Temperature-Jump Time Resolved 2D-IR Spectroscopy of DNA Hairpin Unfolding

Contact *neil.hunt@york.ac.uk*

C.P. Howe
Department of Chemistry
University of York, York, UK

G.M. Greetham
Central Laser Facility
Rutherford Appleton Laboratory, Didcot, UK

B. Procacci
Department of Chemistry
University of York, York, UK

D. J. Shaw
Department of Chemistry
University of York, York, UK

M. Towrie
Central Laser Facility
Rutherford Appleton Laboratory, Didcot, UK

A.W. Parker
Central Laser Facility
Rutherford Appleton Laboratory, Didcot, UK

N.T. Hunt
Department of Chemistry
University of York, York, UK

Introduction

Dynamic processes play important roles in biological function, ranging from the separation of double stranded DNA during transcription and replication to protein conformational changes that constitute part of their biological mechanisms. The ability to observe these dynamic processes in real time would provide not only new insights into biological function but also experimental data for the validation of computational simulations that are used to predict solution phase biomolecular behaviour.

Observing molecular processes in real time requires a trigger to initiate changes in structure, which can then be monitored by a time-delayed probe experiment. For biological molecules, photochemical activating groups can be implanted into protein or nucleic acid structures, but this modifies the structure of the molecule and so risks changing the dynamics that we are seeking to study. [1-2] Temperature jump (T-jump) spectroscopy uses a nanosecond duration infrared laser pulse, tuned to a solvent absorption band, to cause a rapid rise in temperature, followed by a time-delayed probe of the molecule's response.[3-5] The method has the benefit of probing the dynamics of the biological molecule on its natural potential energy surface and has been combined with probe measurements ranging from IR absorption spectroscopy, to fluorescence or circular dichroism.[6-8]

In previously-published work, we have combined high repetition (1 kHz) rate T-jump pumping with time resolved multiple probe (TRMPS) infrared detection to study the melting of double stranded DNA sequences and temperature-induced changes in protein structure.[5, 9-10] A method to characterize the magnitude and dynamics of the temperature jump using a trifluoroacetic acid (TFA) solution was published in a previous annual report.[11]

Here, we report the extension of the T-jump instrument to employ two dimensional infrared (2D-IR) spectroscopy to probe the relaxation dynamics of the sample. 2D-IR spreads the IR spectrum of a molecule over a second frequency dimension, providing access to information on vibrational couplings, energy transfer and molecular dynamics. [12] T-jump-2D-IR was used to observe the unfolding of a short DNA hairpin in real time. The hairpin features a tetraloop motif, which is commonly found in nucleic acid tertiary structures such as RNA-based ribozymes and synthetic nucleic acid aptamer molecules. Establishing proof of concept for T-jump 2D-IR measurements of these structural building blocks will thus pave the way to applications aimed at enhancing our understanding of nucleic acid tertiary structure.

Experimental

The T-jump pump 2D-IR probe spectrometer is an extension of the T-jump-IR experiment[9] and employs the Time Resolved Multiple Probe (TRMPS) strategy that has also been used to study photochemically-activated processes.[5, 10, 13-14] A brief description is given below.

The pump pulses used to generate the T-jump were produced by a home-built Nd:YAG-pumped optical parametric oscillator (OPO). The output pulses had a pulse duration of 4 ns, an energy of 70 µJ and were chopped to a repetition rate of 125 Hz. The T-jump pulses were tuned to a frequency of ~2750 cm^{-1} that coincides with the high frequency edge of the OD stretching vibration of the D_2O solvent used for the DNA hairpin samples. A sample path-length of 12 µm ensured that the pump pulses were not completely absorbed by the sample while maximizing the temporal duration of the T-jump. [9]

The mid-IR pulses used for 2D-IR spectroscopy were produced by the CLF ULTRA-B laser system at a pulse repetition rate of 10 kHz with a pulse duration of 50 fs and a centre frequency of 1630 cm^{-1}, resonant with vibrational modes of the DNA bases. Two separate OPAs generate the pump and probe pulses, with their relative delay controlled by an optical delay line. A phase-cycled, pump pulse pair was generated by passing the pump beam through a mid-IR pulse shaper, controlling their relative timing. An odd number difference between the number of multiple probes (R = 10 kHz / 125 Hz = 80) and pulse shaper waveforms (n, typically a few hundred), so that $n = Rm - 1$ (where m is an integer), created a strobing of the multiple probe pulses across the waveform settings. After n x R probe acquisitions (at 10 kHz), each probe time point had sampled all the waveforms, so a 2D-IR spectrum could be generated for each pump – probe delay, across all of the multiple probes. This rapid acquisition method of a large quantity of 2D-IR spectra has been applied to transient 2D-IR previously. [15] T-jump-2D-IR spectra could be measured at time delays ranging from 1 ns to 4 ms following the T-jump.

