VIBRATIONAL AND EXCITED-STATE DYNAMICS OF DNA BASES REVEALED 
BY UV AND INFRARED FEMTOSECOND TIME-RESOLVED SPECTROSCOPY 

DISSERTATION 

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By 

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* * * * * 

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ABSTRACT

Exposure to UV light, particularly from the sun, is the primary controllable risk factor for the development of skin cancer. The damaging effects of UV photons results from their ability to induced photochemistry in DNA bases. While the many possible photoproducts of DNA are well known, the formation mechanisms for these photoproducts are not. In order to better understand these processes, we seek to better understand the events that occur between photon absorption and photoproduct formation - the photophysics of DNA.

Femtosecond UV pump/UV probe transient absorption spectroscopy was used to study the ground-state vibrational cooling of the DNA base derivative 9-methyladenine (9MA) in solution. Photoexcitation of 9MA to the lowest bright electronic excited state at 267 nm is followed by rapid ($\tau \approx 0.4$ ps) internal conversion to the electronic ground state, generating more than 30,000 cm$^{-1}$ of excess vibrational energy. Transfer of this excess vibrational energy to the solvent was monitored via changes in the ground-state electronic absorption band at 250 and 285 nm. The vibrational cooling time increases in H$_2$O (2.4 ps), D$_2$O (4.2 ps), methanol (4.5 ps) and acetonitrile (13.1 ps) solvents. The studies show that the initial vibrational energy transfer from the hot solute molecule to the first solvent shell determined the thermalization rate. The studies also suggest that
energy transfer between high-frequency solute and solvent modes play a more important role in vibrational cooling than expected.

While the majority of excitation lead to ultrafast internal conversion, in pyrimidine bases additional decay pathways exist involving long-lived, intermediate, $^1\text{n}\pi^*$ and $^3\pi\pi^*$ states. The $^1\text{n}\pi^*$, $^1\pi\pi^*$, $^3\pi\pi^*$ and $S_0$ states of single pyrimidine bases have strongly-overlapping electronic absorption spectra which complicates study of their dynamics with conventional UV and visible techniques. A UV-pump/mid-IR-probe femtosecond transient absorption spectrometer was constructed for the purpose of studying, with high resolution and specificity, the excited-state dynamics of DNA bases.

Unique marker bands for the $^1\text{n}\pi^*$ and $^3\pi\pi^*$ were determined for 1-cyclohexyluracil and thymine, respectively. A marker band for the $^1\text{n}\pi^*$ was observed at 1760 cm$^{-1}$ in acetonitrile and methanol-$d_1$. Marker bands at 1603 and $\sim$1714 cm$^{-1}$ were assigned to the $^3\pi\pi^*$ state of thymine. This assignment is consistent with previous nanosecond TRIR measurements and theoretical calculations. The triplet state is fully formed on a timescale significantly faster than the $^1\text{n}\pi^*$ lifetime, suggesting that intersystem crossing from the $^1\text{n}\pi^*$ state to the $^3\pi\pi^*$ state occurs before the excited population reaches the minimum on the $^1\text{n}\pi^*$ potential surface, supporting previous conclusions from UV-visible transient absorption measurements.

The excited-state dynamics of poly(A) were studied with UV and mid-IR transient absorption. Comparison with mid-IR measurements of AMP allow the quantum yield for ultrafast internal conversion in poly(A) to be estimated. Comparison of transient
spectra with ground-state absorption spectrum of poly(A) provides insight into the location of absorption bands of the long-lived excimer-like state.
DEDICATION

Dedicated to Amber and Alex,
for their support, understanding and love;
the foundation for all that I do
I would like to first thank my advisor, Bern Kohler, for his heartening faith and optimism, his sharp insight and his shining example of scientific integrity and rigor.

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Effects on the Vibrational Cooling Dynamics of a DNA Base Derivative,” Journal of


FIELDS OF STUDY

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CHAPTER 1

INTRODUCTION

Together with genetics, the major risk factor for development of melanoma-type skin cancer is exposure to the sun.\textsuperscript{1} While much is still not understood it is commonly accepted that UV-induced photochemistry of DNA can lead to carcinogenic mutations.\textsuperscript{2} The UV chromophores of DNA and RNA are the nucleobases: adenine, cytosine, guanine, thymine and uracil (see Figure 1.1). DNA bases are known to form a wide variety of photoproducts including cis-syn cyclobutane pyrimidine dimers (CPD), pyrimidine (6-4) pyrimidone photoadducts, valence Dewar isomers, and photohydrates.\textsuperscript{3} However, the microscopic mechanisms for the formation of these photoproducts are poorly understood.

An important step toward better understanding DNA photodamage is an understanding of DNA photophysics. The photophysics of DNA covers the initial events in the life of photoexcited DNA and determines whether or not an excited base reaches a reactive state/conformation or not. The last 10 years have seen a resurgence of interest in DNA photophysics resulting from advances in both experiment and theory. This resurgence has been fueled by the development of sub-ps timed-resolution experimental techniques and was largely triggered by the first direct measurement, using sub-ps time-resolved transient absorption, of the fluorescent lifetimes of single DNA bases in aqueous solution in 2000.\textsuperscript{4} Many theoretical studies have also been conducted showing the impor-
tance of conical intersections for the ultrafast nonradiative decay of bases. In particular, the development by Martinez et. al of combined ab initio and molecular dynamics calculations of excited-state evolution and surface hopping dynamics have allowed for direct simulation of excited-state dynamics as opposed to the more common but less direct approach of interpreting dynamics based upon the energetic accessibility of CIs. Combined, experiment and theory have lead to such a high level of understanding of the decay of the fluorescent excited states of DNA bases that highly-fluorescent DNA base analogs can now be rationally designed.

