Supporting Information:

Ultrafast 2D-IR and Optical Kerr Effect Spectroscopy Reveal the Impact of Duplex Melting on the Structural Dynamics of DNA

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FTIR Principal Component Analysis

Principal Component Analysis (PCA) was applied to the FTIR data using the Multivariate Analysis suite available within Origin Pro 9.0. The results show that the variance in the data could be largely described (99.6%) by two principal components (PC). The major PC (PC1, representing 98.2% of the variance) represented the average IR absorption spectrum across the temperature range studied and was temperature independent. By contrast, (PC2, 1.8%) represented the changes in the spectrum as a function of temperature. The temperature-dependence of PC2 was well-represented by a sigmoidal profile with a melting temperature (T_m) for the ds-DNA of 323±2 K.

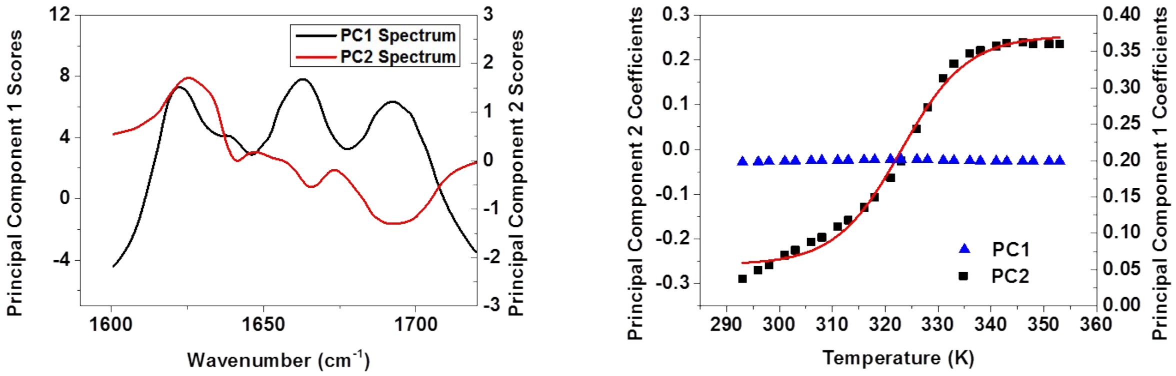

Figure S1 *– Results of applying Principal Component Analysis (PCA) to FTIR spectra of the AT-15mer Left: Plot showing the spectral contributions to PC1 (black) and PC2 (red). To obtain the experimental spectrum at a given temperature the spectra are weighted according to the coefficients (shown in the right hand graph) and summed. Thus, PC1 (temperature invariant) is an average spectrum across the temperature range, PC2 is a temperature-induced difference spectrum. In particular, the loss of intensity of the 1692* cm^{-1} *mode and gain in intensity of the 1622* cm^{-1} *mode at elevated T are clearly visible. Right, temperature-dependence of PC1 and PC2 extracted from PCA of FTIR spectra of the AT-15mer (Fig.2(a) main text). The red curve shows the results of fitting the PC2 data to a Boltzmann Sigmoid that yielded a* T_m *of 323±2 K.*

UV-Visible Spectroscopy

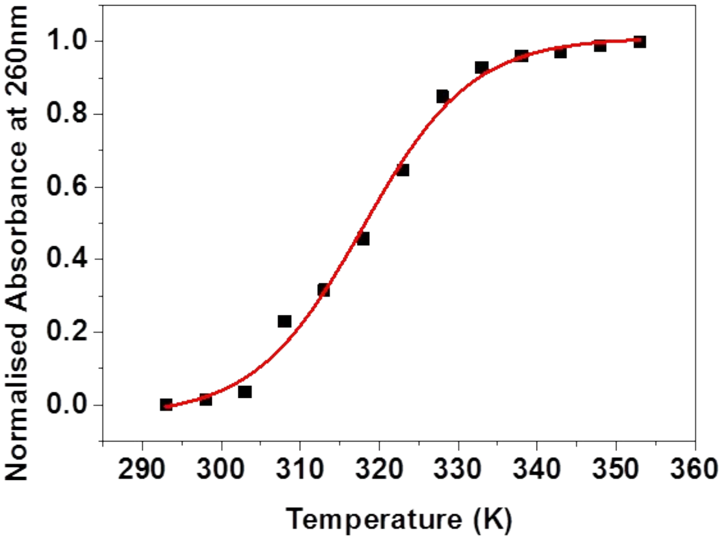

Figure S2 *– UV-Visible melting curve recorded with an excitation wavelength of 260nm for the AT-15mer DNA duplex. Boltzmann Sigmoidal fitting yields a* T_m *value of 318±2 K. A decrease in absorptivity at 260 nm is widely attributed to hypochromic effects due to base stacking in duplex DNA.*

