Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Combining steady state and temperature jump IR spectroscopy to investigate the allosteric effects of ligand binding to dsDNA

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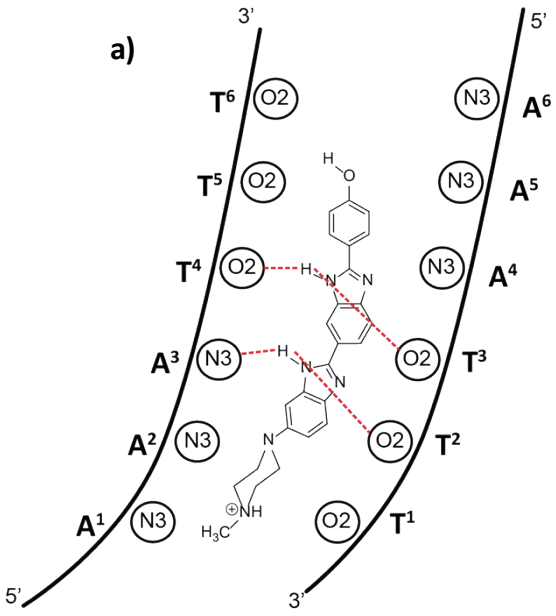

Figure S1: *Schematic diagram of the binding of H33258 to AT-rich dsDNA.*[1,2]

