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Supplemental Information

Stability of the pH-Dependent Parallel-Stranded d(CGA) Motif

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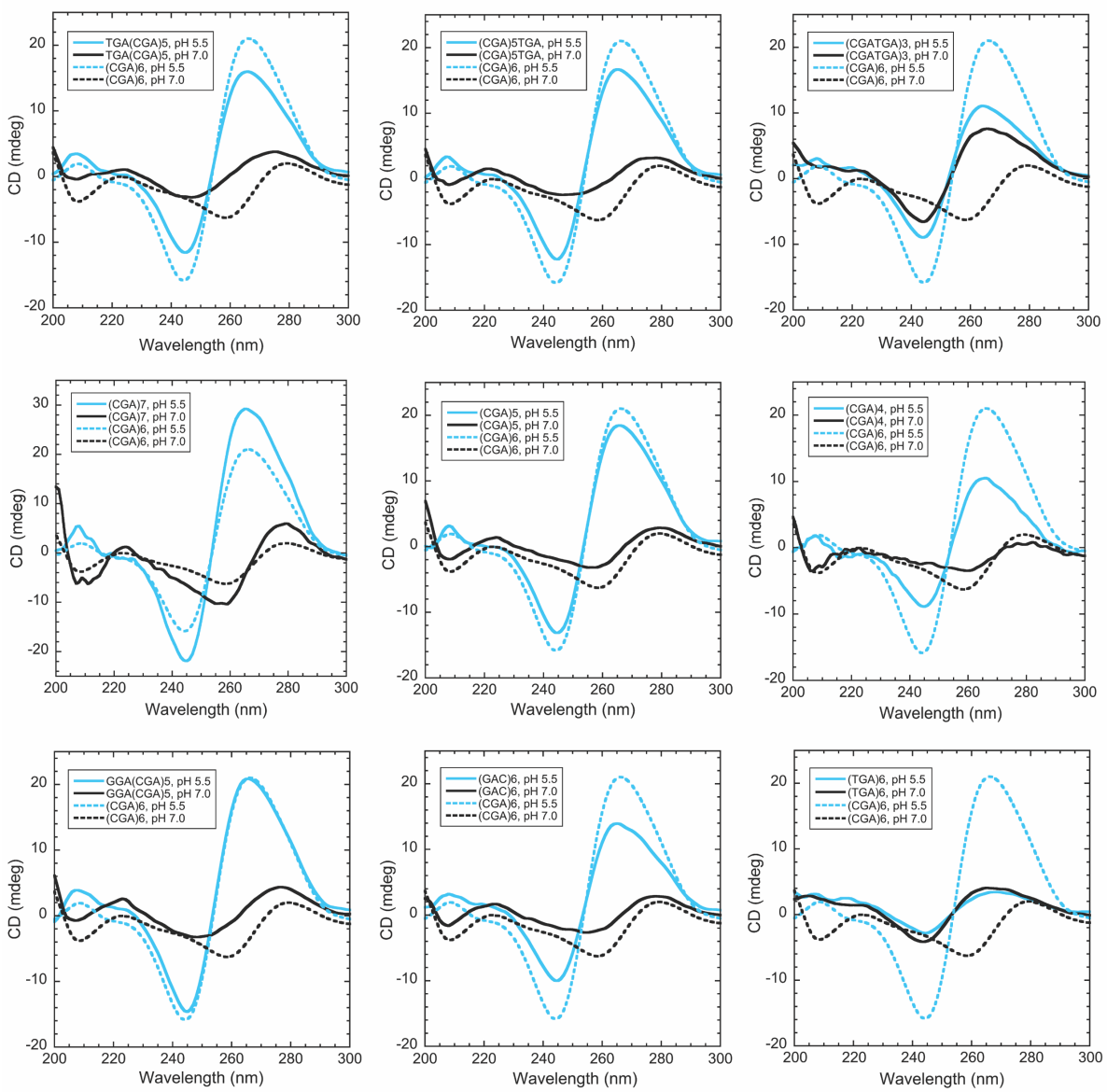

Figure S1. Circular dichroism spectra of 10 μM $(CGA)_6$ variants. The spectra of $(CGA)_6$ at pH 5.5 and pH 7.0 are shown (dashed lines) in each panel for reference. At pH 5.5 (blue curves), the prominent positive band at ~265 nm and the negative band at ~245 nm are characteristic of parallel-stranded duplex formation. As the number of d(CGA) triplets decreases, the intensity of the bands associated with parallel-stranded duplexes also decreases. At pH 7.0 (black curves), the positive band at ~275 nm and negative band at ~258 nm are characteristic of anti-parallel strands. $(TGA)_6$ and $(CGATGA)_3$ have bands characteristic of anti-parallel strands at each pH, suggesting that pH sensitivity is decreased or eliminated with increasing d(TGA) content.

