# Supporting Information

## Unique Length-Dependent Biophysical Properties of Repetitive DNA

Ji Huang and Sarah Delaney*
Department of Chemistry, Brown University, Providence, Rhode Island 02912

Table S1. DSC-Derived Melting Temperatures and Thermodynamic Parameters for $(CTG)_n$ and $(CAG)_n$ Stem-Loop Hairpins under low salt conditions.[1, 2]

| Sequence | $T_m$ Hairpin (°C) | $\Delta H$ (kcal mol$^{-1}$) | $T\Delta S$ [3] (kcal mol$^{-1}$) | $\Delta G$ [3] (kcal mol$^{-1}$) |
|---|---|---|---|---|
| $(CTG)_6$ | 56.1 | 18.5 ± 0.8 | 17.5 ± 0.7 | 1.1 ± 0.1 |
| $(CTG)_7$ | 53.9 | 20.0 ± 1.2 | 19.0 ± 1.1 | 1.0 ± 0.1 |
| $(CTG)_8$ | 58.7 | 35.1 ± 1.9 | 32.8 ± 1.8 | 2.3 ± 0.1 |
| $(CTG)_9$ | 58.3 | 37.0 ± 3.3 | 34.6 ± 3.0 | 2.4± 0.3 |
| $(CTG)_{10}$ | 59.2 | 50.6 ± 3.3 | 47.2 ± 3.1 | 3.4 ± 0.2 |
| $(CTG)_{11}$ | 59.8 | 52.1 ± 3.0 | 48.5 ± 2.8 | 3.4 ± 0.2 |
| $(CTG)_{12}$ | 60.6 | 68.6 ± 2.3 | 63.7 ± 2.1 | 4.9 ± 0.2 |
| $(CTG)_{13}$ | 60.4 | 69.1 ± 4.3 | 64.2 ± 3.9 | 4.9 ± 0.3 |
| $(CTG)_{14}$ | 60.6 | 85.3 ± 1.7 | 79.3 ± 1.5 | 6.1 ± 0.2 |
| $(CAG)_6$ | 59.0 | 7.6 ± 1.0 | 7.1 ± 0.9 | 0.5 ± 0.1 |
| $(CAG)_7$ | 56.6 | 5.6 ± 0.3 | 5.2 ± 0.4 | 0.3 ± 0.1 |
| $(CAG)_8$ | 55.7 | 15.2 ± 0.4 | 14.3 ± 0.5 | 0.9 ± 0.1 |
| $(CAG)_9$ | 55.3 | 12.6 ± 1.0 | 11.9 ± 1.0 | 0.7 ± 0.1 |
| $(CAG)_{10}$ | 54.2 | 22.3 ± 0.4 | 21.1 ± 0.3 | 1.2 ± 0.1 |
| $(CAG)_{11}$ | 54.2 | 21.5 ± 0.2 | 20.4 ± 0.1 | 1.1 ± 0.1 |
| $(CAG)_{12}$ | 54.1 | 34.1 ± 1.3 | 32.4 ± 1.2 | 1.8 ± 0.1 |
| $(CAG)_{13}$ | 54.3 | 36.8 ± 1.5 | 34.9 ± 1.3 | 2.0 ± 0.1 |
| $(CAG)_{14}$ | 54.8 | 42.6 ± 1.5 | 40.3 ± 1.3 | 2.3 ± 0.2 |

[1] In 20 mM potassium phosphate, pH 7.2.
[2] Errors represent standard deviation from the analysis of three scans of a single sample preparation.
[3] Values at 37 °C.

Table S2. DSC-Derived Melting Temperatures and Thermodynamic Parameters for $(CAG)_n/(CTG)_n$ Duplexes.[1, 2]

