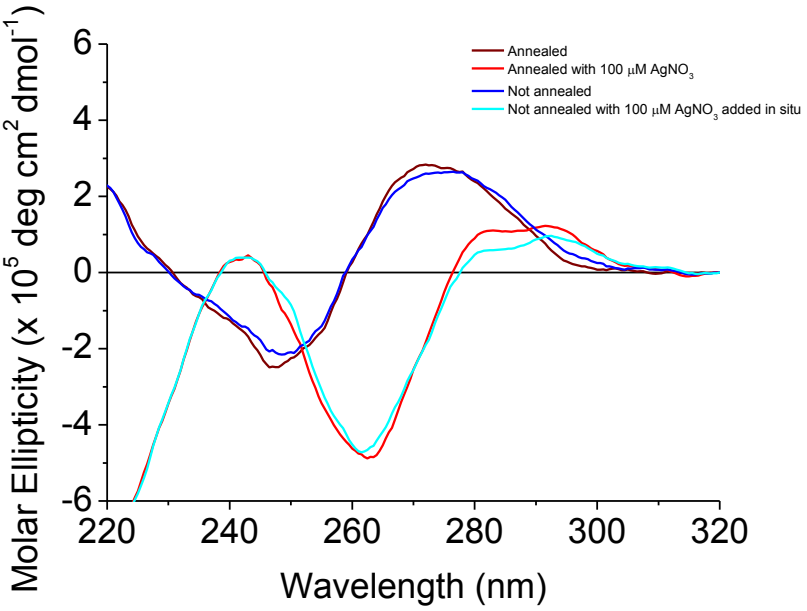

Figure S8: CD spectra of 10 µM hTeloC in 10 mM sodium cacodylate buffer, pH 7.4 supplemented with 5 mM of NaCl and between 0 and 100 µM of $AgNO_3$ as detailed

Determination of Stoichiometry using CD

Stoichiometry was determined using the method of continuous variation binding analysis. 200 µL samples containing hTeloC DNA with Ag^+ were made up in pH 7.4 buffer. The individual concentrations of DNA and Ag^+ varied between 0 and 20 µM but the total concentration of DNA + Ag^+ remained constant at 20 µM. Samples with identical DNA concentrations between 0 and 20 µM but without any Ag^+ were used as controls. Plotting the change in CD signal at 275 nm between samples containing just DNA and those with DNA and Ag^+ versus the mole fraction of DNA gave a Job plot with an inflection point directly related to the stoichiometry. The stoichiometry was determined by fitting the two parts of the data with straight lines and solving their equations to find their point of intersection. This mole fraction was then converted into a DNA concentration and hence an Ag:DNA ratio, which was found to be 4:1 Ag^+:DNA (Figure S9).

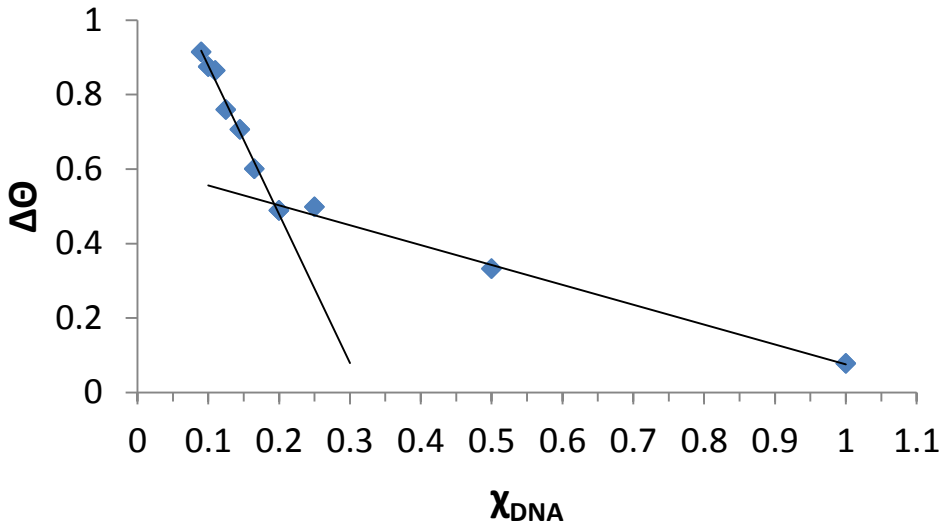

Figure S9: Job plot of hTeloC and $AgNO_3$ in 10 mM sodium cacodylate buffer, pH 7.4 supplemented with 5 mM of NaCl

CD with Silver Cations and Cysteine

To investigate the effect of cysteine on the Ag^+-stabilised DNA structure CD titrations with up to 100 µM (10 eq) of Ag^+ (Figure S10) followed by 100 µM (10 eq) of cysteine (Figure S11) were performed.

