Thermal Difference Spectra:
A specific signature for nucleic acid structures

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURES

Supplementary Figure S1. Raw (S4A, left) and Normalized (S4B, right) TDS of the i-DNA forming $(C_5TA_2)_3C_5$ sequence (1) using various upper temperatures (indicated in the figure). Experiment performed in a pH 7.2 10 mM sodium cacodylate buffer. (Absorbance recorded while cooling the sample; a hysteresis is present, even with a 0.2°C/min temperature gradient).

Supplementary Figure S2: Normalized differential absorbance signatures.

A. ***DNA self-complementary duplexes, 100% AT:***

ATATATATATATATAT (dark red) AATTAATTAATTAATT (orange) AAATTTAATTAAATTT (yellow) AAAATTTTAAAATTTT (green) AATTTTTTAAAAAATT (light blue) TTTTTTTTAAAAAAAA (dark blue) AAAAAAAAATTTTTTTTT (purple); Poly d(A-T) (black). Samples at pH 7.2, 0.1M KCl.

B. ***DNA self-complementary duplexes 100% GC:***

CGCGGGCCCGCG (dark red) CGCGCGCGCGCG (red) CCGGCCGGCCGG (orange) CCCGGGCCCGGG (yellow) CCGGGGCCCCGG (green) CGGGGGCCCCCG (light blue) GGGGGGCCCCCC (dark blue) GGCGCGCGCGCC (purple) CCGGGGCCCCGG (black). Samples at pH 7.2, 0.1M KCl (poly d(G-C) has a Tm above 95°C under these conditions, and could not be compared with oligonucleotides.

C. ***Z-DNA (2-4)***

CGCGCGCGCGCG in 4M KCl (red) or 3M KCl (blue). CGCGCGCGCGCG in 4M NaCl (orange) MGMGMGMGMGMG (where M= 5methyl Cytosine) in 4M NaCl (green).

D. Parallel stranded DNA (5-8)

5'-AAAAAAAAAATAATTTTAAATATT + 5'-TTTTTTTTTTATTAAAATTTATAA (red)

5'-TTATAAATTTTAATAAAAAAAAAA + 5'-AATATTTAAAATTATTTTTTTTTT (orange)

5'-ATTAAATTTTAAAAATTTTTT + 5'-TAATTTAAAATTTTTAAAAAA (green)

5'-TTTTTATTAAATATA + 5'-AAAAATAATTTATAT (blue)

5'-ATATAAATTATTTTT +5'-TATATTTAATAAAAA (purple)

Samples at pH 7.2, 0.10-0.13M KCl with or without 10 mM $MgCl_2$.

E. GA DNA duplexes (9-12)

d-$(GA)_{12}$ (red) d-$(GA)_{15}$ (greeen) and d-$(GA)_{18}$ (blue). Samples at pH 7.0, 0.18M NaCl.

F. Hoogsteen DNA duplexes (13,14)

5' AGAAAGGAGAAGAA + 5' TCTTTCCTCTTCTT at pH 5 (red) or 5.5 (orange)(13)

5' GAAGGAAGAGAGAAAGGAGG 3’ CTTCCTTCTCTCTTTCCTCC at pH 5 (light blue) or 5.5 (dark blue)

Samples in 0.1M KCl

G. i-DNA (15,16)

CCCTAACCCTAACCCTAACCCT (17,18)(dark red) CCCTAACCC (red) CCCCAACCCCAACCCCAACCCC (orange) TCCCTCCTTTTTTTTCCCTCCT (yellow) TCCTCCTTTTCCTCCT (19)(green) CCTTTCCTTTACCTTTCC (20)(dark green)

CCTTGCCTTTACCTTCCC (light blue) CCCCTTCCCCTTCCCCTTCCCC (dark blue) TCCCCTTTCCCCTTTCCCCTTTCCCC (purple) CCTCCTTTTCCTCC (19)(black) CCCTCCCTCCCTCCCTTTTTTTTT (grey) poly dC (black, dotted line)

Samples in 10 mM sodium cacodylate at pH 6.0 or 6.4.

H. Pyrimidine triplexes (21,22)

TCTCTCTCCCCTTCCTCTCTCT + GGAGAGAGA (dark red)(23)

TCTCTCTTCCCTTCTTCTCTCT + 5' GAAGAGAGGAG (red)(23)

3'AGCTCCAGAAAAGAAAAAAGAAAATCCCCC + 5'TCGAGGTCTTTTCTTTTTTCTTTTAGGGGG + 3'TCTTTTCTTTTTTCTTTT (orange)(23)

3'-GAAAGAGAGGAGG 5’ + 5'-CTTTCTCTCCTCC + 3')-CTTTCTCTCCTCC (yellow)

dA$_{18}$ + 2. dT$_{18}$ (green) (24)

GAGAGAGAAACCCCTTTCTCTCTCTTTTCTCTCTCTTT (light blue) (25)

5'-CGAGTTAAGAAGAAAAAAGATTGAGC + 3'-GCTCAATTCTTCTTTTTTCTAACTCG + 5'-TTCTTCTTTTTTCT (dark blue)

5'-CTCCTCTCTTC**CCTT**CTTCTCTCCTC + 5'-GAAGAGAGGAG (purple)

All spectra correspond to triplex to duplex+single strands transitions (hence, the "high" temperature spectra must be recorded below the Tm of the corresponding duplex). One may notice that the different sequences give rise to relatively distinct spectra (the 8 other panels are more homogenous). This difference is explained in large part by the different base content of the triplexes: for example, a negative signal around 295 nm is only observed when a significant number of cytosines are present in the third strand . The two base triplets involved in pyrimidine triplexes (T.A*T and C.G*C+) have different TDS signatures. One should also note that these TDS correspond only to the melting of the third strand, with no disruption of the oligopurine-oligopyrimidine duplex; a direct triplex to single strands transition would give a different TDS (data not shown).

I. *DNA G-quadruplexes in Na+*

TAGGGUTAGGGT (26)(dark red) UAGGGTBAGGGT (26)(red) GGTTGGTGTGGTTGG (27,28)(orange) $(GGGGTTTT)_3GGGG$ (29)(yellow) $(GGGGTT)_3GGGG$ (30)(green) $A(GGGTTA)_3GGG$ (dark green)(31) $(GGGTTA)_3GGG$ (32)(light blue) $A(GGGGTTA)_3GGGG$ (dark blue) $A(GGGGGTTA)_3GGGGG$ (purple) $A(GGTTA)_3GG$ (black). All samples at pH 7.0 or 7.2 in 0.1 M NaCl. U and B correspond to deoxyuracyl and bromo-deoxyuracyl, respectively (26).

