Water Interacting with interfaces, ions and itself

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7 Vibrational relaxation pathways of AI and AII modes in N-methylacetamide

We studied the vibrational energy relaxation mechanisms of the amide I and amide II modes of N-methylacetamide (NMA) monomers dissolved in bromoform using polarization-resolved femtosecond two-color vibrational spectroscopy. The results show that the excited amide I vibration transfers its excitation energy to the amide II vibration with a time constant of $8.3 \pm 1$ ps. The transfer from the excited amide II to the amide I mode has a time constant of $16.6 \pm 2$ ps. In addition to this energy exchange process, we observe that the excited amide I and amide II vibrations both relax to a final thermal state. For the amide I mode this latter process has a time constant of $\sim 1.5$ ps and dominates the vibrational relaxation of this mode. We further find that the vibrational relaxation of the amide I mode depends on frequency which can be well explained from the presence of two subbands with different vibrational lifetimes in the amide I absorption spectrum.

7.1 Introduction

The amide motif ($\text{O} = \text{C}\cdot\text{N} = \text{H}$) is extremely important in living systems as it forms the building block of the backbone of proteins. The structural dynamics of this backbone play a crucial role in the folding and functional conformational dynamics of proteins. These dynamics are very much dependent on the hydrogen-bond interactions among the amide moieties and their interactions with surrounding and solvating molecules like water.

An excellent system for studying the properties of the amide motif is N-methylacetamide (NMA). This molecule forms hydrogen bonds with other NMA molecules which mimic well the intra- and interchain hydrogen-bonding interactions of the backbones of proteins [170]. NMA has thus been widely used to investigate the properties of the amide motif in various molecular environments.

The vibrational relaxation dynamics of the amide modes of NMA have been studied before with infrared vibrational spectroscopy [35, 36, 60, 173] and with
molecular dynamics simulations [18, 83, 157, 176]. All previous studies of the vibrational dynamics of NMA showed that the amide vibrations relax via intramolecular energy transfer, irrespective of the solvent used. The highest frequency amide vibration, amide I, with a frequency of $\sim 1650 \text{ cm}^{-1}$, is always found to relax in two subsequent steps. The first step involves intramolecular energy transfer from the amide I mode to lower frequency amide modes, and the second step involves the further redistribution of the vibrational energy among low frequency intra- and intermolecular modes, leading to a complete thermalization of the vibrational energy.

DeFlores et al. studied the vibrational relaxation mechanism of NMA in D$_2$O and DMSO with two-dimensional vibrational spectroscopy. In both solvents they observe a rapid energy exchange and equilibration between the amide I and the amide II modes. This rapid energy redistribution is followed by a much slower relaxation process that leads to thermalization of the vibrational energy. Interestingly, we did not find any evidence of energy transfer between the amide I and amide II vibrations for hydrogen-bonded NMA clusters dissolved in carbon tetrachloride (see previous chapter). We observed that both the amide I and the amide II mode directly relax on a time scale $< 1$ picosecond to lower frequency intramolecular modes.

The hydrogen-bonded NMA clusters of the previous chapter formed a good model for the helical parts of proteins for which the amide motifs of the amino-acids are connected via hydrogen bonds. So far no experimental work has been reported on the relaxation pathways of isolated non-bonded NMA molecules. Here we study the relaxation mechanisms of the amide I and amide II vibrations of isolated NMA molecules using polarization-resolved femtosecond two-color pump-probe spectroscopy.

### 7.2 Experiment

The polarization-resolved two-color pump-probe experiment employs femtosecond mid-infrared pulses that are generated with the laser system and frequency conversion processes described in sections 3.1 and 3.2. The generated pulses are centered at $\sim 1600 \text{ cm}^{-1}$ ($\sim 6 \mu \text{m}$), have a pulse energy of $\sim 60 \mu \text{J}$, a pulse duration of $\sim 55 \text{ fs}$ and a spectral bandwidth of $\sim 350 \text{ cm}^{-1}$.

