

Supplemental Information

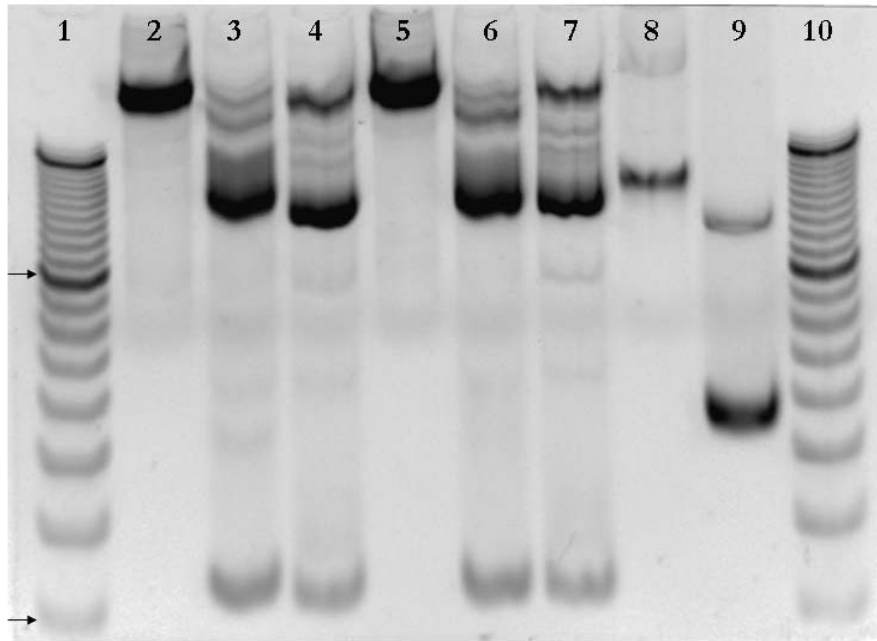


Figure S1. Image of native 12% polyacrylamide gel that structurally characterizes the three-way junction using restriction endonucleases. Lanes 1 and 10 contain a 5bp ladder with 15 (bottom) and 50 (top) base pair oligonucleotides marked with arrows. Lanes 2-4 describe the changes in 1-AP_J. Lane 2 shows the three-way junction before digestion, while Lanes 3 and 4 represent the products of digestion by DraI and BsaAI, respectively. Lanes 5-7 describe the changes in 11-AP_J. Lane 5 shows the three-way junction before digestion, and Lanes 6 and 7 show the products of digestion by DraI and BsaAI, respectively. Digestion in Lanes 3, 4, 6, and 7 yields faster fragments with mobilities between 15 and 20 base pairs, consistent with the cleavage of the 16 base pair termini of the duplex arms (Fig. 1D). Single stranded components are shown in Lanes 8 and 9: the 95 base and the 50 base components of the three-way junction 11-AP_J, respectively. In Lane 9, the lower and upper bands are ascribed to an intramolecularly folded and intermolecularly duplexed species, respectively. Neither of the species from Lanes 8 or Lane 9 is present in Lane 5, indicating stoichiometric formation of the three-way junction.

