

Supplementary Data for

“Distinctive structural motifs of RNA G-quadruplexes composed of AGG, CGG and UGG trinucleotide repeats”

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RNA sample preparation

Commercially available A, C, G and U phosphoramidites with 2'-O-tetrabutyltrimethylsilyl were used for synthesis of RNA (Glen Research, Azco, Proligo). After synthesis, the oligoribonucleotides were removed from the solid support and deprotected by treatment with concentrated ammonia in ethanol (3:1, v/v) at 55 °C overnight. The 2'-silyl protection was removed by treatment with 1.0 M triethylammonium fluoride at 55 °C by 3 hours. Next, the precipitation was made by addition 5 ml of 1-butanol and the samples were stored at -20 °C by 1 hour. The precipitate was separated from the solution by spinning at 5000 rpm, 4 °C by 10 minutes. The crude samples were desalted through a Sep-Pak C-18 cartridge (Waters) and purified on a silica gel 60 F₂₅₄ thin-layer chromatography plate developed with 1-propanol : ammonia : water (55:35:10, v/v). The samples were desalted again with a Sep-Pak C-18 cartridge.

The obtained amounts of oligoribonucleotides were divided into four parts. One part was used for the MS measurements and prepared as described below (Electrospray mass spectrometry section). The second part was dissolved in 50 mM KCl, 10 mM K₂HPO₄/KH₂PO₄, 0.1 mM EDTA, pH 6.8 (potassium buffer) with the single strand concentration 0.01 mM (unless otherwise specified). The third part was dissolved in 150 mM NaCl, 10 mM Na₂HPO₄/NaH₂PO₄, 0.1 mM EDTA, pH 6.8 (sodium buffer) with the single strand concentration 0.01 mM (unless otherwise specified). Finally, the fourth part was prepared in 150 mM NH₄Cl, 10 mM Tris/HCl, 0.1 mM EDTA, pH 6.7 (ammonium buffer) with the single strand concentration 0.01 mM. All the samples were annealed by heating at 90 °C for 5 min and then slowly cooled down to the room temperature and stored at 4 °C. The measurements were done after three weeks in order to allow the formation of the tetramolecular G-quadruplexes.

Electrospray mass spectrometry

The spray capillary was at -2.4 kV, the sampling cone was 40 V, the extraction cone was 4 V, the source and desolvation temperatures were 20°C and 40°C, respectively, with a source backing pressure of 3.40 mbar. The trap and transfer collision voltages were set a 4 V each, with an argon pressure of 2.57×10^{-2} mbar. All these instrumental settings ensured soft desolvation and transfer conditions for non-covalent complexes. The instrument was operated in ion mobility mode, with an IMS cell pressure of 0.532 mbar (in N₂), and traveling waves of 8 V at 300 m/s.

Nuclear magnetic resonance

The ^1H - ^{15}N HSQC spectra were recorded in 90% H_2O /10% D_2O . For $(\text{AGG})_2\text{A}$ sample ^1H - ^{15}N HSQC spectrum was acquired within 4866 Hz spectral width in the ^{15}N dimension and 8013 Hz spectral width in the ^1H dimension; 1024 complex points and 80 FIDs were acquired. For ^1H - ^{15}N HSQC spectrum of $\text{p}(\text{UGG})_2\text{U}$ spectral width of 2838 Hz and 11261 Hz were used in the ^{15}N dimension and ^1H dimension, respectively, with the use of 2048 complex points and 64 FIDs.

The guanosine and uridine imino protons of $\text{p}(\text{UGG})_2\text{U}$ were assigned using two-dimensional NOESY spectrum obtained in 90% H_2O /10% D_2O for 2.2 mM $\text{p}(\text{UGG})_2\text{U}$, at 3 °C with 150 ms mixing time. The spectra were acquired with a sweep width of 11261 Hz in both dimensions. 512 FIDs of 2048 complex points were collected.