As in the previous chapter, we excite the amide vibrations with narrow-band pump pulses that are obtained by spectrally filtering the broadband mid-infrared pulses with a Fabry-Pérot filter. The thus obtained pump pulses have a bandwidth of $\sim 50 - 60 \text{ cm}^{-1}$ and a pulse energy of $\sim 6 \mu \text{J}$.

The pump pulse excites either the amide I (AI) or amide II (AII) vibration of NMA, thereby promoting either vibration from its ground state $\nu = 0$ to the first excited state $\nu = 1$. This excitation results in ground-state depletion and stimulated emission $\nu = 1 \rightarrow 0$, thus leading to a decrease in absorption (bleaching) at frequencies corresponding to the $\nu=0\rightarrow1$ transition. Simultaneously, the population of the $\nu = 1$ state leads to an induced absorption at frequencies corresponding to the $\nu=1\rightarrow2$ transition. A detailed description of the pump-probe setup can be found in section 3.3.
7.3 Vibrational relaxation pathways in NMA

The sample is a \( \sim 10 \) mM solution (1:1000 molar ratio) of NMA in bromoform (CHBr\(_3\)). The advantage of using bromoform over carbon tetrachloride is that there are no vibrational resonances overlapping with the amide modes. For highly diluted solutions as studied in this chapter, the weak vibrational mode of CCl\(_4\) at \( \sim 1530 \) cm\(^{-1}\) overwhelms the AII absorption band. Bromoform possesses a weak absorption at \( \sim 1600 \) cm\(^{-1}\) in between the absorption bands of the AI and AII modes. Hence, these amide modes can be excited without exciting the solvent molecules. The sample is contained in an infrared sample cell consisting of two 2 mm thick CaF\(_2\) windows and a 1.2 mm thick teflon spacer.

7.3 Results and discussion

7.3.1 Linear spectra

In figure 8.4 we show the linear absorption spectrum of a solution of 10 mM NMA in bromoform (CHBr\(_3\)). The two amide modes are centered at \( \sim 1665 \) cm\(^{-1}\) (AI) and \( \sim 1535 \) cm\(^{-1}\) (AII). The small absorption band centered at 1600 cm\(^{-1}\) originates from the solvent. At this low concentration only NMA monomers will be present in solution. The AI absorption band is somewhat asymmetric with a shoulder on the lower frequency side. The AII band is weaker (\( \sim 1/5 \)) and somewhat broader than the AI band. The central frequency of the AI mode (\( \sim 1665-1670 \) cm\(^{-1}\)) is redshifted with respect to the central frequency of the AI mode of NMA dissolved in carbon tetrachloride (\( \sim 1690 \) cm\(^{-1}\)) at the same concentration. This difference likely results from the dipole-dipole interaction between the NMA molecule and the small (\( \sim 3^{-30} \))
Figure 7.2. Transient spectra in the frequency range of the AI and AII modes at different delay times following excitation of the AI mode of a 10 mM solution of NMA in CHBr₃. The solid lines represent the results of a fit to the relaxation model described in the text. For clarity, the response of the AII mode has been multiplied by a factor of 13. The arrows indicate the evolution of the spectra in time.

[C·m]) dipole moments of the solvating bromoform molecules. The frequency splitting of the two amide modes is similar to that of hydrogen-bonded NMA clusters in CCl₄.

7.3.2 Vibrational energy relaxation

Exciting the amide I mode

Figure 7.2 shows transient absorption spectra in the frequency ranges of the AI and AII modes at different delay times after excitation of the AI mode. The spectral response in the AII region has been multiplied by a factor of 13 for clarity. Both the transient spectra of the AI and AII mode are characterized by a negative (bleach) contribution at high frequencies (>1515 and >1660 cm⁻¹ respectively) representing the reduced absorption and stimulated emission of the ν = 0→1 transition, and a positive signal at lower frequencies (<1515 and <1660 cm⁻¹), representing the induced ν = 1→2 absorption. For the AI mode both the bleach and induced absorption decay in time. After ∼30 ps the transient spectrum is nearly zero and does not change anymore with delay time.