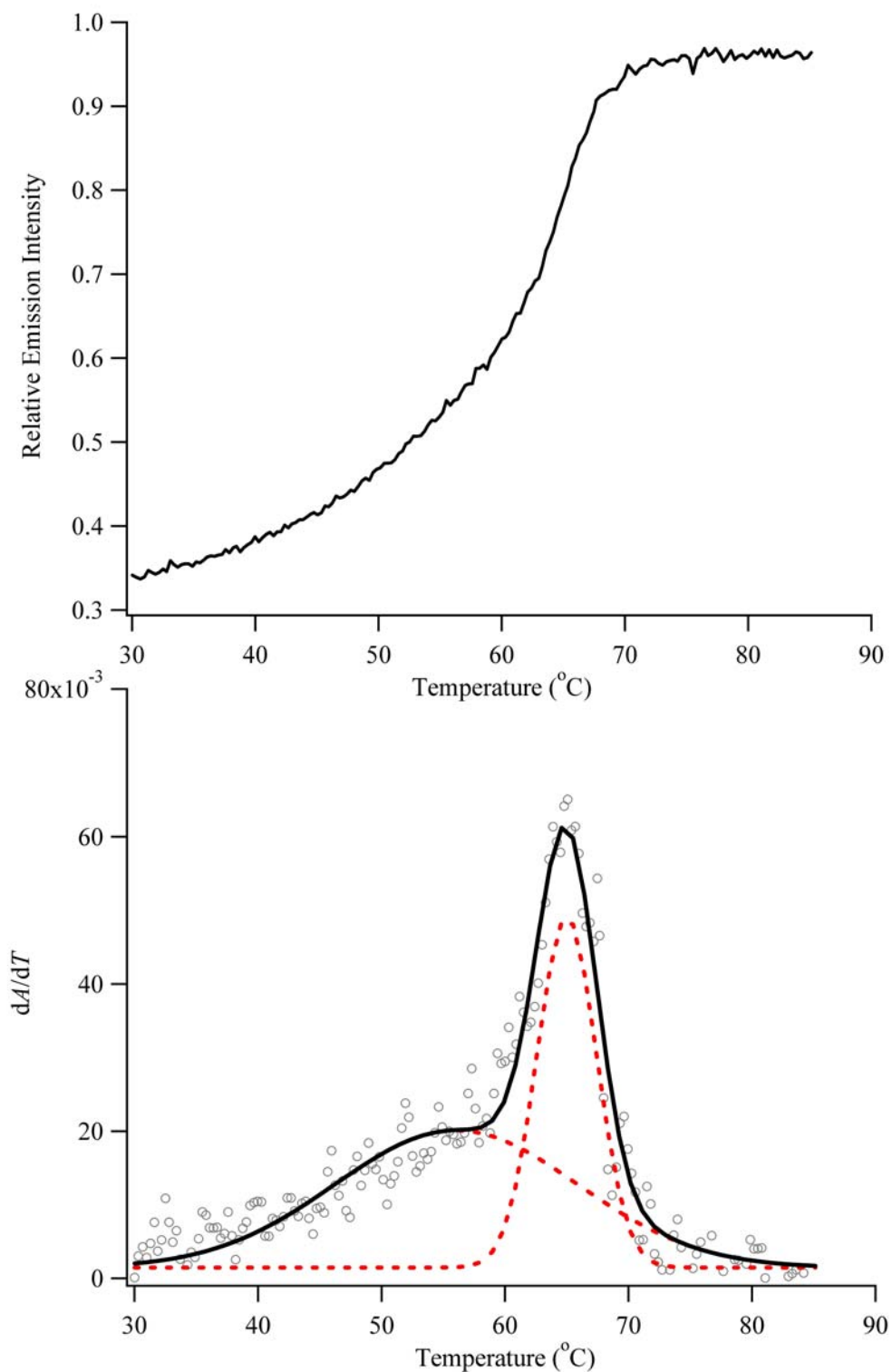


Figure S2. (Top) Fluorescence changes as a function of temperature measured at 370 nm using excitation at 307 nm for α -AP₇ as a function of temperature. (Bottom) Deconvolution of the derivative profile yields two gaussian peaks centered at 55 and 65 °C.

