SUPPLEMENTAL MATERIAL

Nanosecond folding dynamics of an alpha helix: Time-dependent 2D-IR cross peaks observed using polarization-sensitive dispersed pump-probe spectroscopy

Matthijs R. Panman, Chris N. van Dijk, Heleen Meuzelaar, and S. Woutersen

Van ’t Hoff Institute for Molecular Sciences, Universiteit van Amsterdam, Amsterdam, The Netherlands

(Dated: January 12, 2015)
I. EXPERIMENTAL DETAILS

A. Sample preparation

The α-helical peptide (≥95% purity) were purchased from GL Biochem (Shanghai). Residual trifluoroacetic acid (TFA) from the purification method was removed by multiple lyophilizations against a 35% DCl/D$_2$O solution. Stock solutions of 12–16 mM were prepared by directly dissolving the dry peptide in a 50 mM KD$_2$2PO$_4$/K$_2$DPO$_4$ buffer (pH$^*$ = 7.0), resulting in a peptide solution of pH$^*$ = 2.5. The pH$^*$ value of the peptide solution was adjusted to pH$^*$ = 7.0 by addition of NaOD (the volume added was less than 1% of the peptide solution).

The sample was kept in a thermostated IR cell consisting of two 25.4 mm diameter, 1 mm thick, CaF$_2$ windows separated by a 50 µm teflon spacer. The temperature was kept constant with a circulating water bath. The cell was sealed with PTFE paste (Krytox®, DuPont), to limit H$_2$O contamination and evaporation of the sample. All steady-state Fourier-transform infrared (FTIR) spectra were measured on a Bruker Vertex 70 spectrometer (resolution 2 cm$^{-1}$).

B. Time-resolved mid-IR spectrometers

For the transient dispersed pump-probe (t-DPP) experiments we use a commercial Ti:sapphire laser (Coherent Legend Elite, 3.5 mJ, 35 fs FWHM) synchronized with a commercial, pulsed Nd:YAG laser of which we use the second harmonic (Quanta-Ray INDI Spectra-Physics, 532 nm, 20 Hz). The $T$-jump pulse is generated using a β-Barium Borate (BBO)-based optical parametric oscillator pumped by the Nd:YAG laser. A variable, computer-controlled time delay between the two laser outputs is achieved in a similar manner as reported by other groups$^{1,2}$. A signal generated from the Ti:sapphire oscillator (80 MHz) output is amplified and frequency-divided to produce a 1 kHz signal. This signal triggers a pulse generator which provides the triggering for the YLF pump laser and Pockels-cell driver of the regenerative amplifier, an electronically gated amplifier used to record the signals of the MCT-detector array, and a computer-controlled electronic delay generator (Berkeley Nucleonics Corporation Model 575-4C). The latter provides the triggering for the Nd:YAG laser that generates the $T$-jump pulse. The maximum delay between the $T$-jump and mid-IR laser pulses is determined by the repetition rate of the Ti:sapphire laser (1 ms).

The $T$-jump pulse duration, determined from the linear mid-IR response of a D$_2$O sample, is 3.50±0.04 ns. The Nd:YAG laser is pumped and Q-switched at 20 Hz. Using the amplified 800 nm
output of the Legend and an optical setup described elsewhere we obtain mid-IR pulses with a duration of \(\sim 150 \text{ fs}\), a bandwidth of 200 cm\(^{-1}\) and an energy of 20 \(\mu\text{J}\). Probe and reference pulses are obtained from the mid-IR light by reflection off the front and back surfaces of a wedged BaF\(_2\) window. The polarisation of the IR pump pulse is set at 45° with respect to that of the probe pulse using a MgF\(_2\) zero-order \(\lambda\)-half plate. Subsequently, the polarisation of the measured spectrum is selected using a polariser situated directly after the sample set at either 0° (parallel spectrum) or 90° (perpendicular spectrum) with respect to the pump polarisation. To eliminate effects of laser drift, the polarizer is automatically rotated to 0° and 90° every other scan using a computer-controlled rotatory stage. In this way, we alternately measure parallel and perpendicular 2D-IR scans, each of which takes a few minutes. By averaging a large number of the raw 2D-IR scans (typically 10 or more) obtained in this way, we eliminate effects of laser drift. The pump pulses have an intensity envelope that is approximately single-sided exponential with a FWHM of 800 fs, as determined from a cross correlation measured by using two-photon absorption in InAs placed in a sample cell similar to the one used in the t-DPP experiment.