| n | $T_m$ (°C) | $\Delta H$ (kcal mol$^{-1}$) | $T\Delta S$ [3] (kcal mol$^{-1}$) | $\Delta G$ [3] (kcal mol$^{-1}$) |
|---|---|---|---|---|
| 6 | 77.8 | 96.0 ± 3.1 | 84.7 ± 2.7 | 11.2 ± 0.3 |
| 7 | 81.1 | 126 ± 1 | 110 ± 1 | 15.8 ± 0.2 |
| 8 | 83.8 | 154 ± 2 | 131 ± 2 | 20.3 ± 0.2 |
| 9 | 85.6 | 183 ± 2 | 158 ± 2 | 24.9 ± 0.4 |
| 10 | 86.7 | 221 ± 5 | 190 ± 3 | 31.0 ± 1.5 |
| 11 | 88.2 | 239 ± 1 | 204 ± 1 | 35.1 ± 2.2 |
| 12 | 90.1 | 274 ± 6 | 234 ± 5 | 40.2 ± 1 |
| 13 | 90.3 | 295 ± 5 | 252 ± 4 | 43.4 ± 0.7 |
| 14 | 91.0 | 324 ± 8 | 276 ± 7 | 48.2 ± 1.2 |

[1] In 20 mM sodium phosphate, 100 mM NaCl, pH 7.0.

[2] Errors represent standard deviation from the analysis of three scans of a single sample preparation.

[3] Values at 37 °C.

**Figure S1.** Representative example for $(CTG)_9$ of creating 5$^{th}$ order polynomial baseline for overlapped transitions. (A) Three points were placed either prior or post the transitions on the concentration normalized thermogram after buffer scan was subtracted. (B) The thermogram obtained after baseline correction.

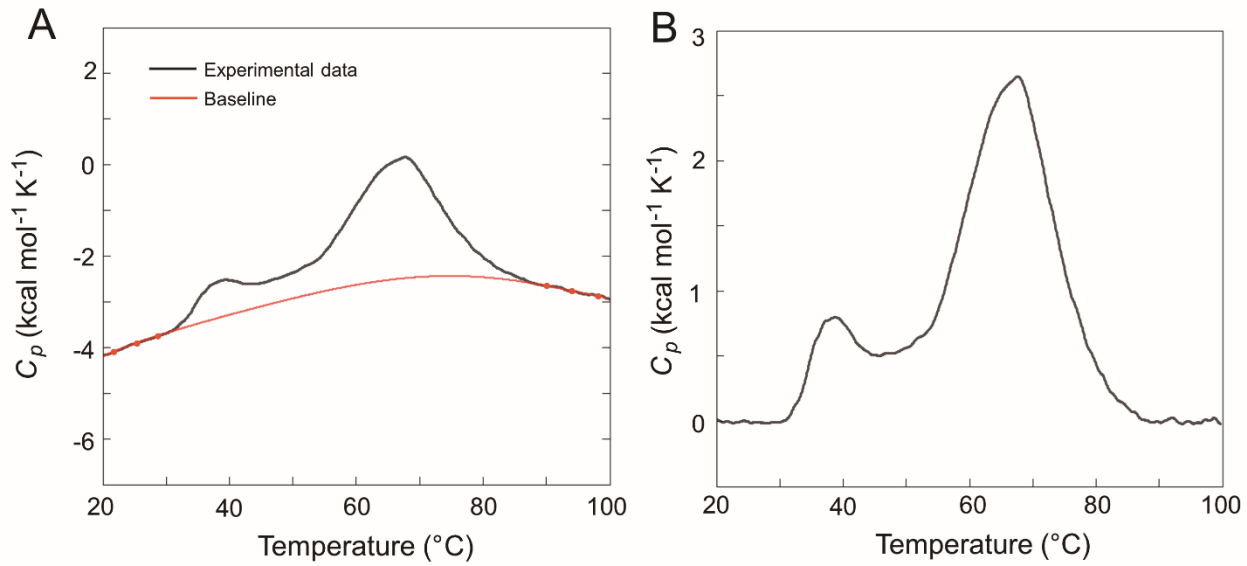
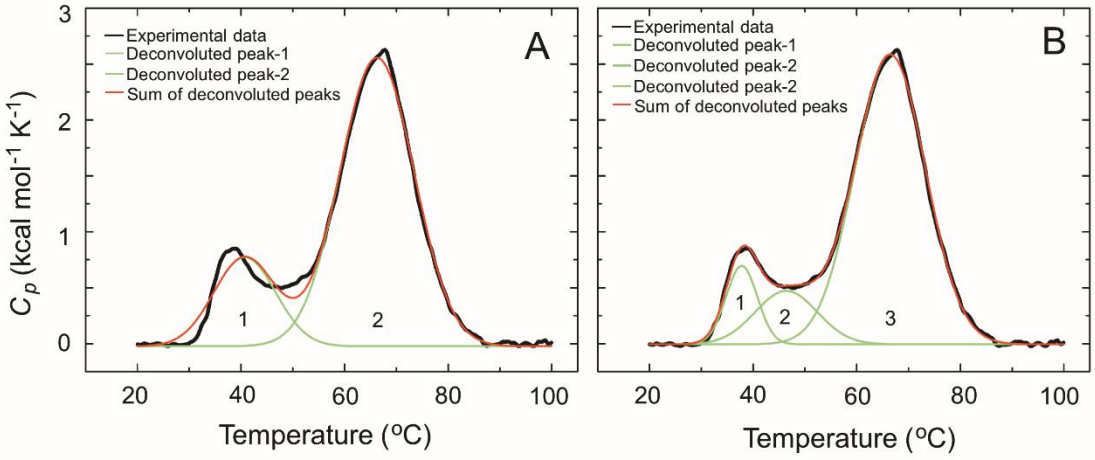


