

Electronic Supporting Information

Long-lived excited states in i-motif DNA studied by picosecond time-resolved IR spectroscopy

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1. Experimental Details

ps-TRIR measurements were performed at the ULTRA and PIRATE ultrafast infrared absorption facilities at the Rutherford Appleton Laboratory, Chilton which have been described in detail elsewhere.^{1,2}

ULTRA¹: The time-resolved IR (TRIR) spectrometer comprises of a 10 kHz repetition rate titanium sapphire dual output amplifier (Thales), producing 0.8 mJ output with 40 fs pulse duration, at 800 nm. Optical parametric amplifiers (Light Conversion, TOPAS) and third harmonic generation of the 800 nm crystals created the mid infrared radiation and 266 nm femtosecond UV pump pulses used in these experiments. The pump pulses at the sample were at magic angle with energy of 1.5 μ J. The IR probe beam was split to form the reference and probe beams which were passed through spectrographs onto MCT array detectors (IR Associates). The 5 kHz 266 nm pump pulses were focussed ($\sim 100 \mu$ m spot sizes) and overlapped with the probe beam ($\sim 50 \mu$ m spot size) in the sample cell. High speed data acquisition systems (Quantum Detectors) allowed 10 kHz acquisition and processing of the probe and reference pulses to generate a pump-on pump-off infrared absorption difference signal. The difference signal was calibrated using the characteristic *cis*-stilbene absorption lines. The sample was raster scanned in *x*- and *y*- directions at an approximate rate of >10 mm/s.

PIRATE²: The sample was excited with 267 nm, 200 fs pulses with 2 μ J of energy at 0.5 kHz repetition rate, generated from the third harmonic of part of the output from a 1 kHz, 800 nm, 200 fs, 1 mJ regenerative amplifier (Spectra Physics Tsunami/Spitfire) and probed with 150 cm^{-1} FWHM broadband infrared pulses generated by difference frequency mixing the signal and idler outputs of a BBO (β -BaB₂O₄) optical parametric amplifier, pumped with some residual 800 nm, in AgGaS₂ at 1 kHz. The spot size was 200 and 150 μ m diameter for the pump and the probe beam, respectively. The difference signal pump-on minus pump-off was normalised on a shot-by-shot basis and typically accumulated for four successive rounds of 30 s data integration for a single time delay. The infrared beams were dispersed by 150 l/mm, 4000 nm blaze, gold grating monochromators and imaged onto 64 element MCT arrays. The data were collected in a number of 150 cm^{-1} spectral windows using the delay line for optical delays between 2 ps and 1.5 ns. The sample was raster scanned in *x*- and *y*- directions at an approximate rate of 100 mm/s. The difference signal was calibrated using water vapour lines present in the probe spectrum, and the spectral windows were interleaved after scaling using overlapping transients recorded at the same delay time.

Samples with an approximate volume of 40 μ L were recorded between two 25 mm diameters CaF₂ plates (Crystran Ltd), separated by a 56 micron Teflon spacer and mounted in a demountable liquid cell (Harrick Corp.) FTIR spectra were recorded on a Perkin-Elmer FTIR2000 spectrometer. CD spectra were recorded on a JASCO J810 spectropolarimeter. dC₃₀ was synthesised by ATDBio (Southampton, UK). dCMP was purchased from Sigma-Aldrich. Oligonucleotide d(CCCTAACCCCTAACCCCTAA) was prepared by Sigma-Genosys in HPLC purified form and desalted on a Nap 5 column.