The spectral changes in the AII frequency region show very different dynamics from the AI frequency region. At early delay times, up to a delay time of ∼4 ps, the amplitude of the AII transient spectrum increases. For delays larger than ∼4 ps the signal slowly decays. After about 40 ps the transient spectrum does not change anymore. The induced absorption shows a much less pronounced ingrowth than the bleaching.

In figure 7.3 we show delay traces (circles and squares correspond to AI and AII modes, respectively) that are recorded at the frequencies corresponding to the maxima of the bleach and the induced absorption signals of both amide modes. These frequencies are marked with black vertical lines in figure 7.2. It is clear that the dynamics of both modes is very different and involves at least...
two distinct regimes. The response of the AI mode shows a fast decay at early delays (<4 ps) and a much slower decay at later delays. The response of the AII mode is characterized by two similar rates, however, in contrary to the AI dynamics, the first fast process leads to an ingrowth of the intensity of the AII signal. This process is followed by a decay with the slower rate.

Upon close inspection of the spectral dynamics of the AI mode we observe a frequency dependence of the decay (see inset in figure 7.3). For delays <5 ps the signal in the blue wing of the AI transient spectrum (dashed line) decays slower than the signal in the red wing of the spectrum (solid line). The high frequency side of the transient spectrum decays with a time constant of ~2 ps, whereas the low frequency side decays faster with a time constant of ~1 ps. This variation in the dynamics across the AI transient spectrum leads to a transient blue shift of the AI spectral response (the red wing decays faster than the blue wing). At later delays this difference vanishes - the transient signal decays uniformly across the spectrum on a much slower time scale. We did not observe any spectral dependence of the dynamics in the AII frequency region.

**Exciting the amide II mode**

In figure 7.4 we show transient spectra recorded in the frequency range of the AI and AII modes for a few delay times after excitation of the AII mode. Both
the AI and AII responses consist of a negative absorption change on the high frequency side of the fundamental absorption band and a positive signal on the low frequency side, similar as was observed when the AI mode was excited. The AII and AI responses have their maximum intensity around 0 ps and both show a similar slow decay. After about 30 ps the transient spectrum does not change anymore, which implies that the system has reached thermal equilibrium.

Figure 7.5 shows time traces (circles and squares correspond to AI and AII mode respectively) at the four frequencies that are marked with solid black lines in figure 7.4. Clearly, the signals at the four different frequencies are very much alike, all showing a decay time constant of about 10 ps.

**INTERPRETATION AND MODELING**

The AI mode shows a fast decay that is faster in the red wing than in the blue wing of the absorption band. This observation indicates that the AI vibrational band contains different subbands with different vibrational relaxation rates. In previous experimental and theoretical work strong indications were found that the absorption band of the AI mode indeed contains two subbands [35, 137]. Therefore we will model the vibrational relaxation dynamics with two AI $\nu = 1$ states with different vibrational relaxation time constants.

The excitation of the AI mode leads to a direct response in the frequency region of the AII mode. This observation indicates that the AI and AII mode are anharmonically coupled. This response consists of a bleaching in the blue wing of the AII absorption spectrum and an induced absorption in the red wing, which means that the excitation of the AI mode induces an anharmonic redshift of the AII mode. The transient spectrum of the AII mode resulting from this redshift shows a smaller frequency difference between the maxima of the bleach and the induced absorption than in case the AII mode is excited. Following this direct anharmonic response of the AII mode, we observe a further ingrowth of...
Figure 7.5. Time traces recorded at four different frequencies corresponding to the maxima of the bleach and the induced absorption signals of the AI and AII modes following excitation of the AII mode. The solid lines result from a fit to the relaxation model described in the text.

the bleach and the induced absorption on a time scale that is similar to the time scale of the decay of the two AI subbands. The complimentary character of these dynamics indicates that the $\nu = 1$ states of the two subbands of the AI mode transfer their energy at least in part to the $\nu = 1$ state of the AII mode. This state constitutes a third level in the relaxation model.