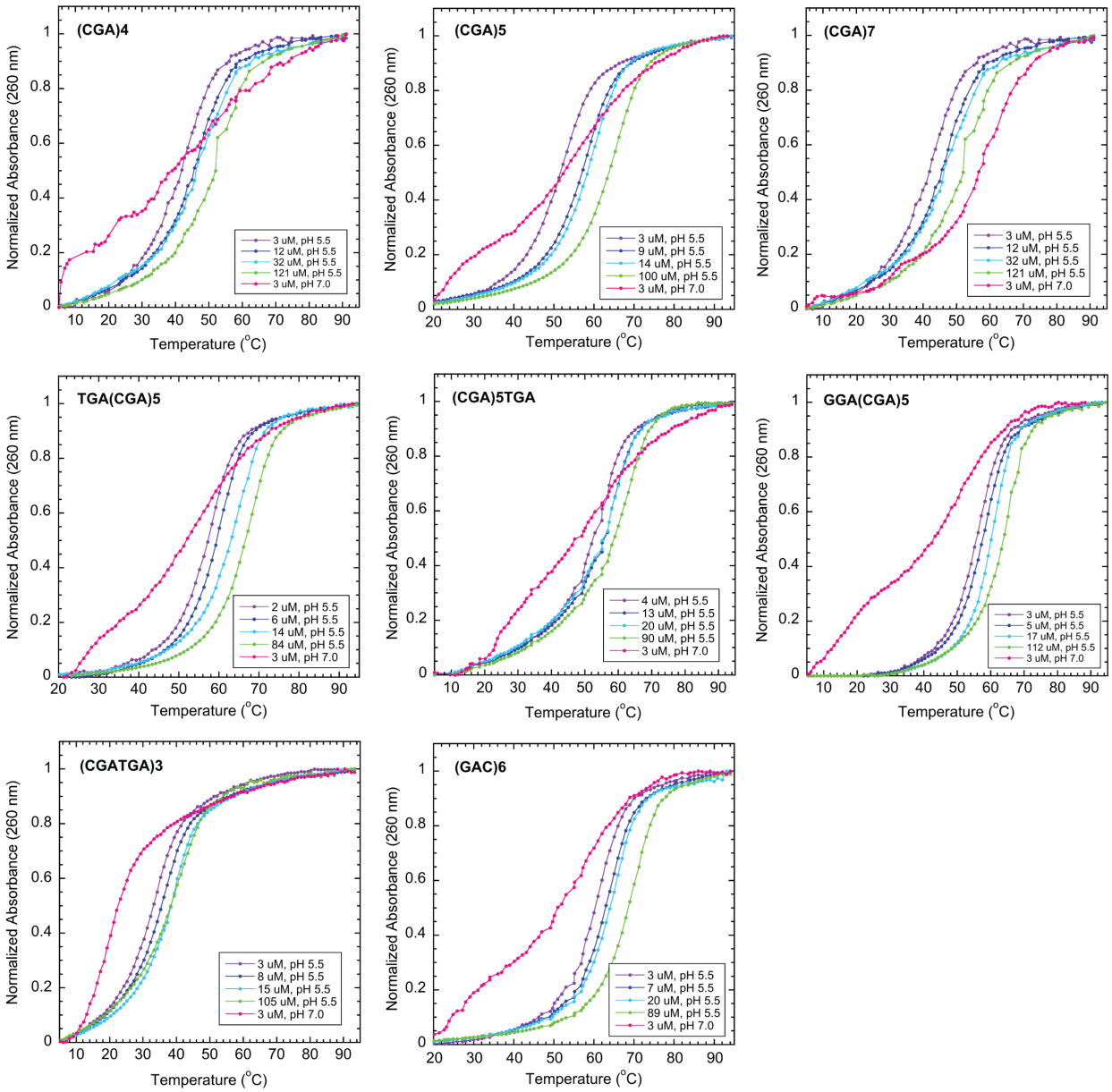

Figure S2. Normalized temperature versus absorbance curves for $(CGA)_n$ variants at pH 5.5 (purple, blue, cyan, green) and pH 7.0 (pink). All $(CGA)_n$ variants have concentration-dependent, two-state melting curves at pH 5.5, indicating bimolecular interactions. At pH 7.0, all variants exhibit non-two-state melting with apparent minor lower-temperature melting transitions between 20-40 °C.

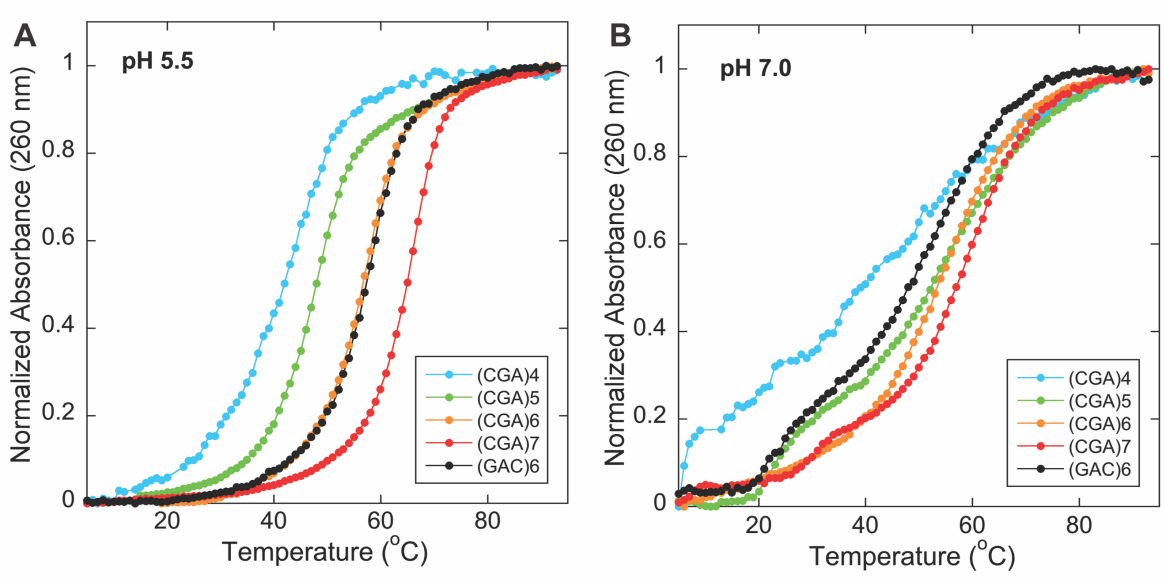

Figure S3. Normalized temperature versus absorbance curves for 2.5 μM $(CGA)_n$ variants, where n = 4 to 7 at (**A**) pH 5.5 and (**B**) pH 7.0. It is unclear why $(GAC)_6$ behaves differently than $(CGA)_6$, as it is a permutation of d(CGA), at pH 7.0. We suspect that the difference could arise from variations in hairpin loop and stem size, as well as the likelihood of interconversion between different hairpin forms. Assuming the d(CGACG) loop is the most stable hairpin form, $(CGA)_6$ could form a 6-bp stem where the d(CGACG) loop is capped by A-A producing the two-state transition seen at pH 7.0. The slight destabilization seen for $(GAC)_6$ could arise from a similar hairpin with a d(CGACG) loop but only accommodating 5-bp. Further, the non-two state melt suggests that $(GAC)_6$ could be dynamically interconverting between two different forms – one with a 5-bp stem and a d(CGACG) loop or 7-bp stem and a d(ACGA) loop.