Polynucleotides appear as dotted lines, oligonucleotides as solid lines. Except where indicated, sequences are provided in the 5'-> 3' direction, and correspond to oligodeoxynucleotides.

Supplementary Figure S3. Circular dichroic spectra of the human telomeric repeat sequence 5'$AGGG(TTAG_3)_3$ in 200 mM NaCl (blue) or KCl (red). Molar ellipticity [ε] is shown.

Supplementary Figure S4. Normalized TDS of the human telomeric repeat sequence 5'$AGGG(TTAG_3)_3$ in NaCl (blue) or KCl (green) (average value ± S.D. at each wavelength)

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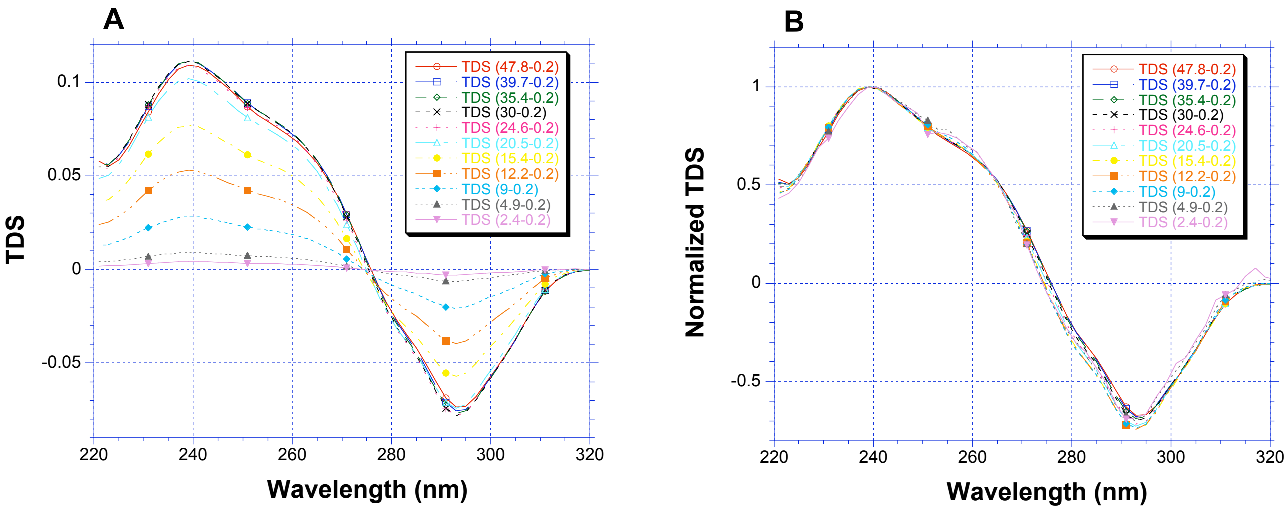
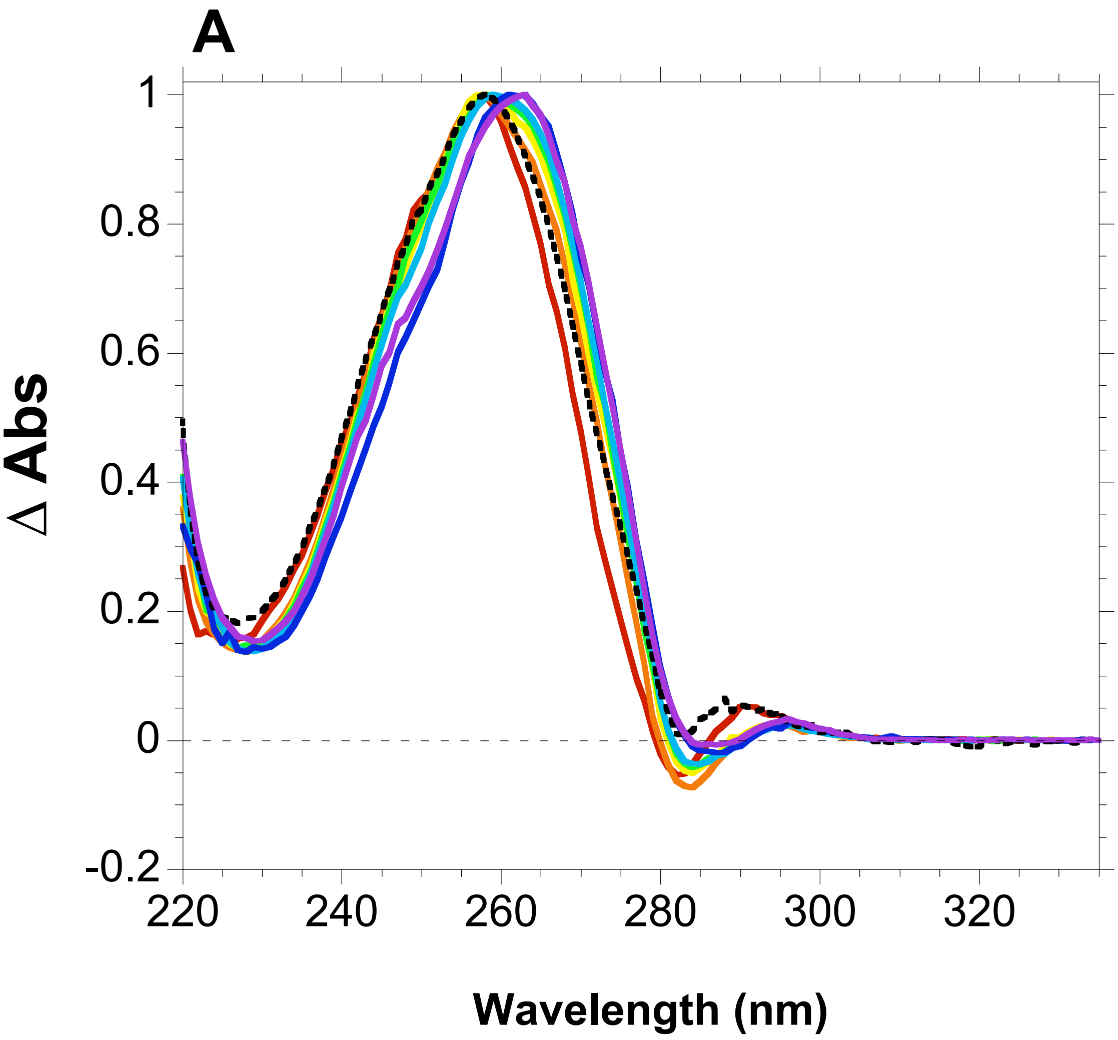
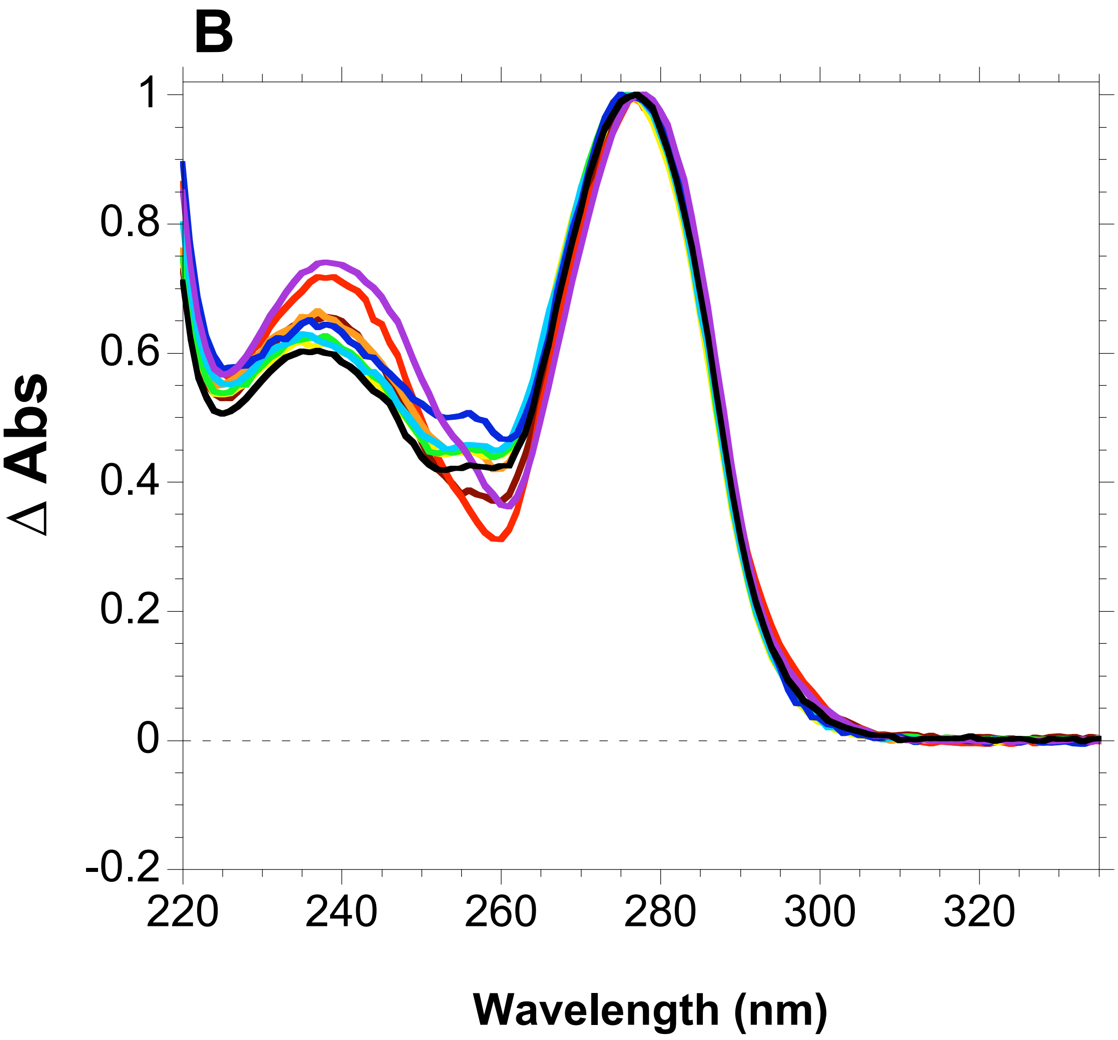