If there is a coupling leading to energy transfer from the $\nu = 1$ states of the two AI subbands to the $\nu = 1$ state of AII, the same coupling will also lead to transfer of energy in the other direction, i.e. from the $\nu = 1$ state of AII to the $\nu = 1$ states of the two AI subbands. Because of detailed balance, the two rates differ by a Boltzmann factor which for the energy exchange between the $\nu = 1$ states of AI and AII ($\Delta E \approx 140 \text{ cm}^{-1}$) equals $\approx 0.5$. The rate at which the energy is equilibrated between the two modes is the sum of the two rates, irrespective of which mode is excited in the first place. This means that the rapid decay observed for the two AI subbands cannot be due to energy transfer to AII because we do not observe a similar partial fast decay when the AII mode is excited. Figure 7.5 clearly shows that excitation of the $\nu = 1$ state of AII only leads to a slow relaxation on a time scale of $\approx 10$ ps. Therefore, we conclude that only a minor part of the excitation of the two AI subbands is transferred to AII. The dominant relaxation channel of the two AI subbands involves transfer of energy to combination tones of other lower-frequency (amide) vibrations. This final state is the fourth and final level of the relaxation and has a spectral response that is not very different from the response that is obtained after the energy of the excitation is fully equilibrated (thermalized).

The excitation of the AII mode is observed to lead to a direct transient spectral response in the frequency regime of the AI mode, which shows that
the AI and AII modes are anharmonically coupled, similar as was observed in the experiment in which the AI mode was excited. Both the responses in the frequency region of the AII and the AI mode decay on a timescale of about 10 ps. This decay is partly due to energy transfer to the $\nu = 1$ states of the two AI subbands and partly due to energy relaxation to combination tones of lower-frequency (amide) vibrations.

The complete relaxation model thus consists of four levels: two levels represent the $\nu = 1$ states of two AI subbands and account for the frequency dependence of the fast relaxation following excitation of the AI mode, the third level represents the $\nu = 1$ state of the AII mode and the fourth level represents the final thermal state. All levels have associated differential spectral responses (difference between the absorption spectrum starting from that level and the linear absorption spectrum) in the frequency regions of the AI and the AII modes. In modeling the data we assume that the spectral responses of the two AI excited states are the same in the frequency region of the AII band, and we also assume that the rate of energy transfer between the two AI subbands and the AII mode is the same for both AI subbands. The relaxation model is illustrated in figure 7.6. We fit all measurement results simultaneously to this relaxation model in a global fit. The results of this fit are represented in figures 7.2, 7.3, 7.4 and 7.5 by the solid lines.

From the fit we obtain the differential spectra associated with each of the levels, as shown in figure 7.7 and the relaxation time constants that connect the states of the relaxation model. The differential spectra associated with two AI excited states are represented by the blue and green spectra in figure 7.7. The spectra show a strong bleaching and induced absorption in the frequency region of the AI mode that are similar in shape for the two levels but shifted by $\sim 10 \text{ cm}^{-1}$ with respect to each other. The response of the two excited AI levels in the frequency region of the AII mode is clearly upshifted with respect to $\Delta \alpha = 0$ and differs from the usual antisymmetric differential spectral shape.
that results from an anharmonic frequency shift. The upshift probably results from the presence of a long low-frequency tail of the \( \nu = 1 \rightarrow 2 \) induced absorption of the excited AII subbands. The total transient spectral response in the frequency region of the AII mode is thus the sum of this low-frequency tail and an antisymmetric signal due to the anharmonic shift of the AII mode induced by the excitation of the AII subbands. Both contributions decay when the AII subbands relax. The level associated with the \( \nu = 1 \) state of the lower-frequency AII subband decays with a time constant \( T_{1,AIIa} \) of \( 1.1 \pm 0.1 \) ps to the final (thermal) level, and the level associated with the higher-frequency AII subband decays with a time constant \( T_{1,AIIb} \) of \( 2.7 \pm 0.4 \) ps. In addition to these relaxation channels, both AII subbands transfer energy to the AII level with a time constant of \( 8.3 \pm 1 \) ps.