**Figure S2.** Example for $(CTG)_9$ of deconvolution of overlapped transitions. (A) Experimental data were deconvoluted into two peaks. (B) Experimental data were deconvoluted into 3 peaks. (C) Summary of $\Delta H$ using either 2 or 3 peaks in deconvolution. Analysis was carried out assuming the low temperature transition is homoduplex to hairpin transition as discussed below in Figure S4.




C

| Deconvolution Method | peak-1 $\Delta H$ (kcal/mol) | peak-2 $\Delta H$ (kcal/mol) | peak-3 $\Delta H$ (kcal/mol) | Total $\Delta H$ (kcal/mol) |
|---|---|---|---|---|
| 2-peak deconvolution (A) | 11.9 | 46.2 | / | 58.1 |
| 3-peak deconvolution (B) | 5.3 | 7.2 | 44.2 | 56.7 |

**Figure S3**. Native PAGE characterization of $(CTG)_7$ under various oligonucleotide concentration and buffer conditions. C1: $(CTG)_7$ stem-loop hairpin control; C2: $(CTG)_7/(CAG)_7$ canonical heteroduplex control. $(CTG)_7$ at various concentrations (1, 15, 30, 50 and 100 μM) were annealed in 20 mM potassium phosphate buffer, pH 7.2 with either no additional KCl or with 100 mM KCl .

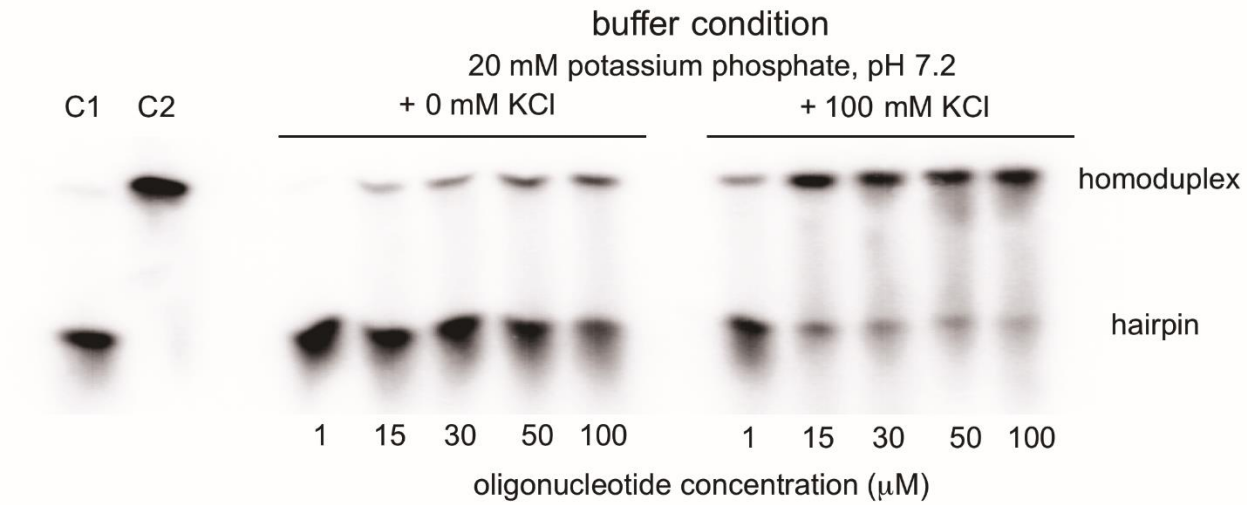
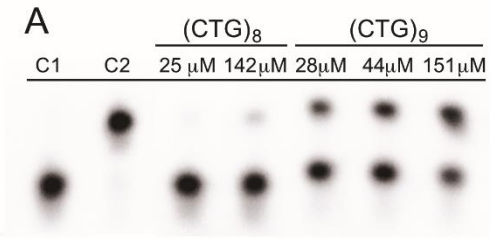