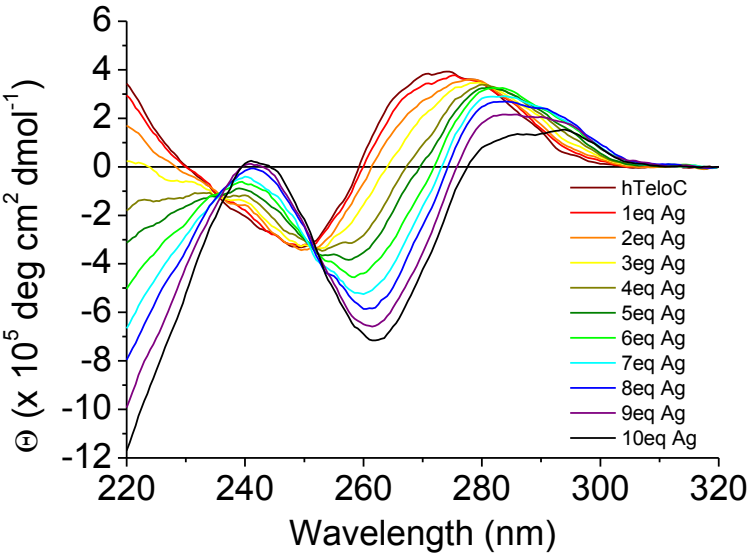

Figure S10: CD spectra of 10 µM hTeloC in 10 mM sodium cacodylate buffer, pH 7.4 supplemented with 5 mM of NaCl and between 0 and 100 µM of $AgNO_3$ as detailed

On addition of up to 100 µM (10 eq) of Ag^+, there are spectral changes indicative of formation of a folded structure. When the reverse titration with cysteine was performed, it indicated complete reversibility. We then analysed the effect of sequential additions of Ag^+ and cysteine, by monitoring the CD signals at 250 and 261 nm, to show that multiple cycling is possible (Figures S12 and S13). These results showed that the formation of the Ag^+-stabilised structure is reversible on addition of the same number of equivalents of cysteine.

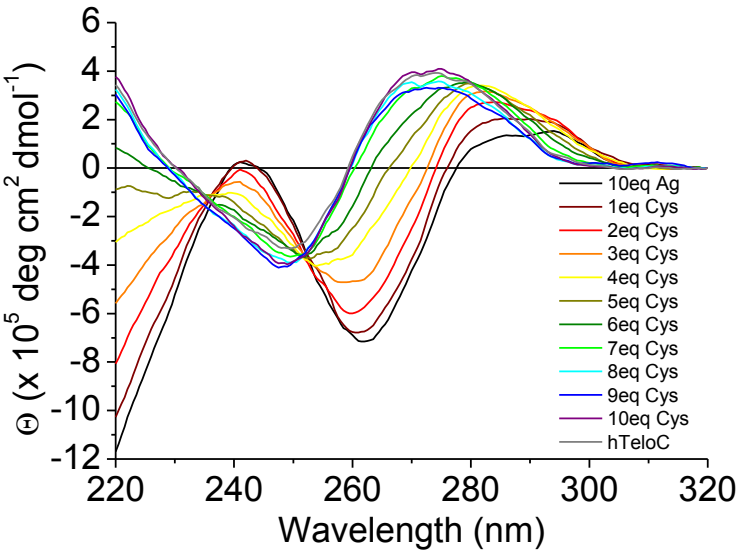

Figure S11: CD spectra of 10 µM hTeloC in 10 mM sodium cacodylate buffer, pH 7.4 supplemented with 5 mM of NaCl, 100 µM of $AgNO_3$, and between 0 and 100 µM of cysteine as detailed