Data analysis was performed on OriginPro8.0/8.5. Biexponential fitting models were applied at single wavenumbers at band maxima

Figures and Schemes

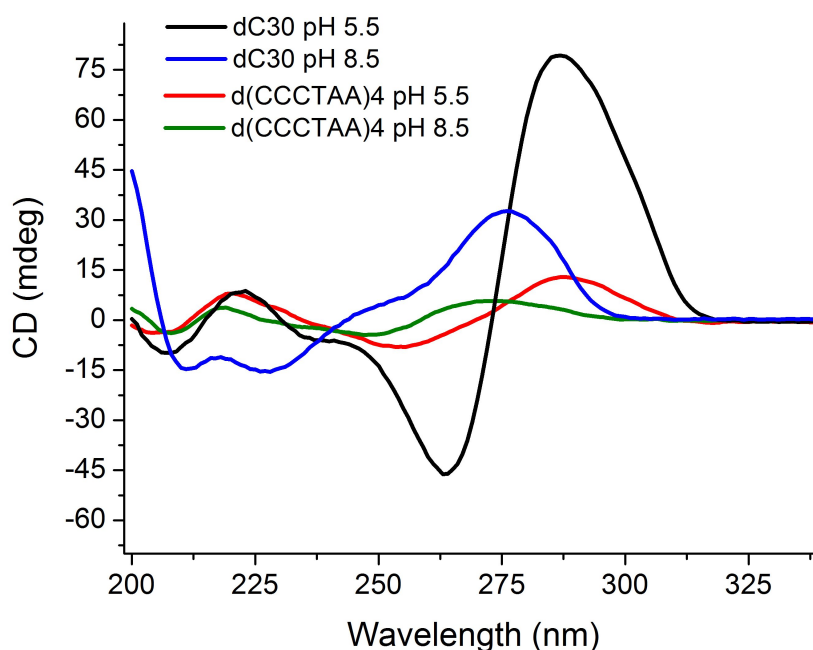


Figure S1. CD spectra of dC₃₀ and d(CCCTAA)₄ under basic and acidic conditions in phosphate buffered D₂O (raw data).

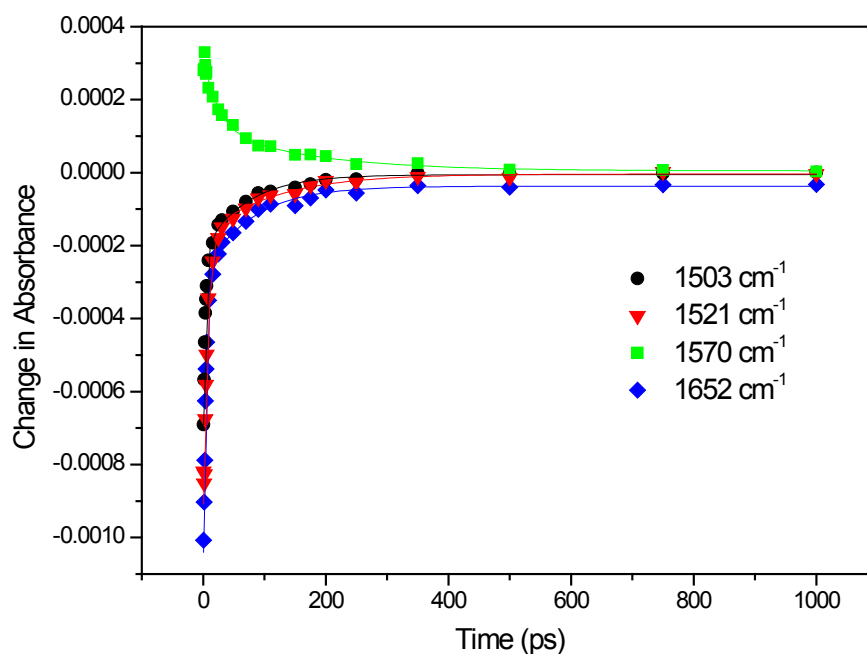


Figure S2. Kinetic analysis of 10 mM dC₃₀ in 50 mM Na₂HPO₄ (pH 8.5, single stranded ss-dC₃₀) in D₂O at 1503, 1521, 1570 and 1652 cm⁻¹. Delays (ps): 1, 2, 3, 4, 5, 6, 9, 11, 25, 31, 49, 70, 90, 110, 150, 175, 200, 250, 350, 500, 750 and 1000.