Peak Assignments

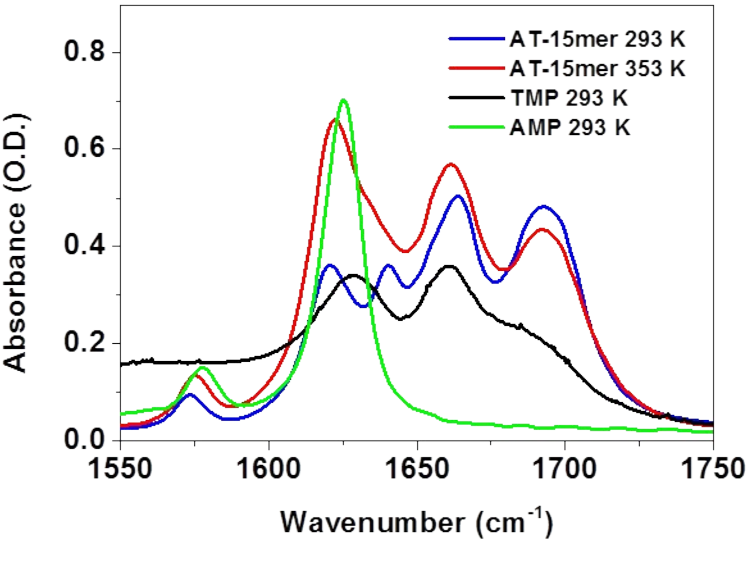

Figure S3 *– Comparisons of FTIR spectra of the AT 15mer DNA sample under dsDNA (blue) and ss(DNA) red conditions with the spectra of adenosine (AMP) and thymidine (TMP) monophosphates.*

2DIR Principal Component Analysis

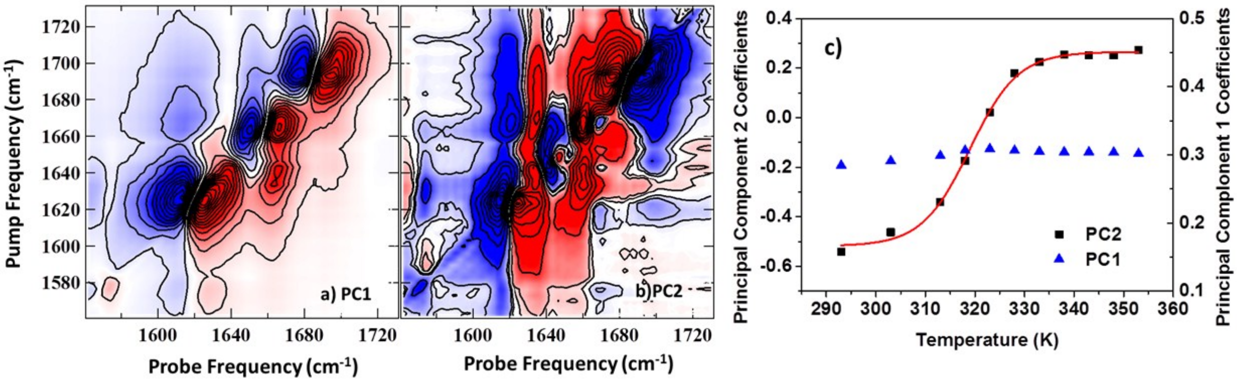

Figure S4: Results of applying Principal Component Analysis (PCA) to 2D-IR spectra of the AT 15mer using the Multivariate Analysis suite available within Origin Pro 9.0. The results show that the variance in the data could be largely described by two principal components (PC). a) The major PC (PC1, 94.9%) represented the average 2D-IR absorption spectrum across the temperature range studied and was largely temperature independent. b) By contrast, (PC2, 4.8%) represented the changes in the spectrum as a function of temperature. c) Shows the temperature-dependence of PC1 and PC2. PC2 was well-represented by a sigmoidal profile with a melting temperature (T_m) for the ds-DNA of 319±2 K.

