Supporting Information

Base-Stacking Heterogeneity in RNA Resolved by Fluorescence-Detected Circular Dichroism Spectroscopy

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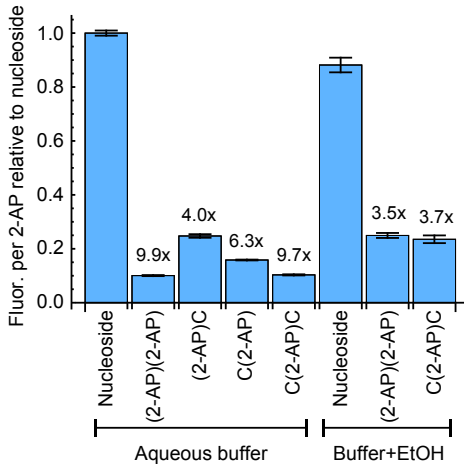

Figure S1. Fluorescence intensities of all samples used in this study, determined from emission spectra recorded under excitation at 295 nm. Intensities per unit 2-AP absorbance were determined and then normalized to the intensity of free 2-AP riboside in aqueous buffer. Error bars are the standard deviation of measurements on three independently prepared samples. Numbers above the bars are the factors by which 2-AP fluorescence is quenched relative to the free nucleoside in the same solvent.

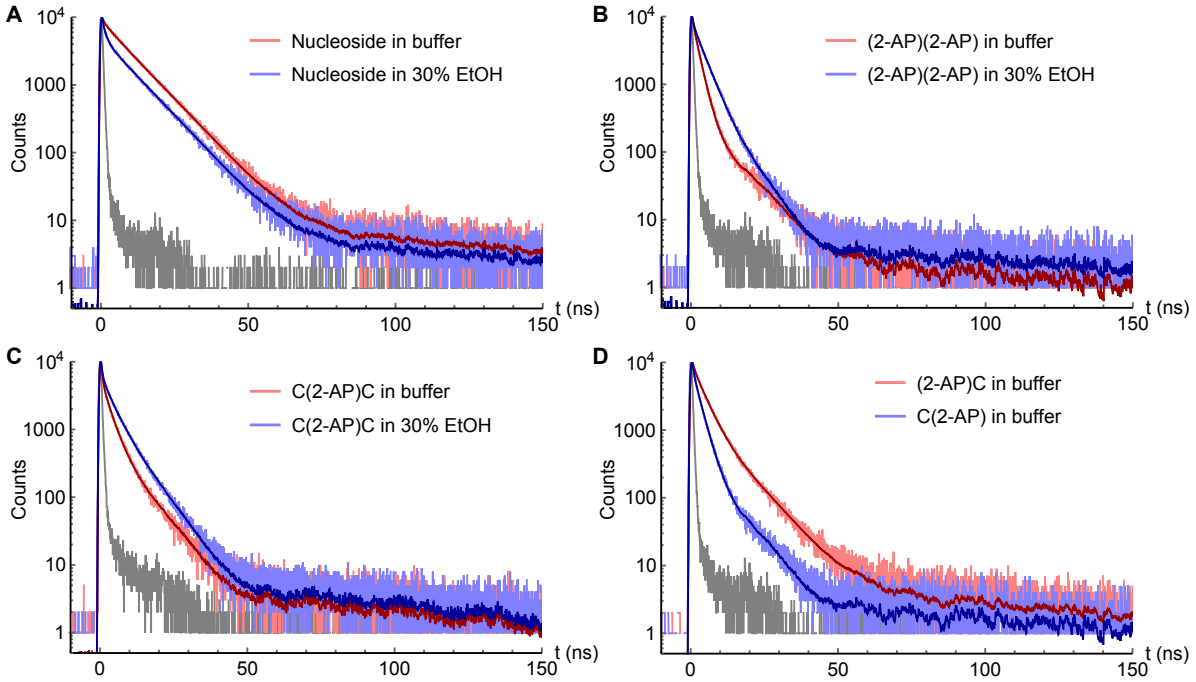

Figure S2. TCSPC data and fits. Gray: instrument response function; Light red and blue: data; Dark red and blue: fits. (A) 2-AP riboside in buffer (red) and buffer containing 30% v/v ethanol (blue). (B) 2-AP dinucleotide in buffer (red) and buffer containing 30% ethanol (blue). (C) C(2-AP)C in buffer (red) and buffer containing 30% ethanol (blue). (D) (2-AP)C in buffer (red) and C(2-AP) in buffer (blue).