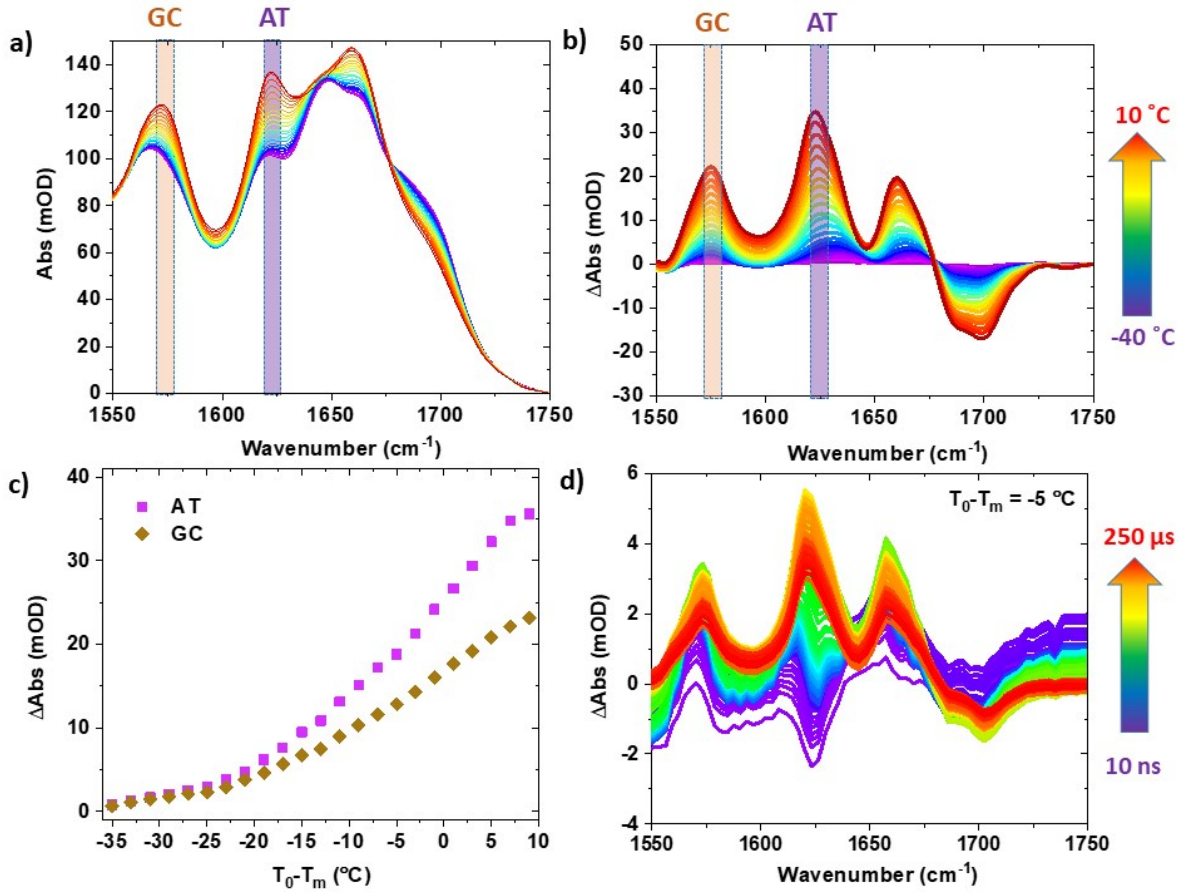

Figure S2: *a) IR absorption spectrum of* ***ODN1·H33258*** *as a function of temperature. Colored arrow shows temperature range of measurements, represented as* T_0-T_m *(see main text) b) Difference spectroscopy reference relative to* T_0-T_m *= -40 ˚C c) Variation in amplitude of the GC and AT marker bands as a function of* T_0-T_m*. d) T-jump IR spectra of* ***ODN1·H3325*** *at* T_0-T_m *= -5 ˚C obtained at a range of T-jump-probe delay times (see arrow). N.B.* T_m *for* ***ODN1*** *= 60 ˚C;* T_m *for* ***ODN1·H33258*** *=* 75 °C.

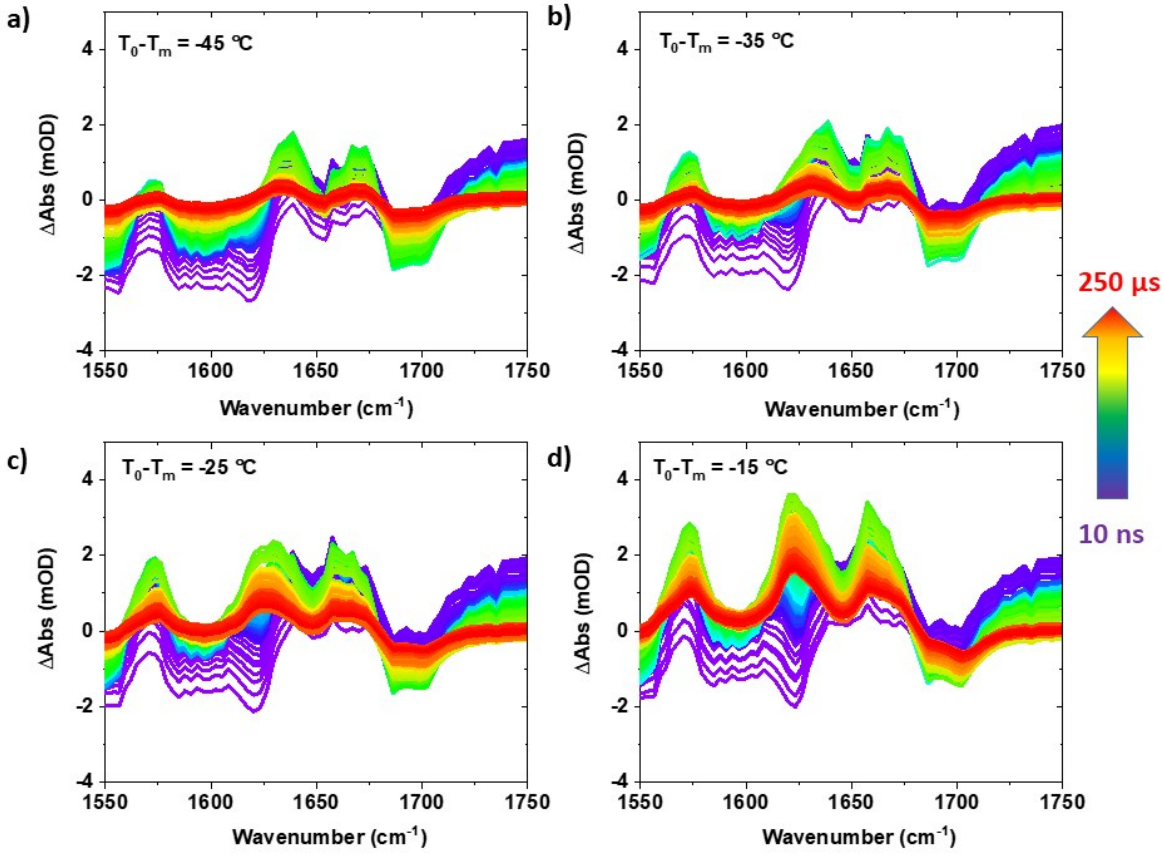

Figure S3: *T-jump IR spectra of* ***ODN1·H33258*** *obtained as a function of pump-probe delay time from 10 ns to 250 µs. Spectra were obtained at initial temperatures (T_0-T_m) of a) -45 ˚C, b) -35 ˚C, c) -25 ˚C and d) -15 ˚C.*