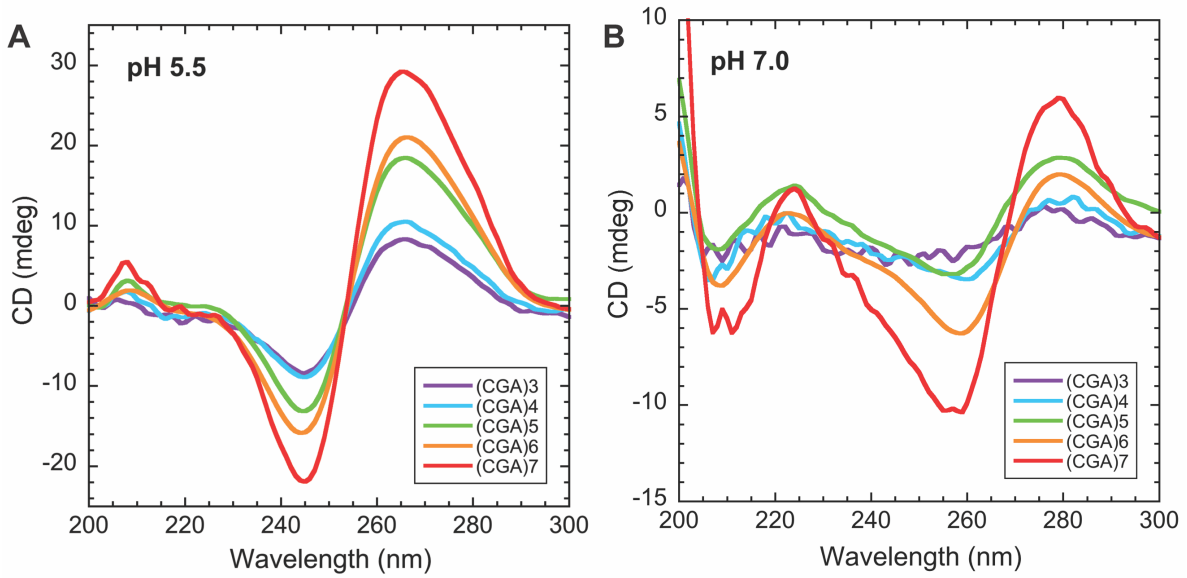

Figure S4. CD spectra of 10 μM $(CGA)_n$ where n = 3 to 7 at (**A**) pH 5.5 and (**B**) pH 7.0. At pH 5.5, the intensity of the bands characteristic of the parallel-stranded duplex (245 nm, 265 nm) decrease as the number of d(CGA) units decrease. This suggests that the parallel-stranded form can exist at all repeat sizes tested. At pH 7.0, the signal intensity corresponding to anti-parallel strands (258 nm, 275 nm) decreases with repeat number, but is lost when $n \leq 4$ suggesting that the hairpin structure cannot form at low repeat number.

