Supporting Information

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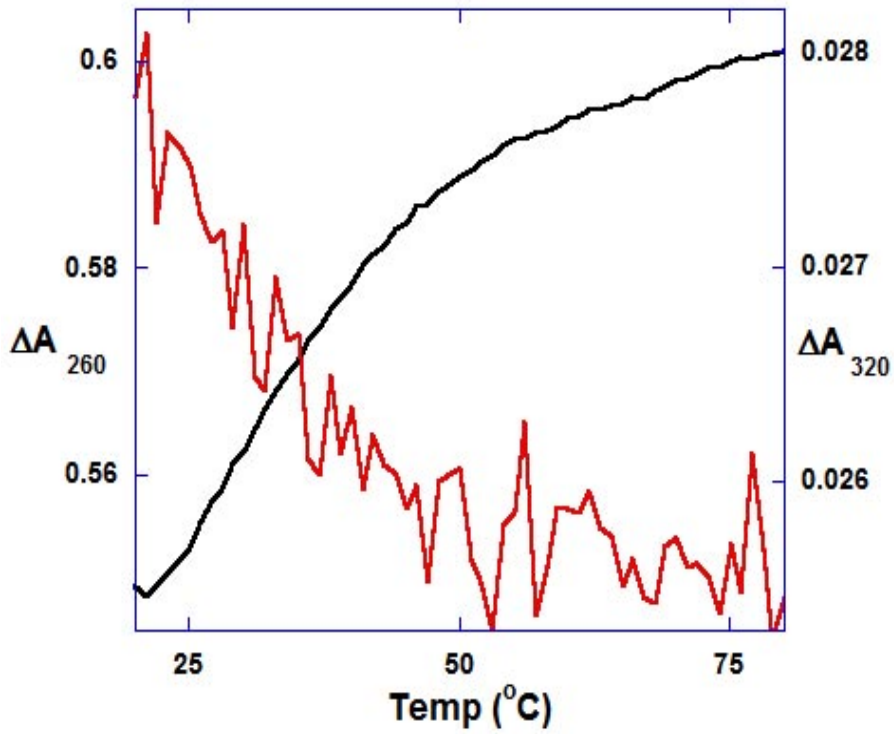

Fig. S1. Melting curves for ssDNA containing a 2-AP dimer probe. UV absorbance measured at 260 nm (black line) and 320 nm (red line), respectively. See Fig. 1 for residue sequence.