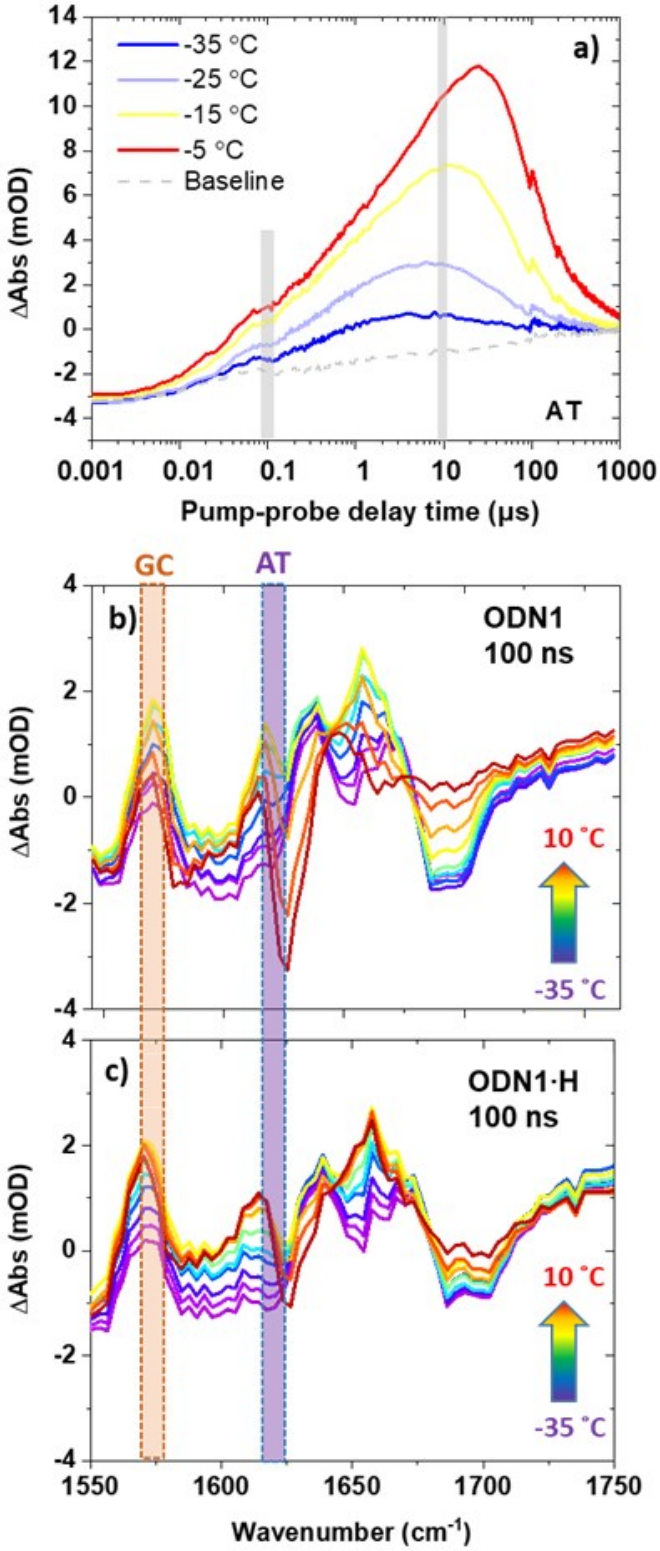

Figure S4: *a) Time trace of AT marker mode of ODN1 at a range of T_0-T_m values. 100 ns and 10 μs points used for reference in text are highlighted by grey boxes. b) and c) Comparisons of T-jump IR spectra obtained at a T-jump IR probe delay time of 100 ns for b)* ***ODN1*** *and c)* ***ODN1·H****. Arrow shows T_0 for the data represented as T_0-T_m (see text).*

