Temperature-Jump Time Resolved Infrared Spectroscopy of Trifluoroacetic Acid Solutions – Characterising the T-Jump

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Introduction

There is an increasing appreciation of the role played by dynamic structural changes in biological function, from the unwinding of double stranded DNA during transcription and replication through to structural changes undergone by proteins as part of their biological mechanisms. The underlying molecular physics of these transitions are therefore of significant interest and the ability to measure them in detail would provide valuable experimental validation for computational Molecular Dynamics methods that are used to predict biomolecular behaviour in the solution phase.

Observing biomolecular structural changes in real time is technically challenging however, with a particular problem lying in the desire to be able to trigger a transition, to allow time-resolved monitoring, while avoiding having to modify the structure of the molecule in question, for example by implanting non-natural photo-activating groups or site-specific probes. [1-2]

Temperature jump (T-jump) time-resolved spectroscopy has thus become a powerful method for studying biomolecular transitions.[3-5] In T-jump spectroscopy, a nanosecond duration infrared laser pulse, tuned to absorptions of the solvent, is used to induce a rapid rise in temperature. This perturbs the biomolecule using its natural potential energy surface and the resulting response can be probed by a time-delayed measurement experiment, which has varied from IR absorption spectroscopy, to fluorescence or circular dichroism.[6-8]

In the application of T-jump strategies however, it is important to carefully calibrate the temperature jump achieved in order to separate biomolecular dynamics from those of the solvent bath. In this report we describe the use of trifluoroacetic acid (TFA) solutions to characterize the magnitude and dynamics of a temperature jump initiated using a high repetition rate pumping and time resolved multiple probe (TRMPS) infrared detection methodology. [5, 9]

Experimental

T-Jump pump-IR probe spectroscopy:
The T-jump pump-mid IR probe spectrometer was based upon the Time Resolved Multiple Probe (TRMPS) strategy demonstrated previously for photochemical activation. [10-11] The spectrometer has been described elsewhere but a brief description is given below.
The excitation 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 500 Hz. The higher repetition rate pumping approach has the advantage of increasing the data acquisition rate over more widely used strategies employing 10-20 Hz pulse repetition rates.[1-2] However, higher repetition rate pumping also limits the maximum accessible T-jump-pump– IR probe window to 2 ms.

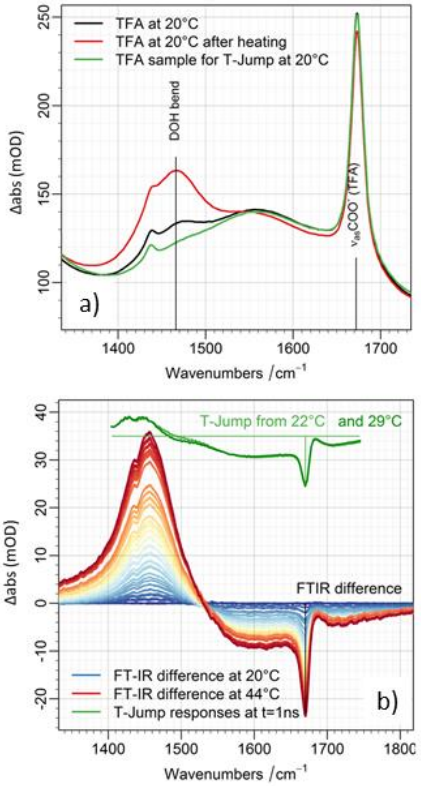

Figure 1: *a) IR absorption spectra of TFA in D_2O solution (black and red). NB the increase in absorbance of the HOD bending vibrational mode after heating due to further exchange with the environment. Green shows the absorption spectrum of the sample used to acquire the data in b). b) Temperature-dependent IR absorption difference spectra of TFA (lower). Colour scale runs from 20 °C (blue) to 44 °C (red). The difference spectra are relative to the spectrum at 20 °C. Green traces show T-jump-IR spectra of the same sample obtained at a T-jump-probe delay time of 1 ns.*

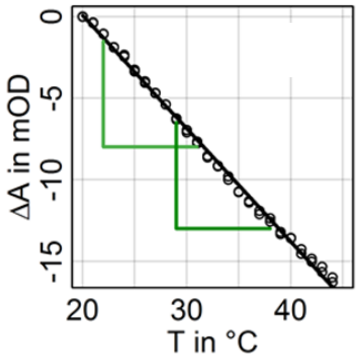

Figure 2: *Calibration plot derived from IR absorption difference spectra in Fig.1(b). Plot shows the change in magnitude of the TFA carboxylate stretching mode difference spectral feature as a function of temperature.*

