Electronic Supplementary Information

to the manuscript:

On the thermodynamics of folding of an i-motif DNA in solution under favorable conditions

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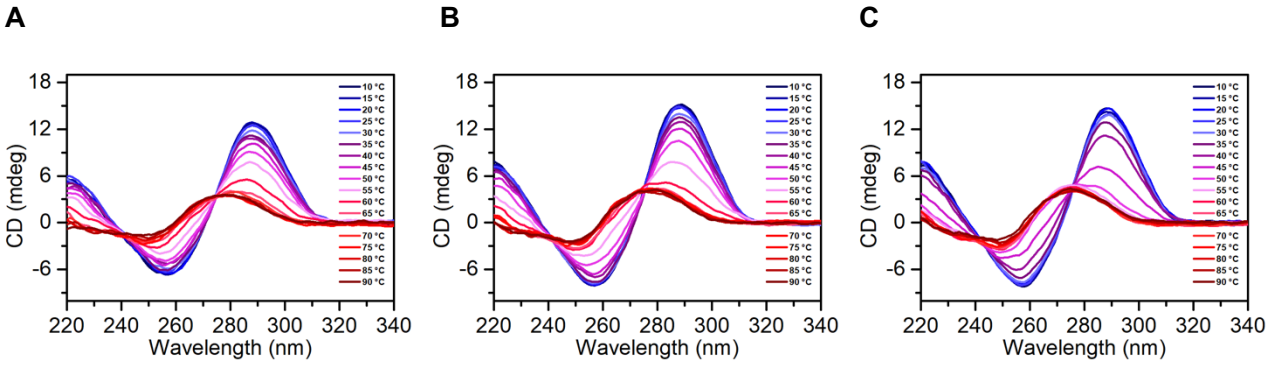

Fig. S1. CD spectra for the iM-forming oligonucleotide d(CCCTAA)$_3$CCCT as a function of temperature in Na^+-containing acetate buffer at pH (**A**) 4.5, (**B**) 5.0, and (**C**) 5.5.

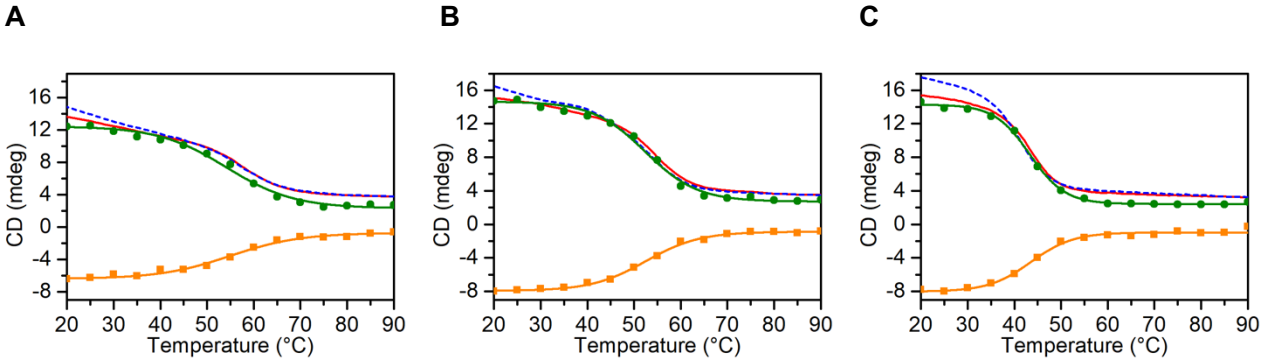

Fig. S2. CD melting (red solid lines) and annealing (blue dashed lines) curves for the iM-forming oligonucleotide d(CCCTAA)$_3$CCCT recorded at 288 nm with a scan rate of 0.5 °C min^{-1}, and CD melting curves at 288 (green lines/circles) and 257 (orange lines/squares) nm derived from the corresponding CD spectra at different temperatures (collected every 5 °C) for the iM in Na^+-containing acetate buffer at pH (**A**) 4.5, (**B**) 5.0, and (**C**) 5.5.