**Figure S4.** (A) Native PAGE analysis of $(CTG)_8$ and $(CTG)_9$ at different concentrations with $(CTG)_8$ hairpin control (C1) and $(CAG)_8/(CTG)_8$ heteroduplex control (C2) and (B) Quantitation of homoduplex and hairpin in $(CTG)_9$ at different concentrations. (C) Analysis of $\Delta H$ based on the two scenarios provided below.

A




B

| $(CTG)_9$ | prep-1 | prep-2 | prep-3 |
|---|---|---|---|
| Total Conc. (μM) | 28 | 44 | 151 |
| Homoduplex %[1] | 32 | 37 | 52 |
| Hairpin %[1] | 68 | 63 | 48 |

C

| | Scenario-1 | | | Scenario-2 | | |
|---|---|---|---|---|---|---|
| $(CTG)_9$ Total Conc. (μM) | 28 | 44 | 151 | 28 | 44 | 151 |
| $(CTG)_9$ hairpin Conc. (μM)[2] | 28 | 44 | 151 | 19 (28×68%) | 28 (44×63%) | 72 (151×48%) |
| $\Delta H$ (kcal/mol)[3] | 45 | 45 | 49 | 66 | 74 | 104 |

[1] Percentage calculated based on the native gel in Figure S4A
[2] Concentration corresponds to the amount of hairpin melt in higher temperature transition
[3] The $\Delta H$ of stem-loop hairpin melting calculated based on two scenarios for $(CTG)_9$

**Figure S5.** Native PAGE characterization of (A) $(CTG)_n$ and (B) $(CAG)_n$ (n = 6-14). The concentrations for $(CTG)_n$ are 15 μM and 30 μM for $(CAG)_n$. Buffer conditions are: 20 mM potassium phosphate, pH 7.2

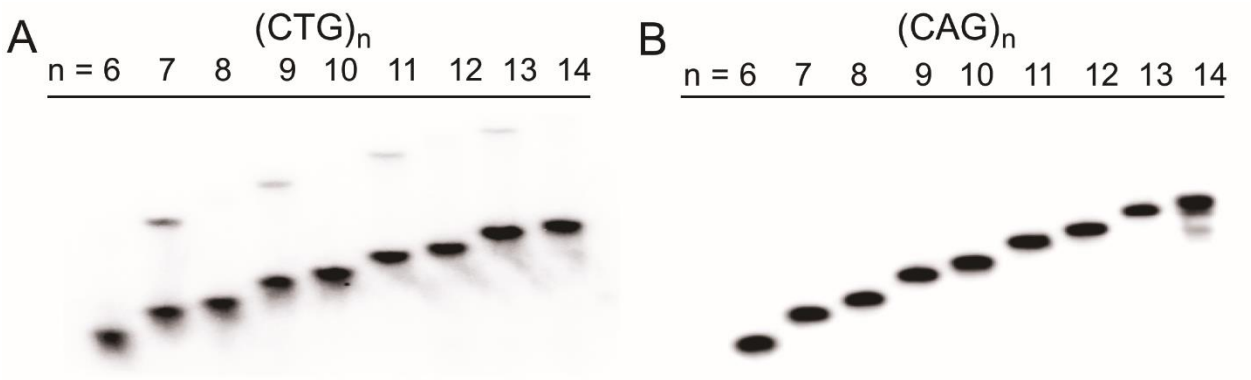


**Figure S6.** DSC thermograms of $(CTG)_n$ (n = 6-14) hairpin after buffer scan subtraction (A) and after both buffer scan subtraction and baseline correction (B). All oligonucleotide concentrations are 15 μM. Buffer conditions are: 20 mM potassium phosphate, pH 7.2