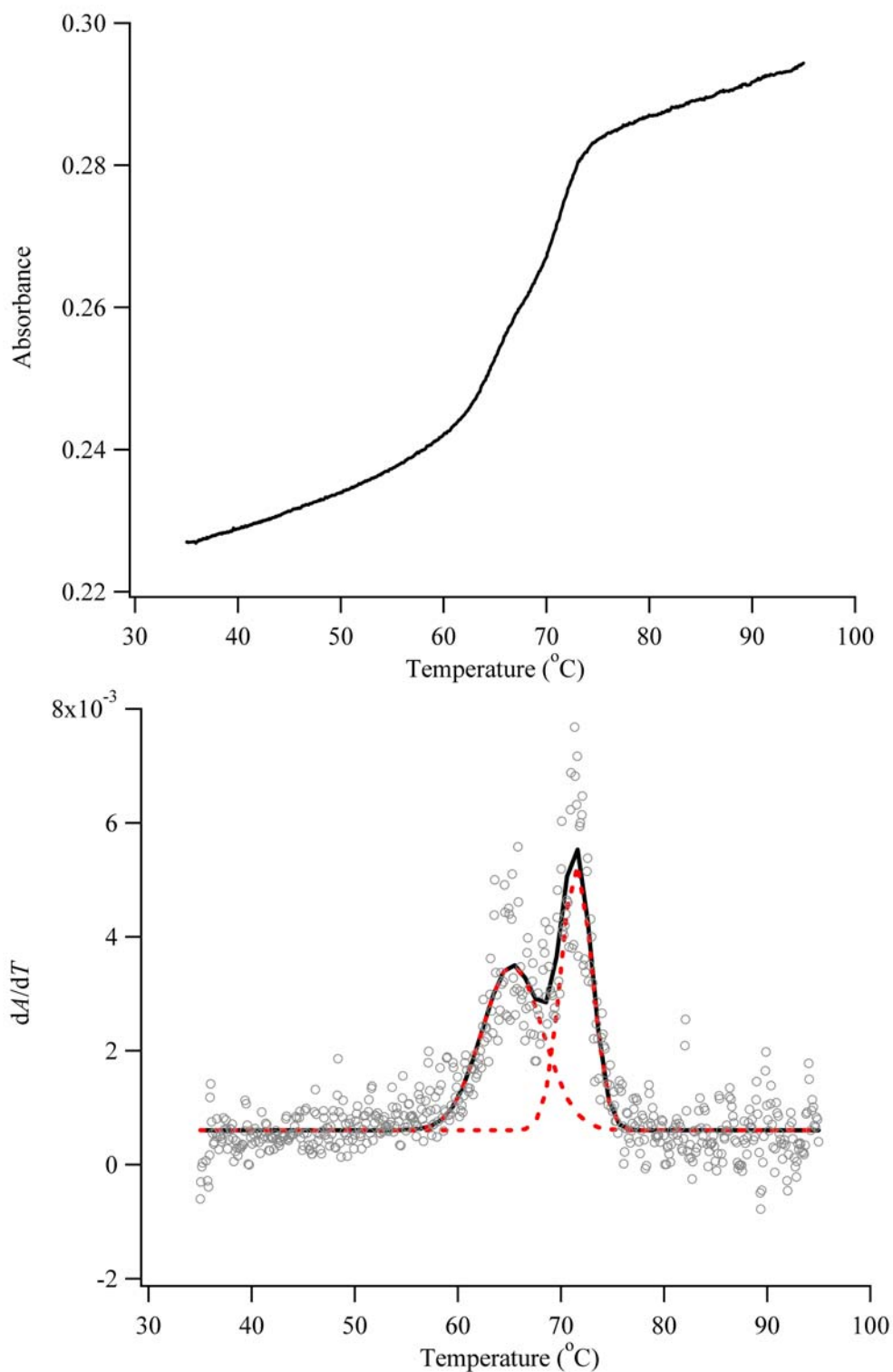


Figure S3. (Top) Absorbance changes at 260 nm as a function of temperature for the unmodified three-way junction. (Bottom) Deconvolution of the derivative profile yields two gaussian peaks centered at 66 and 72 °C.