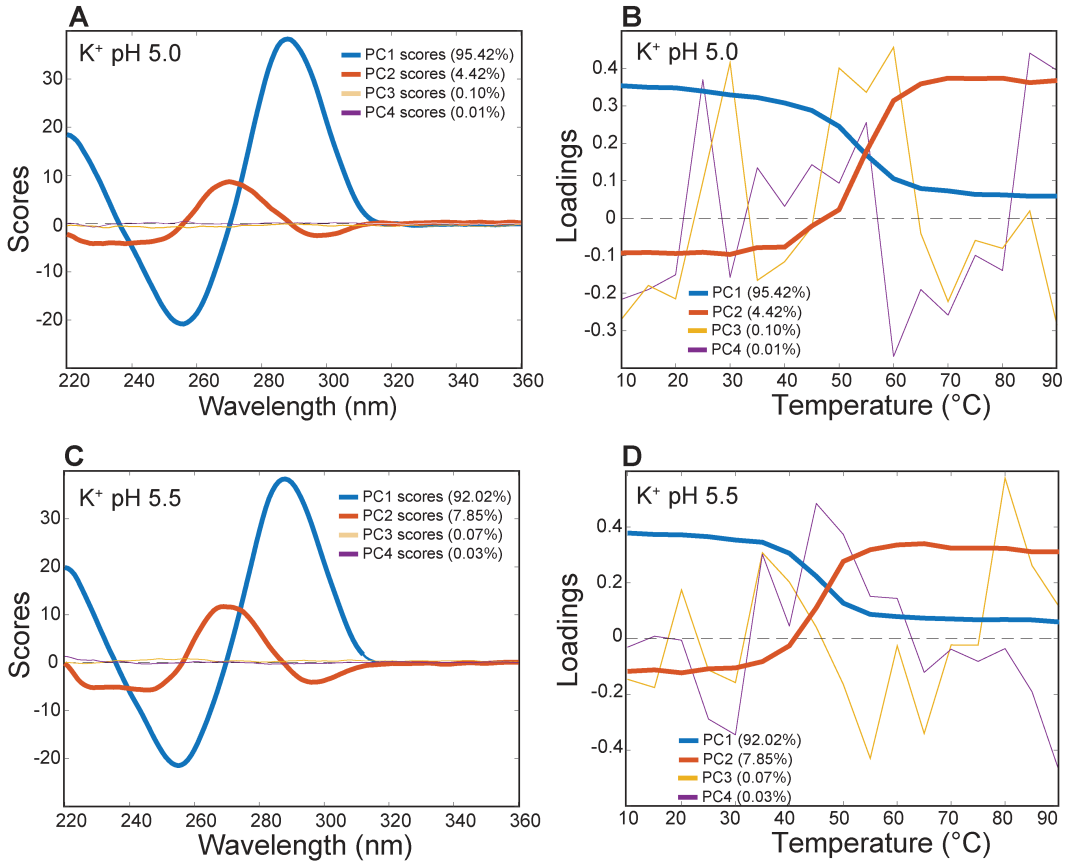

Fig. S3. Score and loadings plot of the four principal components (PC1-PC4) obtained from the PCA models computed using the CD spectra of the iM-forming oligonucleotide d(CCCTAA)$_3$CCCT as a function of temperature in K^+-containing buffer at pH (**A**,**B**) 5.0 and (**C**,**D**) 5.5.