2D-IR off-diagonal peaks

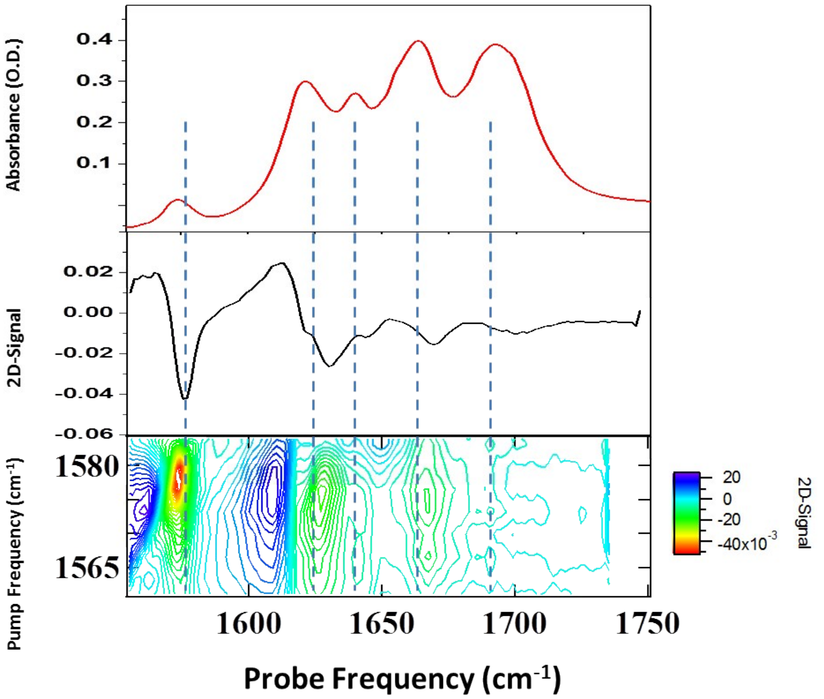

***Figure S5** –FTIR spectrum of the AT 15-mer at 293K alongside a slice through the 2D-IR spectrum at a pump frequency of 1575 cm^{-1} (middle) and an expanded section of the 2D-IR spectrum (bottom) showing the off-diagonal peaks accompanying the diagonal A ring vibration at 1575 cm^{-1}. Peaks indicating coupling to the three high frequency T modes and the A_r mode are visible.*