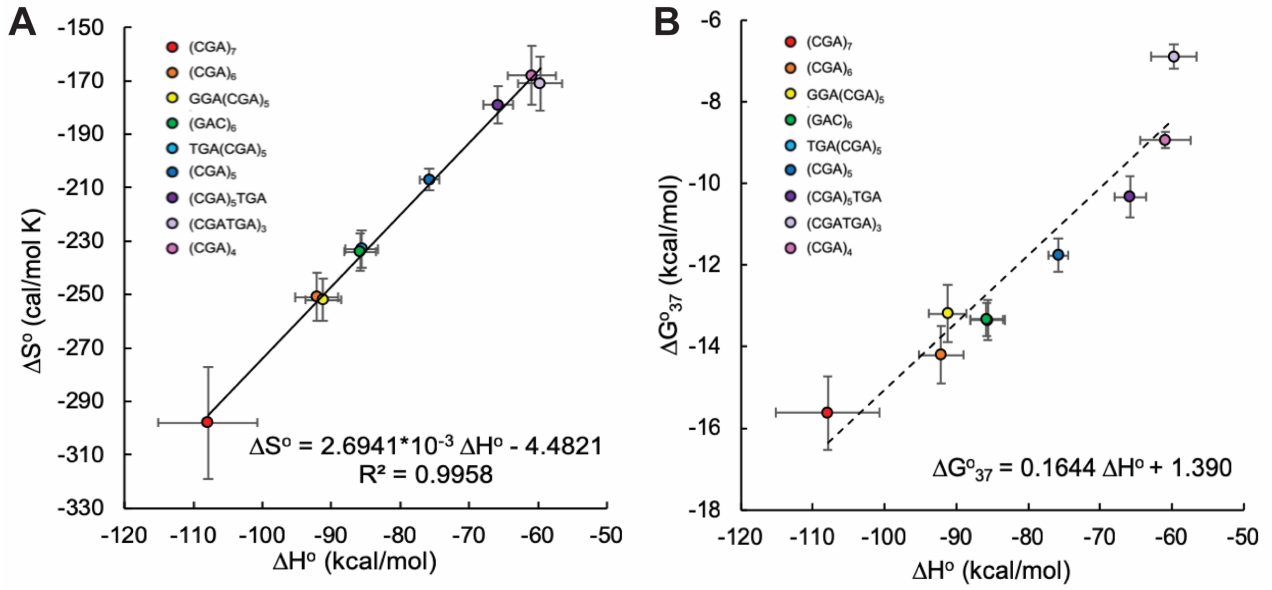

Figure S5. Apparent entropy-enthalpy compensation of all $(CGA)_n$ variants used in this study. (**A**) The positive correlation between ΔH^o and ΔS^o indicates a strong enthalpy-entropy compensation for all oligonucleotides tested. (**B**) The ΔG^o_{37} vs ΔH^o plot suggests that compensation is due to the underlying physical reality, as opposed to experimental error.

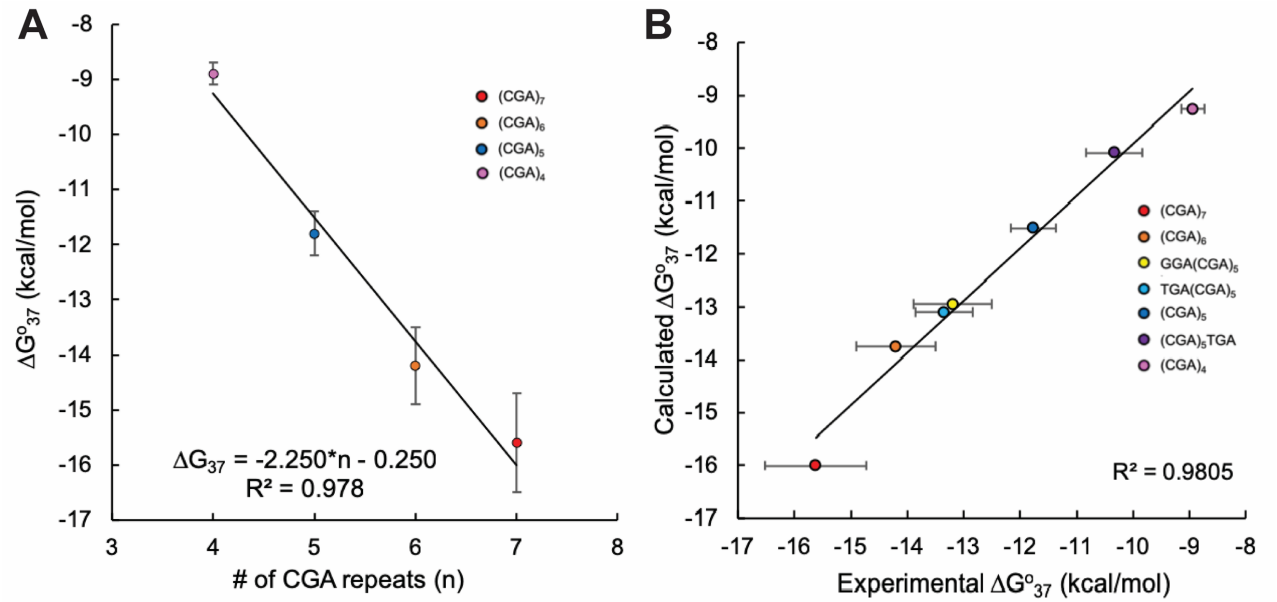

Figure S6. Thermodynamic estimates can be used to predict $\Delta G°_{37}$ values for new triplet repeat sequences. (**A**) Linear fit of $(CGA)_n$ sequences vs experimentally obtained $\Delta G°_{37}$ values. (**B**) Experimental $\Delta G°_{37}$ is highly correlated to calculated $\Delta G°_{37}$ for all sequences, assuming additive contributions from each triplet. Estimates for d(CGA) were obtained from the fit in **A**, while estimates for 5′-d(GGA), 5′-d(TGA), 3′-d(TGA) were obtained from Table 1. All sequences were included except $(TGA)_6$ and $(CGATGA)_3$.

