

Mercury-Thymine interaction with a chair-type G-quadruplex architecture

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SUPPORTING INFORMATION

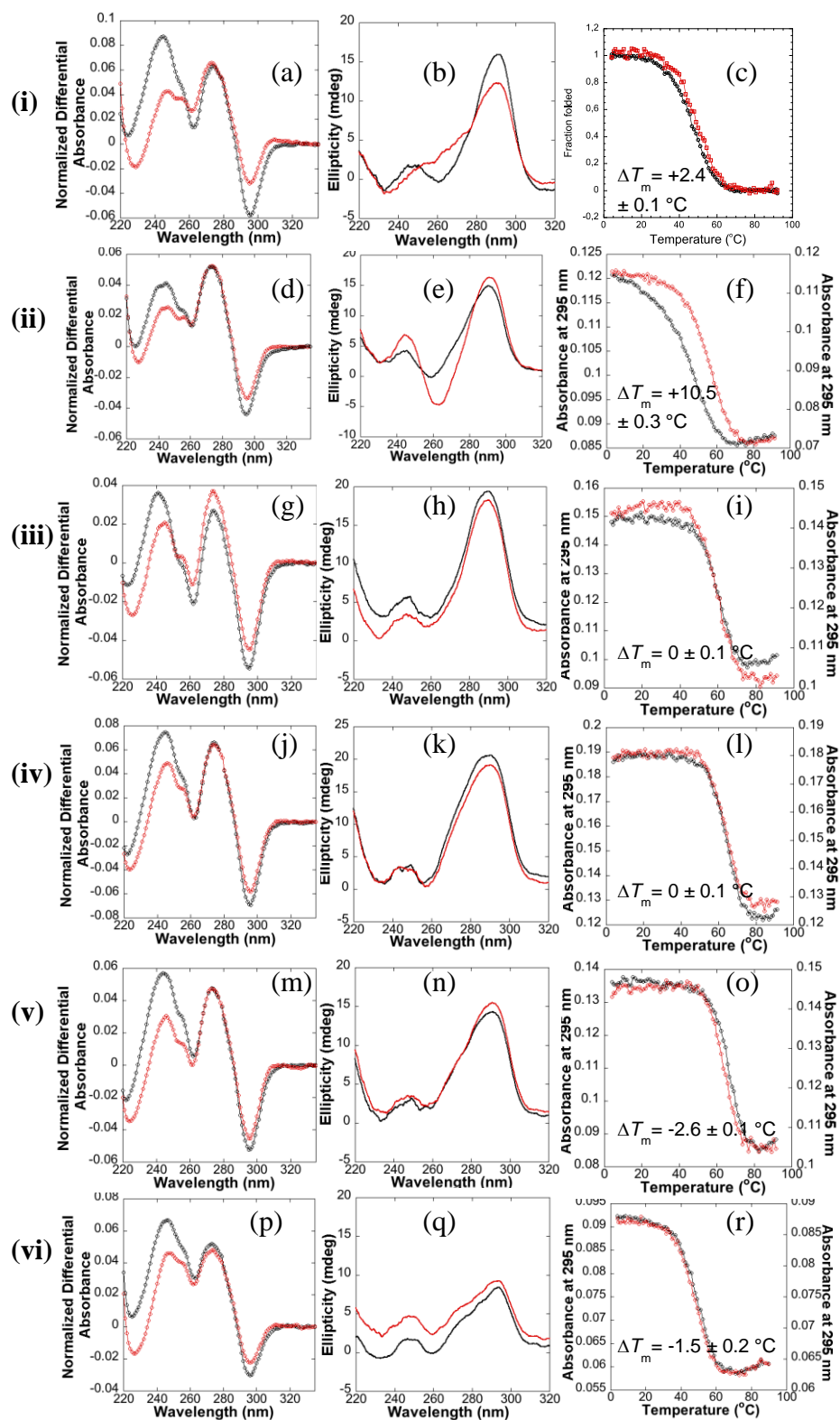
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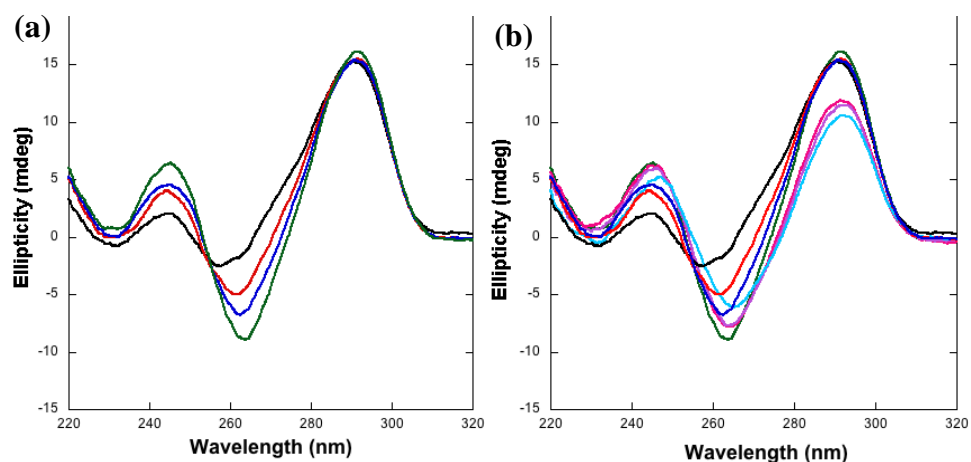
1. TDS, CD and UV Melts Analysis