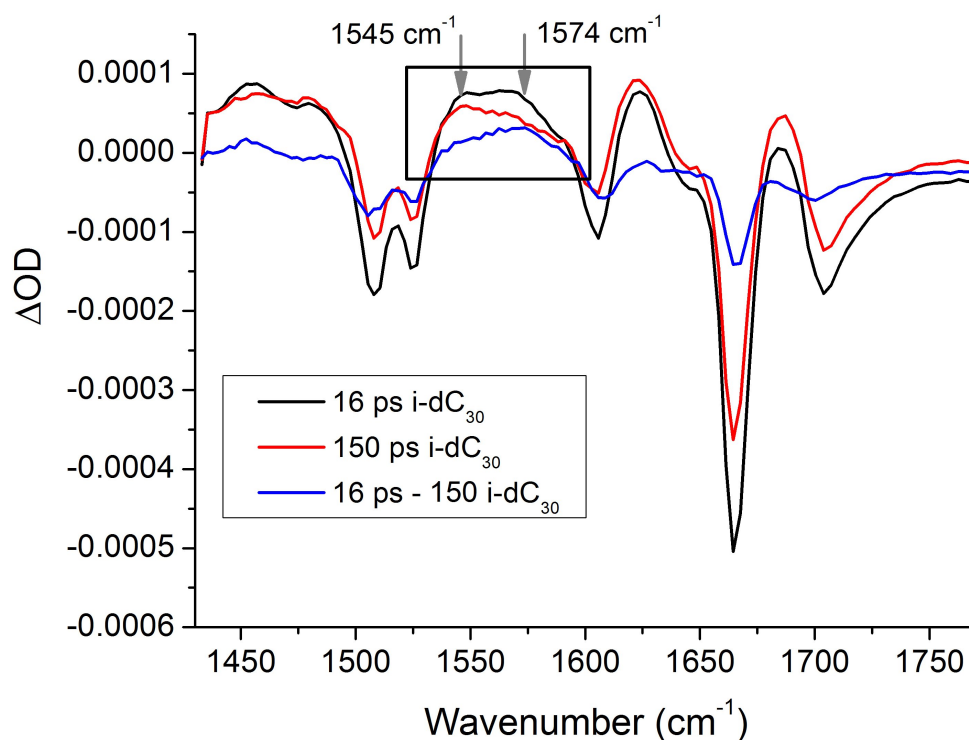


Figure S3. Comparison of the spectral profile of i-dC₃₀ (10 mM nucleotide dC₃₀ in 50 mM Na₂HPO₄ pH 5.5, i-motif) at 16 and 150 ps with subtraction spectra given to demonstrate the nature of band that is giving rise to loss of absorption in the 1500-1650 cm^{-1} region.