OKE water subtraction

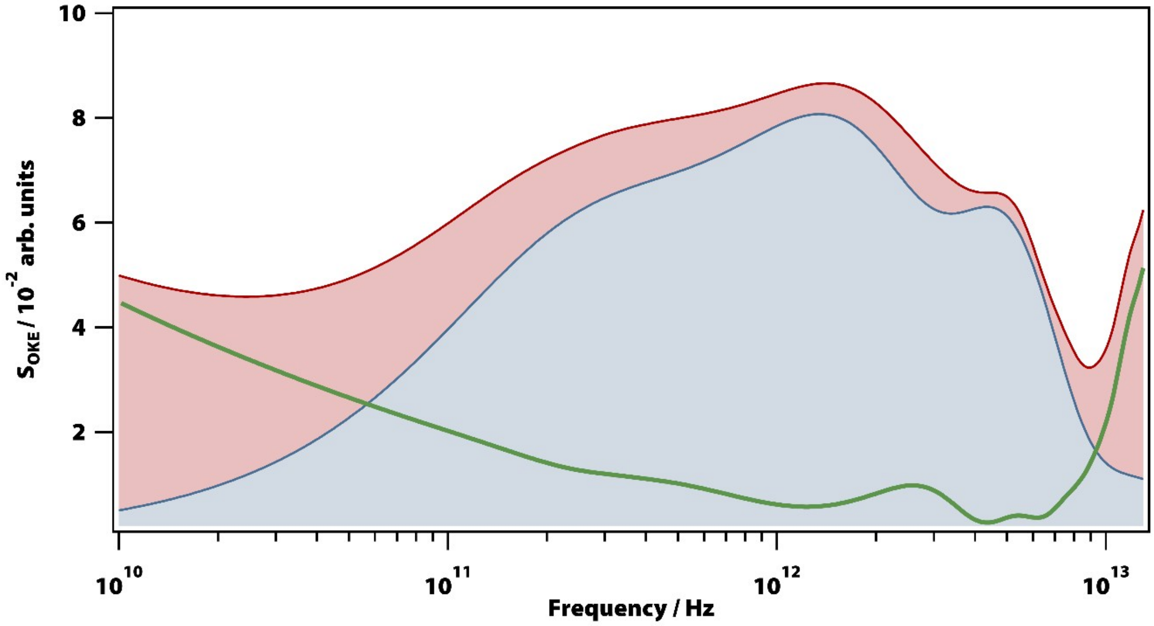

Figure S6 *– Representative OKE spectrum of the AT 15-mer before (pink) and after (green) subtraction of the signal from water/buffer (grey).*

OKE Fitting Parameters

	Cole-Cole functions					Brownian oscillators			
	CC1 (α = 0.87)		CC2			B1 $\omega_0/2\pi$ = 9.9 THz $\gamma/2\pi$ = 1.35 THz	B2 $\omega_0/2\pi$ = 1.4 THz $\gamma/2\pi$ = 0.6 THz	B3 $\omega_0/2\pi$ = 2.4 THz $\gamma/2\pi$ = 0.78 THz	B4 $\omega_0/2\pi$ = 3.04 THz $\gamma/2\pi$ = 0.61 THz
T / K	A_D	τ / ns	A_{CC}	τ / ps	α	A_{BO}	A_{BO}	A_{BO}	A_{BO}
298	2.90	2.7	0.45	5.0	0.82	0.078	0	0.033	0.018
308	2.81	2.1	0.54	4.8	0.81	0.088	0.005	0.040	0.017
318	2.94	1.7	0.61	4.6	0.80	0.089	0.015	0.042	0.015
328	2.82	1.4	0.73	4.5	0.73	0.089	0.036	0.048	0.011
338	3.20	1.1	0.85	4.2	0.70	0.092	0.053	0.051	0.005
348	3.31	0.98	0.95	3.8	0.69	0.104	0.054	0.054	0.002
358	3.35	0.84	1.15	3.3	0.67	0.110	0.055	0.056	0.001