Supplementary Figure 1



Supplementary Figure S2A



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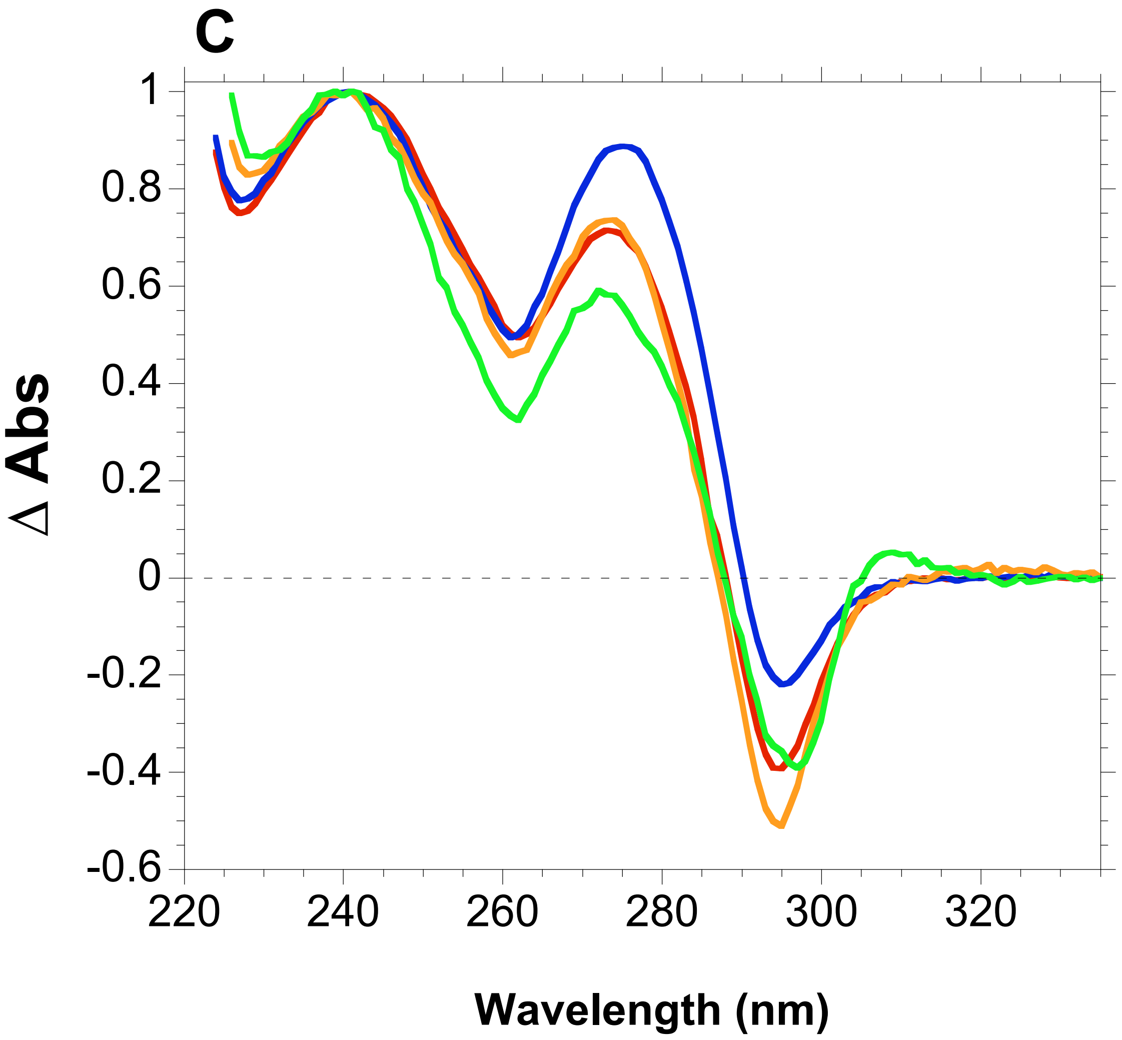

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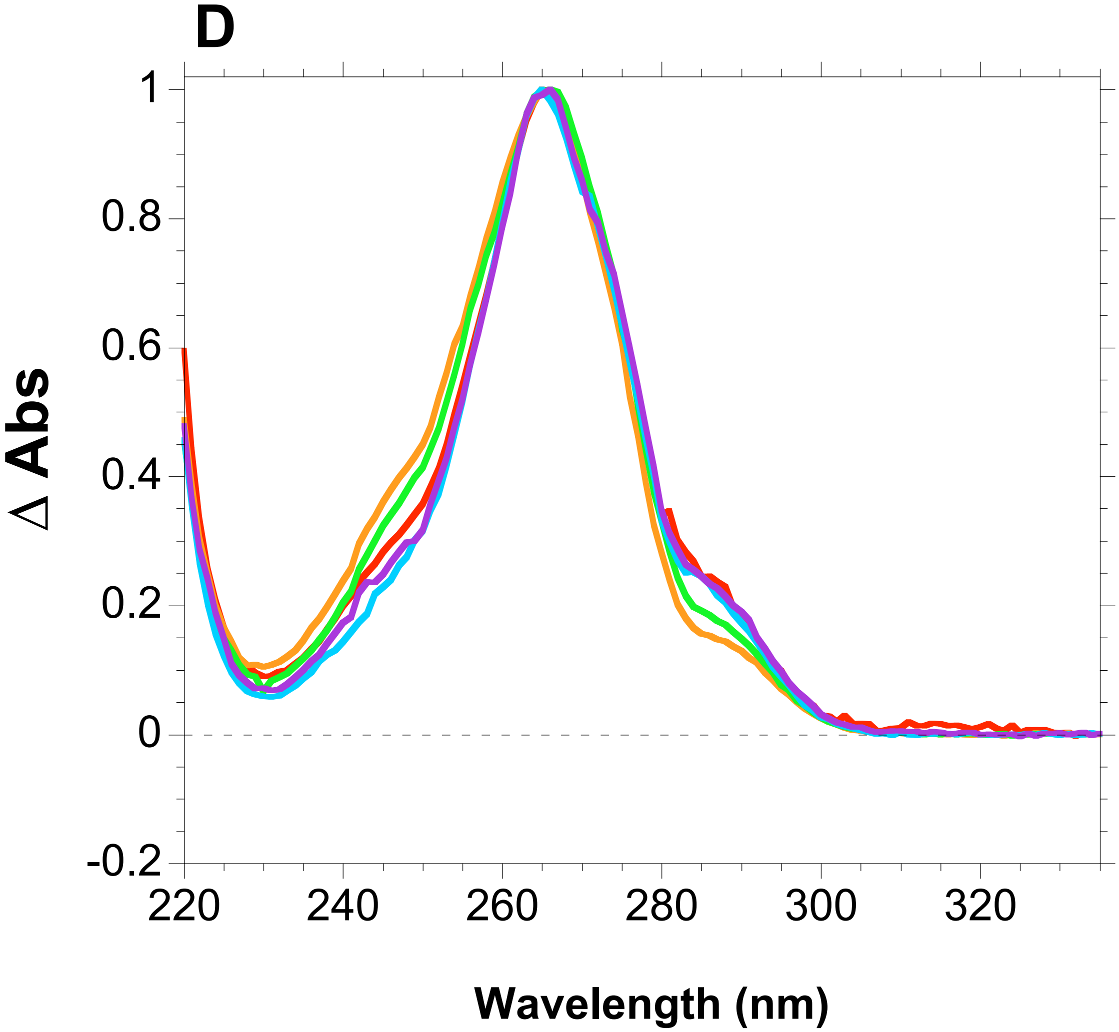