Rapid internal conversion to the ground state reduces the possibility of undergoing destructive chemistry. Many have suggested that this ability was an important selection criterion during the evolutionary development of life’s genetic code. However, the full story of the photostability of cellular DNA is more complex and still remains to be told. Recent studies have discovered that other, relatively long-lived states are also become populated in photoexcited DNA. Almost half of all excitation of single pyrimidine bases in solution lead to population of a $1\text{n}\pi^*$ state with a lifetime of 30 ps to 4 ns, depending on the solvent. Additionally, pyrimidine bases can undergo intersystem crossing to the triplet manifold, which persists on the μs timescale. Compared with the plethora of experimental and theoretical studies conducted on the decay pathways from the $1\pi\pi^*$ state to the ground state, much less is known about the decay pathways involving these intermediate dark states.

The photophysics of bases in single- or double-stranded DNA show even more complexity. Studies have shown that in these systems photoexcitation leads to both the
decay pathways present in single bases as well as new pathways involving intermediate states with lifetimes ranging from tens of picoseconds to many nanoseconds.\textsuperscript{42-47} The nature of these states and their decay pathways are currently an area of active investigation.

One of the difficulties associated with the study of the new states is that they are dark states, i.e. they have a low cross-section for emission. Since they are dark states they are difficult to detect via steady-state fluorescence or phosphorescence. More importantly, they are difficult (though not impossible) to observe in time-resolved fluorescence measurements such as time-correlated single photon counting or fluorescence upconversion. In contrast, excited states that are dark in emission may still have sizable absorptive cross-sections and can be detected via transient absorption. Additionally, transient absorption can be used to monitor the photodepletion and subsequent recovery of the ground-state population and thereby provide an overall picture of the excited-state decay pathways. Transient absorption experiments encompass many possible wavelength probe pulses which can together provide complementary information and a complete picture of the photophysics of the system under study. Pump-probe schemes utilized in this work are depicted in Figure 1.2. For all of these reasons, transient absorption techniques are highly advantageous to the study of DNA photophysics.

A particularly powerful transient absorption spectroscopy involves the use of probe pulses in the mid-IR region to monitor photoinduced changes in the vibrational absorption spectrum of the sample. Vibrational spectra are generally more sensitive to the molecular structure of the probed species than electronic spectra. Vibrational frequencies
involving moieties strongly coupled to the solvent, such as carbonyl or amino groups, can also be sensitive to changes in the environment. Vibrational absorption features are also narrower than electronic absorption features, increasing the likelihood of resolving unique features for different species or states. To date, a very small number of mid-IR transient absorption measurements have been completed on DNA bases. A major goal of this work is to describe some initial results using mid-IR transient absorption to reveal new insights into the photophysics of DNA. These measurements set the foundation for future studies that will more fully utilize the power of this method.

In Chapter 2, the experimental instrumentation and methods are described. A detailed description of the UV pump/mid-IR probe spectrometer and its use is given. In Chapter 3, the ground-state vibrational cooling of DNA bases as derivatives are measured using UV pump/UV probe transient absorption is described. The solvent dependence is discussed in terms of current models for vibrational cooling. The importance of solute-solvent hydrogen bonding is also discussed. Particular focus is given the dependence of vibrational cooling on the isotopic composition of the solvent. The implications of this solvent isotope effect for the mechanism of vibrational cooling as well as intramolecular vibrational redistribution are discussed. In Chapter 4, we describe the use of UV pump, mid-IR probe transient absorption to study the excited-state dynamics of pyrimidine bases. Unique IR marker bands for the triplet state of thymine as well as the $1\pi^*$ state of 1-cyclohexyluracil are identified. The formation dynamics bolster the previously proposed model for intersystem crossing in pyrimidine bases. The $1\pi^*$ band is identified as a combination or overtone band and its possible assignment is discussed. In Chapter 5,
the mid-IR transient absorption of poly(A) is presented and compared with that of AMP. The dynamics probed in the mid-IR are compared with those probed in the UV. Implication for the identification of a marker band for the long-lived excimer state is presented.

In Chapter 6, some final conclusions are presented along with a discussion of future directions based on this work.

1.1. References


2. From DNA Photolesions to Mutations, Skin Cancer and Cell Death; Evelyne Sage; Régen Drouin; Rouabhia, M., Eds.; Royal Society of Chemistry, 2005.


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CHAPTER 2

METHODS

2.1. Steady-state measurements

Steady-state UV-visible absorption measurements were made with a Perkin-Elmer Lambda 25 spectrometer in a 1 mm quartz cuvette. Steady-state IR absorption measurements were made with a Perkin-Elmer Spectrum 2000 FTIR spectrometer in a variable path length CaF\textsubscript{2} cell from International Crystal Labs. It has been assumed that only ground-state species contribute to the steady-state absorption spectra.