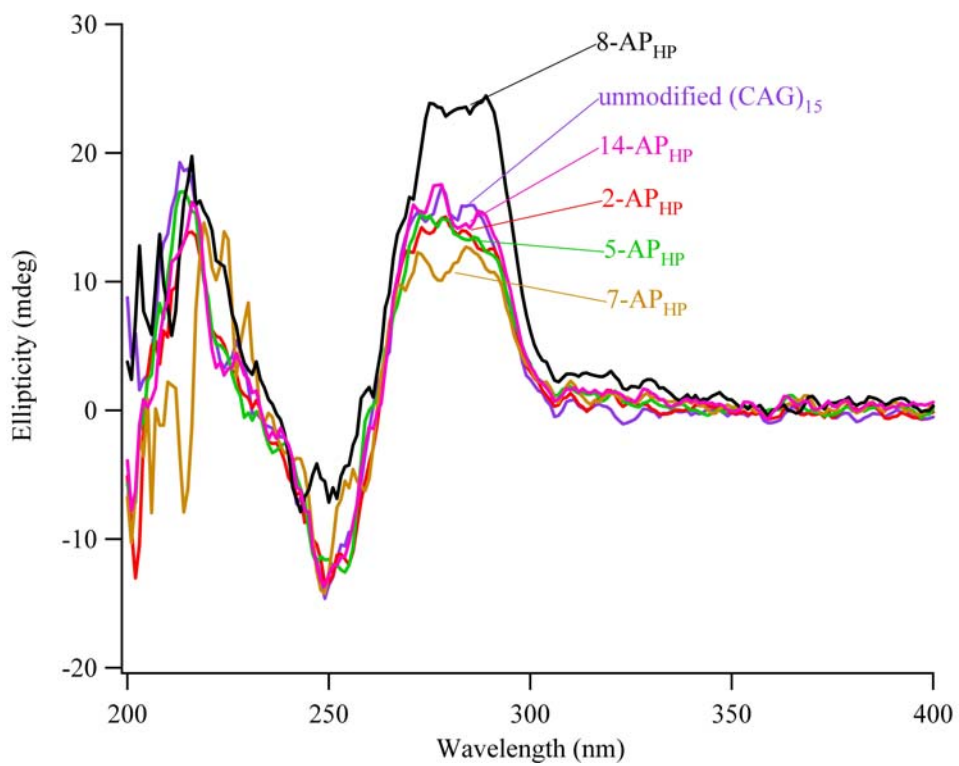


Figure S4. Circular dichroism spectra of modified and unmodified (CAG)₁₅ oligonucleotides sequences at 20 °C.

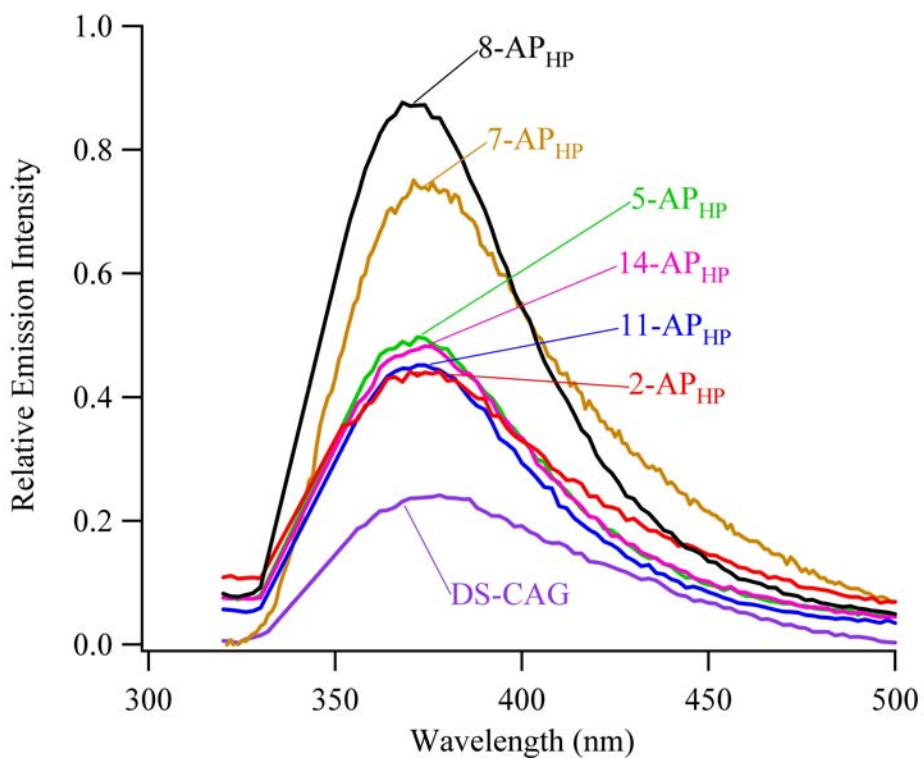


Figure S5. Fluorescence spectra of the modified (CAG)₁₅ oligonucleotides normalized relative to the single-stranded DNA reference (SS-CAG) at 20 °C. Positions of the substitutions follow the notations in Figure 1A.