The idler pulse from the OPO (\(\lambda = 1.98 \mu\text{m}\), 12.5 mJ, 5 ns FWHM) is focused (\(f = 75 \text{ mm}\), CaF\(_2\)) to 500 \(\mu\text{m}\) diameter at the sample, leading to a temperature-rise within the pulse width of the laser. The changes induced in the sample are monitored by the two mid-IR probe pulses, which are spatially overlapped with the pump beam, at various time delays between the \(T\)-jump and mid-IR pulses. The mid-IR reference beam passes through an area of the sample not influenced by the pump. The mid-IR pump, probe and reference beams are focused through the sample by means of an \(f = 100 \text{ mm}\) off-axis parabolic mirror. At the sample, the mid-IR beam diameters is 200 \(\mu\text{m}\). Transient absorption changes are measured by frequency-dispersed detection of the mid-IR pulses using a \(2 \times 32\) HgCdTe (MCT) array detector (Infrared Associates).

For the recording of 2D-IR spectra, the mid-IR pump is passed through an IR Fabry-Perot interferometer, resulting in pump pulses with a bandwidth of 25 cm\(^{-1}\). The centre frequency of the light is varied by adjusting the distance between the parallel mirrors of the etalon using a feedback-controlled piezoelectric mount. In this case, the \(T\)-jump pulse is not used.
C. Transient dispersed pump-probe (t-DPP) IR spectroscopy

The t-DPP spectrum, $\Delta \Delta A(\omega, t_{\text{TJ}}, t_{\text{IR}})$, is the difference between the DPP spectrum after a $T$-jump, $\Delta A_{\text{TJ,IR}}(\omega, t_{\text{TJ}}, t_{\text{IR}})$, and the DPP spectrum in absence of a $T$-jump, $\Delta A_{\text{IR}}(\omega, t_{\text{IR}})$:

$$\Delta \Delta A(\omega, t_{\text{TJ}}, t_{\text{IR}}) = \Delta A_{\text{TJ,IR}}(\omega, t_{\text{TJ}}, t_{\text{IR}}) - \Delta A_{\text{IR}}(\omega, t_{\text{IR}})$$

(1)

$$\Delta \Delta A(\omega, t_{\text{TJ}}, t_{\text{IR}}) = A_{\text{TJ,IR}}(\omega, t_{\text{TJ}}, t_{\text{IR}}) - A_{\text{TJ}}(\omega, t_{\text{TJ}}) - (A_{\text{IR}}(\omega, t_{\text{IR}}) - A_0(\omega))$$

(2)

where $\omega$ is the probe frequency, $t_{\text{TJ}}$ the delay between the $T$-jump and mid-IR pulse pair, and $t_{\text{IR}}$ the delay between the IR-pump and IR-probe pulses.

![Electronic Timing of a t-DPP Experiment](image)

**FIG. 1:** Electronic timing of a t-DPP experiment. The coloured bars represent the probe (red), pump (orange), and $T$-jump (blue) laser pulses. The green line in the middle graph shows the electronic “pump present” signal which encompasses two subsequent pump pulses.

We use the timing scheme shown in Supplementary Figure 1 to obtain the signals necessary to construct $\Delta \Delta A$ as shown in equation 2: the sample is probed at a repetition rate of 1 kHz. The mid-IR pump pulse is optically chopped at a frequency of 250 Hz such that two sequential pulses are allowed to pass and the following two sequential pulses are blocked, effectively pumping the sample at 500 Hz. The $T$-jump laser runs at a repetition rate of 20 Hz (which is not a divider of
250 Hz). In this manner, the following absorption spectra required for the generation of the t-DPP spectrum can be recorded: the ground state absorption, $A_0$, is measured at each laser shot where both $T$-jump and IR pumps are off (occurring at 490 Hz). The vibrationally excited state absorption, $A_{IR}$ at a certain pump frequency and delay time, is measured at each shot where only the IR pump pulse acts on the sample (occurring at 490 Hz). The electronic excited state absorption, $A_{TJ}$ at a certain delay time, is measured at each shot where only the $T$-jump pulse acts on the sample (occurring at 10 Hz). The vibrationally excited absorption of the electronic excited state species, $A_{TJ,IR}$ at a certain IR-pump frequency and delay time, is measured at each shot where both IR and $T$-jump pumps act on the sample (this occurs at 10 Hz).

