**Supporting Information for 'Picosecond Infrared Probing of the Vibrational Spectra of Transients Formed Upon UV Excitation of Stacked G-tetrad Structures'**

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**Method for ps-TRIR measurements:**

Measurements were performed at the PIRATE ultrafast infrared absorption facility at the Rutherford Appleton Laboratory, Chilton which has been described in detail elsewhere.[1] Briefly, the sample was excited with 267 nm, 200 fs pulses with 2 μJ of energy at 0.5 kHz repetition rate, generated from the third harmonic of part of the output from a 1kHz, 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-$BaB_2O_4$) optical parametric amplifier, pumped with some residual 800 nm, in $AgGaS_2$ at 1 kHz. The spot size was 200 μm and 150 μm diameter for the pump and the probe beam, respectively. The difference signal pump-on minus pump-off was normalized 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 array. The data were collected in a number of 150 $cm^{-1}$ spectral windows centred at approximately 1620 $cm^{-1}$, 1624 $cm^{-1}$, 1630 $cm^{-1}$ and 1565 $cm^{-1}$ using the delay line for optical delays between 2 ps and 1.5 ns, normally at 2, 3, 4, 5, 6.5, 8, 10, 12.5, 15, 20, 35, 50, 100, 150, 200, 500, 1000 and 1500 ps . The difference signal was calibrated using water lines present in the probe spectrum, and the spectral windows were interleaved after scaling using overlapping transients recorded at the same delay time. (This was done at 1603 $cm^{-1}$ for 10 mM $Na_2GMP$ in 50 mM sodium phosphate buffer and 1541 $cm^{-1}$ for 11.4 mM Poly(G) in 50mM potassium phosphate buffer.) The sample was raster scanned in *x*- and *y*- directions at an approximate rate of 100 mm/ms.

1. 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, *Applied Spectroscopy*, 2003, **57**, 367.

**Sample Preparation:**

All measurements were preformed in a Harrick demountable liquid cell in which the liquid sample is placed between two 25.4 mm $CaF_2$ plates separated by 12 μm Teflon spacers in the case of low concentration experiments and without the use of Teflon spacers in high concentration experiments. Samples were prepared by weighing out an amount of the nucleotide base, polynucleotide or quadruplex into an eppendorf followed by the addition of a known amount of $D_2O$ or buffer solution. Concentrations were determined by UV/vis spectroscopy. The mixtures were homogenised by vortexing the sample for approximately 2 minutes but in some cases longer agitation was required for complete dissolution. 50 -70 μl aliquots of each sample were placed centrally onto the $CaF_2$ plates for analysis by ps-TRIR, UV/vis and FTIR (Figure 1) spectroscopies.
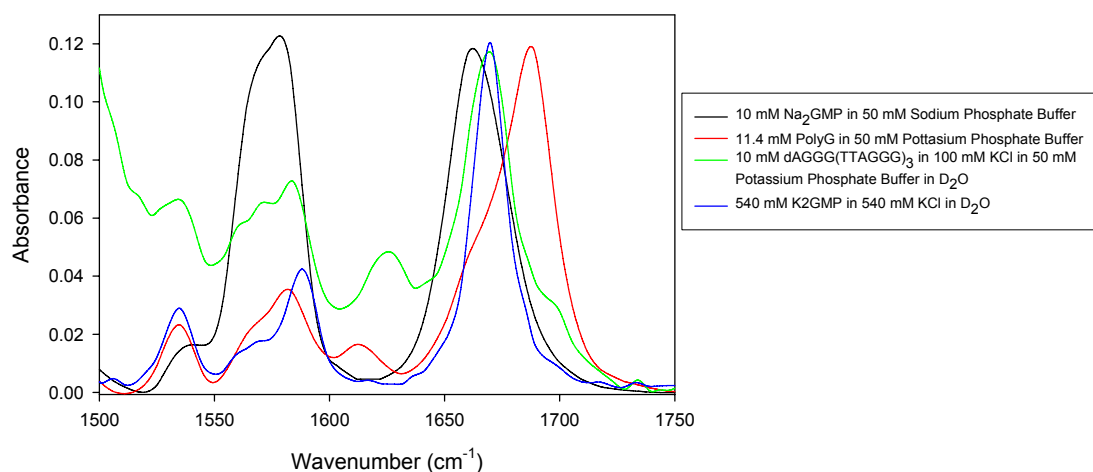