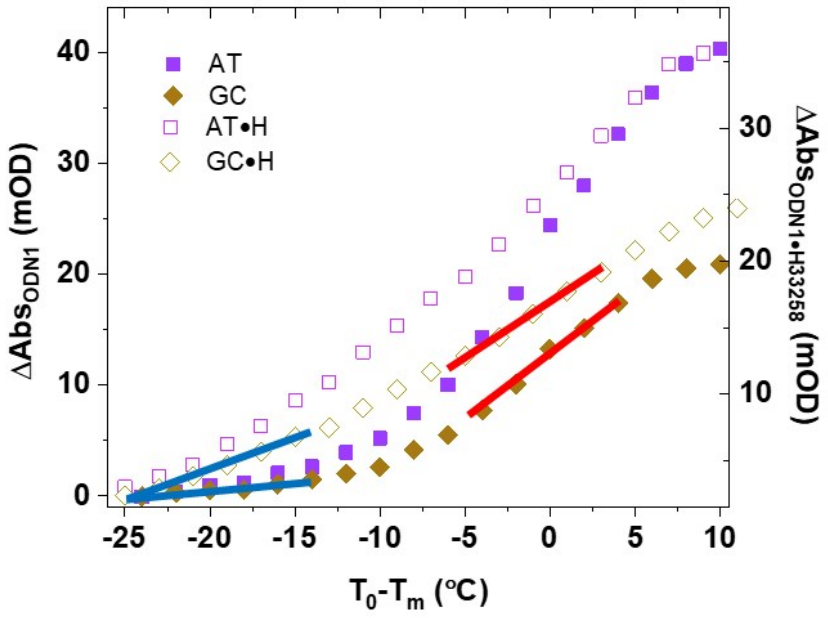

Figure S5: *Direct comparison of FTIR melting curves for ODN1 (left axis) and ODN1·H33258 (right axis) samples. Blue and red bars indicate relative gradients of the melting curves close to (red) and far from (blue) the T_m of the sample.*

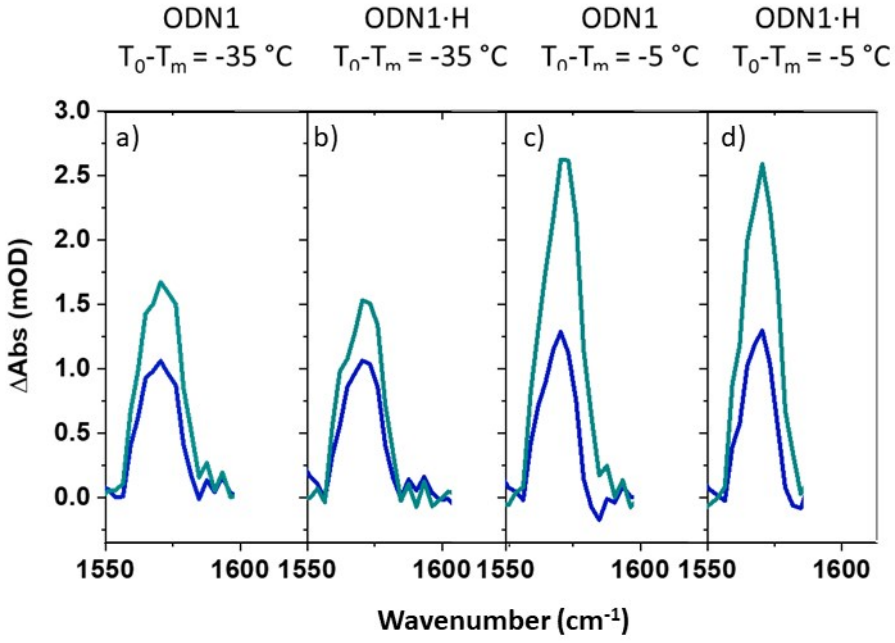

Figure S6: *Comparison of GC marker mode behavior following baseline correction between 5 ns (blue) and 100 ns (green) for ODN1 (a,c) and ODN1·H33258 (b,d) samples at T_0-T_m = -35 °C (a, b) and -5 °C (c, d). Despite the smaller T-jump-probe time delay window (5 ns-100 vs 1-100 ns) and the expected greater sensitivity to increased temperature of the ODN1·H33258 sample far from T_m, based on the respective melting curves (Fig.S5), the suppression of dynamics by H33258 is still clear.*

Notes

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References:

1) Spink, N.; Brown, D. G.; Skelly, J. V.; Neidle, S. Sequence-dependent effects in drug-DNA interaction: The crystal structure of Hoechst33258 bound to the d(CGCAAATTTGCG)2 Duplex. *Nucleic Acids Res*. **1994**, 22, 1607−1612.

2) Ramakers, L. A. I., Hithell, G., May, J.J., Greetham, G.M., Donaldson, P.M., Towrie, M., Parker, A.W., Burley, G.A., Hunt, N.T. 2D-IR Spectroscopy shows that optimized DNA minor groove binding of Hoechst33258 follows an induced fit model. *Journal of Physical Chemistry B* **2017** 121, 1295-1303.