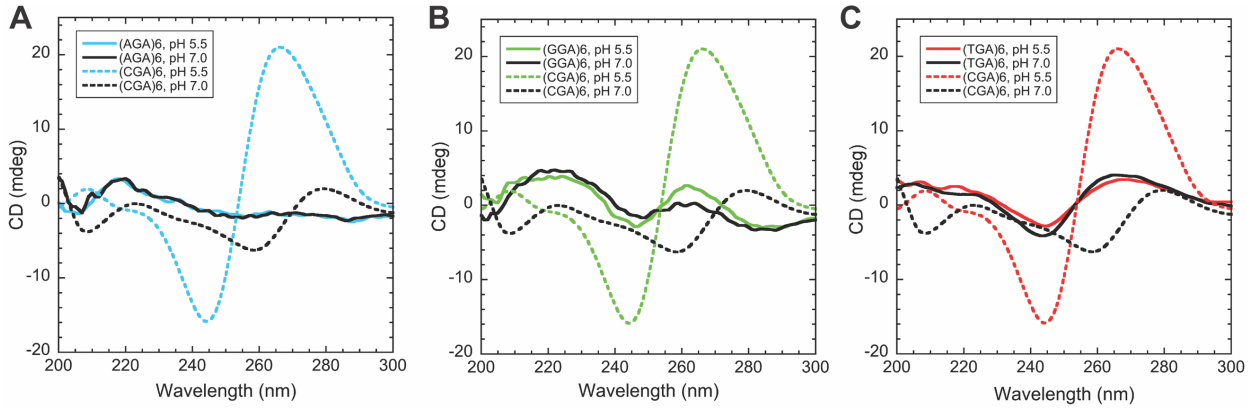

Figure S7. CD spectra of 10 μM $(NGA)_6$ sequences compared to $(CGA)_6$. (**A**) $(AGA)_6$ does not have CD bands characteristic of parallel- or anti-parallel oriented strands and is not pH dependent. The lack of CD signal could suggest a lack of structure at each pH. (**B**) $(GGA)_6$ also does not have CD bands characteristic of parallel- or anti-parallel oriented strands and is not pH dependent. The band at 220 nm could indicate the formation of a different structure at each pH. (**C**) $(TGA)_6$ has CD signal characteristic of the anti-parallel form at each pH, suggesting a lack of pH sensitivity.