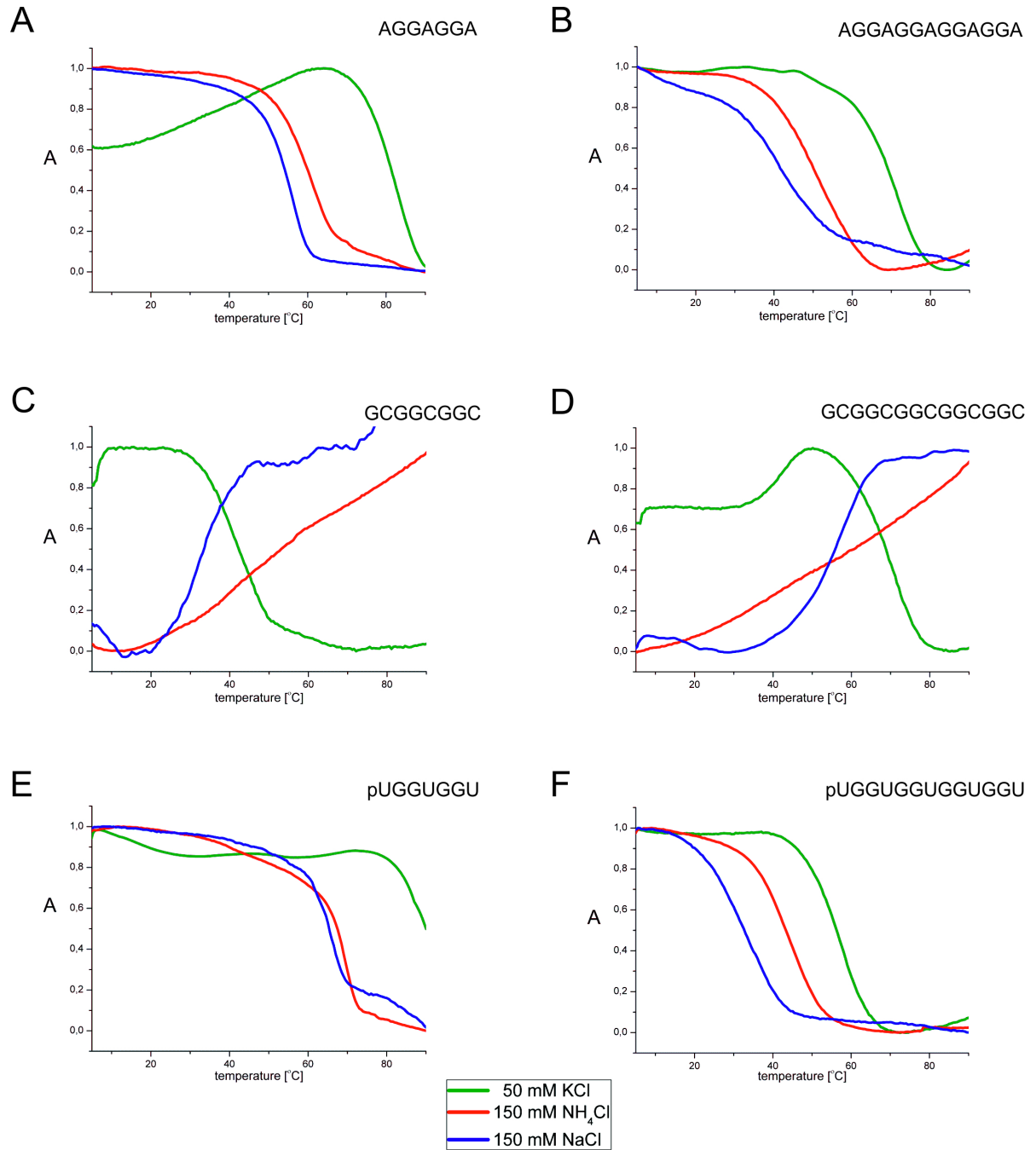


Figure S1. Normalized UV melting profiles of (A) (AGG)₂A, (B) (AGG)₄A, (C) G(CG)₂C, (D) G(CG)₄C, (E) p(UGG)₂U and (F) p(UGG)₄U at 295 nm in the presence of 50 mM KCl, 10 mM potassium phosphate and 0.1 mM EDTA, pH 6.8 (green), 150 mM NH₄Cl, 10 mM Tris/HCl, 0.1 mM EDTA, pH 6.8 (red) and 150 mM NaCl, 10 mM sodium phosphate and 0.1 mM EDTA, pH 6.8 (blue). Panel B is an equivalent to the Figure 1A.