Sample Preparation

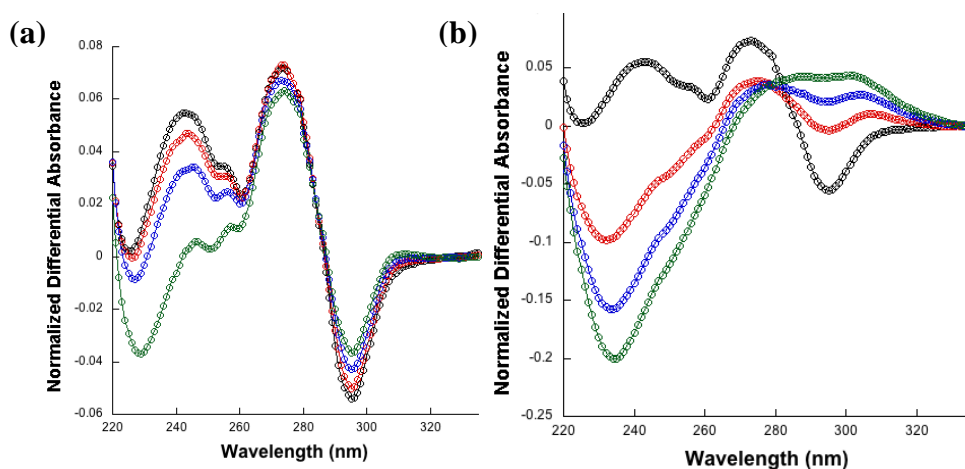
All oligonucleotides were purchased from Eurogentec (Belgium). Stock solutions were made up by diluting the oligonucleotides in purified water to obtain an initial concentration of 100 μM . HgCl_2 was stored as a 0.2 mM stock solution in purified water. All oligonucleotides were then further diluted to a final concentration of 4 μM in the relevant buffer in the presence or absence of HgCl_2 (1 equiv. or 1.5 equiv.) For the CAT_CAT, CAT_AAT, AAT_AAT and CAA_CAA sequences the buffer used was a 10 mM K^+ buffer which consisted of lithium cacodylate (10 mM) at pH 7.2, LiCl (90 mM) and KCl (10 mM). For all other sequences the buffer used was a 70 mM K^+ buffer containing potassium phosphate (20 mM) at pH 7.0 and KCl (70 mM). The solutions were then heated at 90 °C for 10 min and cooled on ice for 2 h before conducting TDS, CD and UV melt experiments. All experiments were conducted in triplicates and the reported ΔT_m values are an average of at least 3 experiments on Uvikon XS/XL spectrophotometers equipped with 2×6 thermostated cuvette holders.



Supplementary Figure 1: Analysis of (i) AAT_AAT (ii) CAT_CAT (iii) TAA_AAT (iv) AAT_TAA (v) TAA_TAA and (vi) CAA_CAA. (a,d,g,j,m,p) TDS in the absence (black) and presence of Hg^{2+} (red). (b,e,h,k,n,q) CD profiles in the absence (black) and presence of Hg^{2+} (red). (c,f,i,l,o,r) Melting curves in the absence (black) and presence of Hg^{2+} (red). In all cases, G4-DNA concentration was 4 μM . For AAT_AAT, CAT_CAT and CAA_CAA Hg^{2+} concentration was 1 equiv (4 μM) in lithium cacodylate buffer (pH 7.2) with 10 mM KCl. For TAA_AAT, AAT_TAA and TAA_TAA Hg^{2+} concentration was 1.5 equiv (6 μM) in potassium phosphate buffer (pH 7.0) with 70 mM KCl.



Supplementary Figure 2: CD titration profile of CAT_CAT (4 μM) in lithium cacodylate buffer (pH 7.2) with 10 mM KCl with various concentrations of Hg^{2+} : **(a)** black (0 equiv.), red (0.5 equiv.), blue (1.0 equiv.), green (2 equiv.). **(b)** black (0 equiv.), red (0.5 equiv.), blue (1.0 equiv.), green (2 equiv.) pink (4 equiv.) light purple (6 equiv.) and light blue (8 equiv.).