Figure 1: FTIR of 10 mM $Na_2GMP$ in 50 mM sodium phosphate buffer, 11.4 mM Poly(G) in 50 mM potassium phosphate buffer, 10 mM dAGGG(TTAGGG)$_3$ in 100 mM KCl in 50 mM potassium phosphate buffer in $D_2O$ and 540 mM $K_2GMP$ in 540 mM KCl in $D_2O$. Normalised to the carbonyl bleach.

**Simulation Studies:**

Decay times determined for 10 mM $Na_2GMP$ in 50 mM sodium phosphate buffer in $D_2O$ are observed to change as the kinetic analysis is performed at points across either the carbonyl or ring-based band (Figures. 2 and 3). To understand this further we have modelled the experimental spectral profile using overlapping transient and depletion Gaussian bands. Using OriginPro 7.5 a series of Gaussian curves were drawn with the FWHM of 7 pixels (ca. 23 cm$^{-1}$). The transient Gaussian absorbance was set at 30% of the bleach absorbance similar to that observed experimentally. For the carbonyl region the resultant convoluted image exhibits similar features to the experimental data. The absorbance for each curve was allowed to decay exponentially over the range of 2 -100 ps using the decay time of 3.1 ps as measured experimentally for the carbonyl bleach. The transient and bleach maxima were initially separated by 7 pixels. In order to take account of cooling, which is observed as a shift to higher wavenumbers with time in the transient band, the separation of the maxima was allowed to decrease with time. The deviation ($\Delta t$) between the computed decay time and that observed experimentally was determined and the best fit taken as that with minimum variance, $(\Delta t)^2$ (Table 1). The best fit was observed when the separation of the 2, 3 and 4ps were set at 7 pixels and the 5 ps and longer was set at 6 pixels for the carbonyl transient/bleach profile (1609 to 1703 cm$^{-1}$) (Figure 4). (Due to the presence of two overlapping Gaussian bleach bands, as evidenced by a lower wavenumber shoulder on the ring bleach band, and presumably two overlapping transients bands, simulation of the ring bleach and associated transient kinetics are not reported here.)
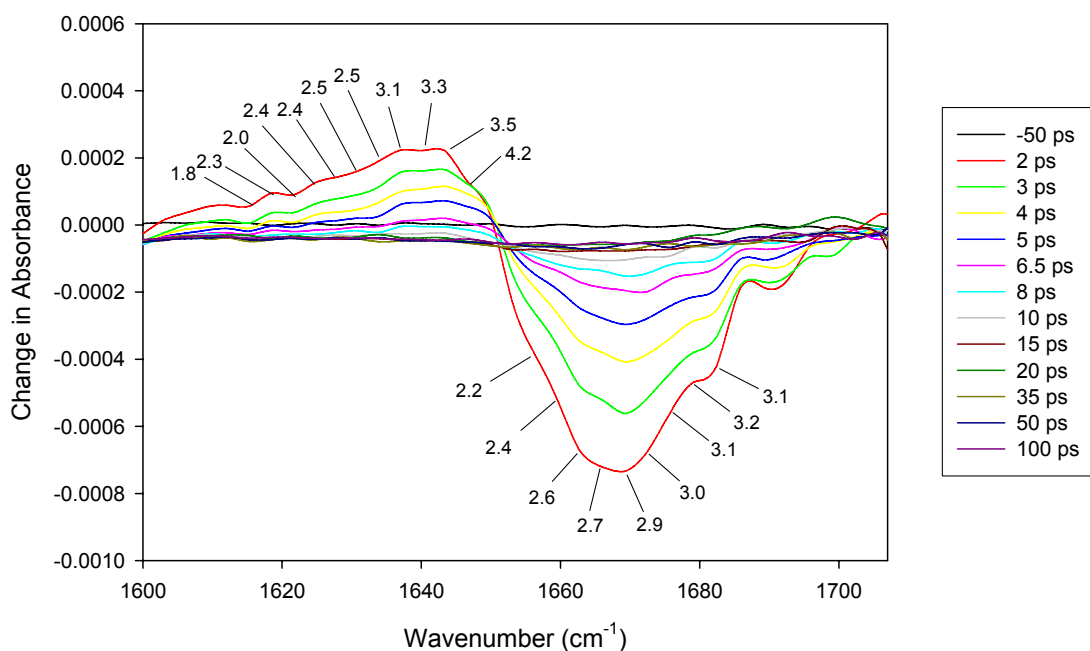
Figure 2: Kinetic decay times measured at various wavelengths on the carbonyl bleach and associated transient for 10 mM $Na_2GMP$ in 50 mM sodium phosphate buffer in $D_2O$, pH=7.0.
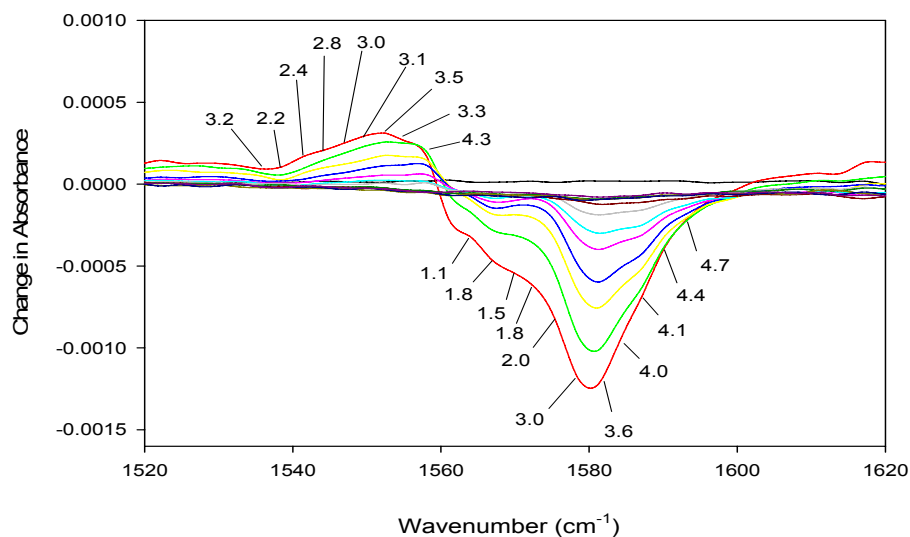