2.2. Sample Handling

During a transient absorption measurement, the illuminated sample volume must be replaced for each laser pulse to avoid undesirable effects including sample heating and re-excitation of long-lived species. Because the repetition rate of the lasers used in this work was 1 kHz, the sample must be replaced every millisecond. For mid-IR transient absorption measurements, a commercial flow cell (Harrick Scientific, DLC-S25) with adjustable path lengths was used. The sample was flowed from a reservoir, through the cell, through a magnetically-coupled gear pump and then returned to the reservoir. The path length was adjusted using Teflon spacers into which a channel was custom cut to
guide the sample through the cell. For a pump pulse spot size (e\(^2\) width) of 500 \(\mu\text{m}\) a linear flow rate of 0.5 m/s is required. Then, for a channel 4 mm wide and a path length of 1 mm, a volume flow rate of 120 mL/min is required.

### 2.2.1 Spinning Sample Cell

A low-volume spinning sample cell was designed and constructed for use with UV probe transient absorption measurements (see Figure 2.1). The minimum sample volume required for use of this cell is 0.4 mL. The sample is contained in a cell consisting of two 1 mm windows, a compressible spacer and a two-piece stainless-steel housing. The windows and spacer sit inside a 3 mm deep well in the cell bottom. Once assembled, screws are used to close the cell and thereby compressing the spacer to a thickness of ~1 mm and sealing the cell. The depth of this well and the thickness of the windows determine the exact path length of the cell. The sample is inserted and removed from the cell with a syringe inserted through one of two small injection ports on the side of the cell (not shown). Once assembled, the cell can be secured inside the cell housing for rotation. The cell is thermally isolated from the motor with a Teflon riser. The height of the cell can also be easily adjusted by using risers of different heights.

The required rotation speed is linearly proportional to pump spot size and inversely proportional to the distance of the excited volume from the rotation axis. Assuming a pump spot size of 400 \(\mu\text{m}\) and a distance of 0.25 in. from the axis, a rotation speed of 600 rpm is required. The rotation speed of the motor is adjustable and can be calibrated to the cell rotation speed using a stroboscope.
2.3. Transient Absorption Measurements

All transient absorption measurements were made at the Center for Chemical and Biophysical Dynamics at The Ohio State University’s Department of Chemistry. High-energy femtosecond pulses were generated with a Ti:Sapphire laser system. The output of a mode-locked Ti:Sapphire oscillator (Coherent Mira-SEED; approx. specifications: 76 MHz, 700 mW, 795 nm max., 45 nm FWHM, 40 fs FWHM) was evenly split and used to seed twin chirped-pulse amplifiers (Coherent Legend-HE; approx. specifications: 1 kHz, 2.45 mJ, 800 nm max., 24 nm FWHM, 60-70 fs FWHM). Amplifier output beams were used to generate pump and probe pulses for UV-visible (see Section 2.4) and mid-IR (see Section 2.5) transient absorption measurements, respectively. All beams were enclosed to prevent any negative effects from air currents.

2.4. UV-pump, UV/visible-probe Spectrometer

Pump pulse for all UV/visible-probe measurements were created by third-harmonic generation (THG) from the fundamental output of the Ti:Sapphire laser system. Approximately 450 μJ was split into two beams for THG. The first arm was passed through a type-I BBO second-harmonic crystal to generate 400 nm pulses. The second arm was sent through a small optical delay line and overlapped with the second-harmonic beam using a dichroic mirror. The combined, collinear beams were passed through a type-II BBO third-harmonic crystal to generate 267 nm pulses. The output beam, containing 800 nm, 400 nm and 267 nm, was passed through a prism-pair pulse-compressor which shorted the 267 nm pulse duration as well as allowed for spatial filtering of the fundamental and second-harmonic. Finally, the pump pulse at 267 nm was mechanically
chopped at 333 Hz, passed through a waveplate-polarizer pair and focused onto the sample. The energy of the pulses was controlled by tuning the relative orientations of the waveplate and polarizer and was typically set to 1 μJ. After passing through the sample, the pump beam was blocked so as to minimize scattering.

UV probe pulses were generated using signal and idler beams from an optical parametric amplifier. For 250-255 nm probe pulse, the signal beam was mixed with residual 800 nm to generate the sum frequency at 500 nm. The second harmonic of the sum frequency was then generated to produce 250 nm probe pulses. For 285 nm probe pulses, the idler beam was mixed with residual 800 nm to generate the sum frequency at 570 nm. The second harmonic of the sum frequency was then generated to produce 285 nm probe pulses. UV signals were detected with a photomultiplier tube.

Visible probe pulses were created via supercontinuum generation in a 1 cm water cell. After passing through the sample, the probe wavelength was selection from the broad continuum through use of a 10 nm bandpass filter (Andover Corporation) for the wavelength of interest. Visible signals were detected with silicon-based joulemeter energy sensor (Molelectron J3S-10).

All probe beams passed through a polarizer and were focused at the sample to a spot size of 100-150 μm. Pump spot sizes were typically 3-5 times larger. The relative polarization of the pump and probe was set to magic angle (54.7°) to eliminate contributions to the signal due to rotational dynamics. Signals from either the photomultiplier tube or joulemeter were collected with a digital lock-in amplifier (Stanford Research Systems SR830) which selectively amplified the frequency component of the analog
signal corresponding to the pump pulse repetition rate at the sample (i.e., after chopping). Amplified signals were retrieved from the lock-in through a GPIB interface.

2.5. UV-pump, mid-IR probe Spectrometer

2.5.1 UV Pump Pulse Generation and Treatment

Pump pulses at 270 nm were produced via second harmonic conversion of 540 nm pulses. The 540 nm light was generated via sum frequency of the idler (1662 nm) from an OPA and the fundamental (800 nm). Approximately 15 mW of 270 nm light was generated for an overall efficiency of ~2%.