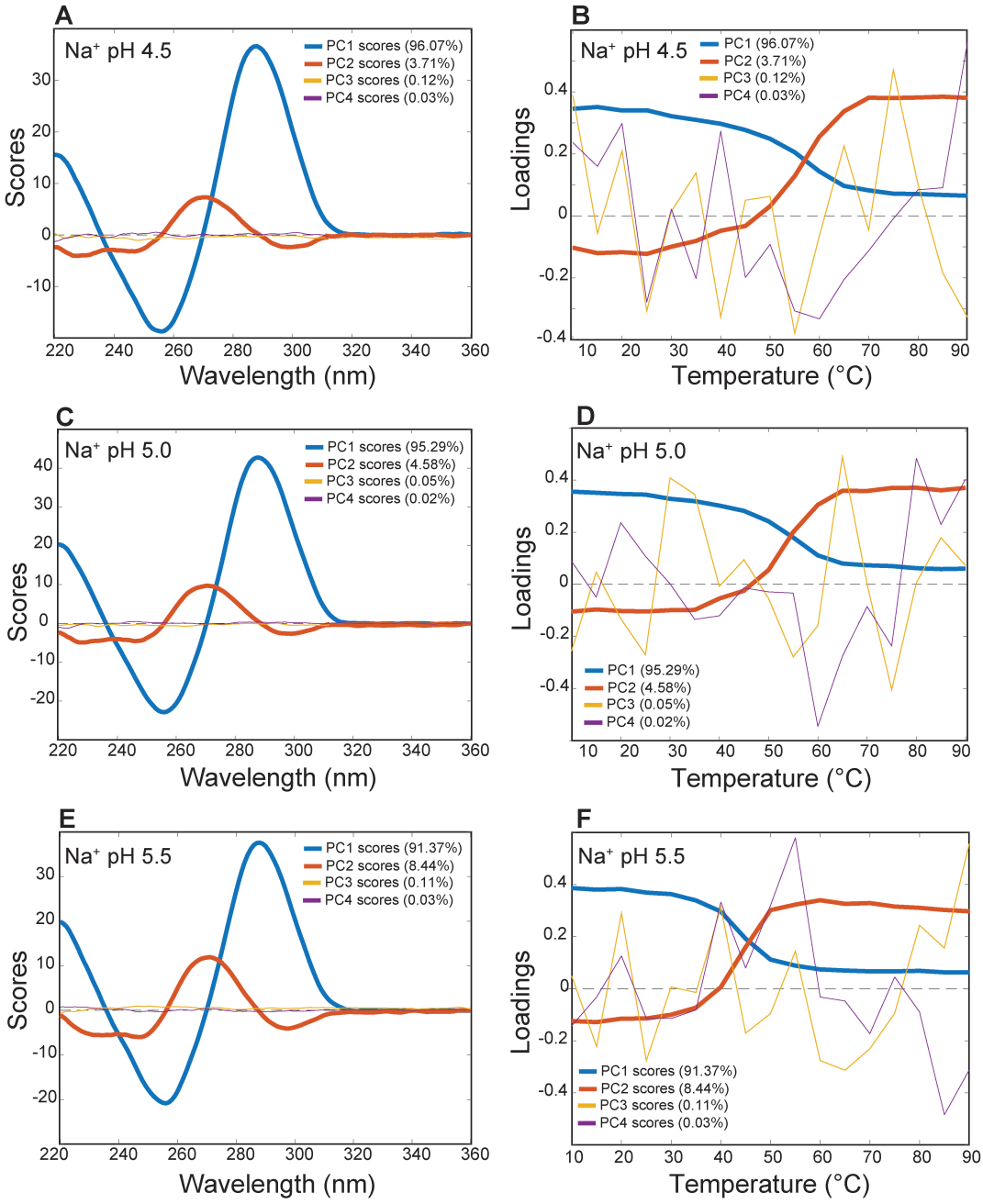

Fig. S4. Score and loadings plot of the four principal components (PC1-PC4) obtained from the PCA models computed using the CD spectra of the iM-forming oligonucleotide d(CCCTAA)$_3$CCCT as a function of temperature in Na^+-containing buffer at pH (**A**,**B**) 4.5, (**C**,**D**) 5.0, and (**E**,**F**) 5.5.