Supplementary Figure 3: TDS profile of CAT (4 μM) in lithium cacodylate buffer (pH 7.2) with 10 mM KCl with various concentrations of Hg^{2+} : **(a)** black (0 equiv.), red (0.5 equiv.), blue (1.0 equiv.), green (2 equiv.). **(b)** black (0 equiv.), red (4 equiv.), blue (6 equiv.), green (8 equiv.).

Supplementary Table 1: Sequences and ΔT_m values for designed G4-DNA sequences (4 μ M) in the presence of Hg^{2+} .

Name	Sequence	ΔT_m
CAT_CAT	G ₃ CAT G ₃ CA G ₃ CAT G ₃	+4.2 ± 0.3 °C (0.5 eq. Hg^{2+}) ^[a] +10.5 ± 0.3 °C (1 eq. Hg^{2+}) ^[a] +12.8 ± 0.3 °C (2 eq. Hg^{2+}) ^[a]
CAT_AAT	G ₃ CAT G ₃ CA G ₃ AAT G ₃	+5.0 ± 0.3 °C (1 eq. Hg^{2+}) ^[a] +7.6 ± 0.3 °C (1.5 eq. Hg^{2+}) ^[a]
AAT_AAT	G ₃ AAT G ₃ CA G ₃ AAT G ₃	+2.4 ± 0.1 °C (1 eq. Hg^{2+}) ^[a] +2.6 ± 0.1 °C (1.5 eq. Hg^{2+}) ^[a]
AAT_CAT	G ₃ AAT G ₃ CA G ₃ CAT G ₃	Not done
ATA_ATA	G ₃ ATA G ₃ CA G ₃ ATA G ₃	+4.0 ± 0.1 °C (1.5 eq. Hg^{2+}) ^[b]
TAA_AAT	G ₃ TAA G ₃ CA G ₃ AAT G ₃	+0.0 ± 0.1 °C (1.5 eq. Hg^{2+}) ^[b]
AAT_TAA	G ₃ AAT G ₃ CA G ₃ TAA G ₃	+0.0 ± 0.1 °C (1.5 eq. Hg^{2+}) ^[b]
TAA_TAA	G ₃ TAA G ₃ CA G ₃ TAA G ₃	-2.6 ± 0.1 °C (1.5 eq. Hg^{2+}) ^[b]
CAA_CAA	G ₃ CAA G ₃ CA G ₃ CAA G ₃	-1.5 ± 0.2 °C (1 eq. Hg^{2+}) ^[a]

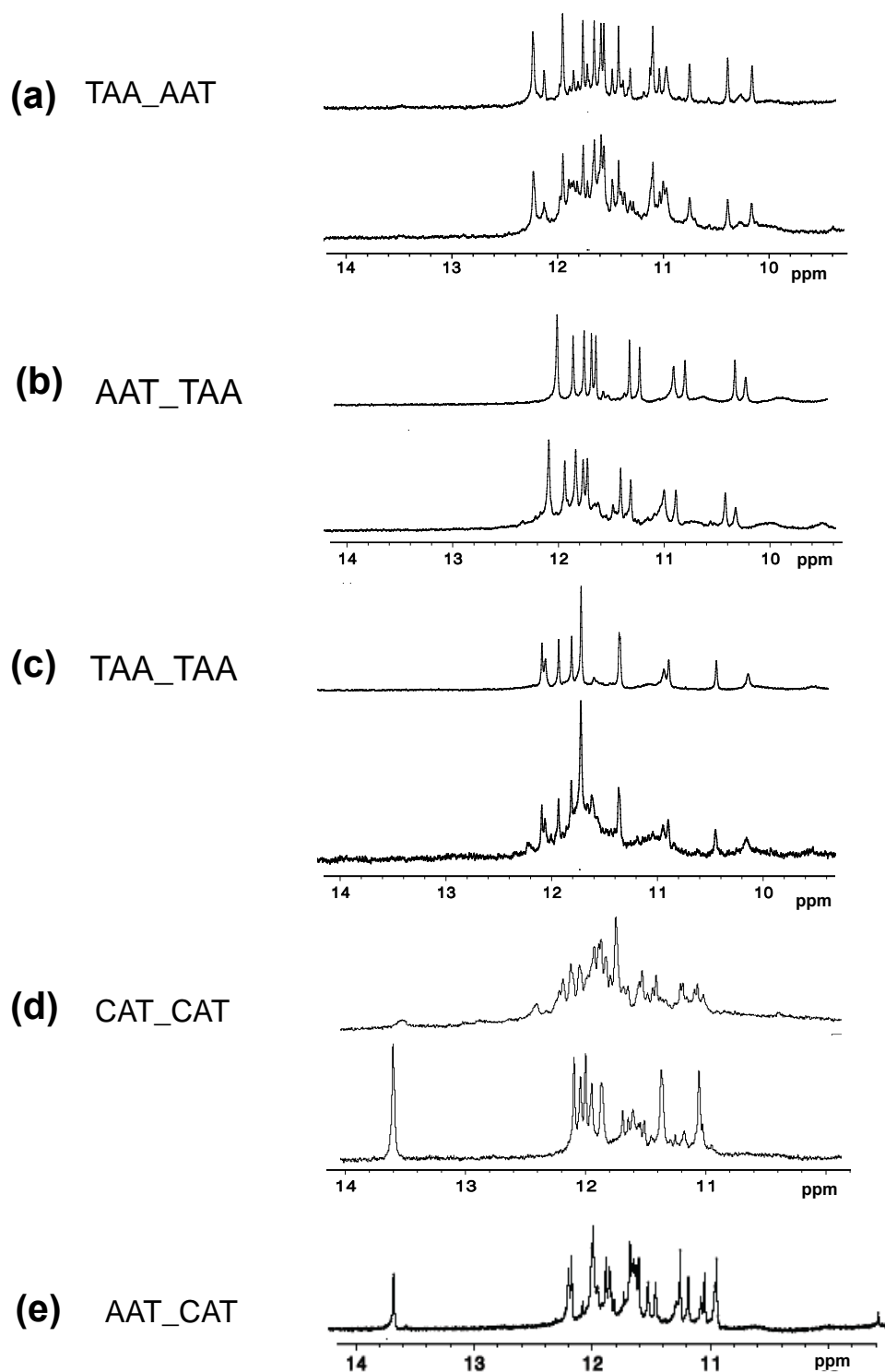
^[a]Melting experiments were conducted in lithium cacodylate buffer (pH 7.2) with 10 mM KCl