Figure 3: Kinetic decay times measured at various wavelengths on the ring bleach and associated transient for 10 mM $Na_2GMP$ in 50 mM sodium phosphate buffer in $D_2O$, pH = 7.0.
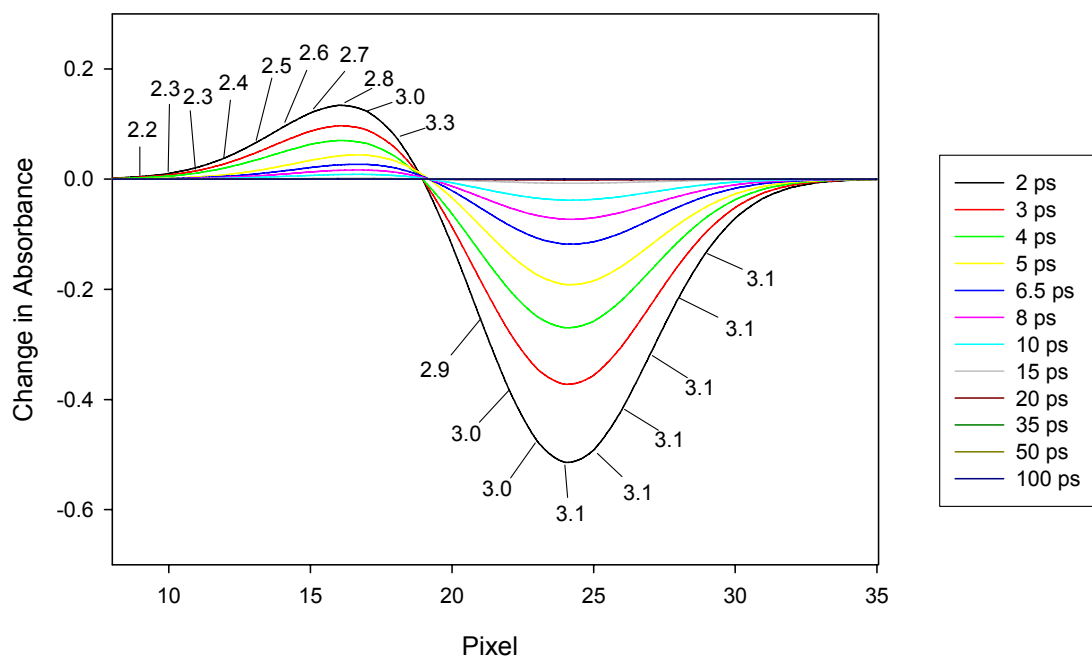
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Figure 4: Simulated kinetic decay times measured at various wavelengths on the carbonyl bleach and associated transient for 10 mM Na$_2$GMP. The transient spectra at 5 ps and at longer times are displaced to higher wavenumbers by 1 pixel compared to those at 2, 3 and 4 ps. (1 pixel = ca. 3.3 cm$^{-1}$)