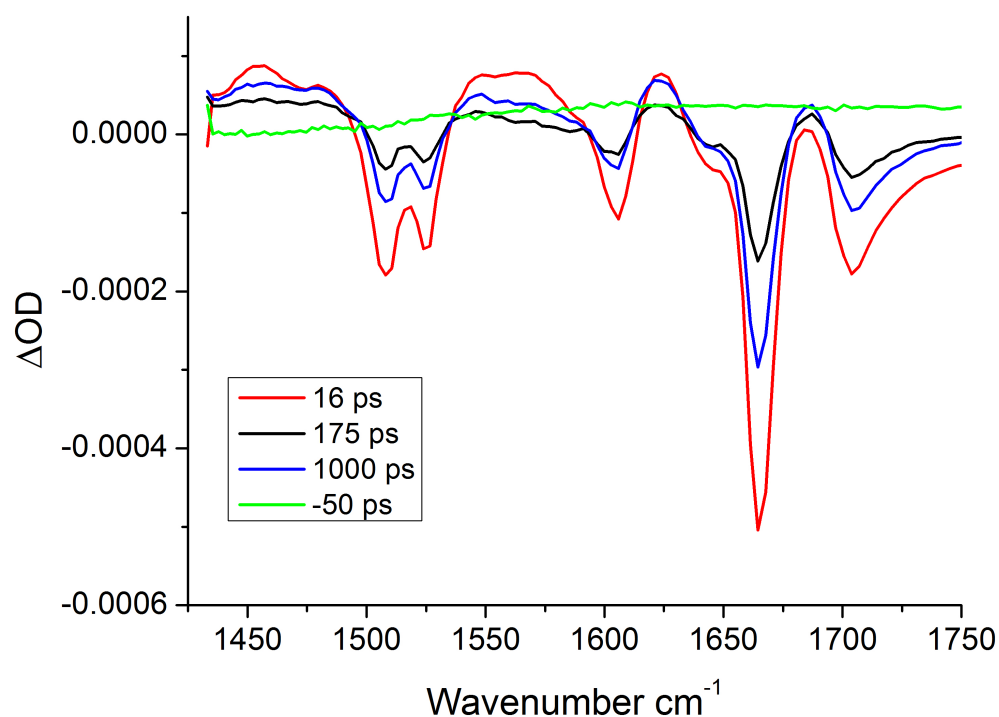


Figure S4. Comparison of the spectral profile of i-dC₃₀ (10 mM nucleotide dC₃₀ in 50 mM Na₂HPO₄ pH 5.5, i-motif) at 16, 175 and 1000 ps in the 1500-1650 cm^{-1} region.

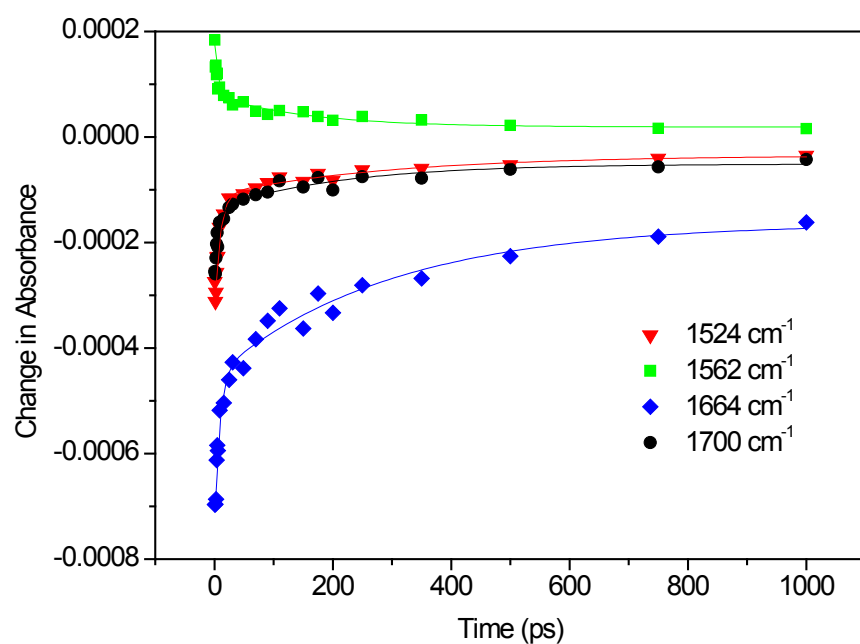


Figure S5. Kinetic analysis of 10 mM i-dC₃₀ in 50 mM NaH₂PO₄ (pH 5.5, i-motif) in D₂O at 1524, 1562, 1664 and 1700 cm⁻¹. Delays (ps): 1, 2, 3, 4, 5, 6, 9, 11, 25, 31, 49, 70, 90, 110, 150, 175, 200, 250, 350, 500, 750 and 1000.