Sample	Solvent	α_1 (%)	τ_1 (ns)	α_2	τ_2	α_3	τ_3
Nucleoside	B	7.3	0.08	2.6	2.58	90.1	9.37
Nucleoside	B+E	13.1	0.08	9.5	1.25	77.4	9.28
(2-AP)(2-AP)	B	19.9	0.16	70.2	2.03	9.9	9.46
(2-AP)(2-AP)	B+E	19.2	0.08	66.8	3.55	14.0	7.05
(2-AP)C	B	8.8	0.29	62.9	3.65	28.3	9.49
C(2-AP)	B	19.9	0.23	71.2	2.43	8.9	8.35
C(2-AP)C	B	30.2	0.16	46.2	2.59	23.6	7.11
C(2-AP)C	B+E	21.8	0.10	26.7	2.77	51.5	6.36

Table S1. Fitting parameter values obtained from TCSPC data. Lifetimes highlighted in red are shorter than the FWHM of the IRF by a factor of 5 or more. These components were thus considered unreliable and were subsumed into the "dark" population α_0 in Table 1. "B": Aqueous buffer. "B+E": Buffer containing 30% ethanol.

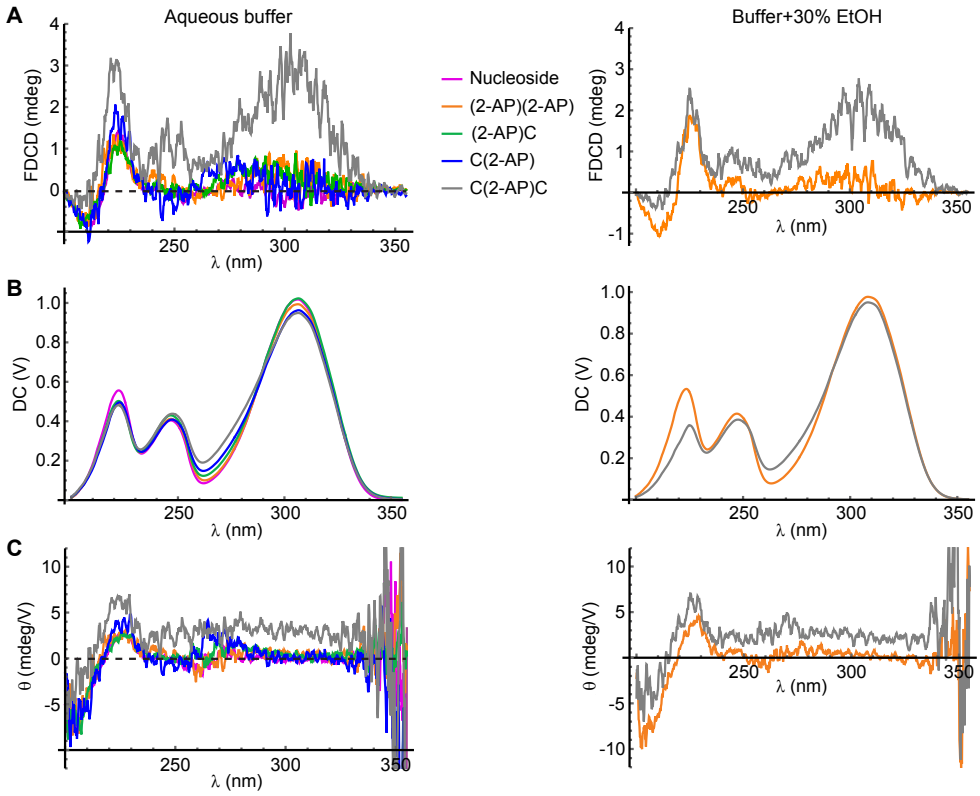

Figure S3. Unprocessed FDCD data. Spectra on the left were recorded in aqueous phosphate buffer and spectra on the right were recorded in buffer containing 30% v/v ethanol. (A) The "FDCD" readout from the instrument after baseline correction using 2-AP nucleobase. This signal is proportional to the difference in fluorescence intensity observed under excitation with left- and right-handed circularly polarized light. (B) The "DC" readout from the instrument after baseline correction using solvent. This signal is proportional to the sum of the intensities observed under excitation with left- and right-handed circularly polarized light. (C) θ = FDCD/DC is the input for processing the FDCD data using the equations in the main text.