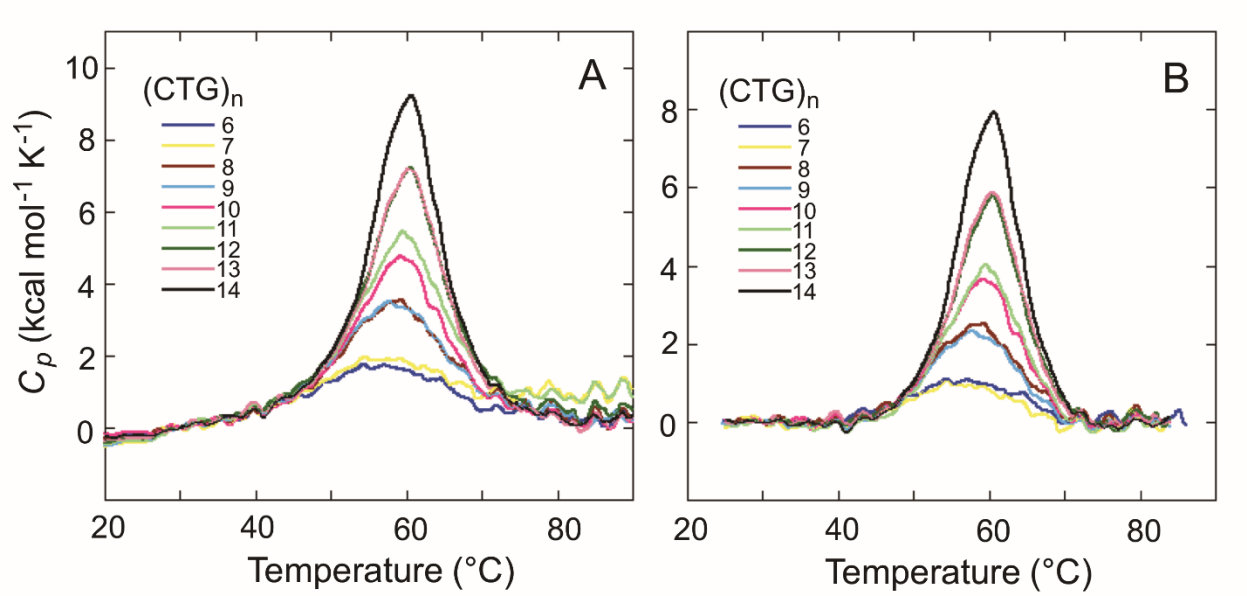


**Figure S7.** DSC thermograms of $(CAG)_n$ (n = 6-14) hairpin after buffer scan subtraction (A) and after both buffer scan subtraction and baseline correction (B). All oligonucleotide concentrations are 30 μM. Buffer conditions are: 20 mM potassium phosphate, pH 7.2.