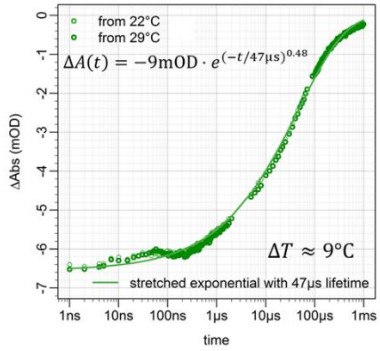

Figure 3: *Temporal dependence of the T-jump-IR spectral response of TFA. The data was well-represented by a stretched exponential fit, shown as a solid green line.*

In the experiments described, the T-jump was achieved by tuning the frequency of the pump pulses to ~2660 cm^{-1}, resonant with the high frequency wing of the OD stretching vibration of the D_2O solvents. At this frequency, using a sample path-length of 6 µm, the pump pulses were not completely absorbed by the sample (absorbance ~1.6) and measurements of the pump energy immediately before and after the sample indicated a reduction in pulse energy from 70 µJ to 18 µJ.

The mid-IR probe pulses were produced by the 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}. The difference in repetition rates of the T-jump pump pulses and the probe pulses along with electronic control of the relative timings of the pump and probe laser systems allowed T-jump pump-IR probe spectra to be measured at time delays ranging from 1 ns to 2 ms. To remove spectral or dynamic features due to the instrument response, a portion of the probe beam was separated before the sample and used as a reference measurement. For each T-jump experiment, 2500 T-jump cycles were averaged per electronic time delay and data was obtained within 12 min.

To characterise the induced T-jump in terms of magnitude and dynamics, solutions of TFA in D_2O were studied. Samples were held between CaF_2 windows separated by a spacer of 6 µm thickness. T-Jump measurements were recorded at different starting equilibrium sample temperatures (T_0) by heating the sample with the temperature-controlled cell. The T-Jump response of the solvent, in the absence of TFA was also acquired. This was scaled and subtracted from the T-Jump response of TFA in order to isolate the response of the calibrant molecule.

Results and Discussion

The infrared absorption spectrum of TFA features an intense asymmetric carboxylate group stretching vibrational mode at 1672 cm^{-1} (Fig.1(a)). Measuring the IR spectrum as a function of temperature showed that this band shifts to higher frequencies upon heating and the construction of difference IR spectra shows that the magnitude of the difference spectral feature increases with temperature in a linear fashion over the temperature range studied (Fig.1(b), lower).

The upper traces in Fig.1(b) show the results of two T-Jump measurements made on TFA solutions, with T_0 = 22°C and 29°C. The difference spectral features shown were obtained with a T-jump-probe delay time of 1 ns and are virtually identical to the difference IR absorption spectra at the same T_0. Both show an increase in amplitude of the DOH bend at 1450 cm^{-1} and a +14 cm^{-1} shift in frequency of the TFA asymmetric carboxylate stretching vibration.

Comparing the magnitude of the change in absorbance of the TFA asymmetric carboxylate stretching band in the T-jump measurements to those obtained via IR absorption spectroscopy under identical sample conditions (concentration, path length, cell holder) enables the production of a temperature calibration plot (Fig.2). This approach indicates that the experimental T-jump magnitude obtained was ~ 9K.

The temporal dependence of the TFA band measured following T-jump activation is shown in Figure 3. Here it is clear that the same dynamics were recovered irrespective of the T_0 value employed. The peak TFA response occurred within the duration of the ns pump pulse and was found to relax to the baseline in a manner that was well-represented by a stretched exponential function. This is consistent with a model in which excitation of the OD stretching vibration of the solvent leads to rapid (sub-picosecond) vibrational relaxation and transfer of vibrational energy to low frequency modes of the water, which are manifest as a rise in temperature on ns timescales. This excess temperature then dissipates primarily through thermal conduction via the CaF_2 windows. This leads to a reduction in the magnitude of the TFA signal with T-jump-probe delay time. Fitting this profile using a stretched exponential function produced a lifetime of ~50 µs with a β value of 0.48. The remaining signal after 1 ms has reduced to ca. 3% of its value at τ = 0 ns. As the change in absorbance measured in the T-jump experiment is directly proportional to the change in temperature of the sample, the need for a stretched exponential function could indicate the presence of some inhomogeneous heating across the sample cell path length, this is the topic of a forthcoming publication.

Conclusion

This report described the use of TFA solutions to calibrate and temporally characterise a T-jump induced by excitation of the OD stretching vibration of a D_2O solvent. This method has been employed to study the melting dynamics of DNA-ligand complexes and the early steps in the unfolding of the C-terminal domain of calmodulin, [5, 9].

Acknowledgements

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