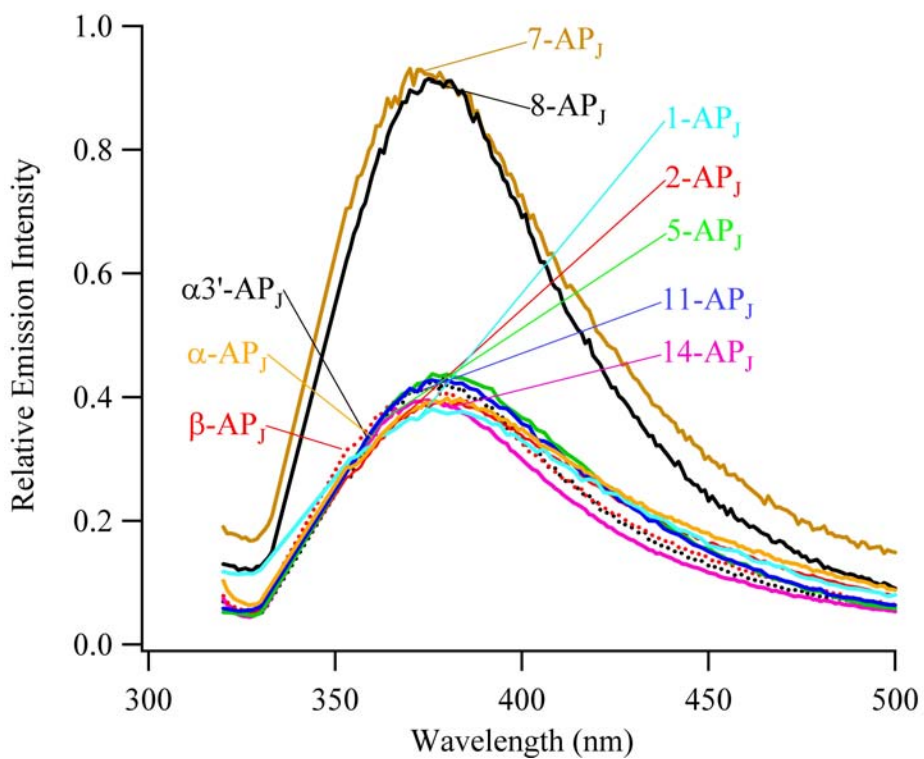


Figure S6. Fluorescence spectra of the modified three-way junctions normalized relative to the single-stranded DNA reference (SS-CAG) at 20 °C. Positions of the substitutions follow the notations in Figure 1D.

Table 1S. Thermodynamic Data for Thermal Denaturation Pertaining to the Three-Way Junction Derived from Absorbance Measurements.^a

position ^b	T _{m1} (°C)	ΔH ₁ (kcal/mol)	ΔS ₁ (cal/mol/K)	T _{m2} (°C)	ΔH ₂ (kcal/mol)	ΔS ₂ (cal/mol/K)
2-AP _J	66.0 ±1.0	115±12	338±33	72.0± 1.0	212±21	617±62
14-AP _J	66.4 ±1.0	120±12	354±35	71.9± 1.0	235±24	713±71
unmodified	65.6 ±0.7	110±9	326±24	71.9± 0.6	235±3	712±8
unmodified duplex				75.4± 1.1	252±12	694±30

^a Standard deviations are derived from a minimum of three measurements. Melting temperatures (T_m), enthalpy changes (ΔH), and entropy changes (ΔS) derived from absorbance changes at 260 nm.

^b See Figure 1D for location of modifications in (CAG)₁₅.