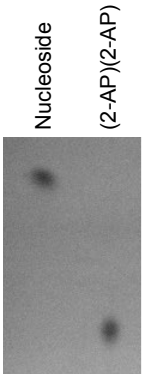

Figure S4. Thin-layer chromatography shows that the 2-AP nucleoside and (2-AP)(2-AP) samples are chemically pure.

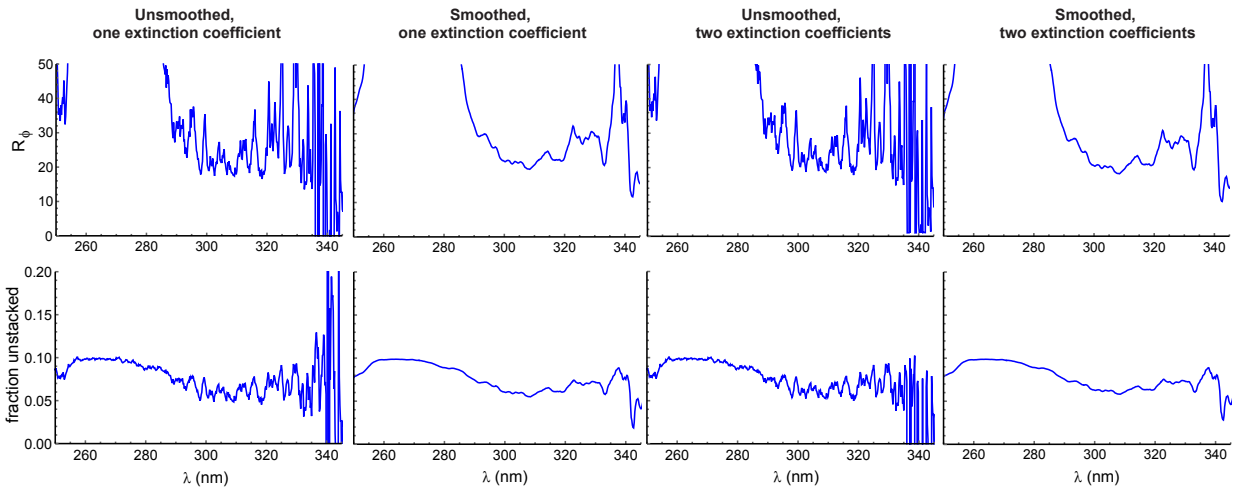

Figure S5. Plots of the quenching ratio R_ϕ (top) and fraction unstacked f_{un} (bottom) obtained for C(2-AP)C samples in aqueous buffer under different model parameters. Results are quantified in Table 2.