Table S1 *– Fitting parameters for the OKE Spectra*

Spectral Diffusion Analysis

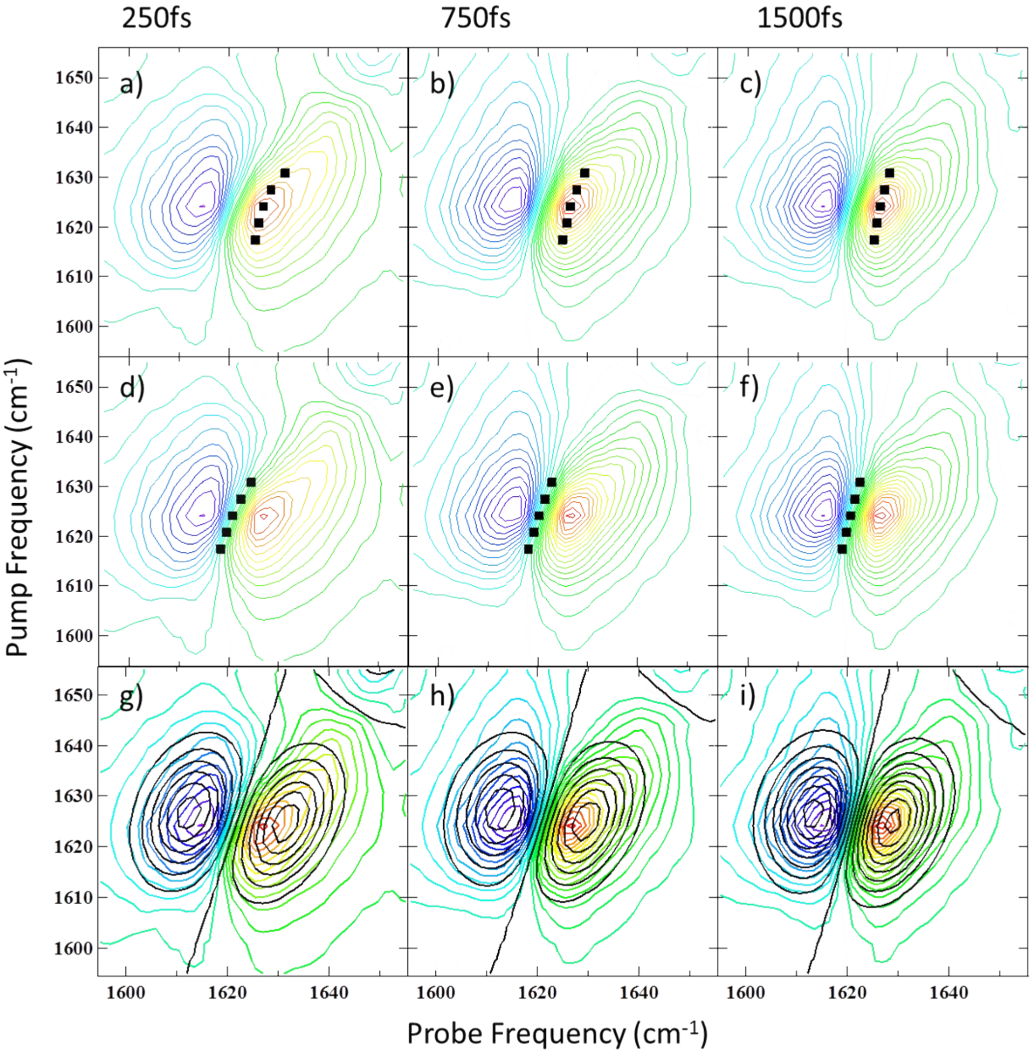

***Figure S7** – Spectral Diffusion Analysis of the A_R mode at 353 K using CLS (a-c), NLS (d-f) and 2D Gaussian fitting (g-i)*

Single Strand Spectral Diffusion

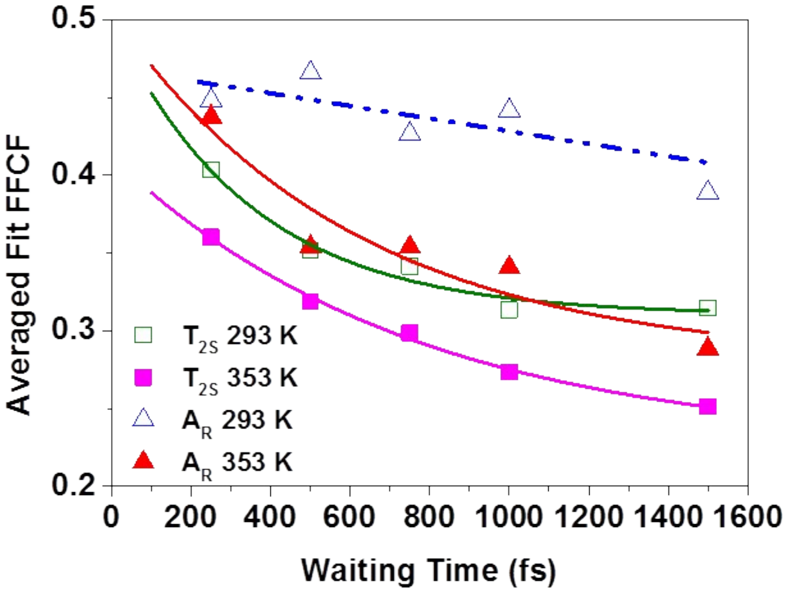

Figure S8 *– Average fit FFCF values as a function of* T_W *for the* T_{2S} *and* A_R *modes of single AT 15mer oligomers at 293 K and 353 K.*

Single Strand Dynamic Parameters

T (K)	Mode frequency (cm^{-1})	Assignment	Vibrational Lifetime (fs)	FFCF (fs)
293	1692	T_{2S}	990	350±120
	1661	T_{4S}	1020	-
	1632	T_R	-	-
	1622	A_R	790	-
353	1692	T_{2S}	860	760±140
	1661	T_{4S}	1080	-
	1632	T_R	-	-
	1622	A_R	750	610±530