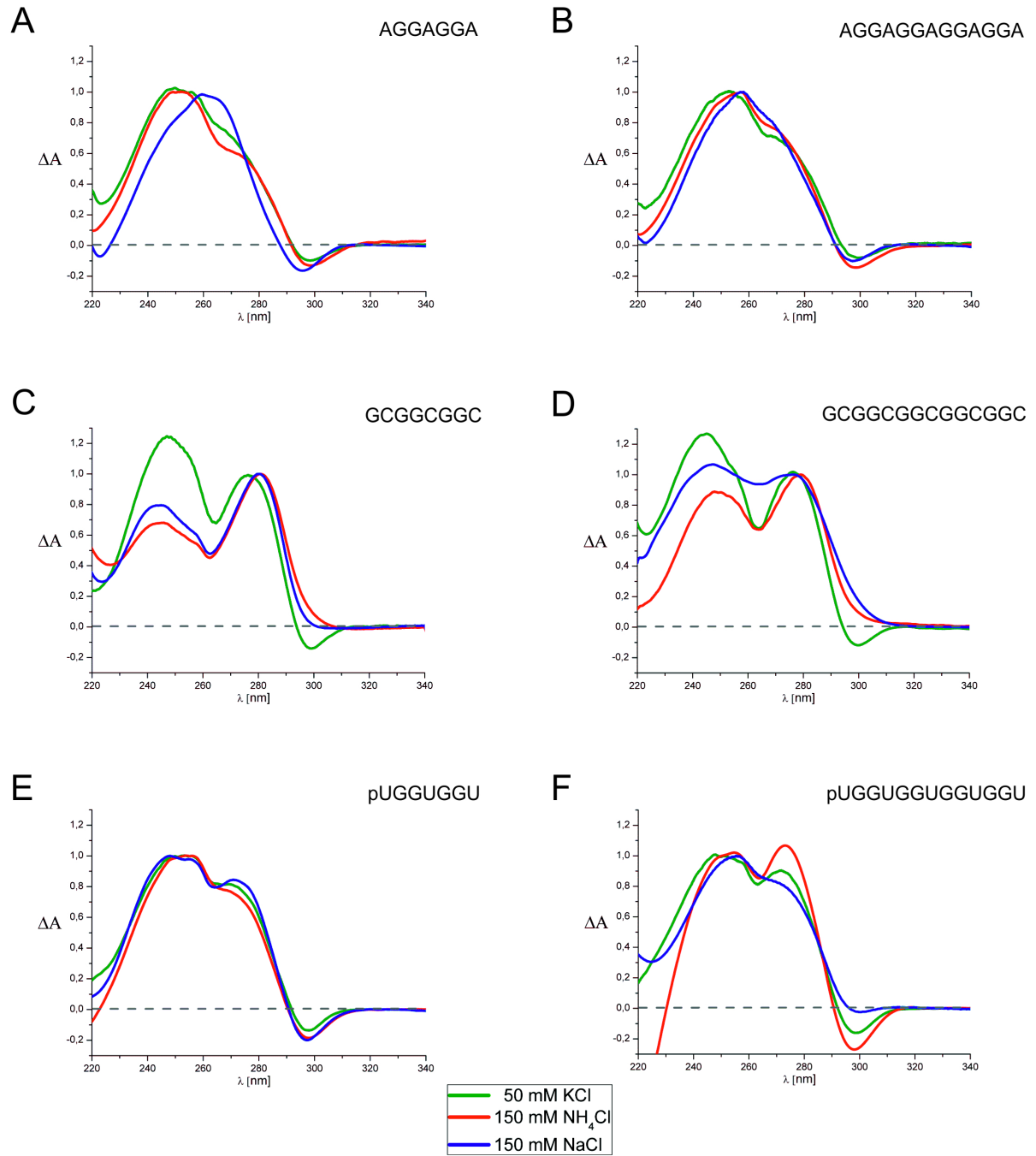


Figure S2. Normalized thermal differential spectra of (A) $(\text{AGG})_2\text{A}$, (B) $(\text{AGG})_4\text{A}$, (C) $\text{G}(\text{CGG})_2\text{C}$, (D) $\text{G}(\text{CGG})_4\text{C}$, (E) $\text{p}(\text{UGG})_2\text{U}$ and (F) $\text{p}(\text{UGG})_4\text{U}$ in the presence of 50 mM KCl, 10 mM potassium phosphate and 0.1 mM EDTA, pH 6.8 (green), 150 mM NH_4Cl , 10 mM Tris/HCl, 0.1 mM EDTA, pH 6.8 (red) and 150 mM NaCl, 10 mM sodium phosphate and 0.1 mM EDTA, pH 6.8 (blue). Panel A is an equivalent to the Figure 1B.