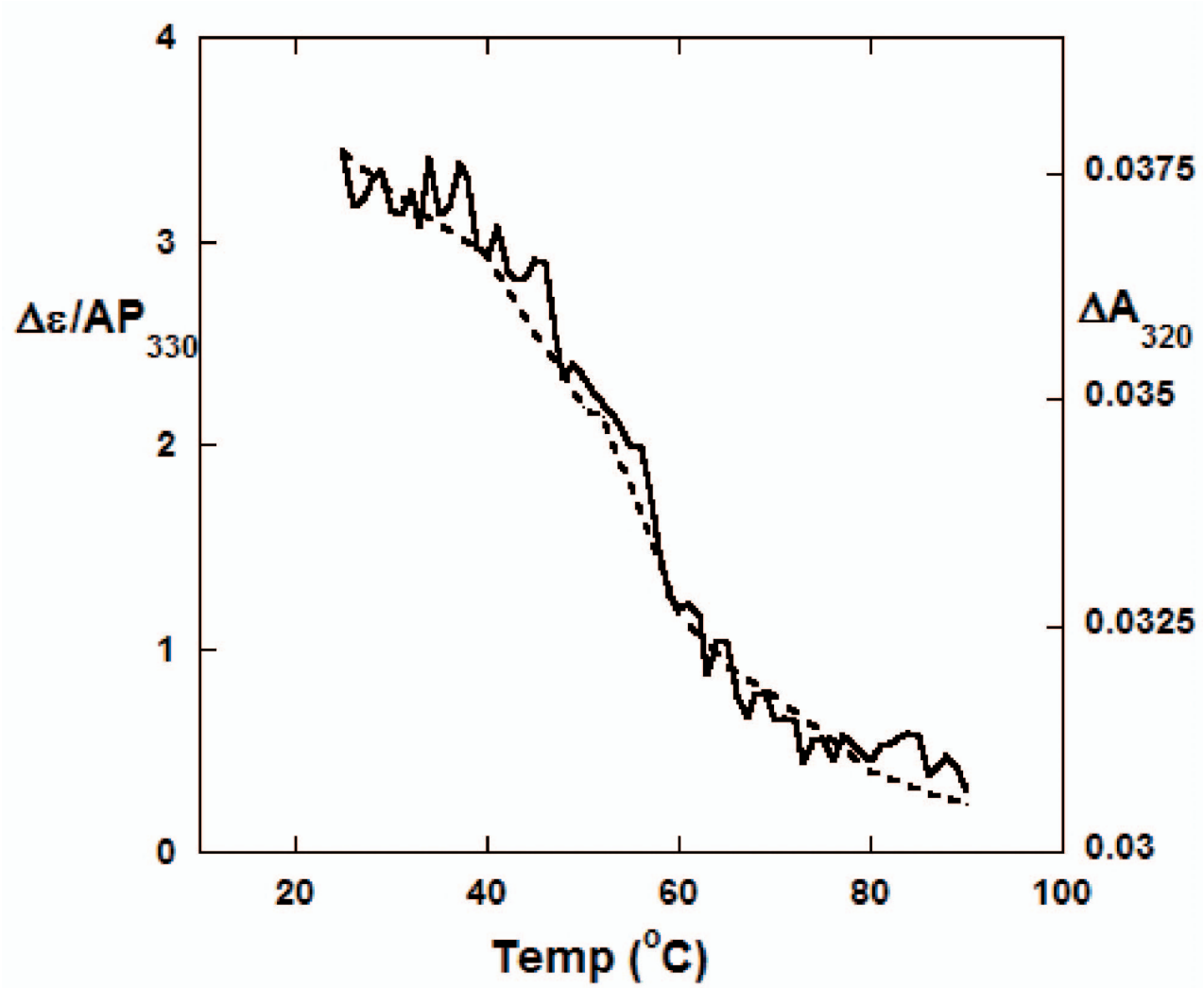

Fig. S2. Melting curves of forked DNA constructs. UV (dashed line) and CD (solid line) melting curves of construct {1,2}, monitored at 320 and 330 nm, respectively.