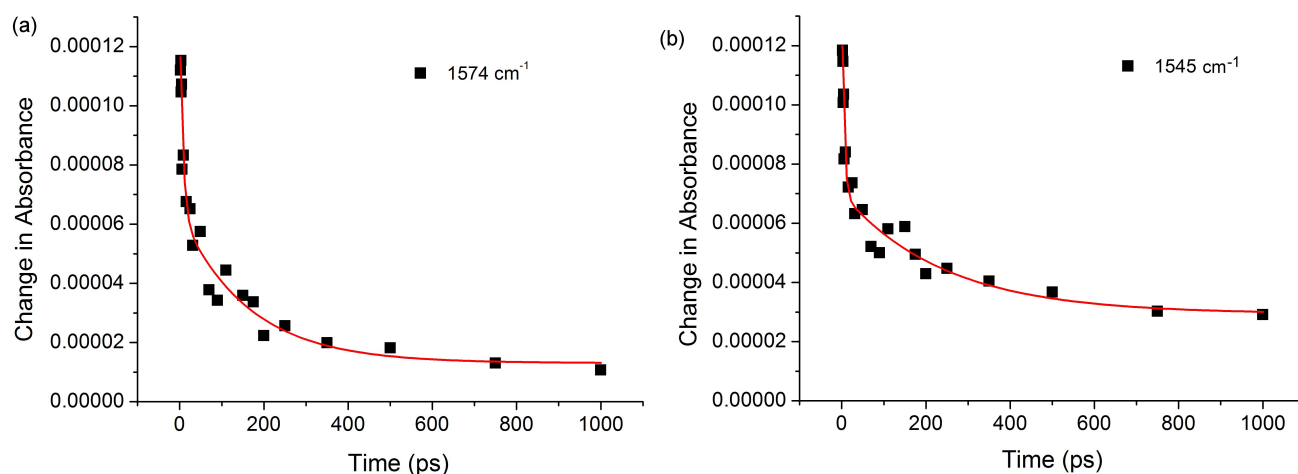


Figure S6. Kinetic analysis of transient bands at (a) 1574 cm⁻¹ and (b) 1545 cm⁻¹ for 10 mM i-dC₃₀ in 50 mM NaH₂PO₄ (pH 5.5, i-motif) in D₂O. Delays (ps): 1, 2, 3, 4, 5, 6, 9, 11, 25, 31, 49, 70, 90, 110, 150, 175, 200, 250, 350, 500, 750 and 1000.

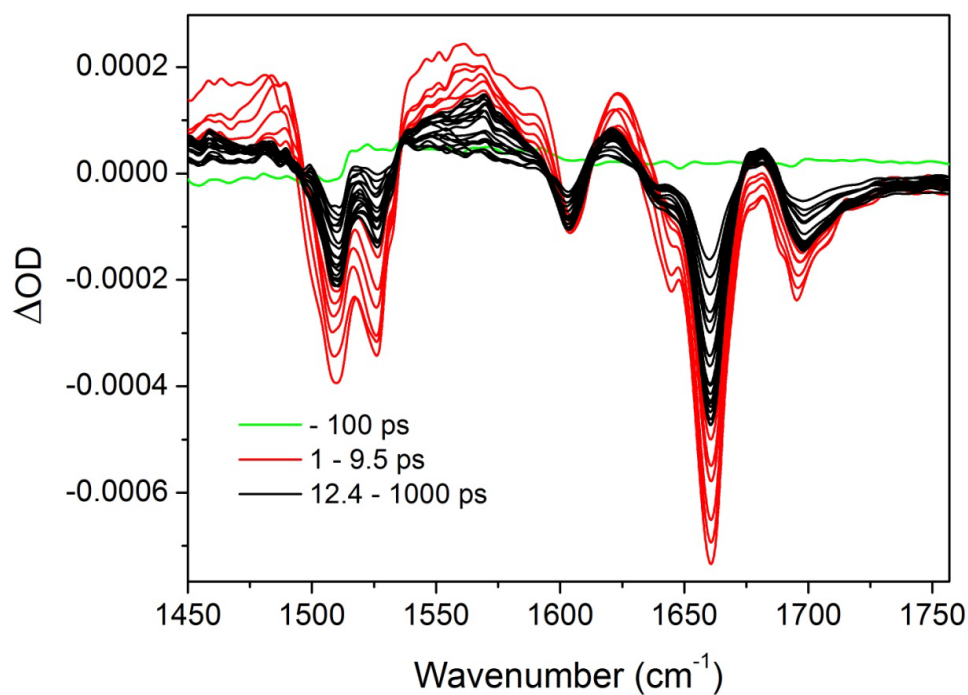


Figure S7 ps-TRIR spectra of dC₃₀ in 50 mM phosphate buffer at pH 7 in D₂O. Delays (ps): 2, 3, 4, 5, 6, 9, 16, 31, 49, 68, 88, 111, 138, 169, 209, 267, 394, 600 and 800.