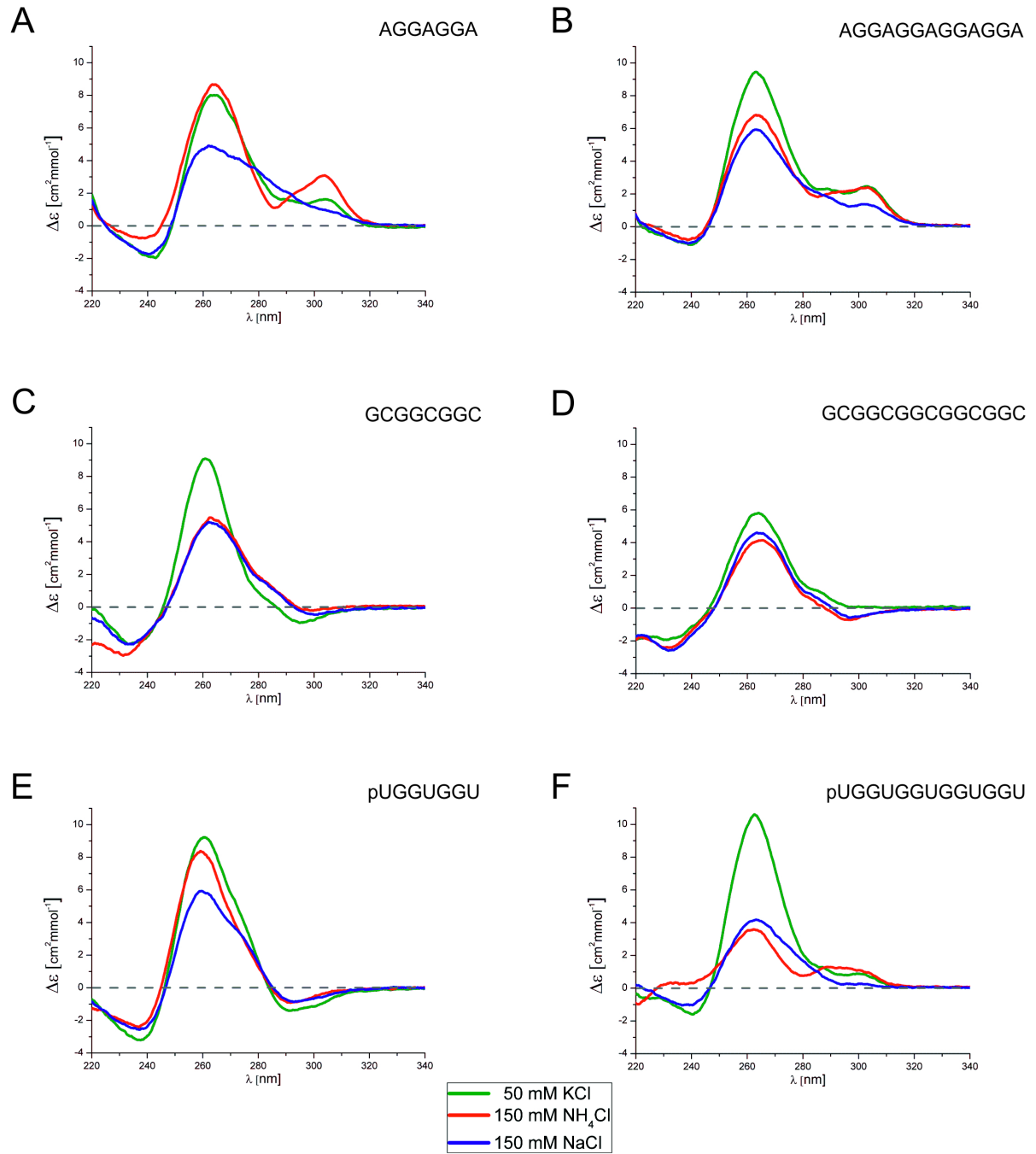


Figure S3. Circular dichroism spectra of (A) (AGG)₂A, (B) (AGG)₄A, (C) G(CGG)₂C, (D) G(CGG)₄C, (E) p(UGG)₂U and (F) p(UGG)₄U at 25 °C in the presence of 50 mM KCl, 10 mM potassium phosphate and 0.1 mM EDTA, pH 6.8 (green), 150 mM NH₄Cl, 10 mM Tris/HCl, 0.1 mM EDTA, pH 6.8 (red) and 150 mM NaCl, 10 mM sodium phosphate and 0.1 mM EDTA, pH 6.8 (blue). Panel A is an equivalent to the Figure 1C.