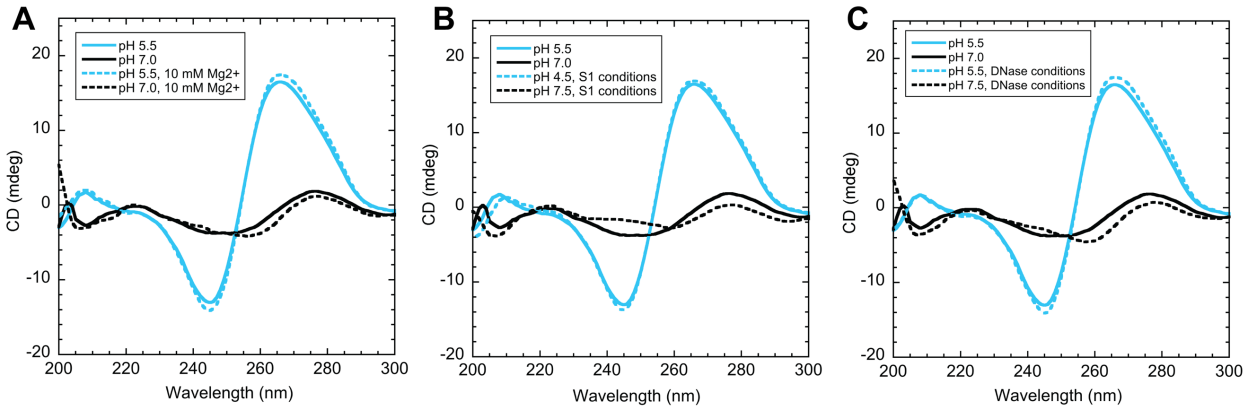

Figure S8. CD spectra of 10 μM GGA(CGA)$_5$ with various divalent cations at pH 5.5 (blue) and pH 7.0 (black). Divalent cations (Mg^{2+}, Zn^{2+}, and Ca^{2+}) do not affect the parallel-stranded structure formed at pH 5.5 or anti-parallel structure formed at pH 7.0. (**A**) The addition of 10 mM magnesium chloride does not affect the structure of GGA(CGA)$_5$ at pH 5.5 or 7.0. (**B**) GGA(CGA)$_5$ retained parallel-stranded structure at pH 4.5 and anti-parallel structure at pH 7.5 in S1 nuclease reaction conditions containing 2 mM zinc sulfate and 300 mM sodium chloride. (**C**) GGA(CGA)$_5$ retained parallel-stranded structure at pH 5.5 and anti-parallel structure at pH 7.5 in DNase I nuclease reaction conditions containing 0.5 mM calcium chloride and 2.5 mM magnesium chloride.

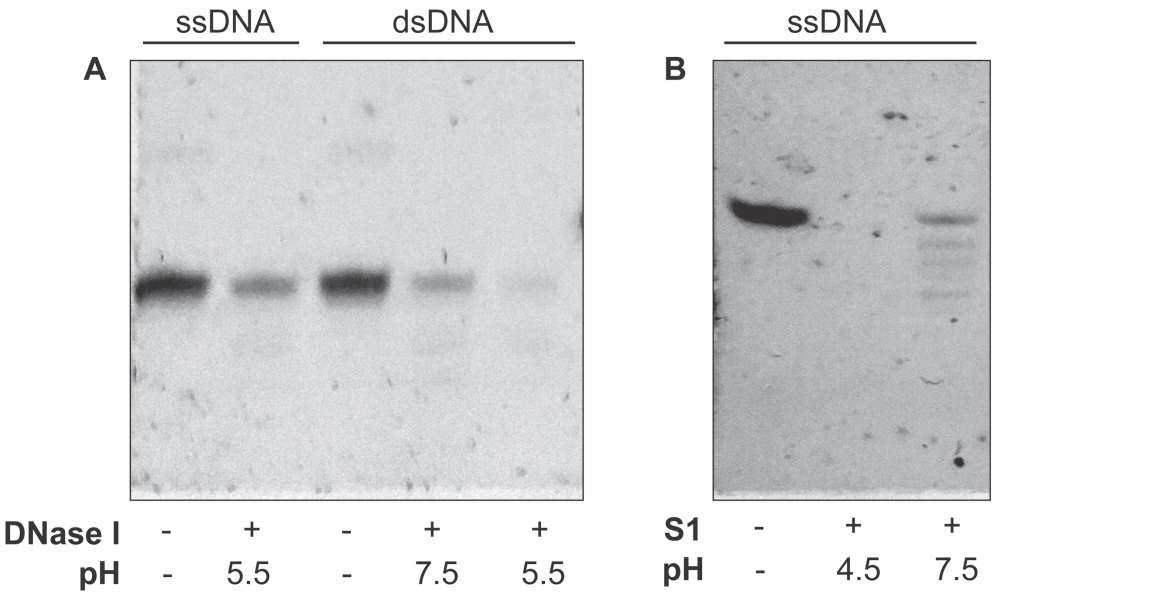

Figure S9. Control DNase I and S1 nuclease sensitivity experiments performed on d(GGACAGCTGGGAG) with Mg^{2+} to form double stranded (ds) DNA or without Mg^{2+} for single stranded (ss) DNA. (**A**) ssDNA and dsDNA exposed to DNase I at pH 5.5 and 7.5. DNase I functioned optimally on dsDNA at pH 5.5. Reduced activity was observed for ssDNA at pH 5.5 and dsDNA at pH 7.5. (**B**) Control ssDNA incubated with S1 nuclease at pH 4.5 and 7.5. S1 nuclease was more active at pH 4.5 than at pH 7.5.