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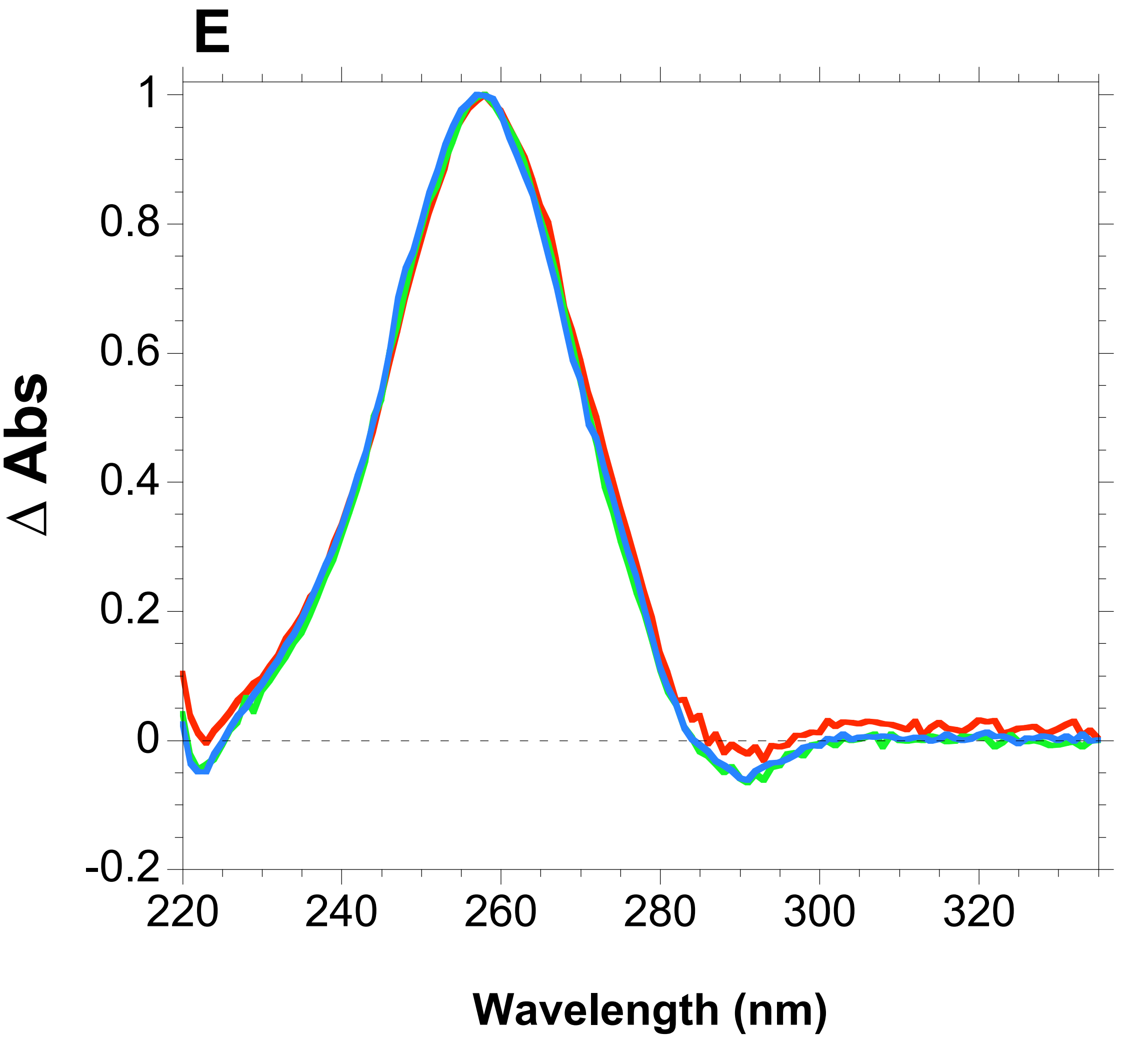

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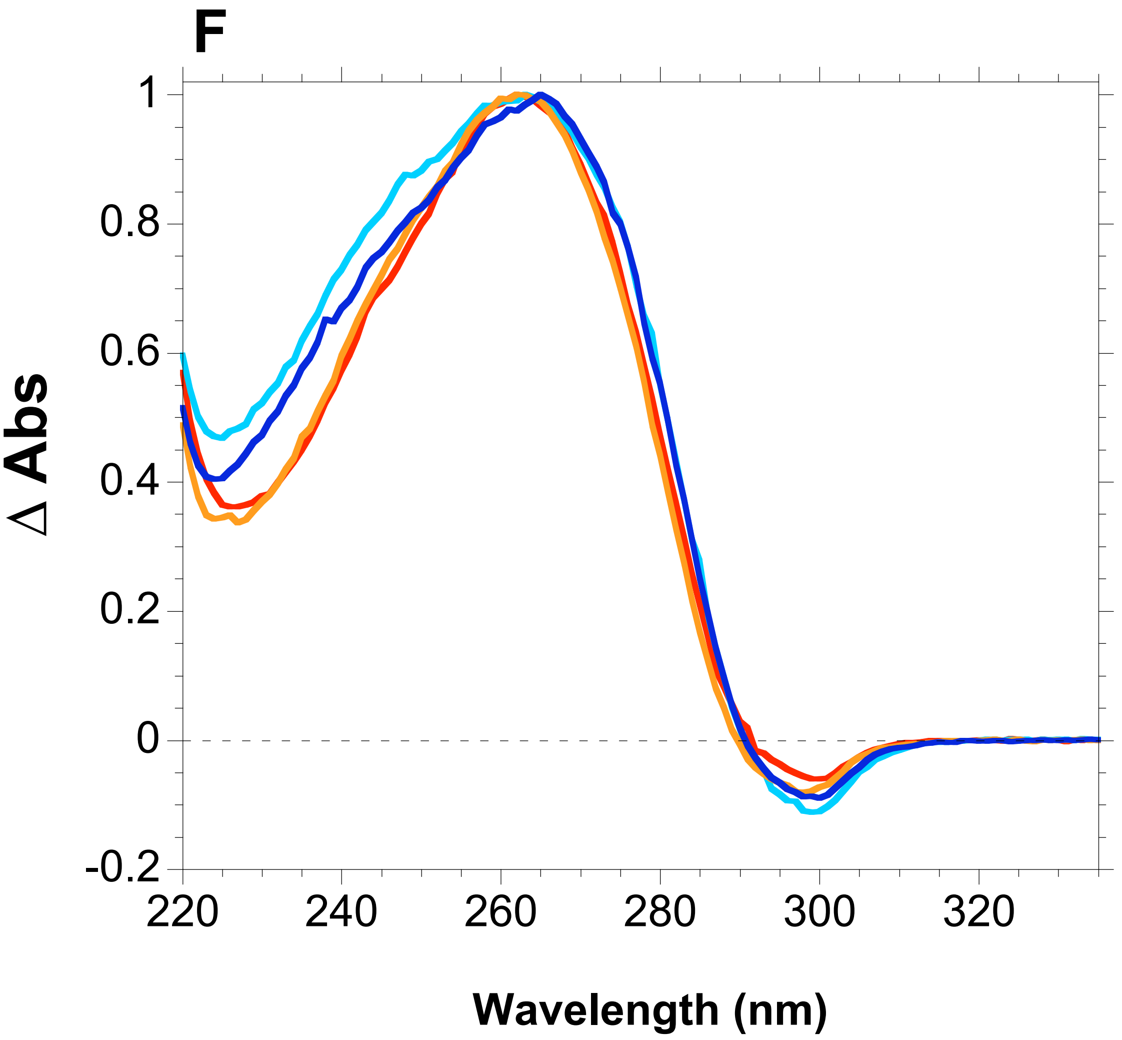