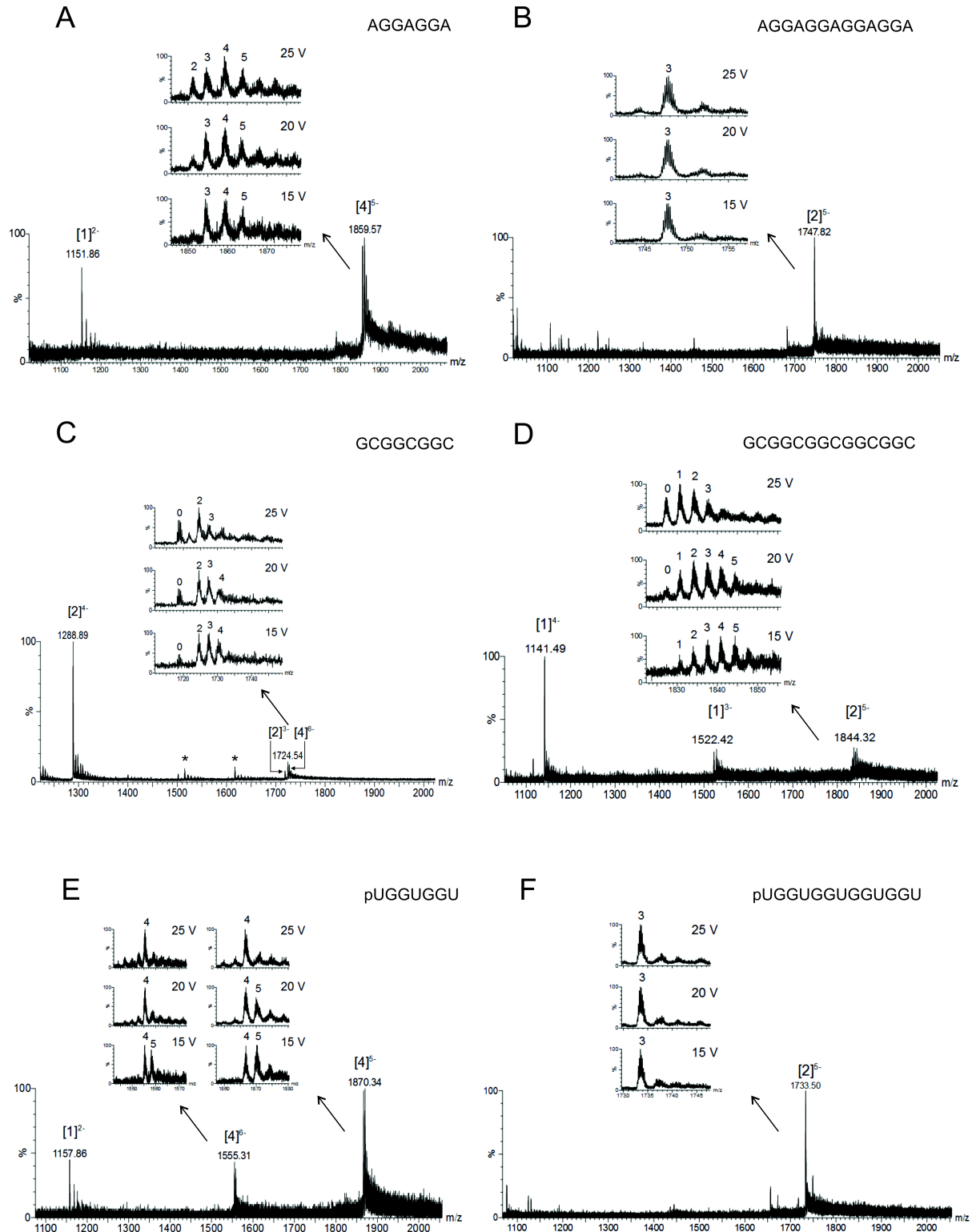


Figure S4. Electrospray mass spectra of (A) $(\text{AGG})_2\text{A}$, (B) $(\text{AGG})_4\text{A}$, (C) $\text{G}(\text{CGG})_2\text{C}$, (D) $\text{G}(\text{CGG})_4\text{C}$, (E) $\text{p}(\text{UGG})_2\text{U}$ and (F) $\text{p}(\text{UGG})_4\text{U}$ in the presence 150 mM ammonium acetate. The final single strand RNA concentration was 18 μM . 10% v/v of methanol was added one hour before measurement. The peak annotation $[n]^z$ indicate the number of strands (n) and the total charge (z). On the insert the distribution of the number of ammonium ions preserved in the structure are shown. The distribution is shown at three bias voltages. Asterisks indicate impurities. Panel B is an equivalent to the Figure 2.