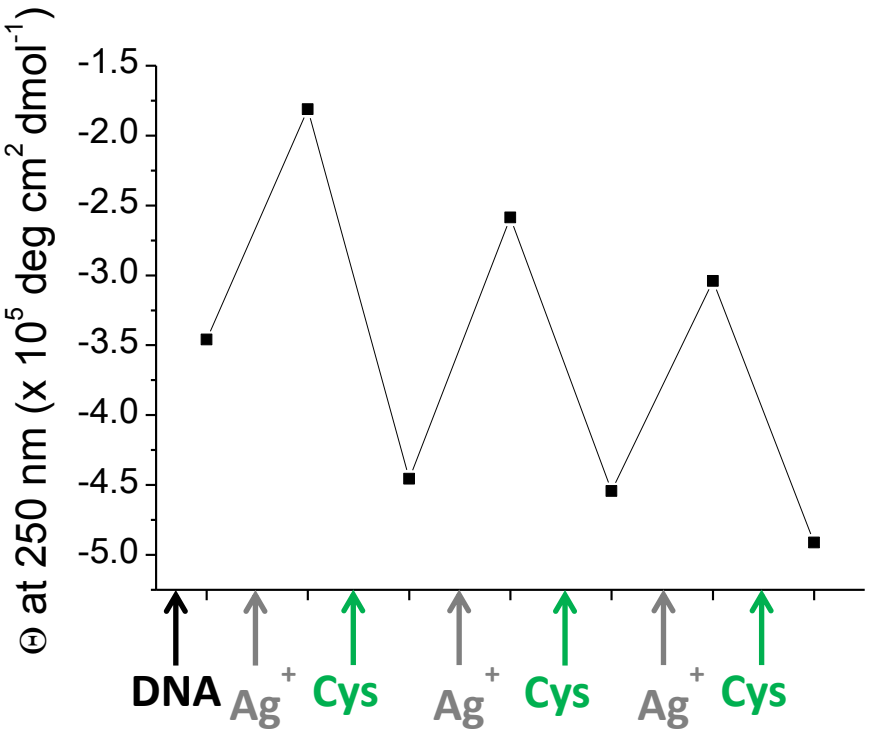

Figure S12: Folding/unfolding of 10 µM hTeloC in 10 mM sodium cacodylate buffer with 5 mM NaCl at pH 7.4 monitored by the ellipticity at 250 nm. Arrows on the x-axis indicate the addition of Ag^+ or cysteine.

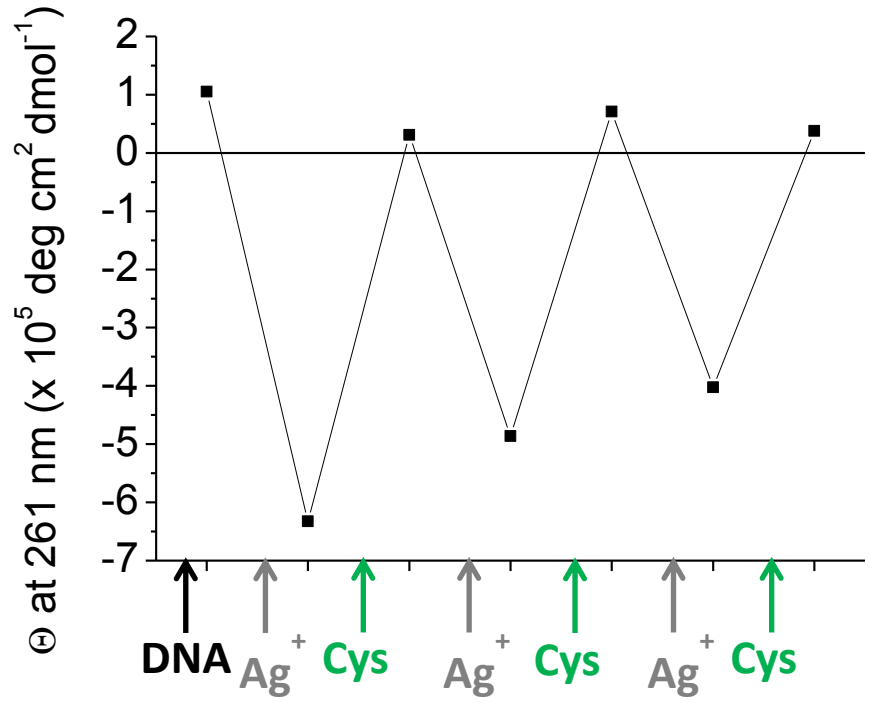

Figure S13: Folding/unfolding of 10 µM hTeloC in 10 mM sodium cacodylate buffer with 5 mM NaCl at pH 7.4 monitored by the ellipticity at 261 nm. Arrows on the x-axis indicate the addition of Ag^+ or cysteine.

