

Investigating early events in protein folding by laser induced temperature jump

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Introduction

Until recently, experimental methods to probe protein folding have suffered from a serious limitation in how early in the folding transition useful data can be measured. For example, the stop flow technique has a dead time of the order of many 100's of μs in the best cases. However, in the past decade there has been an emergence of new techniques that promise to measure protein kinetics with dead times that reach the order of 10 μs and possibly even faster. The two main categories of such techniques are *fast mixing* (see report in this issue by Drysdale *et al*) and *temperature jump*. Such techniques promise to provide new information about the earliest events in protein folding and an important preliminary result is that there are proteins that fold by populating an intermediate state before they reach their folded state, with rate constants of the order of 10 μs .

In Leeds we have been developing a system that can detect protein folding kinetics initialised by a laser induced temperature jump. The detection of such kinetics is done by following the fluorescence of the amino acid Trp in the protein excited by UV laser pulses. The protein system that has been used in the development of the apparatus is apo-myoglobin, a well characterised system on which other T-jump experiments have indicated a three-state protein folding reaction.

The apparatus

Figure 1 shows the temperature jump apparatus. The protein solution lies in the cuvette that is inside a temperature controlled holder (10) within a chamber which can be evacuated to prevent condensation at low temperatures (9). The temperature jump is achieved by an IR pulse at $1.5\mu\text{m}$ (14) produced by Raman shifting (2) an Nd YAG laser (1) pulse. This beam is split into two counter propagating beams (at beam splitter 12) in order to heat the sample evenly from two sides.

The UV pulse train (13) that excites the protein fluorescence is produced by a frequency doubled (6) dye laser (4), pumped by a mode locked Nd YAG laser (3). The fluorescence pulses are detected by a fast PMT (11), whose signal is captured by an oscilloscope (15) and subsequently analysed (16).

Experimental approach

We have initially chosen to study the refolding of horse heart apo-myoglobin from a cold denatured state. It has been shown by Gruebele that at pH 5.9 this protein is about 80% unfolded at -8°C . In our lab we have shown that the protein is nearly completely cold denatured at 2°C in a solution of pH 5.5 and 1.5 M urea. Under the same conditions at 25°C ,

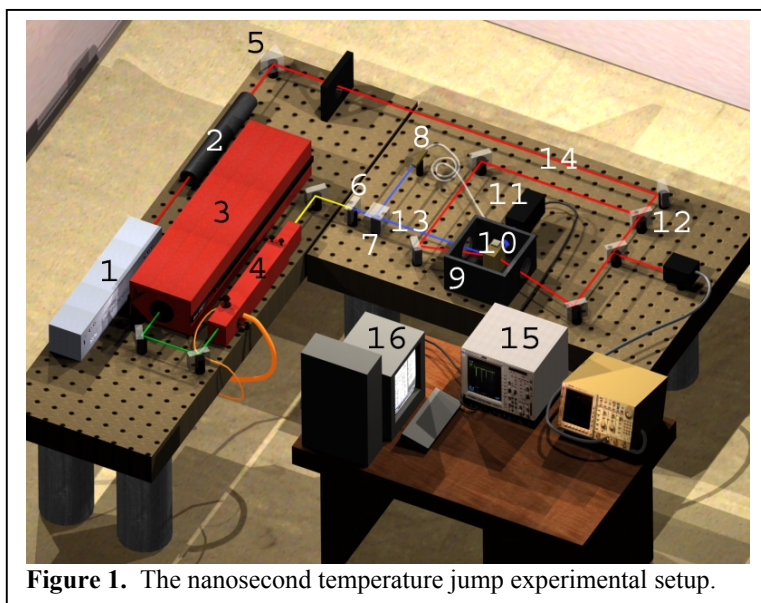


Figure 1. The nanosecond temperature jump experimental setup.

the protein is nearly completely native and therefore a temperature jump of about this magnitude should provide a well populated intermediate (if one exists) for study.

The time resolved fluorescence decays that are detected by our apparatus yield the fluorescence decay time by iterative reconvolution analysis with the instrument function. The experiment can measure the intensity of fluorescence, and the temporal parameters of a fit to a mono-, bi-, or stretched-exponential, thus allowing both the change in intensity and lifetime of the Trp fluorescence to be monitored during folding. The protein fluorescence is excited every 240ns, therefore we can collect 8000 data points that correspond to the first 2 ms of the protein folding. Figure 2 shows the fluorescence intensity change in one such experiment.

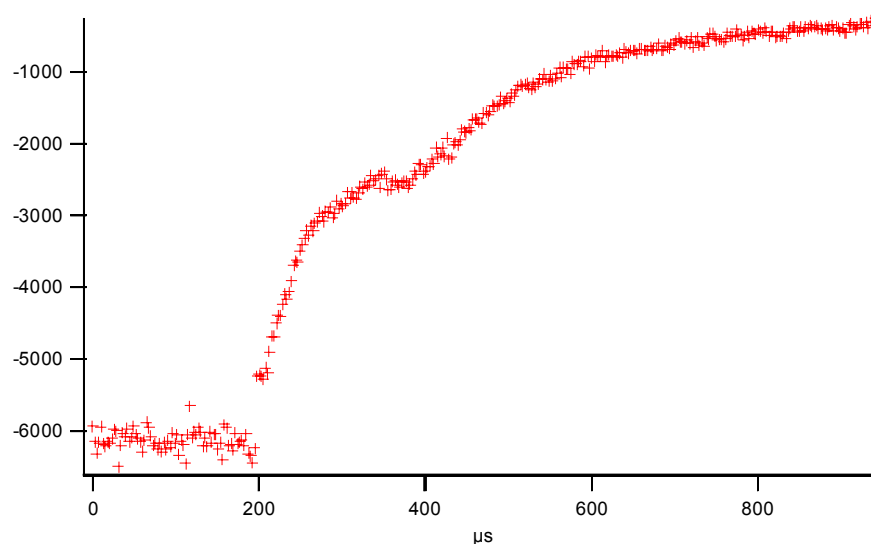


Figure 2 Fluorescence intensity change as a function of time after a 22 degree temperature jump (starting from 2°C under conditions of pH5.5, 1.5M urea). The temperature jump occurred at $t = 200\mu\text{s}$.

Conclusions and future work

We now have a working temperature jump apparatus that is able to resolve the kinetics of protein folding with a dead time of about 250 ns. We shall now further evaluate our experimental technique using apo-myoglobin under different conditions of urea concentration, pH, starting temperatures, and T-jump magnitude. We then plan to study the fast folding of the immunity proteins via equilibrium perturbation experiments and, if possible, refolding experiments from cold denatured states.

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