Every other pulse was chopped with a mechanical chopper operating at 500 Hz and phase locked with the pump pulse train using a TTL output from the chirped-pulse amplifier. The chopped pump beam was then passed through a shutter, 600 mm optical delay line, waveplate and finally a 200 mm focal length, CaF₂ lens which focused the pump beam onto the sample with a spot size of 500 μm. The pump energy at the sample was typically 5 μJ/pulse.

2.5.2 Mid-IR Probe Pulse Generation

Tunable probe pulses in the mid-IR are generated via difference frequency generation (DFG) of signal and idlers beams from an OPA (see Figure 2.11, top). Signal and idler beams are separated by a dichroic filter before exiting OPA. The signal beam is sent through a motorized, computer-controlled, optical delay line and reflected off a dichroic mirror in the direction of the DFG crystal (EKSPLA; AgGaS₂, Type II, θ=50°, φ=0°, 6x6x2mm, anti-reflective coated 1.1-25 μm front, 2.6-11 μm back). The idler beam
is passes through this dichroic mirror and is overlapped collinearly with the signal beam and both are directed through the DFG crystal. The mid-IR pulses typically have an energy of 3 μJ with a spectral FWHM of ~100 cm⁻¹.

After generation of the mid-IR, the signal and idler beams are removed by either a Ge window (Coherent) or a long-pass filter (Spectrogon; LP-2500 nm, cutoff 2500 ± 100 nm). Ge has a refractive index of 4.1-4.6 through the near IR and acts as a filter of signal and idler through strong Fresnel reflection. However, because the signal and idler polarizations are perpendicular, reflections of more than 50% cannot be achieved for both beams simultaneously. Ge, however, has a relatively small band gap of ~0.7 eV (or 2 μm) and, for DFG wavelengths of > 4.0 μm (2500 cm⁻¹), the idler photons have high enough energy to excite a free-carrier electron. The free carriers have strong mid-IR absorption and it is not clear whether excitation of the Ge filter with idler pulses has a detrimental effect on the mid-IR pulses. For these reasons, the long-pass filter is preferred over the Ge filter from removal of signal and idler.

2.5.3 Mid-IR Beam Path

After removal of the signal and idler beams, the mid-IR beam is directed toward the sample area. The mid-IR beam is split into two arms using a 50/50 beamsplitter (Janos Technology A1425M323; CaF₂ substrate, 3 mm thick, 2.5-7 μm range). The transmitted and reflected beams are denoted probe and reference, respectively. The probe beam is focused into the sample using a plano-convex, 250 mm focal length, CaF₂ lens and recollimated ~500 mm after the sample with a matching lens. The reference beam is lowered by ~5 mm using a pair of mirrors and then separated focused through the sample
using another plano-convex, 250 mm focal length, CaF$_2$ lens and is also recollimated ~500 mm after the sample with a matching lens.

After recollimation, the probe and reference arms travel in parallel toward a 320 mm focal length, imaging spectrometer (Jobin-Yvon Triax 320). Probe and reference beams are sent through separate plano-convex, 150 mm focal length, CaF$_2$ lenses so as to focus the beams at the entrance slit of the spectrometer. A tight focus at the entrance slit is beneficial in order to obtain the best spectral resolution and signal-to-noise ratio. In general, reducing the entrance slit width of a spectrometer improves the spectral resolution obtained. However, if the entrance slit clips a portion of the probe (or reference) beams, then any fluctuations of the beam pointing will lead to intensity fluctuations and noise. For this reason it is preferable to keep the entrance slit as wide as possible and focus the beam to a tight focus at the entrance of the spectrometer. In this manner, the beam will diverge into the spectrometer from a small point in space, as if the entrance slit was in place, and good spectral resolution will be retained.

Before entering the spectrometer the beams are recombined in the horizontal direction (parallel to the table surface) but kept separated by 5 mm in the vertical direction (perpendicular to the table surface). It is important to keep the probe and reference beams vertically separated by 5 mm throughout the spectrometer so that the two beams will be correctly imaged on the detector. This is best done with a HeNe laser (see Section 2.5.7) and a small piece of paper on which a 5 mm grid of thin lines has been printed.

The spectrometer houses three gratings on a motorized turret for automated selection. The gratings differ in their resolving power and blaze wavelength. Their properties
are summarized in Table 2.3. The selected grating spectrally disperses the probe and reference beams onto the detector.

2.5.4 Detection and Acquisition

The detector is a liquid-nitrogen cooled, dual 32-element array, mercury-cadmium-telluride (MCT) detector (InfraRed Associates). The pixels are 1.0 mm long, 0.2 mm wide, and separated by a gap of 0.05 mm (see Figure 2.3). The two arrays are separated vertically by 5.0 mm. The detector is coupled to the spectrometer using custom-designed mounting hardware that allows for both translation perpendicular to and rotation parallel with the spectrometer focal plane. The position of the detector perpendicular to the focal plane can be optimized by minimizing the linewi dths of water-vapor absorption bands (see Section 2.5.6) in the IR pulse spectrum. The proper rotation of the detector can determined by matching the shape of IR pulse spectra of probe and reference beams on the two arrays, assuming that the alignment of the probe and reference beams, particularly through the spectrometer, is correct. During measurements, the detector dewar is refilled with liquid nitrogen approximately every 12 hours. If the dewar is not refilled when empty, the background noise level of the detector rapidly increases.