Table S2 *– Mode assignments and dynamic information from AT-15mer single strand 2D-IR data*

Coupling-Energy Transfer

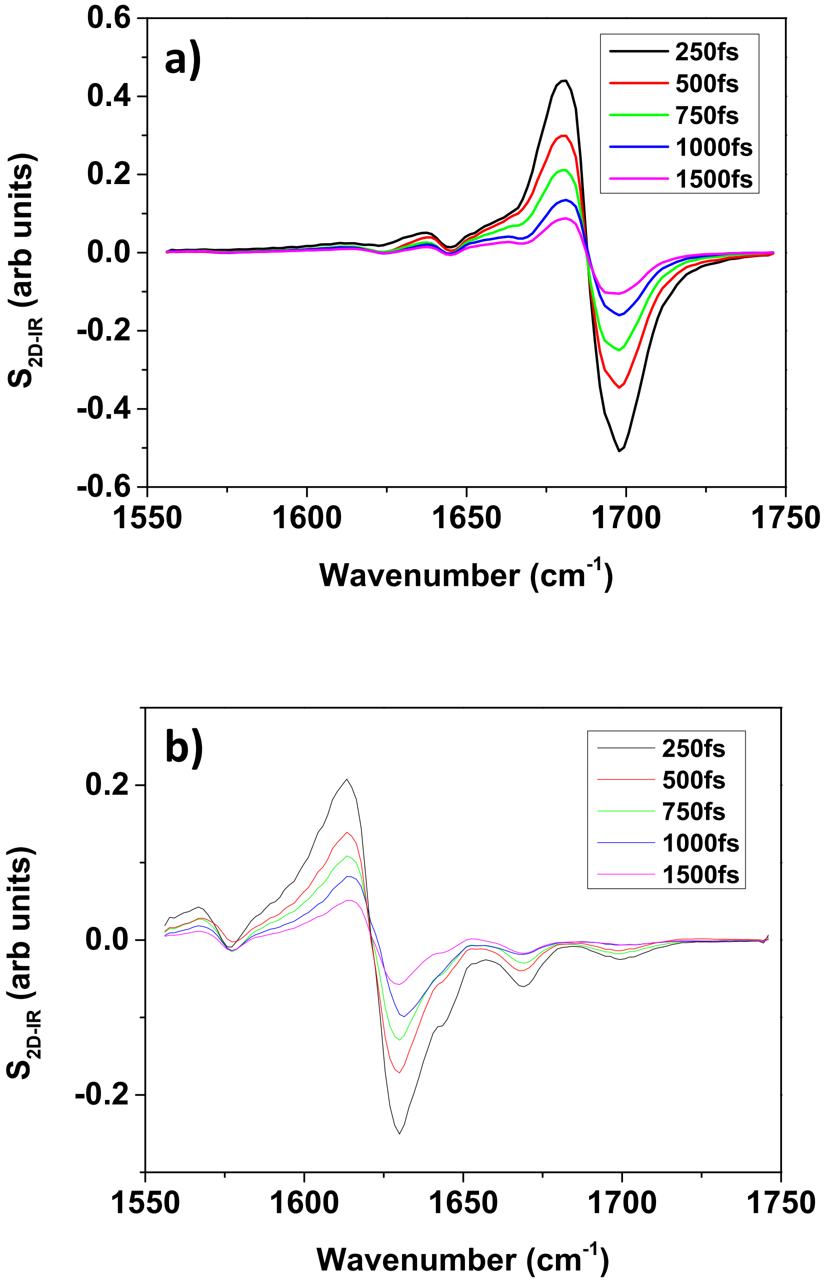

Figure S9*– Slices through the 2D-IR spectra of ds-AT-15mer at a series of waiting times, taken at pump frequencies of a) 1693cm*$^{-1}$ *and b) 1626cm*$^{-1}$