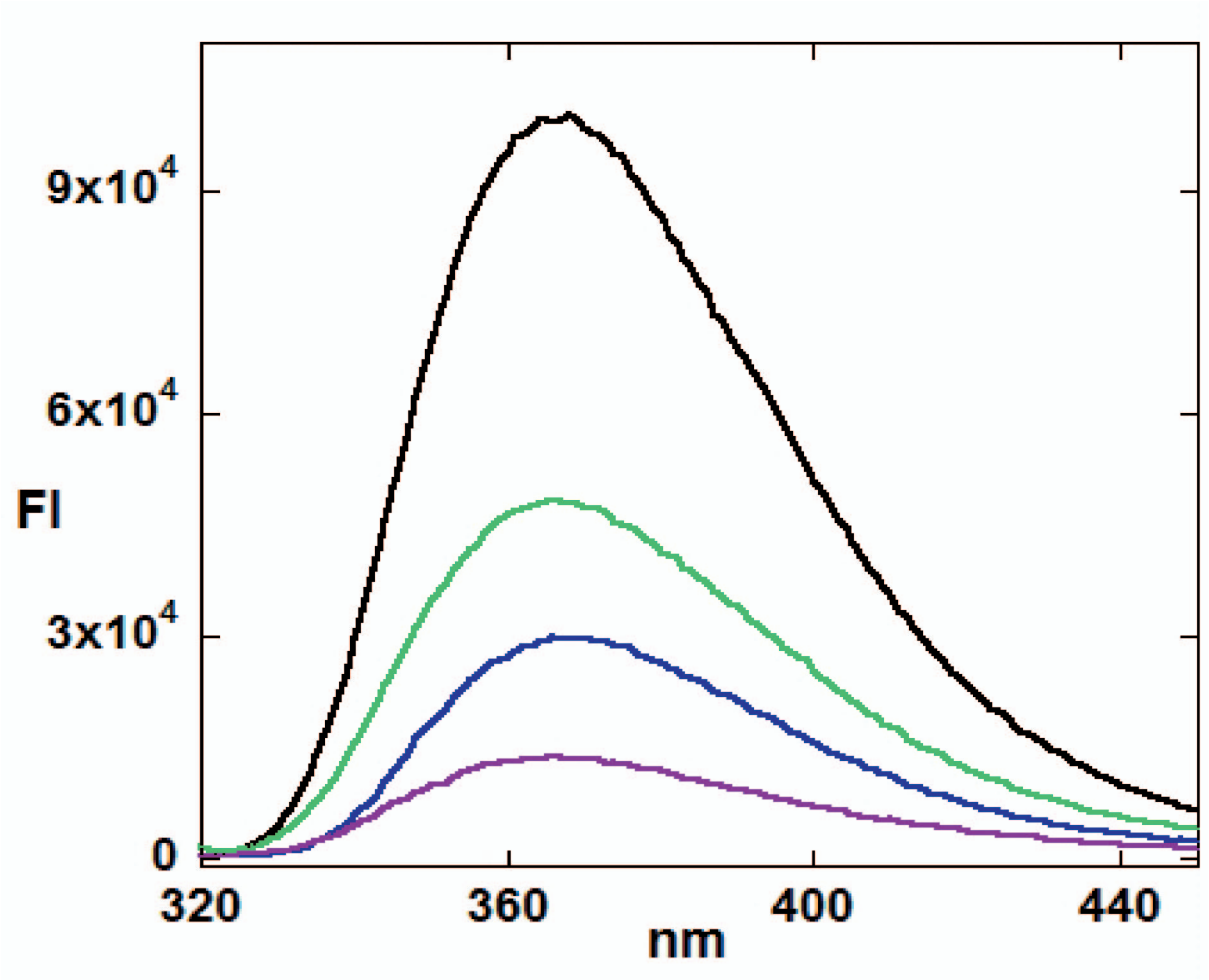

Fig. S3. Representative fluorescence spectra of ssDNA and dsDNA constructs. A single 2-AP base (black) and a 2-AP dimer probe (green) located within a ssDNA construct; a single 2-AP base (blue) and a 2-AP dimer probe (purple) within a dsDNA construct. The fluorescence excitation wavelength was set at 315 nm, and emission spectra were measured from 300 to 450 nm.