^[b]Melting experiments were conducted in potassium phosphate buffer (pH 7.0) with 70 mM KCl

2. NMR Analysis

Sample Preparation

All oligonucleotides were purchased from Eurogentec (Belgium). HgCl_2 was stored as a 20 mM stock solution in purified water. NMR samples were made up by dissolving the oligonucleotides (in the presence or absence of HgCl_2 (1.5 equiv.)) in a 70 mM K^+ buffer containing potassium phosphate (20 mM) at pH 7.0 and KCl (70 mM). The solutions were then heated at 90 °C for 10 min and cooled on ice for 2 h. D_2O (5 %) was added to the solutions before conducting the NMR experiments. The ^1H 1DJR NMR experiments were performed at 298K on a 700 MHz Bruker NMR spectrometer equipped with a TXI probe.

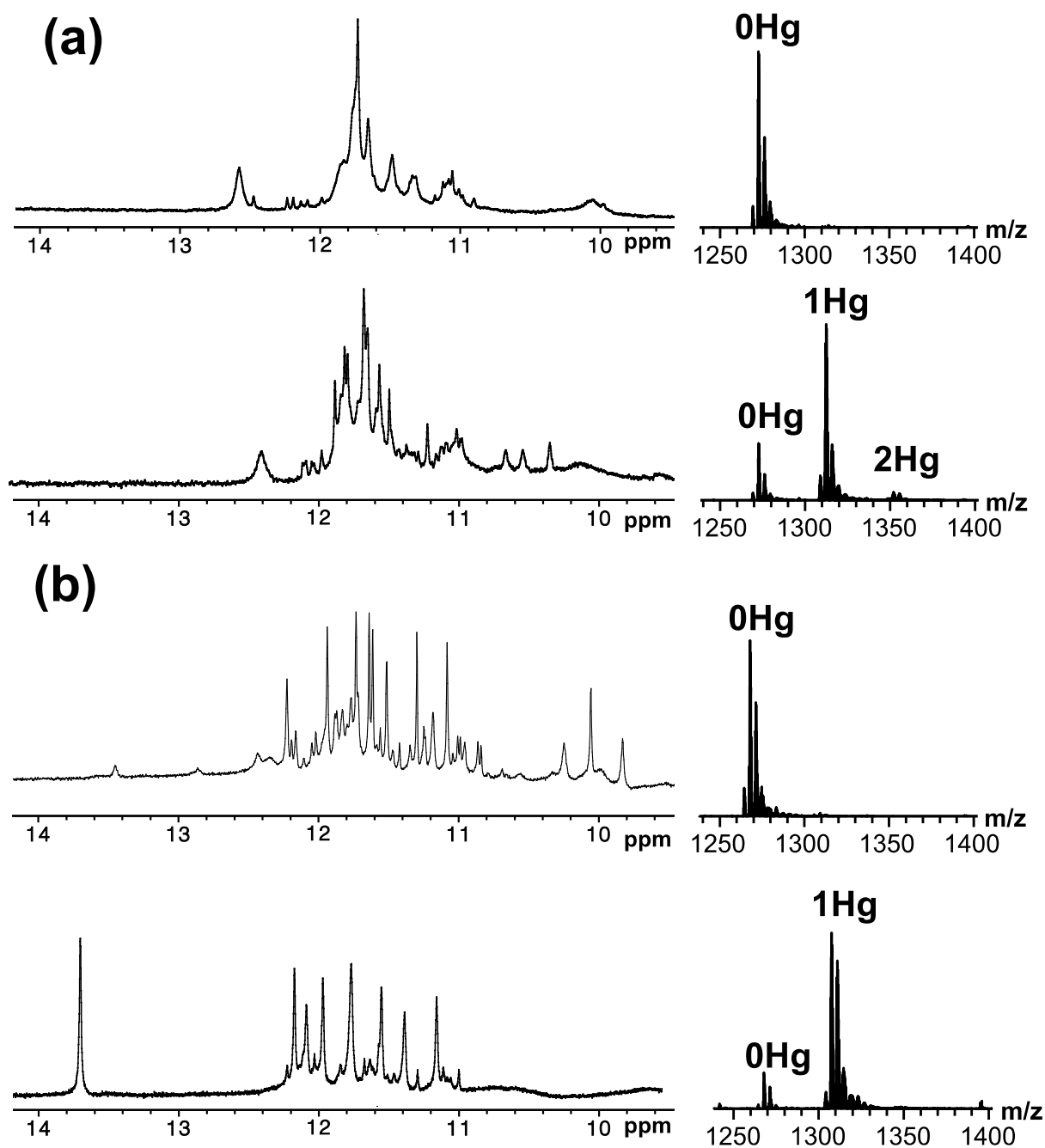


Supplementary Figure 4: ^1H NMR spectra of the imino region for (a) TAA_AAT (concentration: 200 μM) (b) AAT_TAA (concentration: 150 μM) (c) TAA_TAA (concentration: 200 μM) and (d) CAT_CAT (concentration: 210 μM). (e) AAT_CAT (concentration: 250 μM). For each sequence the top spectrum is in the absence of Hg^{2+} and the bottom spectrum is in the presence of Hg^{2+} (1.5 equiv.). For AAT_CAT only spectrum in the presence of Hg^{2+} is shown. All ^1H NMR spectra were collected at 25 $^\circ\text{C}$ on a 700 MHz spectrometer in potassium phosphate buffer (pH 7.0) with 70 mM KCl.