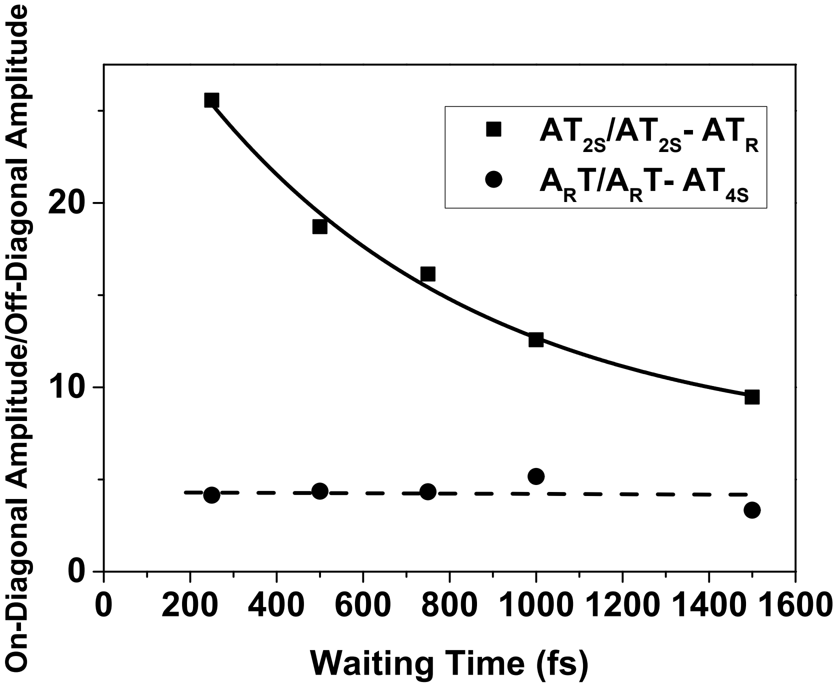

Figure S10 *– Relative signal sizes of on and off-diagonal features in the 2D-IR spectra of the AT 15-mer. The off-diagonal feature between the* AT_{2S} *diagonal and the* AT_R *modes in the upper left quadrant of the spectrum, denoted* AT_{2S}/AT_{2S}-AT_R *(Fig S5(a)), shows behaviour characteristic of energy transfer with a decay of the on-diagonal/off-diagonal peak amplitude ratio of 650±150 fs (squares).The line indicates the results of fitting to an exponential decay function. By contrast while the off-diagonal peak between the* A_RT *and* AT_{4S} *modes in the lower right quadrant, denoted* A_RT */*A_RT-AT_{4S} *(Fig S5(b)) shows behaviour consistent with coupling with the relative signal sizes between on and off-diagonal signals remaining constant with* T_w *(circles).*

Experimental Details

IR Spectroscopy: 2D-IR spectra were obtained using the FT-2D-IR method using the ULTRA laser system described previously.[1-3] Two collinear pump pulses (100 fs duration; 1650 cm^{-1} centre frequency; >250 cm^{-1} bandwidth; 10 kHz repetition rate) with a variable time delay, τ, between them were overlapped with a probe pulse at the sample position using a pseudo pump-probe geometry. For a given spectrum acquisition, τ was scanned in 3 fs steps for a given waiting time (T_w, the delay between the second pump pulse and the probe). The resulting signal was emitted collinearly with the residual probe enabling self-heterodyne-detection of the signal via dispersion of the combination in a spectrometer equipped with 128 pixel mercury-cadmium-telluride (MCT) array detector (resolution ~ 1.5 cm^{-1}). The pump frequency axis was obtained via Fourier Transformation of the signal as a function of the time τ. The experiments employed an all-parallel (ZZZZ) pulse polarisation geometry.

OKE Spectroscopy: The OKE spectra of the samples were obtained by Fourier transforming the time-domain data collected using two standard time domain step-scan pump-probe configurations.[4,5] Both configurations were similar and the most significant difference was the light source. For the short time scale measurements, a laser oscillator (Coherent Micra) was employed. This oscillator provides ~10 nJ pulses (0.8 W average power) with a nominal wavelength of 800 nm at a repetition rate of 82 MHz and the pulse length in the sample was 20 fs. For the longer-timescale relaxation measurements, a regeneratively amplified laser (Coherent Legend Elite USX) with pulses of 1 μJ at a repetition rate of 1 kHz was used.

References

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