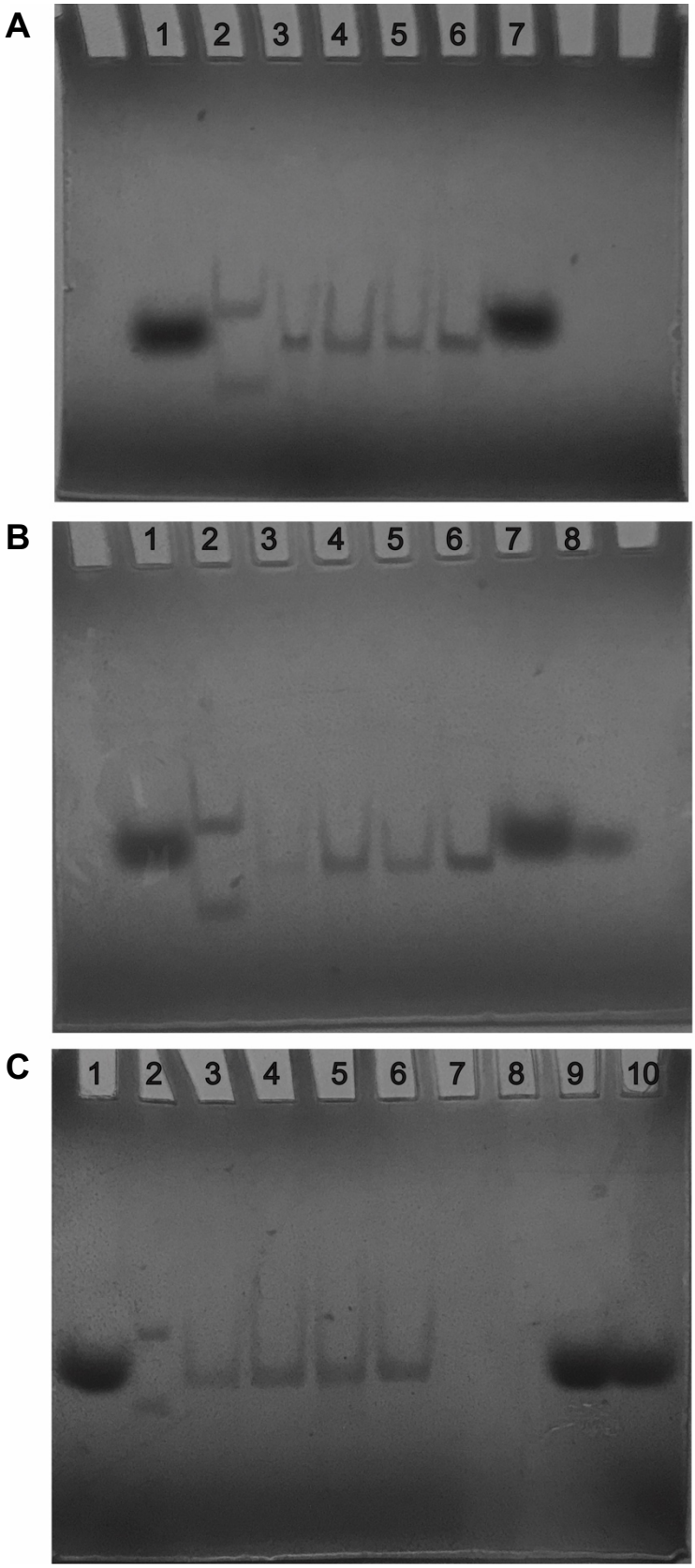

Fig. S5. Uncropped, untouched, full original images of non-denaturing PAGE in K^+- or Na^+-containing acetate buffer at pH (**A**) 4.5, (**B**) 5.0, and (**C**) 5.5, 4 °C. Lane 1: bromophenol blue (run marker). Lane 2: mixture of T_{10} and T_{20} oligodeoxynucleotides (size markers). Lanes 3–6: $d(CCCTAA)_3CCCT$ iM-forming oligonucleotide in the presence of K^+ (3,4) or Na^+ (5,6) from CD (3,5) or DSC (4,6) samples. Lanes 7–10 (if present): bromophenol blue (run marker).

Table S1. PC1–PC4 eigenvalues extracted from the six PCA models.

Cation	pH	PC1	PC2	PC3	PC4
	4.5	2.95E+02	1.10E+01	2.04E-01	4.44E-02
K^+	5.0	2.53E+02	1.17E+01	2.68E-01	3.28E-02
	5.5	2.56E+02	2.19E+01	1.84E-01	7.18E-02
	4.5	2.26E+02	8.72E+00	2.78E-01	7.67E-02
Na^+	5.0	3.16E+02	1.52E+01	1.82E-01	8.11E-02
	5.5	2.45E+02	2.27E+01	2.97E-01	8.08E-02