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Developing a temperature-jump apparatus for the Ultra laser system

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Introduction

Over the last decade there has been increased interest in the use of the laser induced temperature-jump (T-jump) technique[1] to perturb equilibrium conditions in order to probe the dynamics of structural reorganization or reactions such as dimerization.[2] Particular attention has been paid to the study of protein folding on the nano- to microsecond timescale,[3] the aim of much of the research being to predict a protein's secondary and tertiary structures from its primary sequence in the biological relevant solution phase environment. This still remains one of the hottest topics and more challenging research areas in biochemistry.

The T-jump process involves the rapid heating of a sample by means of electrical discharge,[4] microwave[5,6], or optical heating using laser radiation.[1,3] Electrical and microwave heating are both restricted to longer timescales; however, optical heating can be achieved on a very fast timescale, being dependent on the width of the excitation pulse, the relaxation times of the absorbing species, the response of any solvent to heating, and the time delay in obtaining a uniformly heated solution. Wavelengths coincident with a solvent absorption peak may be used or an absorbing species can be added to the solvent to act as a heat-transducer. Clearly the use of a heat-transducer may lead to unwanted interactions with samples eg proteins and thus the optimum conditions would be a natural aqueous environment.

This report describes new developments within the LSF's Molecular Structure & Dynamics section employing the Ultra laser to T-jump on the picosecond timescale.

Technique and experimental

Ultra

The method chosen for T-jump generation in this development was direct excitation of the water solvent using light with a wavelength of 1.9 μm which is coincident with the peak of a water absorption band, see Figure 1. The vibrational-translational relaxation time of water is fast, 50 fs,[7] and therefore with a 1.9 μm pump with a 100 fs or longer pulse length the T-jump occurs in a time determined by the laser pulse length with temperature equilibration in the excitation volume used here occurring in ca 20 ps.

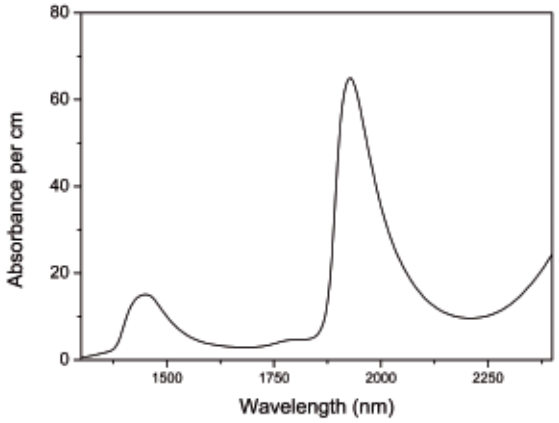

Figure 1. Water absorption spectrum in the near-infrared spectral region. A_{max} ca 70 per cm at 1.9 μm.

To generate the 1.9 μm pump wavelength an optical parametric amplifier (Topas, Light Conversion Ltd) was pumped with 4 W, 800 nm, 2 ps pulses at 10 kHz from the picosecond arm of the ULTRA chirped pulse amplifier (Alpha 10000, Thales Laser) giving ca 28 μJ at 1.9 μm. This was further amplified in a BBO Type 1 crystal using ca 2 W of 800 nm from the same amplifier output yielding 55 μJ at 1.9 μm. This beam was delivered to the sample with the femtosecond mid-IR probe beam through a dry-nitrogen purged optical system, providing ca 23 μJ at the sample. Details of the time-resolved infrared set-up can be found in reference[8].

Due to the decrease in pump intensity as the beam passes through the absorbing sample a temperature gradient will be generated in the sample along the direction of travel of the laser. In an attempt to reduce this and also to raise the induced T-jump a double pass pump system was employed. The pump and probe beams were spatially overlapped in the sample with spot sizes of 120 and 50 μm respectively. The 6 μm pathlength non-flowing aqueous sample (DLC-S25 demountable flow cell, Harrick Scientific) was kept in continuous motion using a home-built raster system.