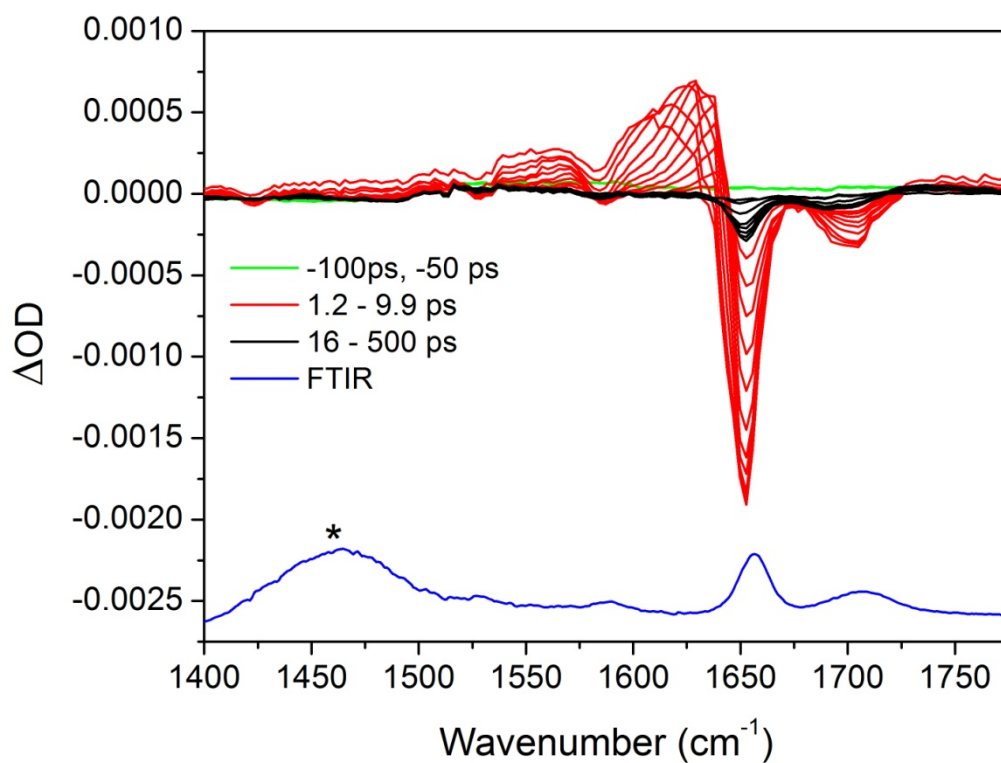


Figure S8. ps-TRIR spectra of 10 mM 5' -dCMP in 0.132 M H₃PO₄ (pH 2). Delays (ps): -100, -50, 1.2, 1.6, 2, 2.5, 3, 3.6, 4.3, 5.1, 6.2, 7.6, 9.9, 16, 20, 25, 35, 50, 100, 250 and 500. * The peak at 1460 cm⁻¹ is due to the presence of HOD)