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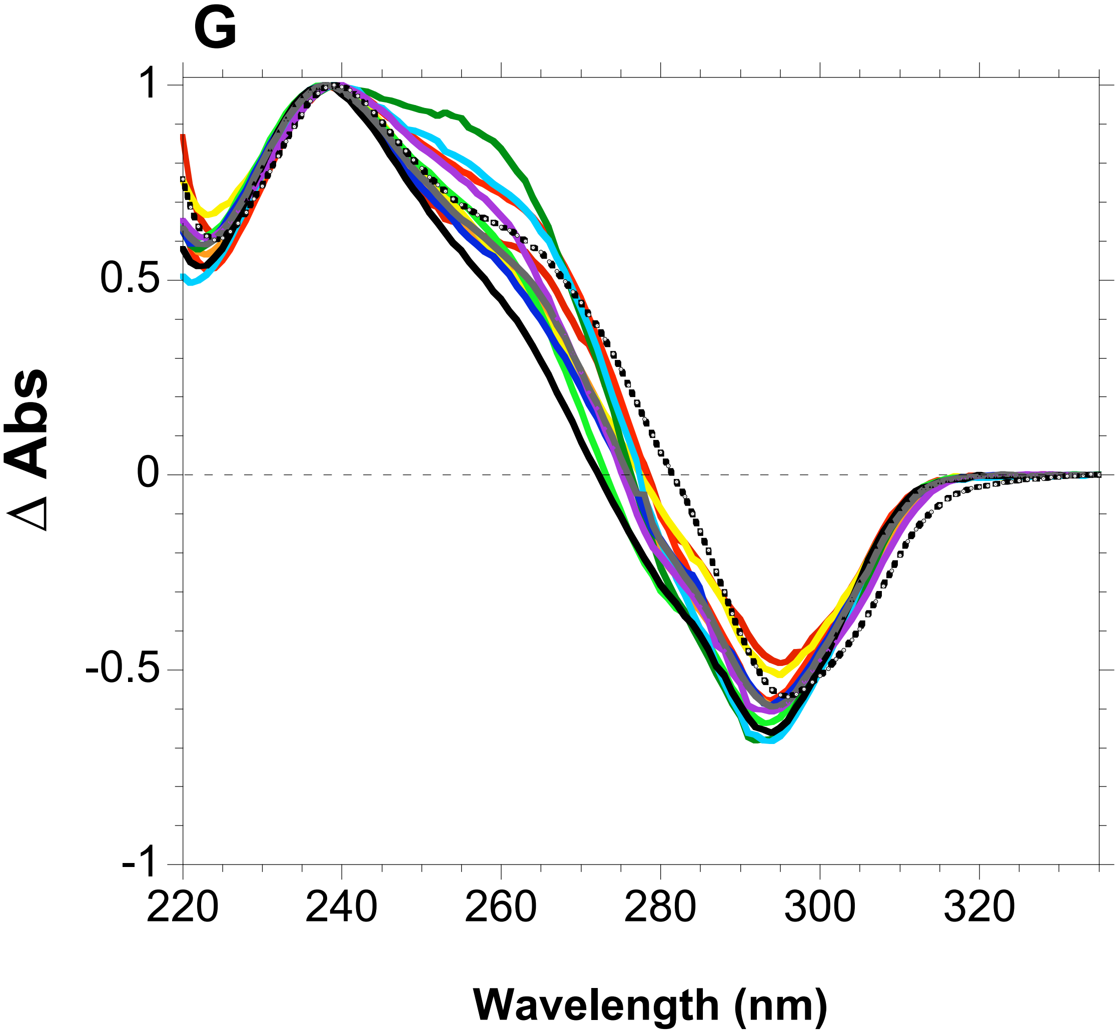