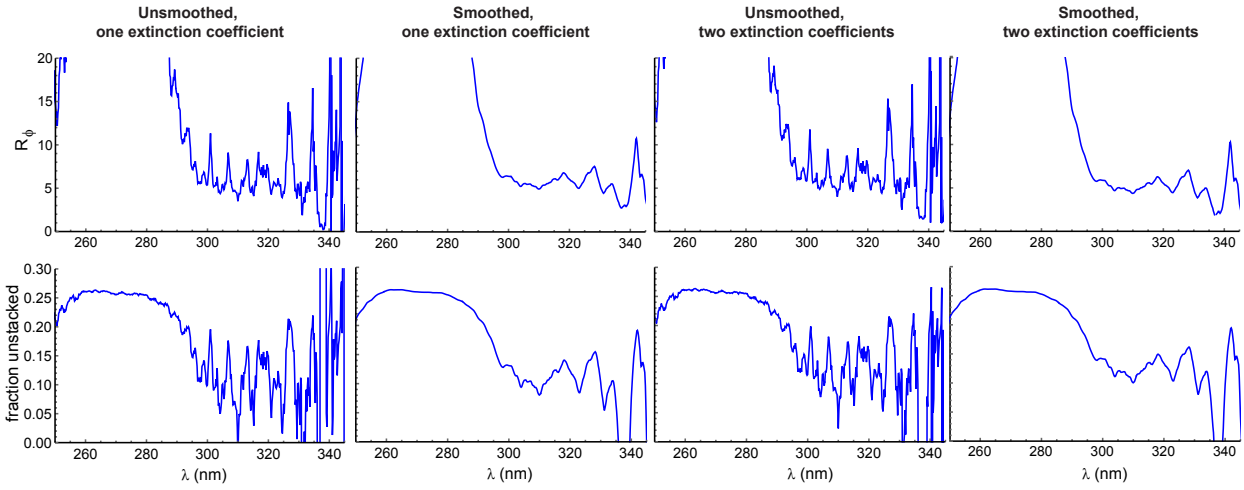

Figure S6. Plots of the quenching ratio R_ϕ (top) and fraction unstacked f_{un} (bottom) obtained for C(2-AP)C samples in buffer containing 30% ethanol under different model parameters. Results are quantified in Table 2.

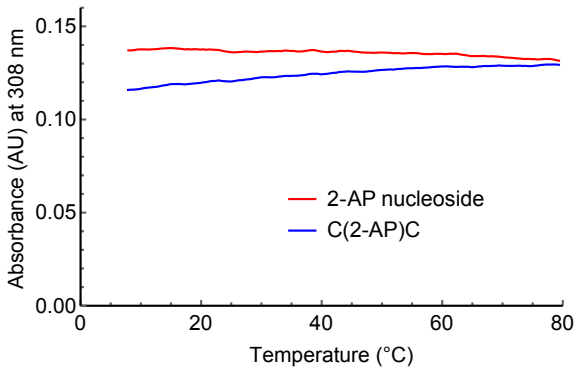

Figure S7. Thermal ramp monitoring absorbance by 2-AP nucleoside and C(2-AP)C at 308 nm.

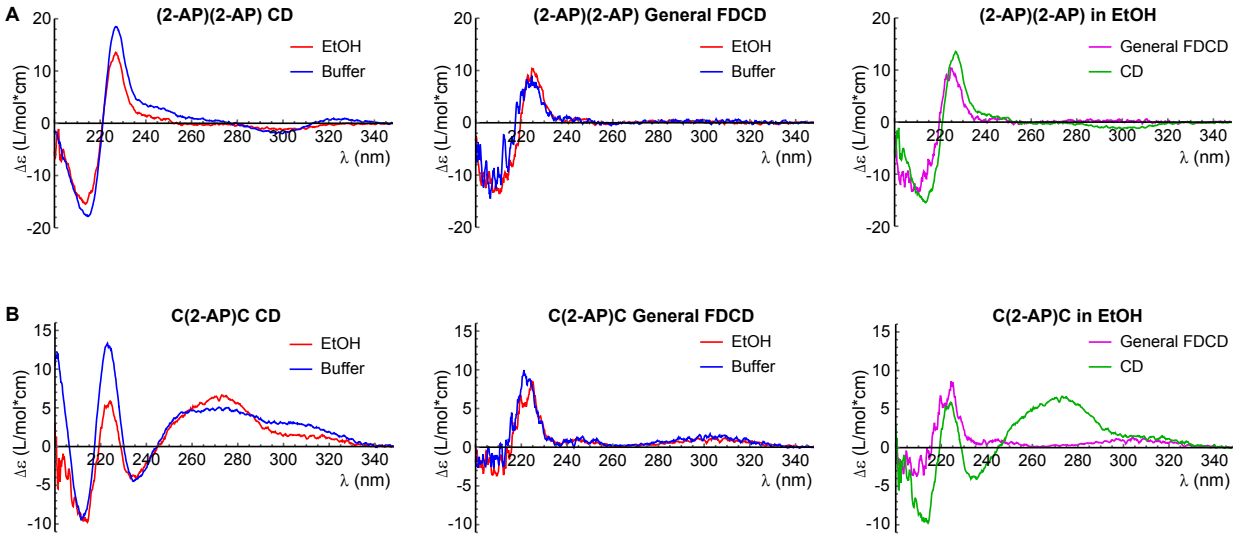

Figure S8. Comparison of spectra recorded in buffer (blue) and buffer+30% ethanol (red) for (A) (2-AP)(2-AP) and (B) C(2-AP)C. Left: Standard CD spectra. Middle: FDCD spectra processed with equation 2. Right: Comparison of FDCD (magenta) and CD (green) spectra for samples in 30% ethanol.