The signal from each channel is sent through independent pre-amplifiers and then sent to boxcar integrators in the acquisition unit (Infrared Systems Development IR-6416). The detector is connected to the acquisition unit by a pair of cables, each containing 32 wires. These wires are thin and so proper care must be taken to not bend the cables with a small radius. Also, because the manufacturer customizes the resistance on each channel in order to obtain a consistent response from each pixel, these cables are not
interchangeable. It should be noted that two detector channels (63 and 64) have been damaged, as determined by comparing the resistance of each detector pin to the resistance measured at the factory. Because of this damage, these pixels, at times, exhibit background noise levels greater than normal.

The boxcar integration is triggered by a TTL signal from the laser system so that it has (1) the same frequency as, and (2) a constant phase relative to, the laser pulse train. The start (relative to the triggering TTL signal) and duration of the integration can be controlled via computer. The parameters are optimized by displaying the gating signal on an oscilloscope along with an analog output for a single pixel from the acquisition unit. The start delay is generally set to zero and instead controlled by adjusting the relative phase of the TTL signal. This is set so that integration begins ~160 ns before the rise of the analog signal. The duration of the integration is set so that the entire rise and decay of the analog signal is captured, about 1300 ns. The relative timing of the TTL, gating and signal pulse is shown in Figure 2.4. The integrated signal is then sampled and held for analog-to-digital conversion (ADC). Sixteen additional external channels are available for input into the acquisition unit. Eight of these channels (labeled 1-8) are integrated in the same manner as the MCT channels. The remaining channels (9-16) are simply sampled. Sampling of all channels occurs 40 μs after the TTL trigger.

ADC is performed on all 80 channels at a rate of 1 kHz. The conversion is 16 bit from -10 V to +10 V providing resolution of $3.1 \times 10^{-4}$ V. Assuming an I0 signal level of 8 V, the corresponding obtainable $\Delta A$ resolution is $\sim 17 \times 10^{-6}$. The signals are then transferred to a computer through a digital input/output card for software processing.
2.5.5 Data Acquisition

The data acquisition is conducted using custom-built software written with National Instruments Labview. The data acquisition software (DAS) automates movement of the optical delay line through a GPIB interface with the translation stage controller. It also controls the state of shutters in the pump and probe beam paths through the parallel port. It controls the spectrometer though a GPIB interface including grating selection, wavelength position, and entrance slit width. Finally the software receives the digital signal from the acquisition unit, through DIO card, and then processes, displays and records the data.

The transient absorption signal at each time delay and at each probe pixel was calculated using Equation 2.1.

\[
\Delta A = -\log\left(\frac{I_{pr,\text{on}}}{I_{pr,\text{off}}} \cdot \frac{I_{\text{ref, on}}}{I_{\text{ref, off}}}\right)
\]

where \(\Delta A\) is the transient absorption and \(I_{i,j}\) is the mean intensity from the probe \((i = pr)\) and reference \((i = ref)\) arrays, with \((j = on)\) and without \((j = off)\) the presence of the pump at the sample. Determination of \(on\) vs. \(off\) signals was based on a TTL signal produced by the mechanical chopper which was sampled using an external input of the acquisition unit (see Section 2.5.4). If the TTL signal and the pump pulse train are incorrectly phased, the calculated \(\Delta A\) will be the negative of the true value. The phase can be determined using a sample with a known transient absorption signal. An option in the acquisition software allows for correction of this phase, if necessary. Once determined the phase should be stable unless significant changes are made to the experimental setup.
An average $\Delta A$ spectrum is collected at each specified time delay, in order, starting at the most negative time delay and ending with the most positive time delay. In general, multiple scans of the specified time delays are made and averaged to reduce long-term noise. By default, alternating scans are made in reverse order although this behavior can be disabled in the DAS. Alternating the delay order can reduce systematic effects of the signal due to delay stage imperfections.

The time delays can be specified by four different methods. (1) A single time delay can be specified to easily obtain a single well-averaged transient spectrum. (2) A custom list of time delays can be specified and can be essentially unlimited in both size and form. This can be useful for emphasizing certain temporal ranges or matching the time delays to those used in other experiments. (3) A linear set of equally-spaced time delays can be specified by entering the value of the first and last desired time delay and a constant step size. (4) The most commonly used method of specifying time delays uses a nonlinear algorithm.

This algorithm produces more time delays per unit time at smaller time delays where higher temporal resolution is necessary. The previously used algorithm was improved in three ways. First, the algorithm was adjusted so that the time delays were centered on and symmetric about zero time delay. Second, the algorithm was improved so that adjustment of the initial and final time delay did not alter the values of the intermediate time delays. This leads to greater consistency in the time delays for different data sets and allows different measurements to be compared more easily. Finally, a secondary algorithm was introduced which allows to the user to minimize the number of negative
time delays to the minimum necessary while maintaining a high number of positive time
delay. This allows for faster data collection and therefore greater signal averaging and
signal-to-noise.

The data from each scan of the time delays is saved to a text file with 32 tab-
separated columns and a number of rows equaling the number of time delays scanned. The first column contains a list of the time delays scanned, from most negative to most positive, in picoseconds. The remaining columns provide the $\Delta \lambda$ measured at each pixel from (left to right) lowest wavelength to highest wavelength. However, the wavelength associated with each pixel is not provided. This must be obtained through spectral cali-
bration techniques.