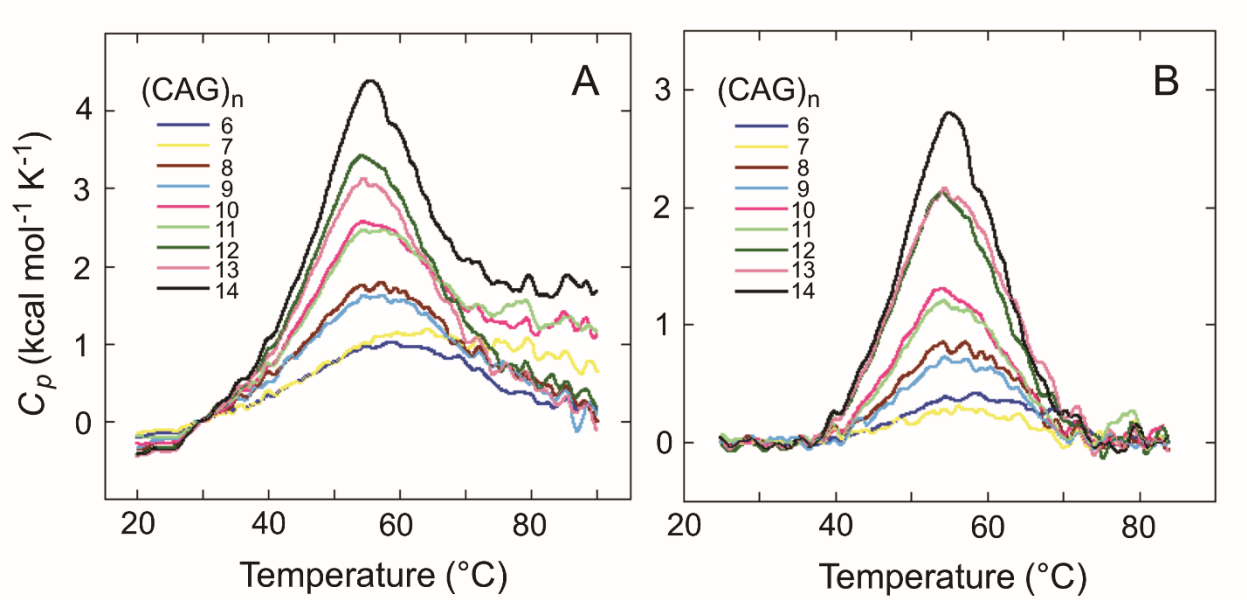


**Figure S8.** DSC thermograms of (A) $(CTG)_n/(CAG)_n$ (n = 6-14) duplex after both buffer scan subtraction and baseline correction. (B) Thermodynamic parameters obtained from duplex to unstructured single-stranded DNA transitions. Sample concentrations are 12.5 μM in duplex form. Buffer conditions are: 20 mM sodium phosphate, 100 mM NaCl, pH 7.0.

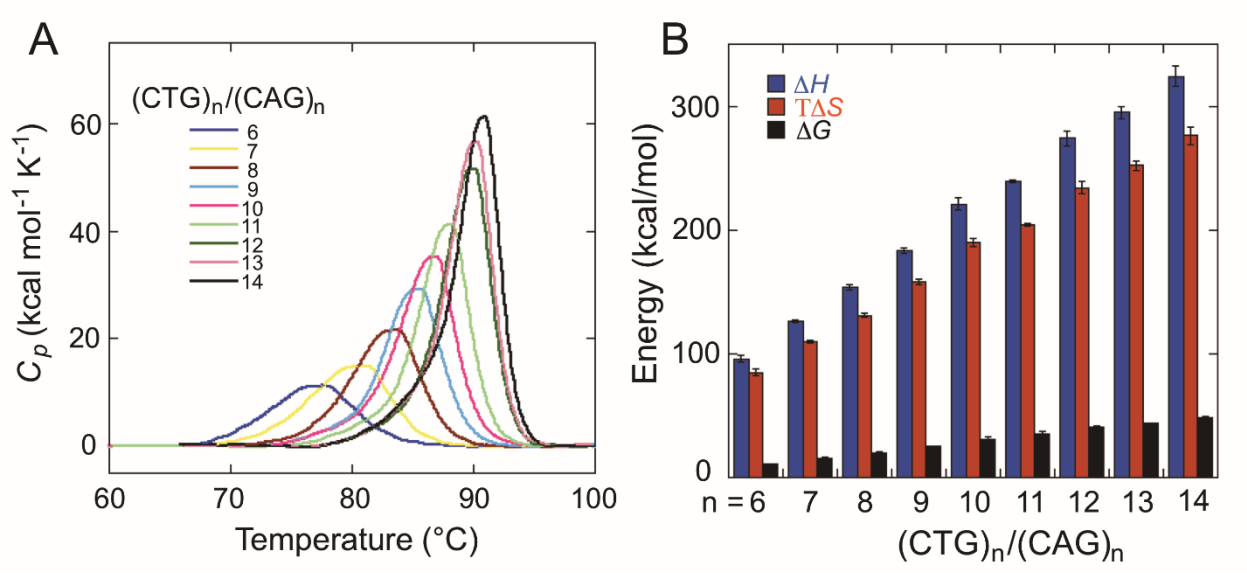


**Figure S9.** Determining the $\Delta H^{\ddagger}$ by Eyring equation for $(CTG)_n$ and $(CAG)_n$ stem-loop hairpin to $(CAG)_n/(CTG)_n$ duplex conversion for n= 6, 7, 10, 11, 14. Each data point represents an average of at least three experiments performed at a given temperature. Lines represent the least square linear fits. Temperatures were chosen below the melting temperature of the stem-loop hairpins.