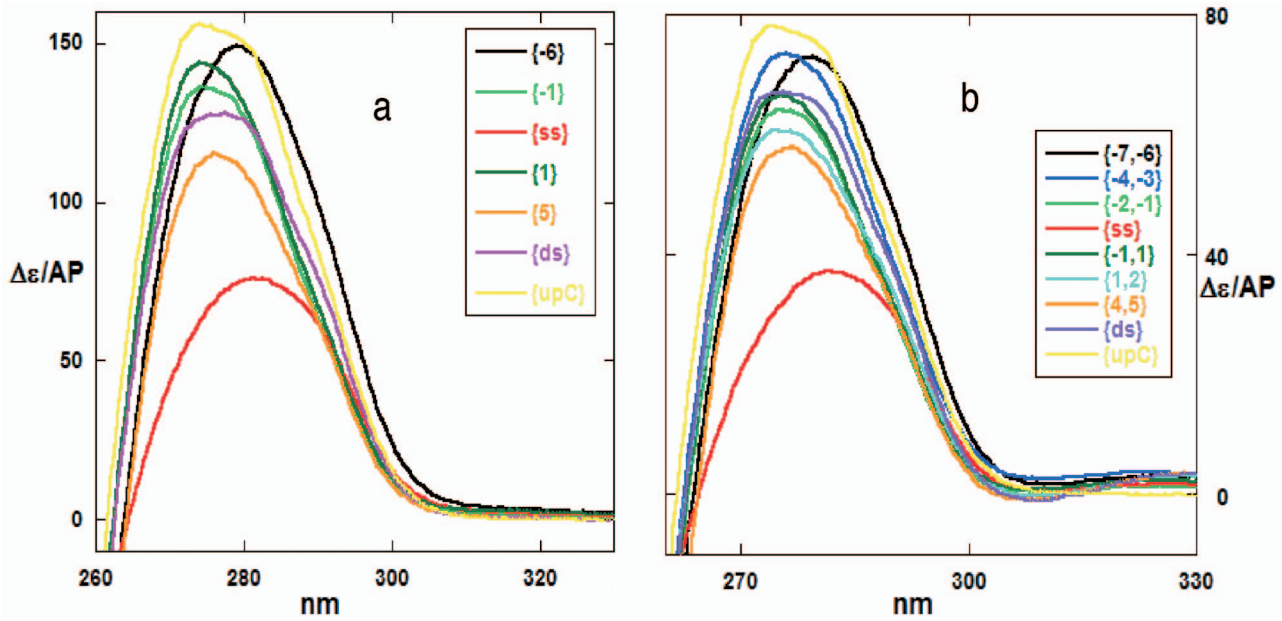

Fig. S4. Representative CD spectra of forked DNA constructs containing 2-AP probes in various positions, showing ''spillover'' above 300 nm. (*a*) Spectra from 260 to 330 nm for constructs containing single 2-AP bases. (*b*) Spectra from 260 to 330 nm for constructs containing pairs of 2-AP bases. The spectrum labeled ''UpC'' was obtained with a dsDNA molecule containing no 2-AP bases. See Fig. 1 for construct sequences.