The differential spectrum associated with the \( \nu = 1 \) state of the AII state is represented by the red line. This differential spectrum consists of a strong \( \nu = 0 \rightarrow 1 \) bleaching and \( \nu = 1 \rightarrow 2 \) induced absorption in the AII frequency region, and a much weaker bleaching and induced absorption in the frequency region of the AII mode. The latter differential spectral response represents the anharmonic redshift of the two AII subbands due to the excitation of the AII mode. The \( \nu = 0 \rightarrow 1 \) bleaching and \( \nu = 1 \rightarrow 2 \) induced absorption of the AII mode have a much larger amplitude than the differential spectrum that results from the anharmonic shift induced by the excitation of the two AI subbands. Therefore, the energy transfer from the two AII subbands to AII leads to an ingrowth of the signal in the AII frequency region. The \( \nu = 1 \) state of the AII mode decays both as a result of energy transfer to the two AII subbands with a time constant of \( 16.6 \pm 2 \) ps, and because of relaxation to the final (thermal) state with a time constant of \( 25 \pm 5 \) ps. The two relaxation channels together explain the observed relaxation time scale of \( \sim 10 \) ps.

The differential spectrum of the final thermalized state is represented by the black line. This differential spectrum has very little amplitude in both the frequency regions of AI and AII. Therefore the relaxation to the final thermal
Vibrational relaxation pathways in NMA

Figure 7.8. Anisotropy time traces of the AI mode (open circles) and the AII mode (solid circles) after excitation of the AI mode. The solid lines represent an exponential function fitted to the AI mode anisotropy and a constant value for the anisotropy of the AII mode.

state leads to a decay of the responses in both the AI and the AII frequency regions.

Anisotropy Dynamics

From the two recorded signals $\Delta \alpha_{\parallel}$ and $\Delta \alpha_{\perp}$ we constructed the anisotropy parameter $R(t)$. In figure 7.8 we show the anisotropy decay for the AI mode (open circles) and for the AII mode (black circles) following excitation of the AI mode. The anisotropy of the AI mode decays with a time constant $\tau_{\alpha}$ of $9\pm1$ ps, which reflects the time scale of the reorientation of this vibration. The initial value of the anisotropy of the AII mode reflects the relative orientation of the transition dipole moment of the AII mode with respect to that of the excited AI mode. Using equation 6.4 we find an angle of $\sim55^\circ$, the same value as we found in the previous chapter for the relative orientation of the AI and AII transition dipole moments of hydrogen-bonded NMA clusters.