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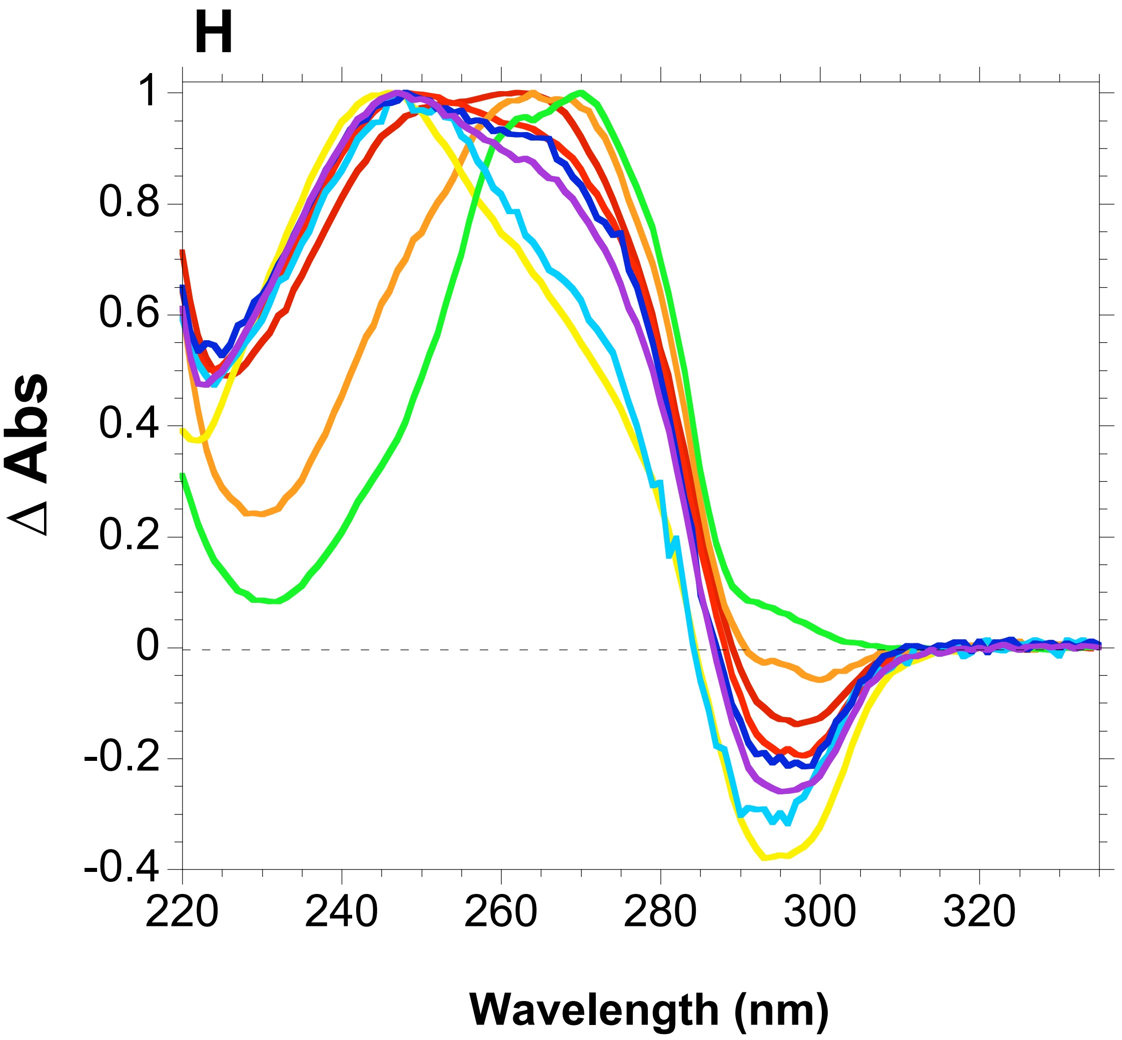

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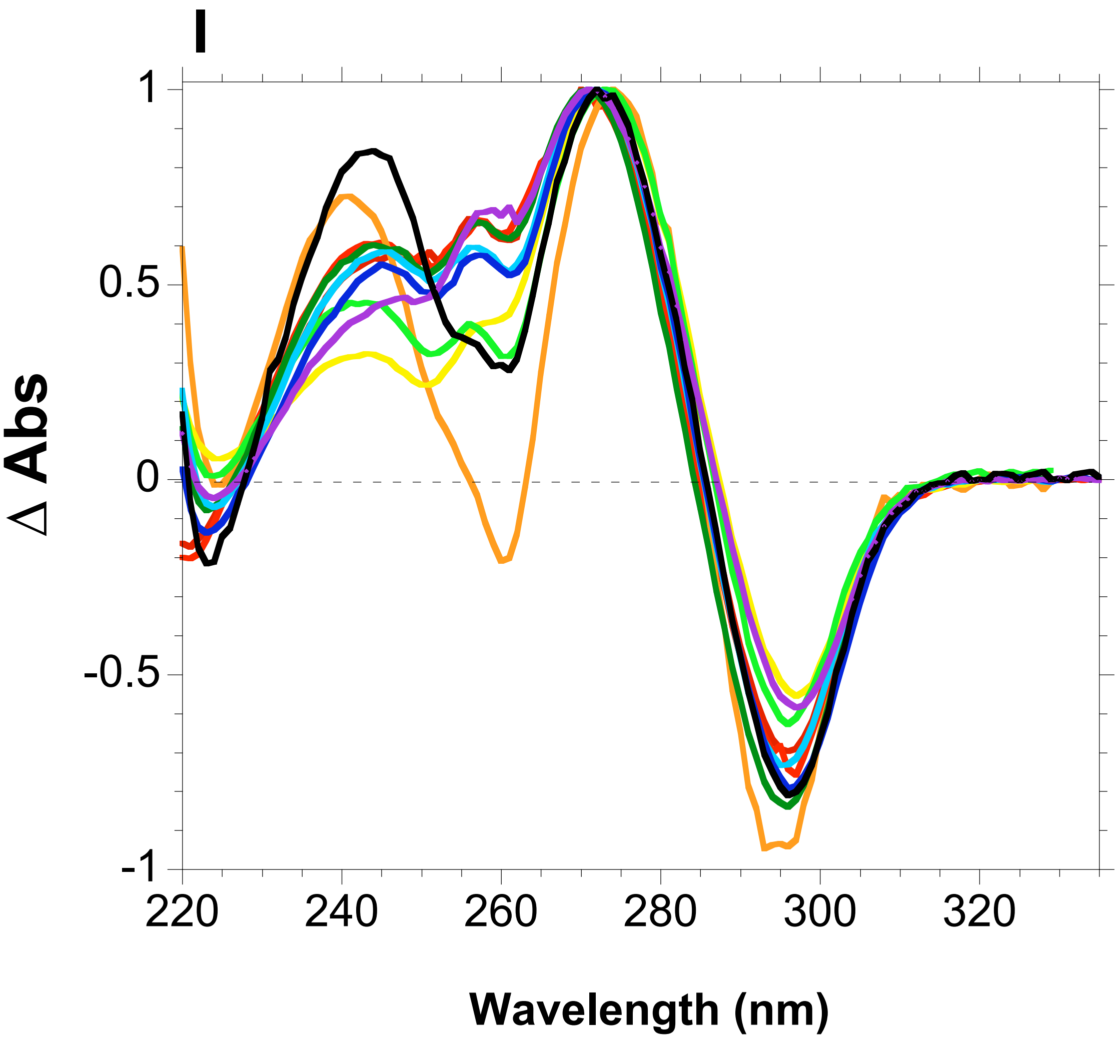