2.5.6 Spectral Calibration

Initial calibration of the spectrometer over the entire mid-IR spectral range (~0-
4000 cm$^{-1}$) can be performed using a broadband mid-IR source (such as a glow bar), a
high-frequency mechanical chopper and a sample with a broad range of preferably sharp
absorption bands. Polystyrene film is a useful calibration sample.

Spectral calibration should be performed for each experiment. In the range of
~1200-2000 cm$^{-1}$, water vapor absorption can be utilized for calibration. Calibration is
performed by obtaining spectra of the mid-IR pulse using each pixel, with and without
the presence of water vapor in the mid-IR beam path. The data is collected by recording
the signal on the MCT arrays while stepping the spectrometer grating angle. An example
of spectra obtained from just one array is shown in Figure 2.5. The resulting spectra are
centered at varying (uncalibrated) wavelengths according to their different positions in
the image plane. While data from all pixels is obtained simultaneously, in practice only four spectra are required for calibration; pixels 0, 15, 16 and 31. However, these four spectra must be recorded both without (Figure 2.6) and with (Figure 2.7) the presence of water vapor in the mid-IR beam path.

These spectra can be used to obtain the three parameters needed for calibration of transient spectra: scaling factor, offset and spectral range. The first two are used for linear calibration of the recorded center wavelength to the true center wavelength, as in Equation 5.1,

\[ \lambda_{\text{calib}} = f(\lambda_{\text{uncalib}} + o) \]

where \( f \) is the scaling factor, \( o \) is the offset and \( \lambda_{\text{calib}} \) and \( \lambda_{\text{uncalib}} \) are the true, calibrated and factor, uncalibrated center wavelengths, respectively. The spectral range is needed to determine the wavelength at each pixel position for a given center wavelength, as in Equation 5.1,

\[ \lambda_i = f[\lambda_{\text{uncalib}} + \Delta\lambda(i \frac{1}{31} - \frac{1}{2}) + o] \quad i = 0, 1, \ldots, 31 \]

where \( \Delta\lambda \) is the spectral range and \( \lambda_i \) is the calibrated wavelength at pixel \( i \).

The spectral width can be determined by comparing the location of spectral features in the mid-IR pulse spectrum measured at pixels 0 and 31. As shown in the example in Figure 2.8, the water vapor absorption lines are, in this case, separated by 181 nm. To determine the scaling factor and offset, spectra obtained under purged and unpurged conditions can be used to a spectrum of water vapor absorption and compared to a reference water vapor absorption spectrum. In principle, this can be done every pixel and the scaling factors and offsets averaged for high accuracy but in practice, use of one of the
near-center pixels (15 or 16) is generally sufficient. The water vapor spectrum is calculated as

\[
A = -\log \left( \frac{I_{\text{unpurged}}}{I_{\text{purged}}} \right)
\]

where \(A\) is the absorbance, and \(I_{\text{unpurged}}\) and \(I_{\text{purged}}\) are the spectra of the mid-IR pulse taken under unpurged and purged conditions, respectively. As an example, Figure 2.9 shows the spectrum under purged and unpurged conditions (top) as well as the resulting water vapor absorption spectrum (bottom). Note that the quality of the absorption spectrum deteriorates in spectral regions of low mid-IR intensity.

To complete the calibration the water absorption spectrum is plotted together with a reference water absorption spectrum obtained with a calibrated FTIR instrument. The uncalibrated wavelengths of the pulse-derived spectrum are then linearly scaled according to Equation 2.3, and the calibration parameters adjusted until the two spectra overlap as in Figure 2.10. The parameters can then be used to calibrate transient spectra obtained under the same conditions.

2.5.7 General Techniques for Alignment of mid-IR Light

Alignment of mid-IR light is more challenging than alignment of near-IR, visible or UV light because mid-IR light is not visible by eye, cannot be detected by near-IR viewing devices and does not induce fluorescence. However there are a number of techniques that can be used to aid alignment.

Thermochromic liquid crystal films with a range of 25-30 °C (Edmund Scientific R25C5W) will change color when illuminated with a mid-IR beam due to heat generated.
While useful, the mid-IR beam must have a power of roughly 2 mW or more to see a significant effect. Also, the response time of the film, while dependent on the power, is generally slow, on the order of seconds. The lower color temperature of the film (25 °C) is close to the typical laboratory temperature (24 °C) and therefore a significant background color change is typical.

Because the difference frequency generation process that produces the mid-IR beam is in a collinear geometry (see Section 2.5.2), in principle the signal and idler beams should travel through the experimental setup along the same path as the mid-IR beam. In practice, the validity of this assumption depends on the quality of the alignment of the DFG setup. Under conditions that the signal and/or idler beams travel collinearly with the mid-IR beams, the long-pass filter in the DFG setup can be removed and the signal/idler beams can be used for alignment. This leads to two advantages. First, the signal and idler beams have an order of magnitude more power than the mid-IR beam and so will induce a strong and fast change in the thermochromic liquid crystal films. Additionally, the signal and idler wavelengths are suitable for observation with a near-IR viewer. Both advantages greatly ease alignment.