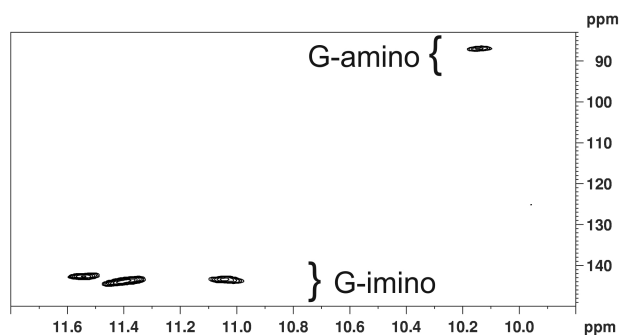


Figure S5. Imino-amino region of ^1H - ^{15}N HSQC spectrum of $(\text{AGG})_2\text{A}$ (1.14 mM) at 25 °C in the presence of 50 mM KCl, 10 mM potassium phosphate and 0.1 mM EDTA, pH 6.8.

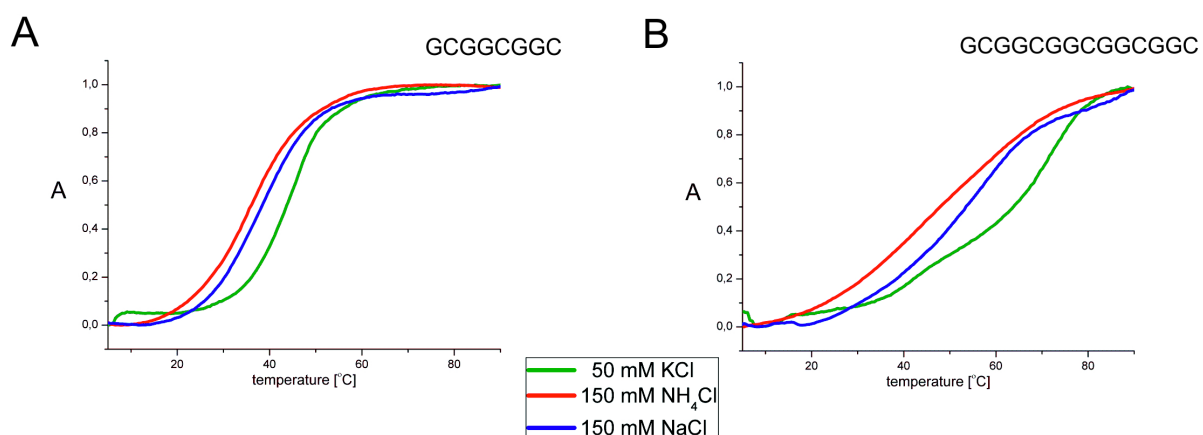


Figure S6. Normalized UV melting profiles of (A) $\text{G}(\text{CG})_2\text{C}$, (B) $\text{G}(\text{CG})_4\text{C}$ at 260 nm in the presence of 50 mM KCl, 10 mM potassium phosphate and 0.1 mM EDTA, pH 6.8 (green), 150 mM NH_4Cl , 10 mM Tris/HCl, 0.1 mM EDTA, pH 6.8 (red) and 150 mM NaCl, 10 mM sodium phosphate and 0.1 mM EDTA, pH 6.8 (blue).

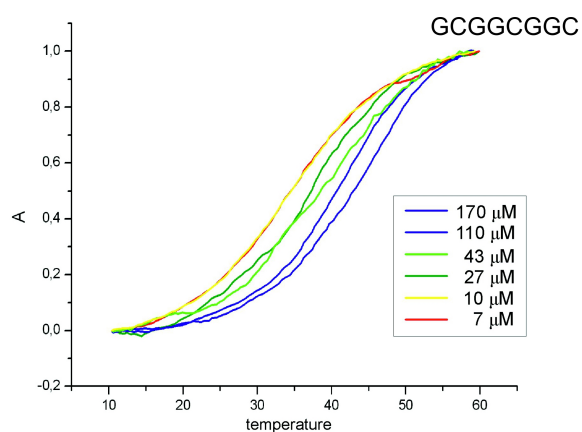


Figure S7. Concentration dependence of the UV melting profiles of G(CGG)₂C at 260 nm in the presence 150 mM NaCl, 10 mM sodium phosphate and 0.1 mM EDTA, pH 6.8.