3. Electrospray mass spectrometry (ESI-MS)

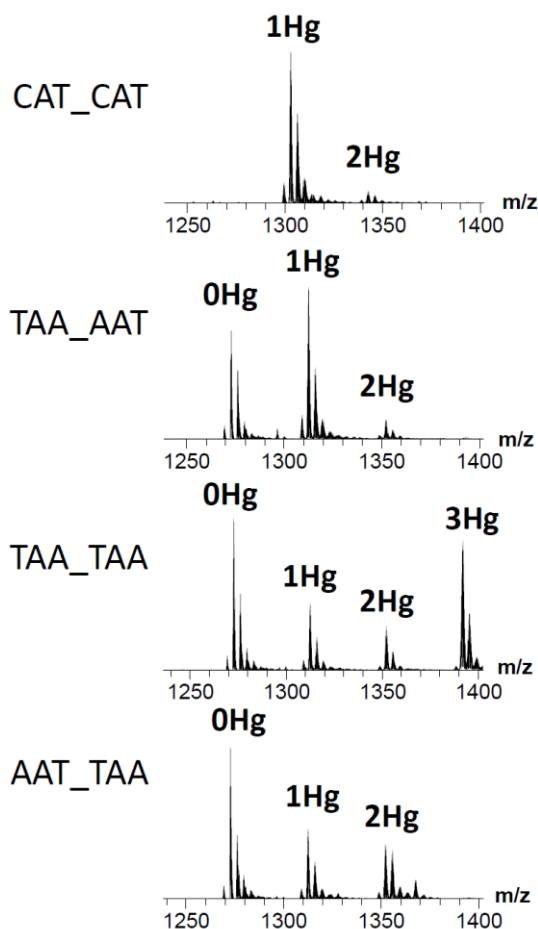
Experimental details

The samples were prepared by annealing (heating at 90 °C for 10 min and then cooling on ice for 2 hours) 500 µL of a 20 µM oligonucleotide solution in 100 mM NH₄OAc, either in absence or in presence of 30 µM HgCl₂ (1.5 equivalents). Then 500 µL of MeOH was added and the sample was stored in the fridge before use as is for mass spectrometry. Mass spectrometry experiments were carried out on a LTQ-FT (Thermo, Bremen, Germany) equipped with the standard electrospray source. The experiments were performed in negative ion mode (capillary voltage = 2.6 kV) in soft source conditions. These conditions were established by injecting the dimeric G-quadruplex d(GGGGTTTTGGGG) and selecting voltages and temperatures that preserve mainly three ammonium ions in the dimer at charge state 5- [1]. The capillary temperature was 190 °C, the skimmer voltage was -10 V and the tube lens offset was -25 V.