7.3.3 Discussion

The relaxation dynamics observed for NMA monomers strongly differs from the relaxation behavior observed for the hydrogen-bonded NMA clusters we studied in the previous chapter. In the first place the relaxation of the AI mode and in particular of the AII mode is much faster for the clusters than for the monomers (0.85 and 0.6 ps versus $\sim1.5$ ps and $\sim10$ ps). It thus appears that the presence of hydrogen bonds between the NMA monomers leads to a strong acceleration of the vibrational relaxation. This acceleration can be due to the fact that the hydrogen bonds take up part of the vibrational energy, thereby compensating for the energy mismatch between the excited AI and AII vibrations and the energy accepting combination tones of lower frequency vibrations.
Another interesting difference between the relaxation behavior of the monomers and the clusters is that for the monomers we observe a partial relaxation of the excited $\nu = 1$ state of the two AI subbands to the $\nu = 1$ state of the AII mode. For the clusters this transfer was not observed, probably because the direct relaxation to combination tones of lower-frequency vibrations was much faster. The energy transfer from AI to AII leads to a rise of the response of the AII spectral response on a time scale that corresponds to the time scale of the decay of the AI vibrations. This time scale is in fact much shorter than the characteristic time scale of the energy transfer of $\sim 8$ ps, because the vibrational relaxation of the two AI subbands is dominated by another much faster relaxation channel. The reason that the time scale of the latter process is observed in the ingrowth of the AII response is because the AI modes can no longer further populate the AII mode after they have relaxed through the fast relaxation channel. The observation of energy transfer between the excited AI and AII vibrations does not agree with the results of Zhang et al. [176] who found that for isolated NMA molecules the AII mode does not participate in vibrational relaxation of the AI mode. It should be noted that we find the fraction of NMA molecules for which the two AI subbands relax via the AII mode to be quite small. From the rate constants we derive that this fraction is $\frac{(0.12/(0.12+0.90)+0.12/(0.12+0.37))}{2} = 0.18$. Hence, less than 20% of the NMA molecules relax via the AII mode. In a molecular dynamics study by Bloem et al. evidence was found for a direct transfer between AI and AII, but on a time scale that is $\sim 10$ times shorter than we observed.

Another interesting observation is that we find the vibrational lifetime of the AI mode to be frequency dependent. NMA molecules absorbing on the high frequency side ($\sim 1680 \text{ cm}^{-1}$) relax slower than molecules absorbing in the red wing of the spectrum ($\sim 1660 \text{ cm}^{-1}$). One possible explanation for this observation is that the NMA spectrum is inhomogeneously broadened due to a variation in conformation. Previous molecular dynamics simulations showed that the trans-NMA molecule can exist in four conformations of planar symmetry with different orientations of the C- and N- methyl groups [29]. If this variation in conformation would be the origin of the frequency dependence of the vibrational lifetime one would expect the lifetime to vary continuously over the AI absorption band. If the variation in conformation would be the origin of the frequency dependent vibrational lifetime one would expect a similar effect for the AII mode of NMA.

However, for the AII mode we do not observe such a frequency dependence of the vibrational lifetime. We also find that a relaxation model involving only two different rates is fully sufficient to describe the experimental data at all delay times. This finding suggests that the frequency dependence may be due to the presence of two different NMA species, thus leading to two subbands in the vibrational spectrum. This finding agrees with the results of a previous experimental study [137] and with the results of molecular dynamics simulations [35]. Rubtsov et al. investigated the coupling between different amide modes of small peptides dissolved in chloroform with two-dimensional infrared spectroscopy. They found strong indications for the presence of two subbands in the spectrum of the AI mode, both in the linear absorption spectrum and in the transient nonlinear vibrational response. They attributed the two subbands to
the splitting of the AI mode by a Fermi resonance of this mode with an overtone of a lower-frequency vibration. DeCamp et al. performed molecular dynamics simulations on solutions of NMA in CDCl$_3$, and they attributed the presence of two subbands to a difference in the number of hydrogen bonds formed between NMA and CDCl$_3$ solvent molecules. They found that singly hydrogen-bonded NMA molecules absorb at the high-frequency side of the absorption spectrum, and that doubly hydrogen-bonded NMA molecules absorb at the red side of the absorption band. Figure 8.4 shows that the linear absorption spectrum of the AI mode of NMA in CHBr$_3$ possesses a shoulder on the low-frequency side. Interestingly, this shoulder is not present in the linear absorption spectrum of the AI mode for a solution of 10 mM NMA in CCl$_4$ solution. This result suggests that the shoulder arises from NMA molecules interacting with CHBr$_3$ solvent molecules, in line with the explanation of DeCamp et al. for the presence of two subbands in the absorption spectrum of the AI mode of NMA dissolved in CDCl$_3$.