II. ADDITIONAL 2D-IR SPECTRA

The parallel ($\Delta A_{||}$) polarized 2D-IR spectra of the peptide at 278 K and 338 K are shown in supplementary figure 2B and G, respectively. The features of the spectrum are very similar to the ones observed in a perpendicularly polarized 2D-IR spectra (main text figure 2B and F, supplementary figure 2B and G), and are discussed in the main text.

The steady-state difference between the 278 K and 338 K 2D-IR spectra with parallel ($\Delta A_{||}$) and perpendicular ($\Delta A_{\perp}$) polarization are shown in supplementary figures 2L and M, respectively. We use the projections of the parallel and perpendicular polarized steady-state difference spectra in supplementary figures 3F and 4F, respectively, to verify the dispersed pump-probe (DPP, $\Delta A_{DPP}(t_{TJ})$) spectra.

The difference between the polarization-weighted difference spectra at 338 K and 278 K is shown in supplementary figure 2N. This spectrum is dominated by an inverted cross peak, which closely resembles the inverse of the $\alpha$–helix cross peak in supplementary figure 2D. The inverted cross peak is caused by the disappearance of the coupling of the $\alpha$-helix due to unfolding of the protein. The cross peaks belonging to the Arg$^+$ modes have disappeared as a result of the subtraction, testifying that these spectral features do not change as the peptide unfolds. The projection of the spectrum shown in supplementary figure 2N ($\int \Delta A_{\text{cross-peak}}$) and the t-DPP spectrum ($\Delta \Delta A_{3\perp-\parallel}$) at 1000 ns is shown in supplementary figure 2O. We discuss this feature in greater detail below.
FIG. 2: (A, F, K) Solvent corrected FTIR spectrum of the protein at 278 K, 338 K, and difference between 278 K and 338 K, respectively. (B, G, L) 2D-IR spectrum, with parallel polarization of the pump and probe, of the protein at 278 K, 338 K, and difference between 278 K and 338 K, respectively. (C, H, M) 2D-IR spectrum, with perpendicular polarization of the pump and probe, of the protein at 278 K, 338 K, and difference between 278 K and 338 K, respectively. (D, I, N) The polarization-weighted difference spectrum $(3 \times \Delta A_{\perp} - \Delta A_{\parallel})$ of the protein at 278 K, 338 K, and difference between 278 K and 338 K, respectively. (E, J, O) Projection of the polarization-weighted difference spectrum in the panels above onto the probe axis. and the DPP spectrum at 288 K. For the 2D-IR spectra: negative signals are indicated in blue, and positive signals are indicated in red; the interval between contour lines is 0.25 mOD; the delay between pump and probe pulses is 1 ps. The delay between pump and probe pulses for the DPP and t-DPP spectra is 200 fs.
III. ADDITIONAL T-DPP SPECTRA

Here we show the signals measured in the t-DPP experiment. Supplementary Equation 2 shows we need to measure three difference absorption signals to perform this experiment. The signals are measured at different delays between the $T$-jump pulse and the mid-IR pulse pair. Each delay point is measured twice with the mid-IR pump pulse polarized parallel and perpendicularly to the probe pulse. In this section we discuss the spectra of the various difference absorption signals and the time dependence of the amplitude of these signals.

A. Transient spectra

Supplementary figures 3 and 4 show the various difference absorption signals with the mid-IR pump pulse polarized parallel and perpendicularly with respect to the probe pulse, respectively. In this subsection, the panel numbering refers to both supplementary figures 3 and 4, unless stated otherwise. Panels A and B show the solvent-corrected steady-state Fourier-transform (FTIR) spectrum at 288 K and 298 K, and the corresponding difference spectrum, respectively. The 10 K difference between temperature of the FTIR spectra reflects the magnitude of the $T$-jump. The difference FTIR spectrum shown in panel B therefore mirrors the spectral shape and magnitude of the difference absorption signal caused by the $T$-jump pulse ($\Delta A_T$, see supplementary figure 5A for the comparison).