Table 1: A comparison of the decay times for the experimental and simulated data (a) for the transient and (b) for the bleach in the carbonyl region of 10 mM Na$_2$GMP.

**(a) Carbonyl Transient**

| Simulation Pixel Position | Equivalent Position (cm$^{-1}$) | Simulated Time (ps) | Experimental Time (ps) | Δt (ps) | (Δt)$^2$ (ps)$^2$ |
|---|---|---|---|---|---|
| 9 | 1618 | 2.2 | 2.3 | 0.0 | 0.0 |
| 10 | 1622 | 2.3 | 2.0 | 0.3 | 0.1 |
| 11 | 1625 | 2.3 | 2.4 | 0.0 | 0.0 |
| 12 | 1628 | 2.4 | 2.4 | 0.0 | 0.0 |
| 13 | 1631 | 2.5 | 2.5 | 0.0 | 0.0 |
| 14 | 1634 | 2.6 | 2.5 | 0.1 | 0.0 |
| 15 | 1637 | 2.7 | 3.1 | -0.4 | 0.2 |
| 16 | 1640 | 2.8 | 3.3 | -0.5 | 0.2 |
| 17 | 1643 | 3.0 | 3.5 | -0.5 | 0.2 |
| 18 | 1647 | 3.3 | 4.2 | -0.9 | 0.8 |

**b) Carbonyl Bleach**

| Simulation Pixel Position | Equivalent Position (cm$^{-1}$) | Simulated Time (ps) | Experimental Time (ps) | Δt (ps) | (Δt)$^2$ (ps)$^2$ |
|---|---|---|---|---|---|
| 21 | 1656 | 2.9 | 2.2 | 0.7 | 0.5 |
| 22 | 1659 | 3.0 | 2.4 | 0.6 | 0.3 |
| 23 | 1663 | 3.0 | 2.6 | 0.5 | 0.2 |
| 24 | 1666 | 3.1 | 2.7 | 0.3 | 0.1 |
| 25 | 1669 | 3.1 | 2.9 | 0.2 | 0.0 |
| 26 | 1673 | 3.1 | 3.0 | 0.0 | 0.0 |
| 27 | 1676 | 3.1 | 3.1 | 0.0 | 0.0 |
| 28 | 1679 | 3.1 | 3.2 | -0.1 | 0.0 |
| 29 | 1682 | 3.1 | 3.1 | 0.0 | 0.0 |