Supplementary Figure S5: ^1H NMR spectra of the imino region and mass spectrometry spectra for (a) ATA_ATA and (b) CAT_AAT. For each sequence the top spectrum is in the absence of Hg^{2+} and the bottom spectrum is in the presence of Hg^{2+} (1.5 equiv.). All ^1H NMR spectra were collected at 25 °C on a 700 MHz spectrometer in potassium phosphate buffer (pH 7.0) with 70 mM KCl.

Additional ESI-MS spectra (sequences not shown in the main text)

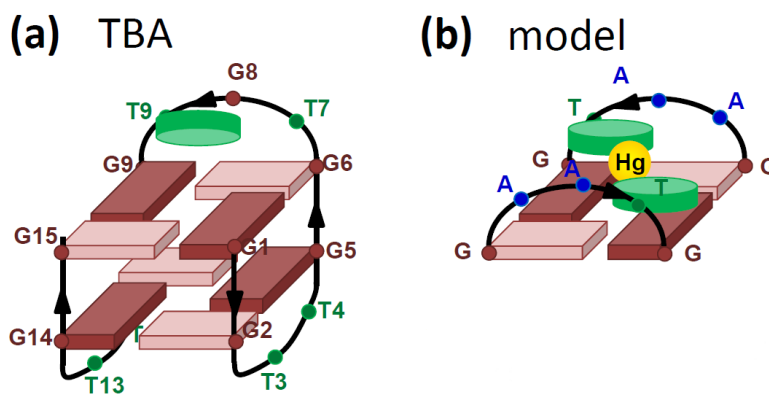


Supplementary Figure S6: ESI-MS spectra recorded for the sequences CAT_CAT, TAA_AAT, TAA_TAA, AAT_TAA in the presence of 1.5 equivalents of Hg²⁺. The spectra recorded in the absence of Hg²⁺ displayed only the peak with zero Hg bound.

4. Molecular modeling

Model building for the AAT_AAT loops

Loop 1 and loop 3 in the anticipated structure bridge wide grooves of an antiparallel G-quadruplex. To start with plausible structures of these loops, we used the second loop of the thrombin binding aptamer structure (PDB code 1QDG) [2]. The thrombin binding aptamer structure is shown in Figure SX(a) and our model is shown in Figure SX(b). We replaced T7 and G8 by adenines, then duplicated the resulting AAT loop and connected it to G15 and G1 of the thrombin binding aptamer. Only the top G-quartet was conserved and the guanine positions were frozen. The thymines in the loops were deprotonated in N3. Importantly, compared to the thymine conformation in the TBA loop, we had to manually impose an unfavourable *anti*→*syn* conformational change on the thymines to have the N3's facing each other and be able to place Hg²⁺ between them.