The DNA sample studied was a 12 base pair oligomer (GCGCTACGGCGC), which forms a tetraloop structure with a stem comprising four GC base pairs (Figure 1). The oligomer was obtained from Eurogentech and dissolved in 1M phosphate buffer solution without further purification.

Results

The results of 2D-IR experiments carried out as a function of temperature on the DNA hairpin sample under equilibrium (non T-jump) conditions are shown in Figure 1(a & b). The melting

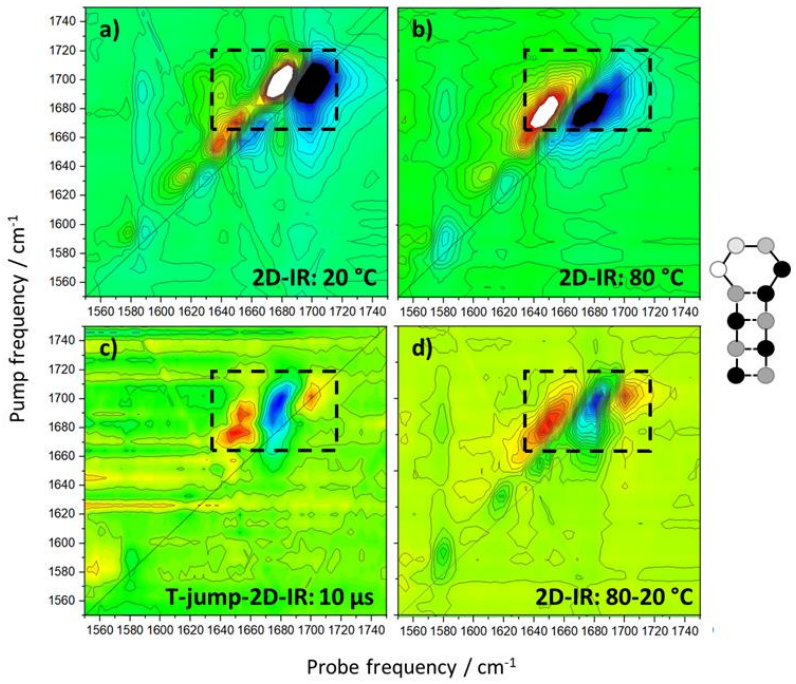

Figure 1: *2D-IR spectra of DNA hairpin (structure on right) under equilibrium conditions at a) 20 °C and b) 80 °C. c) T-jump-2D-IR spectrum obtained at T_0 of 70 °C and a T-jump-2D-IR delay time of 10 µs. d) Difference spectrum produced by subtracting spectrum in a) from that in b).*

temperature of the hairpin was determined by IR absorption studies to be 75 °C. Upon increasing the temperature from 20 °C (Figure 1(a)) to 80 °C (Figure 1(b)) there is a clear shift in the frequencies and intensity distributions of bands that are assignable to vibrational modes of the DNA bases (see black boxes in Figures 1(a) and 1(b)). These changes are consistent with previous studies of DNA samples and are caused by a loss of base pairing and stacking in the hairpin stem as the structure melts. [5, 16] The spectral changes are highlighted by calculating the difference between the spectra in Figures 1(a) and 1(b), which is shown in Figure. 1(d).

A series of T-jump 2D-IR spectra were obtained using the same DNA sample. In these experiments the DNA sample was held at an equilibrium temperature (T_0) and the T-jump pump laser was used to apply a temperature jump of ~10 °C. 2D-IR spectra were then obtained at T-jump-2D-IR delay times from 100 ns to 1 ms.