A flip-mount in the DFG setup allows for a visible beam from a Helium-Neon (HeNe) laser to pass through (see Figure 2.11, bottom). The HeNe beam can be aligned to the same alignment tools as the signal and idler and is thereby set collinear with the path of the mid-IR beam. Since the HeNe beam is visible to the eye its use can greatly expedite alignment of the experimental setup. This method is particularly useful for aligned through the spectrometer. After alignment is complete, the DFG setup can be returned to
the normal configuration for generating mid-IR pulses (Figure 2.11, top). Generally some small alignment is still required due to inexact overlap of the HeNe and mid-IR beam paths. A number of the mid-IR optics will not properly transmit the NeHe beam and must be adjusted, removed or replaced with visible-wavelength equivalents. The long-pass filter in the DFG setup (item 8 in Figure 2.11 and Table 2.4) must be removed. Due to the wavelength-dependence of the refractive index of CaF$_2$, the focal lengths of the lenses in the experimental layout are slightly different in the mid-IR and visible, and the separation between focusing and recollimating lenses needs to be adjusted. Finally, the mid-IR beamsplitter (item 18 in Figure 2.2 and Table 2.2) needs to be replaced with a CaF$_2$ window. In order to maintain a consistent alignment when switching between HeNe and mid-IR beams, the CaF$_2$ windows should be the same thickness as the beamsplitter and mounted identically. Because of these caveats, the HeNe alignment is best used for initial, semi-course alignment followed by use of the other methods for fine alignment.

**2.5.8 Achieving High Sensitivity (Signal-to-Noise)**

Use of a referencing beam results in significantly improved signal-to-noise. However, for effective noise reduction the probe and reference beams should be as identical as possible: The two beams should pass through the sample as close as possible while avoiding overlap of the pump beam with the reference. They should be focused similarly through the sample as well as through the entrance slit of the spectrometer. It is important that both beams are well aligned through the spectrometer (though vertically separated by 5 mm).
For the best results, the spectrum of the probe and reference pulses measured with the MCT array detector should be matched in both shape and intensity. If the mid-IR is tuned between ~1200 and 2000 cm\(^{-1}\) or between ~3400 and 4200 cm\(^{-1}\), it can helpful to temporarily expose the IR beam path to atmospheric water vapor. Water vapor absorption of the mid-IR pulse produces sharp features in the pulse spectrum that provide excellent metrics for matching the probe and reference spectra. The strong absorption of CO\(_2\) at ~2350 cm\(^{-1}\) may also be helpful. The RMS noise levels of the probe and reference pixels can also be used. The acquisition software displays the RMS noise at each pixel across the arrays, corresponding to the noise for different wavelengths in the pulse spectra, or noise spectra. If the probe and reference spectra are well matched, these noise spectra should also be matched. This is also an important factor in obtaining good sensitivity.
<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cell top</td>
</tr>
<tr>
<td>2</td>
<td>Window, 1mm thick</td>
</tr>
<tr>
<td>3</td>
<td>Gylon spacer, compressible to 1 mm thick</td>
</tr>
<tr>
<td>4</td>
<td>Window, 1 mm thick</td>
</tr>
<tr>
<td>5</td>
<td>Cell bottom, 3 mm deep well</td>
</tr>
<tr>
<td>6</td>
<td>Cell housing</td>
</tr>
<tr>
<td>7</td>
<td>Bearing</td>
</tr>
<tr>
<td>8</td>
<td>Bearing housing</td>
</tr>
<tr>
<td>9</td>
<td>Belt, 1/8 inch diameter, cut and melded to length</td>
</tr>
<tr>
<td>10</td>
<td>Pulley</td>
</tr>
<tr>
<td>11</td>
<td>Motor, rate specifications: 1/8 HP/90 W power, 2000 rpm speed, 63 oz.-in. torque</td>
</tr>
<tr>
<td>12</td>
<td>Teflon riser</td>
</tr>
<tr>
<td>13</td>
<td>Motor housing</td>
</tr>
<tr>
<td>14</td>
<td>Base plate</td>
</tr>
</tbody>
</table>

Table 2.1. Description of items in spinning sample cell as labeled in Figure 2.1.
<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diode-pumped, intra-cavity frequency-doubled, Nd:YAG, CW laser, 532 nm, 4.8 W</td>
</tr>
<tr>
<td>2</td>
<td>Ti:Sapphire mode-locked oscillator, 76 MHz, 700 mW, 795 nm, 45 nm FWHM</td>
</tr>
<tr>
<td>3-4</td>
<td>Collimating telescope</td>
</tr>
<tr>
<td>5</td>
<td>Beamsplitter, 55% reflected, 45% transmitted</td>
</tr>
<tr>
<td>6</td>
<td>Diode-pumped, intra-cavity frequency-doubled, Nd:YLF, Q-switched laser, 1 kHz, 527 nm, 19 W</td>
</tr>
<tr>
<td>7</td>
<td>Ti:Sapphire chirped-pulse amplification system, 1 kHz, 2.45 W, 800 nm, 20 nm FWHM, 60-70 fs FWHM</td>
</tr>
<tr>
<td>8</td>
<td>Beamsplitter, 50/50% reflected/transmitted</td>
</tr>
<tr>
<td>9</td>
<td>Supercontinuum-seeded, two-stage, tunable, computer-controlled, optical parametric amplifier, three outputs: (1) 800 nm (residual fundamental), 600 mW, (2) 1150 – 1600 nm (signal), 40 mW, (3) 1600 – 2630 nm (idler), 100 mW</td>
</tr>
<tr>
<td>10</td>
<td>Nonlinear conversion setup, sum-frequency generation of idler and fundamental (533 - 613 nm) followed by second harmonic generation, (267 - 307 nm), 15 mW</td>
</tr>
<tr>
<td>11</td>
<td>Chopper, operating at 500 Hz</td>
</tr>
<tr>
<td>12</td>
<td>Shutter</td>
</tr>
<tr>
<td>13</td>
<td>Optical delay line, 600 mm travel translation stage mounted with a hollow corner-cube retroreflector</td>
</tr>
</tbody>
</table>