We observed that the transition dipole moments of the AI and AII modes of NMA monomers have the same relative angle of $\sim 55^\circ$ as we observed in the previous chapter hydrogen-bonded clusters. This angle is consistent with the findings of previous experimental studies [36]. Apparently, the hydrogen-bond interactions between the NMA molecules have very little effect on the normal mode character and thus the direction of the transition dipole moment of the AI and AII vibrations. The reorientation time $\tau_{\text{or.}} = 9 \pm 1$ ps and is comparable with the time constant found by DeCamp et al. for NMA dissolved in D$_2$O and CDCl$_3$ and with the time constant by Hamm et al. for deuterated NMA dissolved in D$_2$O. The reorientation time constant of $\sim 9$ ps is somewhat longer than the reorientation time constant of 6 ps that we observed for hydrogen-bonded NMA clusters in CCl$_4$. This result may seem surprising as one would expect that single NMA molecules reorient faster than NMA oligomers. One reason for this unexpected result may be the difference in viscosity of the solvent. The viscosity of bromoform is approximately twice as high ($\sim 1.85$ mPa-s) than that of carbon tetrachloride ($\sim 0.97$ mPa-s). According to hydrodynamic theory [12] the reorientation time constant is proportional to the viscosity of the solvent: $\tau_{\text{or.}} = V \eta / k_B T$, with $V$ the volume of the solute, $\eta$ the viscosity, $k_B$ Boltzmann’s constant, and $T$ the temperature. Hence, the difference in reorientation time constant between NMA monomers in CHBr$_3$ and NMA oligomers in CCl$_4$ can be explained from the difference in viscosity of the solvent. Even so, it remains surprising that the effective larger volume of an NMA cluster in comparison to an NMA monomer does not lead to a strong increase of the reorientation time constant of the AI mode. This observation suggests that the reorientation involves the orientational motion of NMA within the hydrogen-bonded cluster.

7.4 Conclusions

We studied the vibrational energy relaxation mechanisms of the amide I and amide II vibrations of monomeric N-methylacetamide (NMA) molecules dissolved in bromoform using polarization-resolved femtosecond two-color pump-
probe spectroscopy. We excited both the amide I and the amide II vibration to the $\nu = 1$ excited state with a short, narrow-band mid-infrared pump pulse and we monitored the vibrational relaxation in the frequency regions of both modes with a short, broadband mid-infrared probe pulse.

We observe that the vibrational relaxation of the amide I mode depends on frequency. This frequency dependence can be well explained from the presence of two subbands with different vibrational lifetimes in the absorption spectrum of the amide I mode. The presence of two subbands agrees with the observation of a low-frequency shoulder in the linear absorption spectrum of the amide I mode. The two subbands are likely caused by a difference in the interaction strength of NMA with the bromoform solvent molecules.

We find that the excited amide I mode relaxes via two vibrational relaxation channels. In one channel the vibration relaxes to a final state with an absorption spectrum that corresponds to a full equilibration of the vibrational excitation energy. The time constants for this relaxation are found to be $1.1 \pm 0.1$ ps for the subband at the red side of the amide I absorption band and $2.7 \pm 0.4$ ps for the subband at the blue side of the amide I absorption band. In the other channel the two subbands transfer their energy to the $\nu = 1$ state of the amide II mode. This process has a much longer time constant of $8.3 \pm 1$ ps, which implies that only for $\sim 20\%$ of the NMA molecules the amide I mode relaxes via energy transfer to the amide II mode. The excited amide II mode relaxes via two similar relaxation channels, i.e. to a final thermalized state with a time constant of $25 \pm 5$ ps, and via energy transfer to the amide I mode with a time constant of $16.6 \pm 2$ ps.