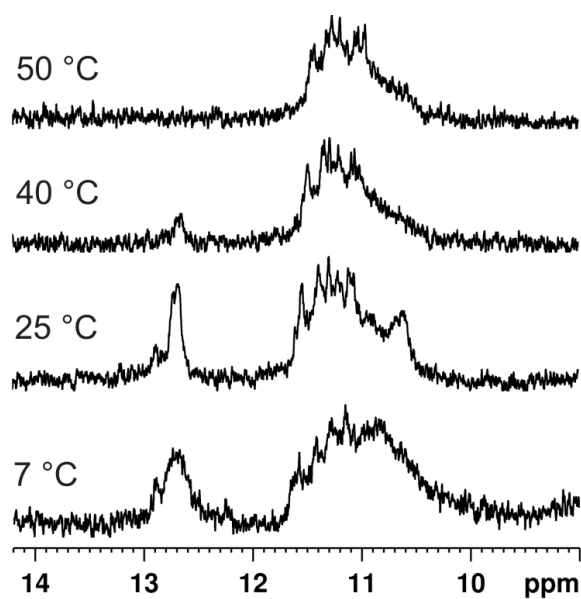


Figure S8. Temperature dependence of the ¹H NMR spectra of G(CGG)₄C (0.45 mM) in the presence of 50 mM KCl, 10 mM potassium phosphate and 0.1 mM EDTA, pH 6.8.

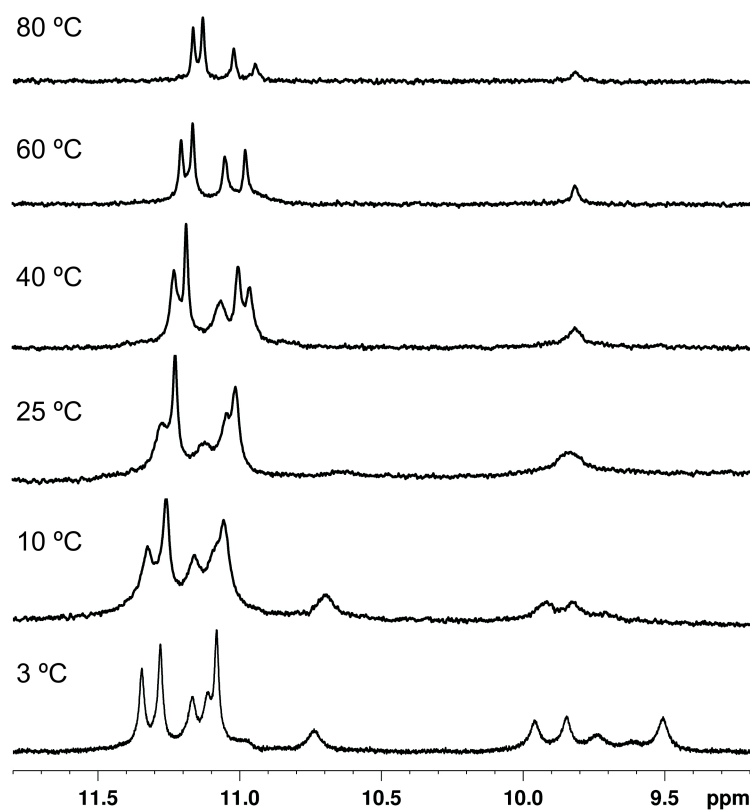


Figure S9. Temperature dependence of the ^1H NMR spectra of $\text{p(UGG)}_2\text{U}$ (0.95 mM) in the presence of 50 mM KCl, 10 mM potassium phosphate and 0.1 mM EDTA, pH 6.8.

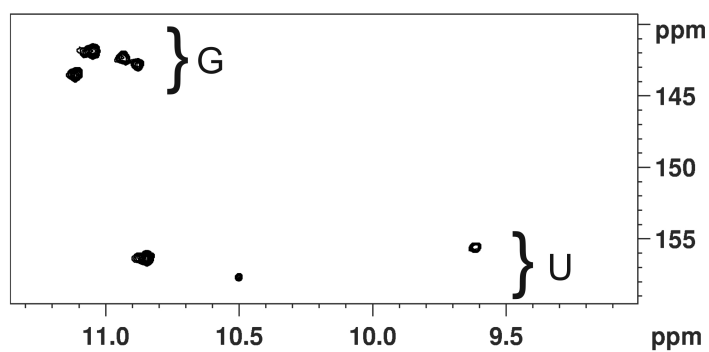


Figure S10. Imino region of ^1H - ^{15}N HSQC spectrum of $\text{p(UGG)}_2\text{U}$ (2.2 mM) at 3 °C in the presence of 50 mM KCl, 10 mM potassium phosphate and 0.1 mM EDTA, pH 6.8.