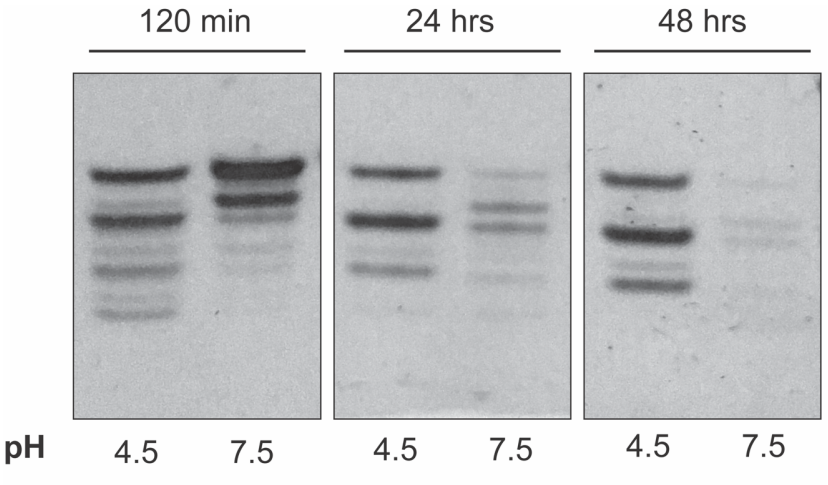

Figure S10. Extended S1 digestion of TGA(CGA)$_5$. The bands are almost completely digested at pH 7.5 when TGA(CGA)$_5$ was exposed to S1 nuclease for 2 days, suggesting that the structure formed at this pH is dynamic or not resistant to S1 digestion over time. In contrast, the intensity of the 18, 15, and 12-nt bands corresponding to digestion by S1 nuclease at pH 4.5 does not change over two days of exposure. This suggests that the parallel-stranded duplex formed is stable and resistant to S1 degradation at room temperature.

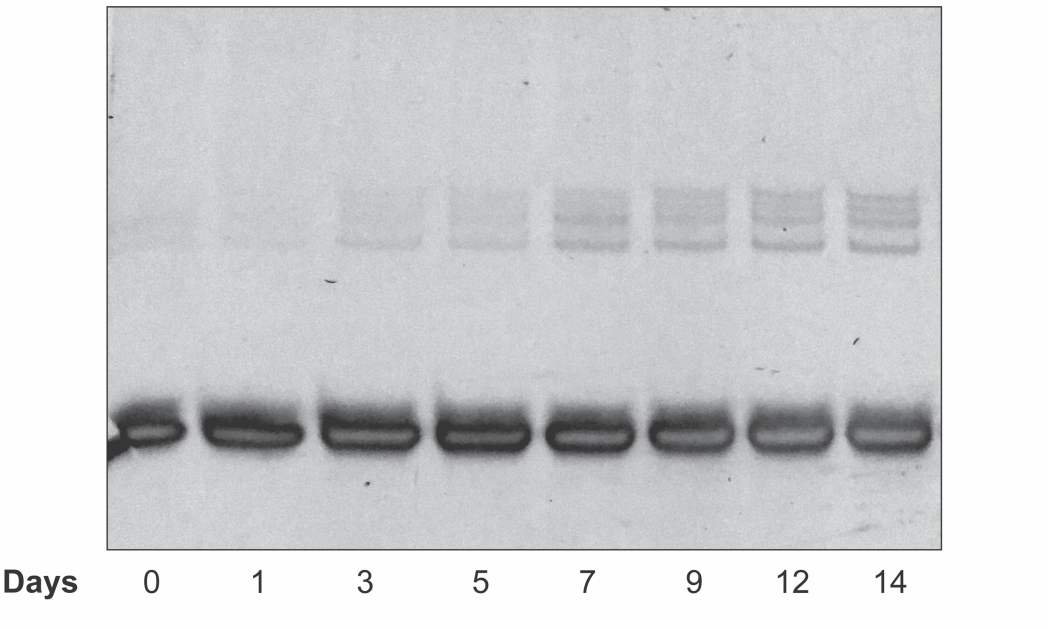

Figure S11. High molecular weight (HMW) bands formed over time when $(CGA)_6$ is incubated in deionized water, at room temperature, for up to 2 weeks, as shown by denaturing PAGE. All variants, except $(TGA)_6$, contain HMW bands in 8 M urea denaturing gels (not shown). HMW band formation increases with time, lower pH, storage temperature and oligonucleotide concentration.