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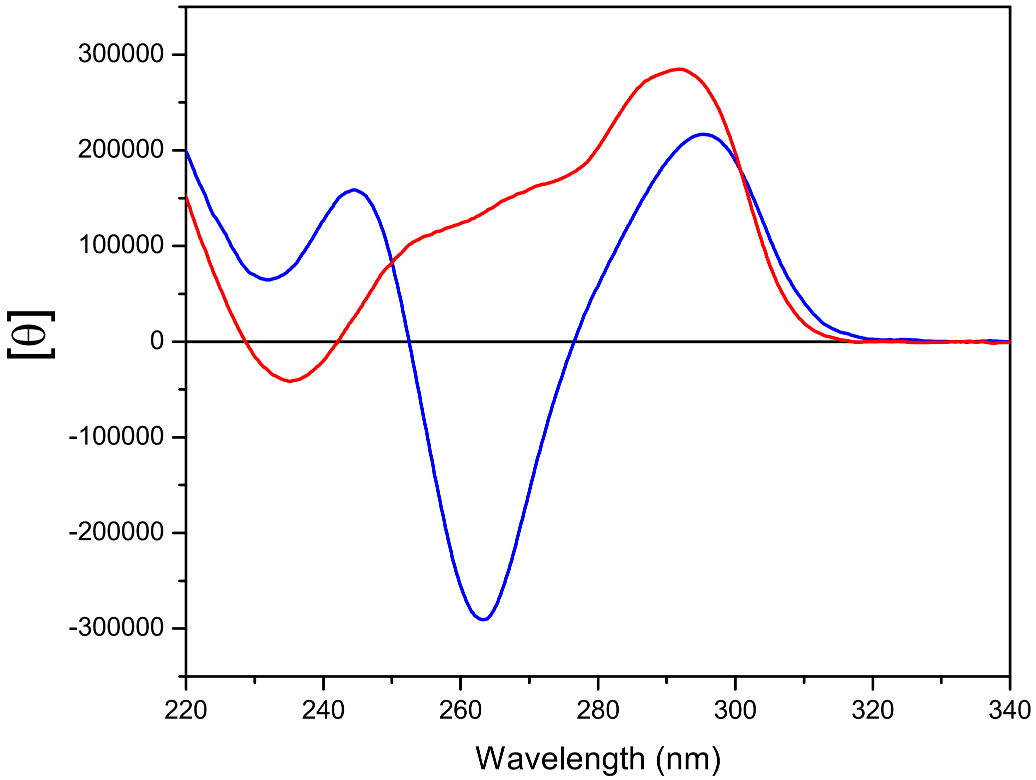
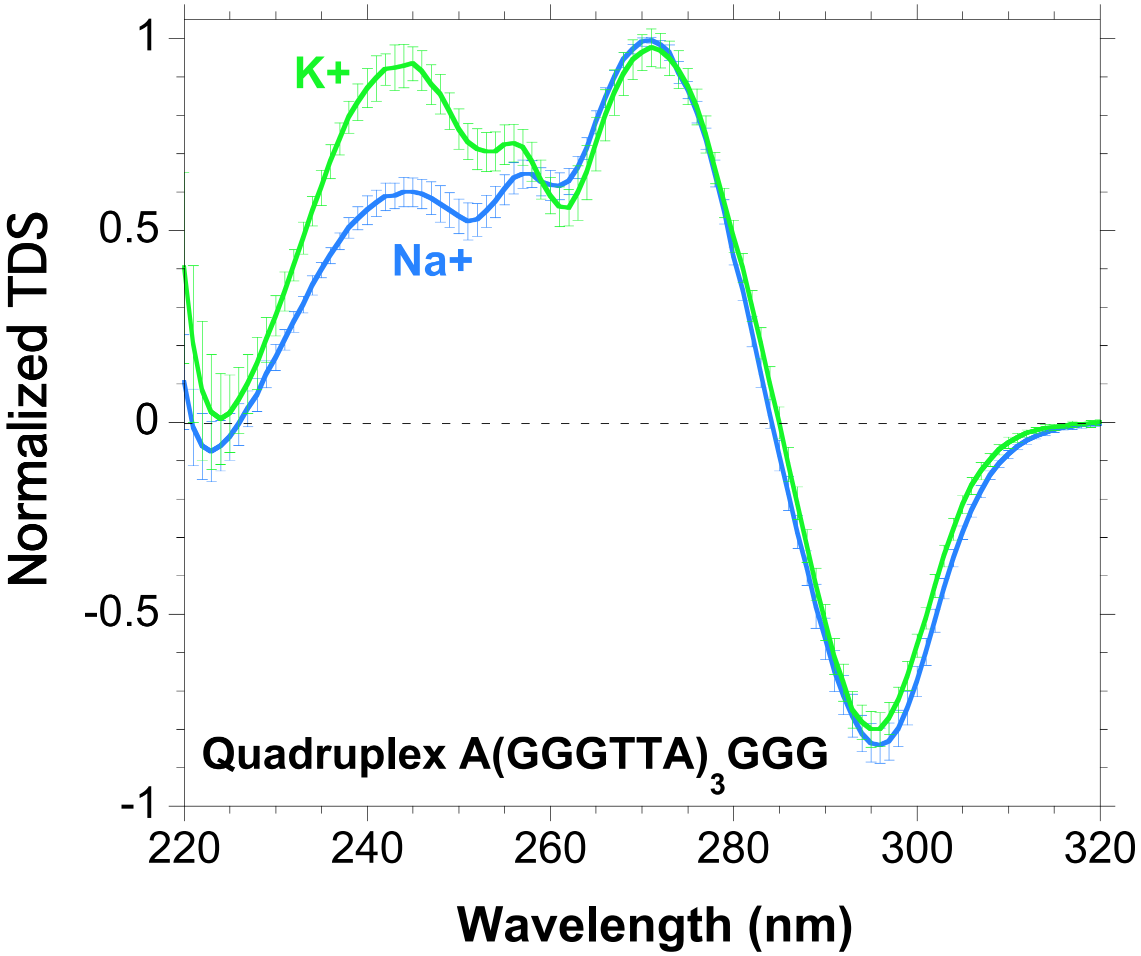


Supplementary Figure S3. Circular dichroic spectra of the human telomeric repeat sequence 5'AGGG(TTAG$_3$)$_3$ in 200 mM NaCl (blue) or KCl (red). Molar ellipticity [Θ] is shown.



Supplementary Figure S4