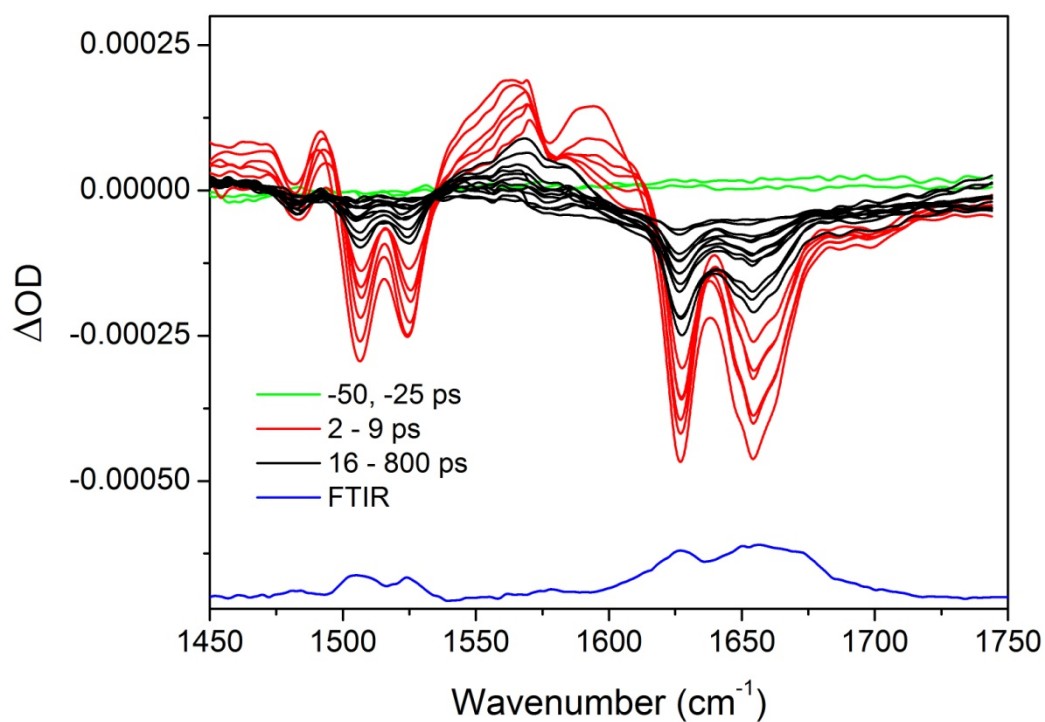


Fig S9 ps-TRIR spectra of 10 mM d(CCCTAA)₄ in 50 mM Na₂HPO₄ (pH 8.5, single stranded)

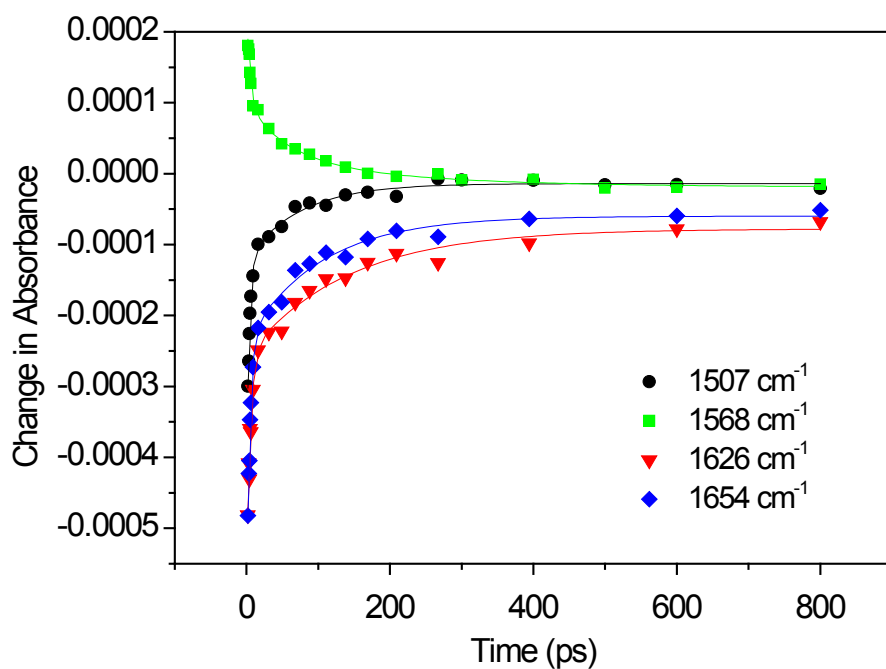


Figure S10. Kinetic analysis of 10 mM d(CCCTAA)₄ recorded in 50 mM Na₂HPO₄ (pH 8.5, single stranded). Delays (ps): -50, -25, 2, 3, 4, 5, 6, 9, 16, 31, 49, 68, 88, 111, 136, 169, 209, 267, 394, 600 and 800.