Panel C shows the change in absorption caused by the broadband mid-IR pump pulse in the absence of the $T$-jump pulse ($\Delta A_{\text{DPP}}$, or DPP signal) and therefore does not show any time dependence as a function of $t_{TJ}$. This signal contains the collective response of all the amide I’ modes of the peptide but lacks any significant contribution from the solvent (see main text for explanation).

Panel D shows the change in absorption caused by the $T$-jump pump pulse in the absence of the mid-IR pump pulse ($\Delta A_{TJ}$, or $T$-jump IR signal). The solvent response has not been removed from the $\Delta A_{TJ}$ signal, which accounts for the offset from the baseline compared to the FTIR spectrum in panel B. As $t_{TJ}$ increases, the absorption corresponding to the folded confirmation of the $\alpha$-helical peptide (bleach at 1630 cm$^{-1}$) decreases in intensity and absorption of the unfolded confirmation (induced absorption at 1655 cm$^{-1}$) increases in intensity (see main text for detailed discussion).

Panel E shows the effect of the $T$-jump on the DPP signal at different delays of $t_{TJ}$. The observed signal is described by the term in square brackets in supplementary equation 2. The
contribution of linear change in absorption caused by the $T$-jump ($\Delta A^{TJ}$) has been subtracted (which removes the contribution of $D_2O$ from the signal). We are left with the $\Delta A^{DPP}(t_{TJ})$ response of the $\alpha$–helical peptide as it relaxes to the new thermodynamic equilibrium. The spectra in panel E of supplementary figures 3 and 4 are used to calculate the polarization-weighted DPP spectra ($\Delta A^{DPP}_{3\perp-\parallel}(t_{TJ})$) shown in figure 2 of the main text.

If only the difference in the $\Delta A^{DPP}(t_{TJ})$ signal is desired, the $\Delta A$ (panel F) signal can be calculated by subtracting the un-pumped $\Delta A^{DPP}$ (panel C) from the $\Delta A^{DPP}(t_{TJ})$ signal (panel E, see supplementary equation 2). The $\Delta A^{DPP}_{3\perp-\parallel}$ signal contains the change in both the diagonal and off diagonal response of the $\alpha$–helical peptide as it relaxes to the new thermodynamic equilibrium. Panel F also shows the projection of the corresponding steady-state difference 2D-IR spectrum in supplementary figure 2 (panel L and M for parallel and perpendicular polarization of the mid-IR pump and probe pulses, respectively). The discrepancy between the projection ($\int \Delta A_{\text{cross-peak}}$) and the $\Delta A^{DPP}_{3\perp-\parallel}$ signal at 1000 ns is caused by the different changes in temperature of the $T$-jump and steady-state 2D-IR experiments. The spectra in panel F of supplementary figures 3 and 4 are used to calculate the polarization-weighted t-DPP spectra ($\Delta A^{DPP}_{3\perp-\parallel}(t_{TJ})$) shown in supplementary figure 5C.
FIG. 3: (A) Solvent corrected FTIR spectra of the peptide at 288 K and 298 K. (B) Difference between the solvent corrected FTIR spectra of the peptide at 288 K and 298 K (scaled by a factor of 5). (C) DPP spectra of the peptide in the absence of the $T$-jump pulse, for parallel polarization of the IR-pump and probe pulses. (D) $T$-jump IR spectra of the peptide at different delays after the $T$-jump pulse. (E) DPP spectra of the peptide at different delays after the $T$-jump pulse, for parallel polarization of the IR-pump and probe pulses. (F) t-DPP spectra of the peptide at different delays after the $T$-jump pulse and difference between the projection spectra of the peptide at 278 K and 338 K. In all spectra, the mid-IR pump pulse is polarized parallel to the probe pulse.
FIG. 4: (A) Solvent corrected FTIR spectra of the peptide at 288 K and 298 K. (B) Difference between the solvent corrected FTIR spectra of the peptide at 288 K and 298 K (scaled by a factor of 5). (C) DPP spectra of the peptide in the absence of the T-jump pulse, for perpendicular polarization of the IR-pump and probe pulses. (D) T-jump IR spectra of the peptide at different delays after the T-jump pulse. (E) DPP spectra of the peptide at different delays after the T-jump pulse, for perpendicular polarization of the IR-pump and probe pulses. (F) t-DPP spectra of the peptide at different delays after the T-jump pulse and difference between the projection spectra of the peptide at 278 K and 338 K. In all spectra, the mid-IR pump pulse is polarized perpendicular to the probe pulse.
B. Protein relaxation dynamics