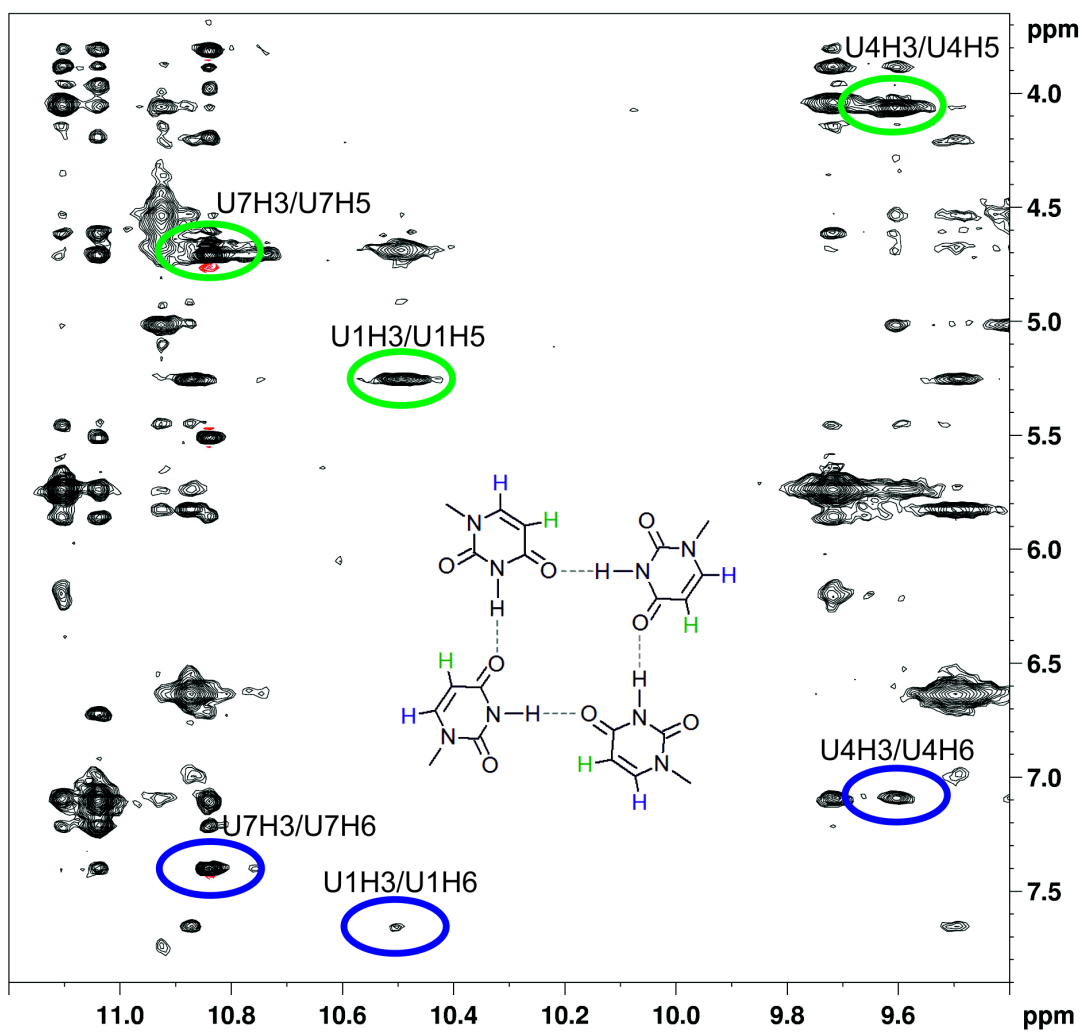


Figure S11. 2D NOESY spectrum of p(UGG)₂U (2.2 mM) at 3 °C in H₂O/D₂O (90%/10%), 50 mM KCl, 10 mM potassium phosphate and 0.1 mM EDTA, pH 6.8. NOE cross-peaks characteristic of U-tetrads are marked by circles.