The T-jump-2D-IR spectrum obtained at a T_0 of 70 °C and a T-jump-2D-IR delay time of 10 µs is shown in Figure 1(c). Previous studies have shown that the melting of double stranded DNA oligomers occurs on timescales of tens of microseconds.[5] Thus, in the event of hairpin melting being observed, the T-jump-2D-IR spectrum in Figure 1(c) would be expected to show similar features to the equilibrium difference 2D-IR spectrum in Figure 1(d). Focusing on the area outlined in the black box shows that the two datasets are in good agreement. The T-jump difference spectrum has a slightly lower signal to noise ratio as a result of the need for a shorter sample path length in the T-jump study compared to the equilibrium 2D-IR spectra (50 µm). As a result the peak shapes are less clear in Figure 1(c) than in Figure 1(d), but the main spectral components are present in the expected intensity ratios showing that the experiment is capable of detecting hairpin melting.

Conclusions

T-jump 2D-IR spectroscopy on the UTRA spectrometer is capable of detecting DNA hairpin melting, delivering both the required sensitivity and access to the relevant experimental timescales. This experiment therefore establishes proof of concept for more detailed studies of the melting process.

Acknowledgements

We are grateful to the STFC Central Laser Facility for access to the ULTRA suite of spectrometers. CPH thanks the University of York and STFC for funding.

References

1. Backus, E. H. G.; Bloem, R.; Donaldson, P. M.; Ihalainen, J. A.; Pfister, R.; Paoli, B.; Caflisch, A.; Hamm, P., *J Phys Chem B* **2010,** *114*, 3735-3740.
2. Kolano, C.; Helbing, J.; Kozinski, M.; Sander, W.; Hamm, P., *Nature* **2006,** *444*, 469-472.
3. Petty, S. A.; Volk, M., *PhysChemChemPhys* **2004,** *6*, 1022-1030.
4. Chung, H. S.; Ganim, Z.; Jones, K. C.; Tokmakoff, A., *Proc Nat Acad Sci* **2007,** *104*, 14237-14242.
5. Fritzsch, R.; Greetham, G. M.; Clark, I. P.; Minnes, L.; Towrie, M.; Parker, A. W.; Hunt, N. T., *J Phys Chem B* **2019,** *123* 6188-6199.
6. Rigler, R.; Rabl, C. R.; Jovin, T. M., *Rev Sci Instrum* **1974,** *45*, 580-588.
7. Khuc, M. T.; Mendonca, L.; Sharma, S.; Solinas, X.; Volk, M.; Hache, F., *Rev Sci Instrum* **2011,** *82*, 054302.
8. Williams, S.; Causgrove, T. P.; Gilmanshin, R.; Fang, K. S.; Callender, R. H.; Woodruff, W. H.; Dyer, R. B., *Biochemistry* **1996,** *35*, 691-697.
9. Greetham, G. M.; Clark, I. P.; Young, B.; Fritsch, R.; Minnes, L.; Hunt, N. T.; Towrie, M., *Appl Spectrosc* **2020,** *74*, 720-727.
10. L. Minnes; G.M. Greetham; D. J Shaw; I. P. Clark; R. Fritzsch; M. Towrie; A. W. Parker; A.J. Henry; R. J. Taylor; Hunt, N. T., *J Phys Chem B* **2019,** *123*, 8733-8739.
11. Fritzsch, R.; Minnes, L.; Greetham, G. M.; Clark, I. P.; Shaw, D. J.; Towrie, M.; Parker, A. W.; Hunt, A. J., *CLF Annual Report* **2019**.
12. Fritzsch, R.; Hume, S.; Minnes, L.; Baker, M. J.; Burley, G. A.; Hunt, N. T., *Analyst* **2020,** *145*, 2014.
13. Greetham, G. M.; Donaldson, P. M.; Nation, C.; Sazanovich, I. V.; Clark, I. P.; Shaw, D. J.; Parker, A. W.; Towrie, M., *Appl Spectrosc* **2016,** *70*, 645-653.
14. Greetham, G. M.; Sole, D.; Clark, I. P.; Parker, A. W.; Pollard, M. R.; Towrie, M., *Rev Sci Instrum* **2012,** *83*, 103107.
15. Hamm, P., *J Chem Phys* **2021,** *154*, 104201.
16. Hithell, G.; Gonzalez-Jimenez, M.; Greetham, G. M.; Donaldson, P. M.; Towrie, M.; Parker, A. W.; Burley, G. A.; Wynne, K.; Hunt, N. T., *PhysChemChemPhys* **2017,** *19*, 10333-10342.