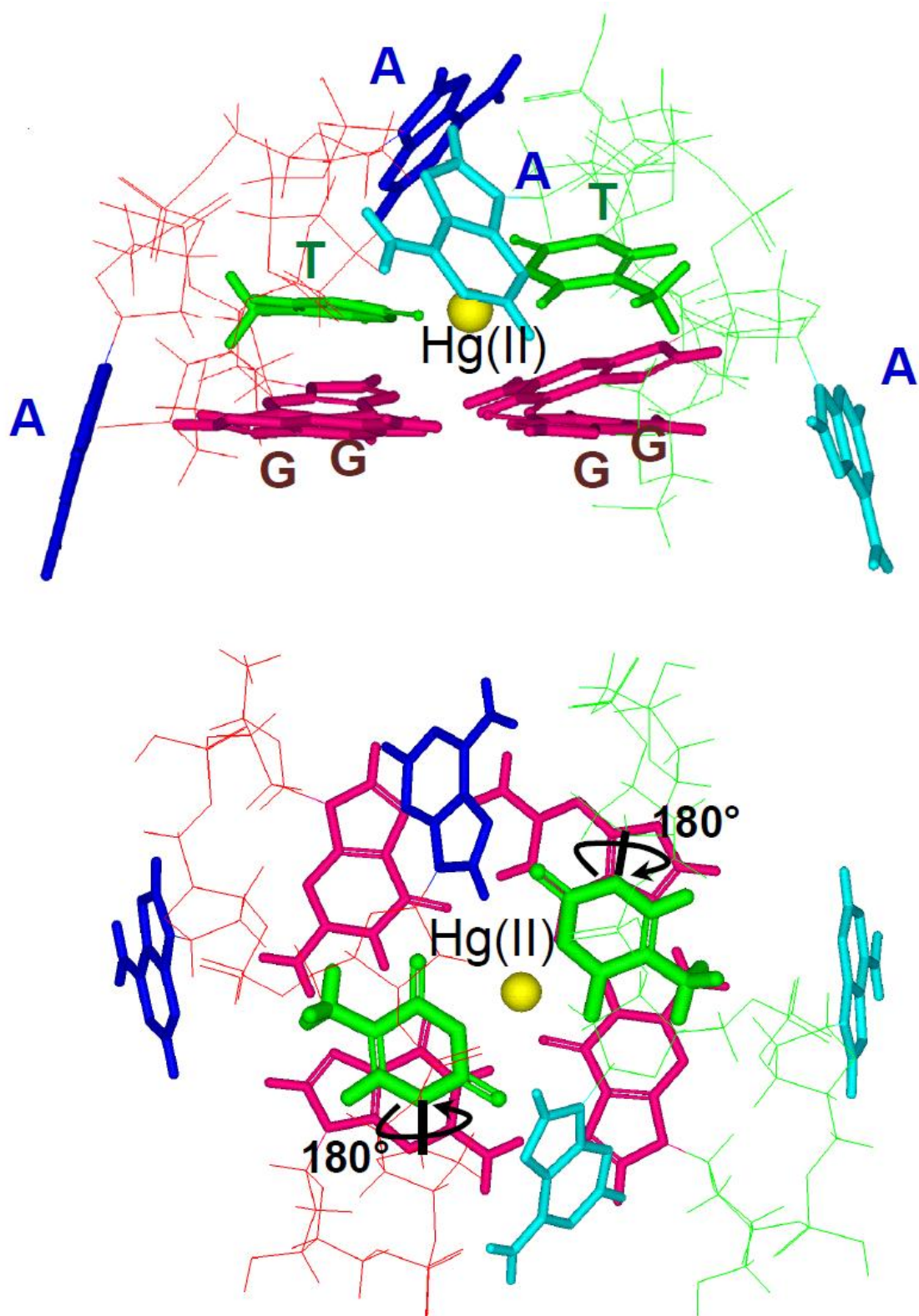
Supplementary Figure S7: Building of the AAT_AAT loop system (b) from the thrombin binding aptamer structure (a).

Theoretical calculations

The loops were fully optimized at the DFT B3LYP 4-31G* level of theory, with a LANL2DZ pseudopotential on the mercury. The Gaussian 09 rev.A02 software suite (www.gaussian.com) was used for the optimization.

Geometry optimization on the two loops

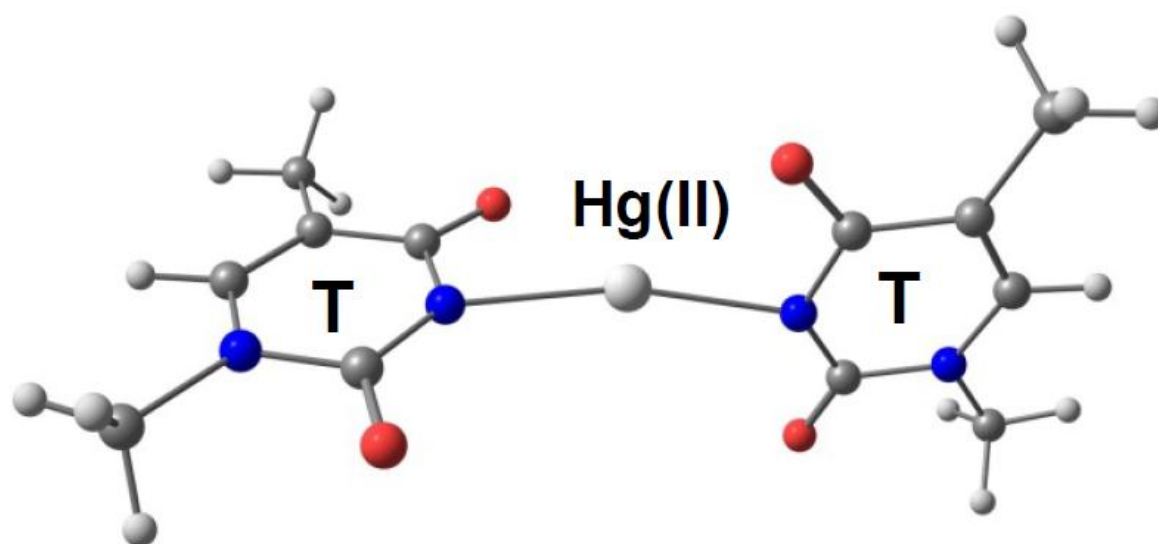
Figure SX is a larger version of the figure shown in the main text, and the bond around which the thymines were rotated by 180° before optimization is highlighted.