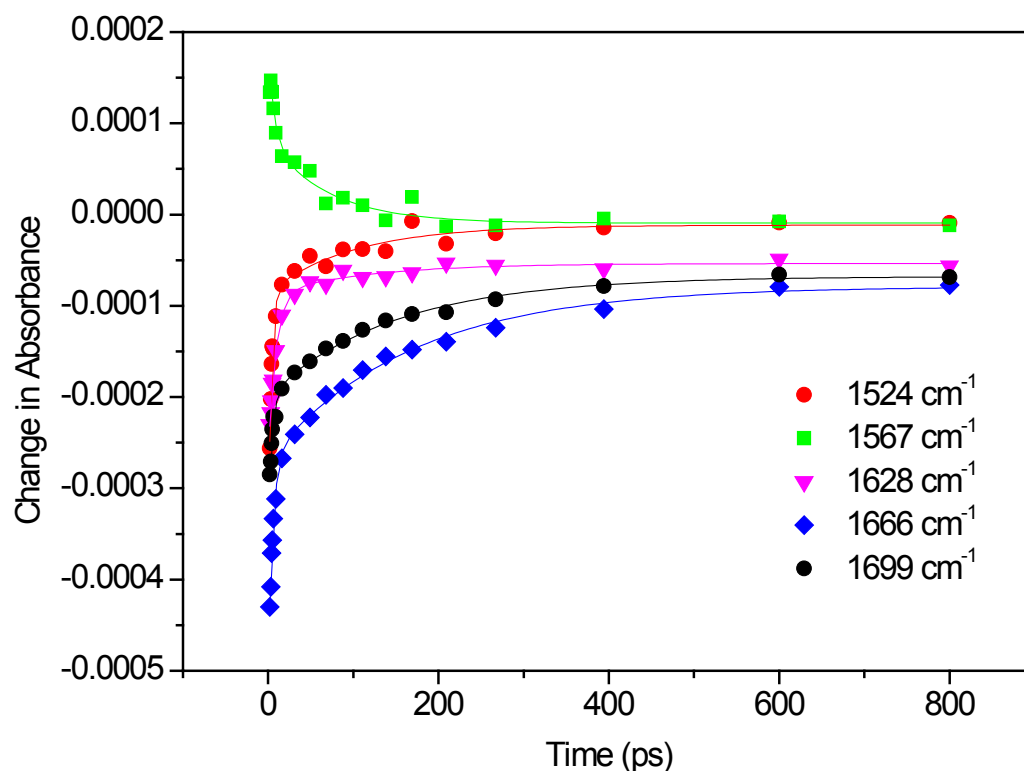


Figure S11. Kinetic analysis of 10 mM d(CCCTAA)₄ in 50 mM NaH₂PO₄ (pH 5.5, i-motif) in D₂O. Delays (ps): 2, 3, 4, 5, 6, 9, 16, 31, 49, 68, 88, 111, 138, 169, 209, 267, 394, 600 and 800.

3. References

1. G. M. Greetham, P. Burgos, Q. Cao, I. P. Clark, P. S. Codd, R. C. Farrow, M. W. George, M. Kogimtzis, P. Matousek, A. W. Parker, M. R. Pollard, D. A. Robinson, Z. J. Xin and M. Towrie, *Appl. Spectrosc.* 2010, **64**, 1311.
2. M. Towrie, D. C. Grills, J. Dyer, J. A. Weinstein, P. Matousek, R. Barton P. D. Bailey, N. Subramaniam, W. M. Kwok, C. S. Ma, D. Phillips, A. W. Parker and M. W. George, *Appl. Spectrosc.* 2003, **57**, 367.