In order to obtain the relaxation dynamics of the peptide from the T-jump IR spectra, we subtract the $\Delta A_{TJ}$ signal of pure D$_2$O (measured in a separate experiment) from that of the $\alpha$-helical peptide. The solvent corrected $\Delta A_{TJ}$ of the $\alpha$–helical peptide is shown in supplementary figure 5A. The steady-state difference FTIR spectrum matches the $\Delta A_{TJ}$ signal at the longest delay point which indicates that the relaxation of the peptide to the new T-jump induced thermodynamic equilibrium is fully realized after 1000 ns.

Supplementary figure 5B shows the delay dependence of the $\Delta A_{TJ}$ signal of the $\alpha$–helical peptide (both with and without solvent correction) at 1630 cm$^{-1}$. We modeled the solvent-corrected signal with a least-squares fit of a single exponential decay. The relaxation rate ($k_r$) was found to be $5.75(2) \times 10^{-3}$ ns$^{-1}$, which is in good agreement with our previous studies.$^4$ We also show the $\Delta A_{TJ}$ response of N-methyl acetamide (NMA, solvent corrected) and D$_2$O to demonstrate the

![Graph showing T-jump IR spectra](image_url)
response of a system that does not change after the initial temperature jump and thereby verify that the kinetics observed in the α–helical protein are indeed caused by the relaxation of the system.

The polarization-weighted t-DPP spectra of the peptide at different delays after the $T$-jump pulse is shown in supplementary figure 5C. This signal is a measure of integrated cross-peak intensity. The magnitude of the $\Delta \Delta A_{3\perp -\parallel}$ signal increases in intensity as time progresses, as opposed to the more intuitive behavior of $\Delta A_{3\perp -\parallel}$ (main text figure 3B) which decreases in intensity. This is because the t-DPP signal represents the change in the $\Delta A_{DPP}^{3\perp -\parallel}$ signal which increases as the system moves away from the original equilibrium. The steady-state difference projection ($\int \Delta A_{\text{cross-peak}}$) has a slightly broader positive feature (on the high-frequency side) than the $\Delta \Delta A_{3\perp -\parallel}$ signal, but otherwise, the spectra match well. This slight discrepancy is caused by the different changes in temperature of the $T$-jump and steady-state 2D-IR experiments.

The delay-dependent intensity of the $\Delta \Delta A$ response of the peptide, NMA, and D$_2$O at various polarizations are shown in supplementary figure 5D. We use the difference between the maximum and minimum intensity of the signals, this was 1620 cm$^{-1}$ and 1640 cm$^{-1}$ for both the peptide and NMA. The most important advantage of using either the $\Delta A_{DPP}^{3\perp -\parallel}$ (main text figure 3B) or the $\Delta \Delta A_{3\perp -\parallel}$ signal is that these signals do not contain any contribution from the solvent and therefore represent purely the response of the system of interest. This advantage is clearly demonstrated in supplementary figure 5D where we observe that the $\Delta \Delta A$ of pure D$_2$O is zero. Also shown is the $\Delta \Delta A_{3\perp -\parallel}$ signal of NMA, which (like its 1D-IR response) shows no relaxation dynamics. To obtain the relaxation dynamics of the α–helical peptide, we performed a least-squares fit of a single-exponential decay to the data shown in supplementary figure 5D. The relaxation rates ($k_r$) for the protein $\Delta \Delta A_{\parallel}, \Delta \Delta A_{\perp}, \Delta \Delta A_{3\perp -\parallel}$ were found to be $5.66(2) \times 10^{-3}$ ns$^{-1}$, $5.66(2) \times 10^{-3}$ ns$^{-1}$, and $5.62(4) \times 10^{-3}$ ns$^{-1}$ respectively.