PIRATE

Temperature jump measurements were also performed using the PIRATE infrared system to monitor the

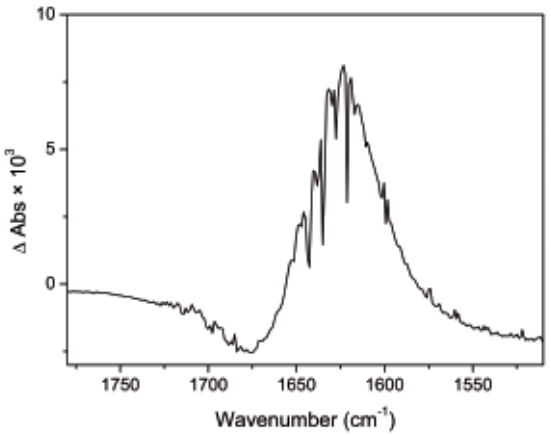

Figure 2. Time-resolved infrared spectrum of water at 10 ps following excitation at 1.9 μm, 23 μJ. The sharp spectral features between 1600 and 1650 cm^{-1} are water vapour absorptions from vapour in the optical set-up remaining following N_2 purge.

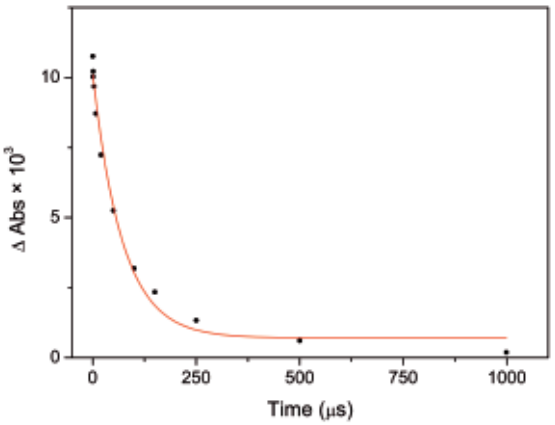

Figure 3. Plot of change in absorbance versus time-delay after excitation. Saturated solution of Neutral Red (heat-transducing dye), excitation at 532 nm, 1 μJ.

lifetime of the temperature increase. However these measurements required excitation with a nanosecond pulsed laser and the use of a heat-transducing dye as the longest achievable time-delay at 1.9 μm is 4 ns (limited by the length of the optical delay line). A saturated solution of Neutral Red dye (Sigma-Aldrich) was used with excitation at 532 nm with pump and probe spot sizes of 150 and 80 μm. Again a rastered 6 μm pathlength non-flowing sample was used.

Results

The infrared spectral features of water can be used to dynamically probe the temperature of the local environment. In this instance Ultra infrared beam line was used to probe the shift and width changes in the sharp water absorption band at 1650 cm^{-1} over the 3 ns following excitation. Figure 2 shows an example spectrum illustrating the broad bleach and transient typical of samples undergoing a temperature increase. From calibration measurements carried out using an FTIR spectrometer the ΔAbs equates to a temperature rise of ca 3 K.

Measurements of the decay profile at 1625 cm^{-1} from the 532 nm pumped neutral red experiment, shown in Figure 3, indicate a lifetime of ca 70 μs. This figure is however very dependent on cell path length, volume of water irradiated and cell window material, due to the heat loss mechanisms. In the case of these experiments the window material used was 2 mm thick CaF_2 which is a good heat conductor thus decreasing the length of time for which the elevated temperature is maintained.

Conclusions

This report demonstrates the use of the Ultra laser system to induce a temperature-jump in an aqueous sample with picosecond time-resolution. The 3 K temperature increase recorded here is the first step towards time-resolved infrared measurements of the unfolding/refolding of biologically relevant species. Whilst this temperature may seem rather modest it is nonetheless feasible to use when the component under investigation is brought to within a degree of the temperature imposed phase change. This approach has been used, for example, by Phillips *et al*. for thermal induced phase change of RNase.[10]

Acknowledgements

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