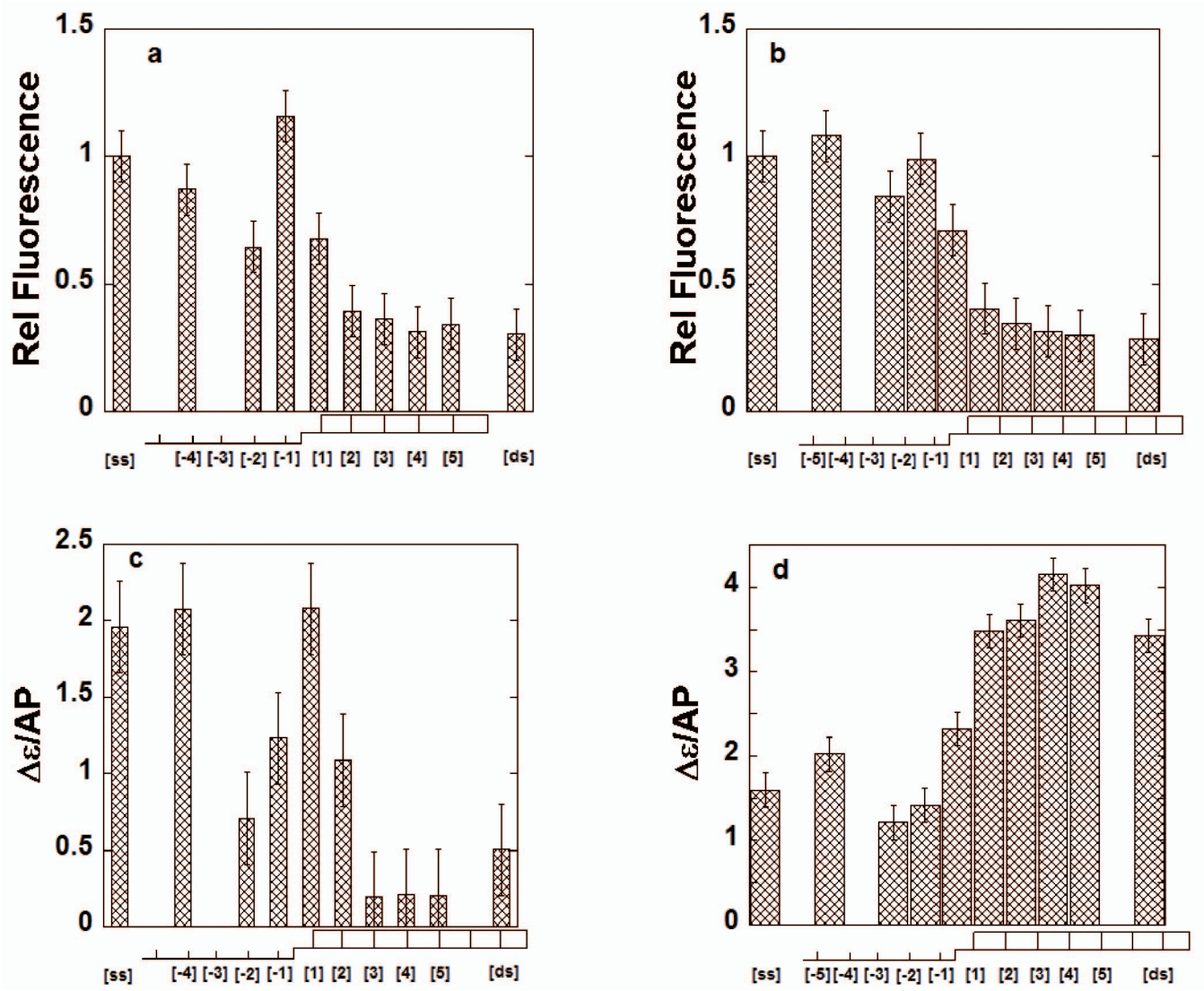

Fig. S5. Spectroscopic properties of P/T DNA constructs as a function of position relative to the ssDNA–dsDNA junction. (*a*) Fluorescence intensity changes at 370 nm for constructs containing single 2-AP probes. (*b*) Fluorescence intensity changes at 370 nm for constructs containing 2-AP dimer probes. (*c*) Ellipticity changes at 320 nm for constructs containing single 2-AP probes. (*d*) Ellipticity changes at 330 nm for constructs containing 2-AP dimer probes. Intensities for probes in ssDNA or dsDNA are at the left and right of each panel, respectively. Ellipticity changes are per mol 2-AP. Fluorescence intensity changes have been normalized to the intensity of the probe signal in the control ssDNA. The bars corresponding to the single 2-AP probes are centered on the probe position. The bars corresponding to the 2-AP dimer probes are broader and bracket the two positions involved.

Table S1. T_m values for various forked DNA constructs from CD and UV melting curves

Forked construct	T_m, °C ($\Delta\varepsilon_{330nm}$)	T_m, °C (ΔA_{320nm})	T_m, °C (ΔA_{260nm})
{−2,−1}	No T_m	No T_m	57 (± 2)
{−1,1}	51 (± 3)	-	57 (± 1)
{1,2}	55 (± 2)	55 (± 3)	57 (± 2)
{3,4}	57 (± 2)	57 (± 2)	57 (± 2)
{4,5}	57 (± 2)	-	57 (± 2)

Data are from Fig. 2.

Table S2. Percentage of double-stranded character at positions 1 and 2 of forked and P/T junction DNA constructs based on CD and fluorescence intensities

Construct	(−1,1), %	(1,2), %	(1), %	(2), %
CD (forked DNA)	50 ± 3	82 ± 3		
Fluorescence (forked DNA)	53 ± 4	87 ± 4	34 ± 3	93 ± 3
CD (P/T DNA)	45 ± 3	85 ± 3		
Fluorescence (P/T DNA)	53 ± 4	87 ± 4	53 ± 4	87 ± 4

Data are from Fig. 4 and Fig. S5.

Table S3. Percentage of double-stranded character at various positions on forked DNA constructs at different temperatures from CD melting curves

Temperature, °C	{−1,1}, %	{1,2}, %	{2,3}, %	{4,5}, %
25	48 ± 3	80 ± 32	100	100
37	46 ± 3	73 ± 3	92 ± 2	92 ± 2
55	32 ± 4	42 ± 3	51 ± 2	51 ± 3
65	14 ± 3	14 ± 4	15 ± 4	14 ± 3

Data are from Fig. 2*B*.