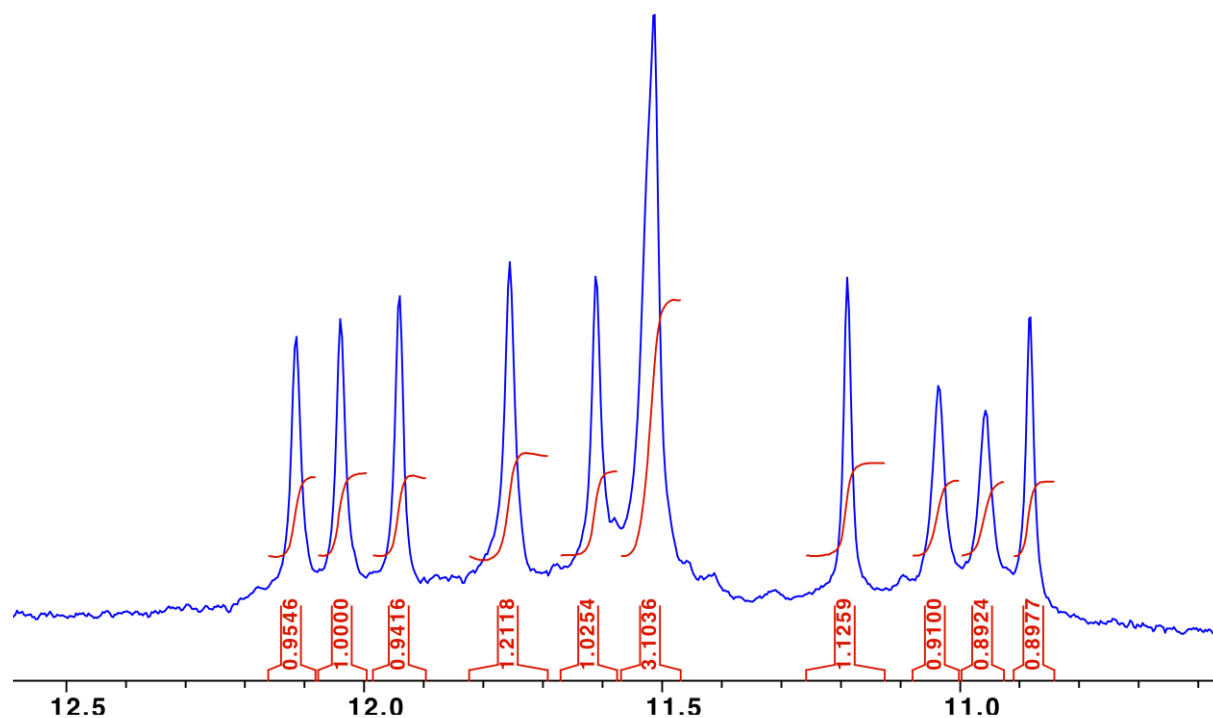
Supplementary Figure S8: Two different views of the structure of the AAT_AAT loops, optimized at the DFT B3LYP 4-31G* level of theory, with a LANL2DZ pseudopotential on the mercury. (This is a larger version of Figure 4 shown in main text).

Geometry optimization of Hg-bound thymines at the same level of theory

To check whether the tilting between the two thymines in the optimized structure of the two loops was due to quantum effects or to strains in the loops, we also optimized the T-Hg-T base pair only. In each thymine, the sugar was replaced by a methyl, and the base was deprotonated in N3. The resulting complex T-Hg-T is therefore neutral. The resulting structure (Figure S9) shows a similar tilting between the two thymines: they are not coplanar in the optimized structure.



Supplementary Figure S9: optimized structure of the T-Hg-T base pair at the DFT B3LYP 4-31G* level of theory, with a LANL2DZ pseudopotential on the mercury.



Supplementary Figure S10: Integrals of the imino peaks for AAT_AAT sequence in presence of Hg^{2+} showing the presence of 12 imino peaks.

5. Supplementary References

1. Rosu F., Gabelica V., Houssier C., Colson P., De Pauw E., Triplex and quadruplex DNA structures studied by electrospray mass spectrometry, *Rapid Commun.Mass Spectrom.* 16 (2002) 1729-1736.
2. Marathias V.M., Wang K.Y., Kumar S., Pham T.Q., Swaminatham S., Bolton P.H., Determination of the number and location of the manganese binding sites of DNA quadruplexes in solution by EPR and NMR in the presence and absence of thrombin, *J.Mol.Biol.* 260 (1996) 378-394.