4. UV DIFFERENCE EXPERIMENTS

UV spectroscopy experiments were performed on a Hitachi U-3010 spectrophotometer and recorded using a low volume quartz cuvette. Human telomeric i-motif (hTeloC, 5'-[TAACCCTAACCCTAACCCTAACCC]-3') or our control sequence capable of forming a hairpin (hairpinC, 5'-[CTCTCTTCTCTTCATTTTTCAACACAACACAC]-3') was diluted in a buffer containing sodium cacodylate (10 mM, pH 7.4) and NaCl (5 mM) to achieve a concentration of 2.5 μM and a total volume of 200 μL. The DNA was not annealed and used immediately after dilution. A solution of $AgNO_3$ was added in small aliquots up to 6 eq of $AgNO_3$ using a Hamilton syringe. Spectra were recorded over a wavelength range of 400 - 200 nm at 20 °C in the absence of any $AgNO_3$ and then in the presence of up to 6 eq of $AgNO_3$. Spectra of the buffer and $AgNO_3$ were recorded and subtracted from the spectra of the DNA. The difference spectrum was calculated by subtraction of the folded (in the presence of $AgNO_3$) spectrum from the unfolded spectrum (in the absence of $AgNO_3$) and normalised so the maximum change in absorption was set to +1 as previously described.[5]

5. FRET TITRATION EXPERIMENTS

Fluorescence titration experiments were performed on a Perkin-Elmer LS-55 fluorescence spectrometer and recorded using a 1 cm path length quartz cuvette. Dual-labelled human telomeric i-motif ($hTeloC_{FRET}$, 5'-FAM-d[TAACCCTAACCCTAACCCTAACCC]-TAMRA-3') was diluted in a buffer containing sodium cacodylate (10 mM, pH 7.4) and NaCl (5 mM) to achieve a concentration of 0.1 μM and a total volume of 200 μL. The DNA was not annealed and used immediately after dilution. Solutions of $AgNO_3$ and L-cysteine were added in small aliquots to the desired equivalent proportions using a Hamilton syringe. The overall dilution was less than 10%. Excitation of the sample was at 490 nm and the emission spectra were recorded over a wavelength range of 500 - 650 nm at 20 °C. The excitation and emission slits were open at a width of 5 nm. The FRET efficiency (E_{FRET}) was calculated by $1-(FI_d/FI_d^0)$ where FI_d^0 is the fluorescence intensity of the donor in the absence of the acceptor. The experiment was performed in triplicate and the error bars represent the standard deviation.

Addition of 5 μM of Ag^+ to $hTeloC_{FRET}$ caused an increase in E_{FRET} (see main article) but further addition of 0 - 5 μM of cysteine to the Ag^+-folded DNA caused a decrease in E_{FRET} (Figure S14). These results are consistent with unfolding of the structure induced by chelation of the Ag^+ by cysteine.

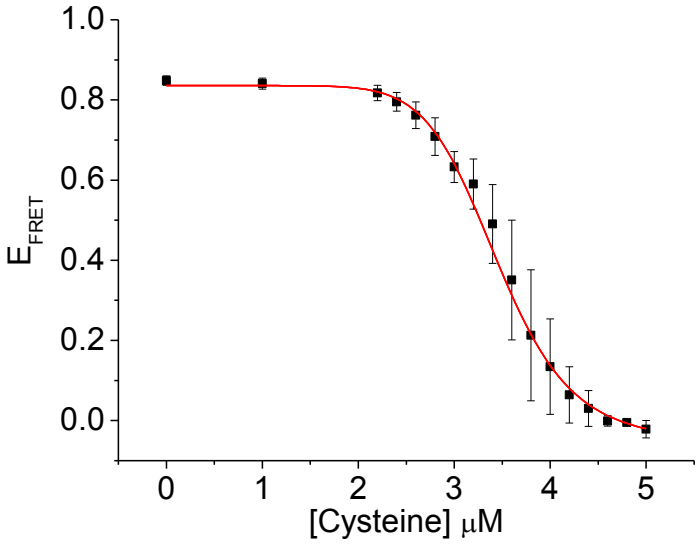

Figure S14: FRET efficiency of 0.1 μM $hTeloC_{FRET}$ in 10 mM sodium cacodylate buffer with 5 mM NaCl at pH 7.4 and 5 μM of $AgNO_3$ with 0 - 5 μM of cysteine titrated in. The error bars represent the standard deviation from the average of three experiments.

6. SUPPORTING INFORMATION REFERENCES

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