Table 2.2. Description of items in UV pump/mid-IR probe transient absorption spectrometer as labeled in Figure 2.2. Specified powers and wavelengths are typical values only.
<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Waveplate, $\lambda/2$</td>
</tr>
<tr>
<td>15</td>
<td>Nonlinear conversion setup, difference-frequency generation of signal and idler, 2400 – 10000 nm, 3-4 mW typical (see also Figure 2.11 and Table 2.4)</td>
</tr>
<tr>
<td>16</td>
<td>Shutter</td>
</tr>
<tr>
<td>17</td>
<td>Variable circular neutral density filter, 0-2 ND, zinc selenide substrate</td>
</tr>
<tr>
<td>18</td>
<td>Beamsplitter, 50/50% reflected/transmitted</td>
</tr>
<tr>
<td>19,20</td>
<td>Lens, plano-convex, CaF$_2$, $f = 150$ mm</td>
</tr>
<tr>
<td>21</td>
<td>Lens, plano-convex, CaF$_2$, $f = 200$ mm</td>
</tr>
<tr>
<td>22</td>
<td>Sample position</td>
</tr>
<tr>
<td>23,24</td>
<td>Lens, plano-convex, CaF$_2$, $f = 150$ mm</td>
</tr>
<tr>
<td>25,26</td>
<td>Mid-IR ND filters and/or Si windows</td>
</tr>
<tr>
<td>27,28</td>
<td>Lens, plano-convex, CaF$_2$, $f = 100$ mm</td>
</tr>
<tr>
<td>29</td>
<td>Imaging spectrometer, $f = 320$ mm, triple grating</td>
</tr>
<tr>
<td>30</td>
<td>Dual 32-element array, liquid nitrogen cooled, MCT detector</td>
</tr>
<tr>
<td>31</td>
<td>Enclosure for dry air purging of mid-IR beam path</td>
</tr>
<tr>
<td></td>
<td>Grating 1</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Grooves/mm</td>
<td>100</td>
</tr>
<tr>
<td>Blaze Wavelength / μm</td>
<td>3.0</td>
</tr>
<tr>
<td>Spectral Dispersion&lt;sup&gt;a&lt;/sup&gt; / nm·mm&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>25</td>
</tr>
<tr>
<td>Spectral Window&lt;sup&gt;a,b&lt;/sup&gt; / cm&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>50</td>
</tr>
<tr>
<td>Minimum Spectral Resolution&lt;sup&gt;b&lt;/sup&gt; / cm&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>1.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Assuming a spectrometer with a focal length of 320 mm

<sup>b</sup> Assuming use of the detector described in text and a spectral window centered at 1600 cm<sup>-1</sup>

Table 2.3. Properties of gratings used in the spectrometer for mid-IR transient absorption.
<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Idler beam generated by optical parametric amplifier</td>
</tr>
<tr>
<td>2</td>
<td>Signal beam generated by optical parametric amplifier</td>
</tr>
<tr>
<td>3</td>
<td>Flip-mounted mirror, in upright position</td>
</tr>
<tr>
<td>4</td>
<td>Optical delay line</td>
</tr>
<tr>
<td>5</td>
<td>Dichroic mirror, reflects signal, transmits idler</td>
</tr>
<tr>
<td>6</td>
<td>Removable alignment tools</td>
</tr>
<tr>
<td>7</td>
<td>AgGaS$_2$ crystal, Type II, $\theta=50^\circ$, $\varphi=0^\circ$, 6x6x2 mm, anti-reflective coated for 1.1-25 $\mu$m on front surface and 2.6-11 $\mu$m on back surface</td>
</tr>
<tr>
<td>8</td>
<td>Long-pass filter, 2500 nm</td>
</tr>
<tr>
<td>9</td>
<td>Mid-IR beam generated by difference frequency mixing of signal and idler, 3 mW typical</td>
</tr>
<tr>
<td>10</td>
<td>Shutter</td>
</tr>
<tr>
<td>11</td>
<td>Helium-Neon laser</td>
</tr>
<tr>
<td>12</td>
<td>Flip-mounted mirror, in down position</td>
</tr>
</tbody>
</table>

Table 2.4. Description of items in DFG setup as labeled in Figure 2.11.
Figure 2.1. Model of spinning sample cell, assembled (left) and exploded (right) views. Numerically labeled items are described in Table 2.1.
Figure 2.2. Schematic layout of the UV pump/mid-IR probe transient absorption spectrometer. Numerically labeled items are described in Table 2.2.
Figure 2.3. Schematic drawing of the dual-array mercury cadmium telluride detector. Inset shows a close up of select pixels. Numeric labeling of the pixels is presented from the point of view of looking through the detector window.
Figure 2.4. Relative timing of pulses used for data acquisition. TTL pulse generated by the Ti:sapphire amplifier and synchronized with pulse ejection, used to initiate acquisition sequence (black, highest amplitude), integration gating pulse (blue, intermediate amplitude), and signal pulse (red, lowest amplitude). Amplitudes have been scaled for comparison.
Figure 2.5. Intensity of mid-IR pulse measured on pixels 0-31 (right to left) as a function of the uncalibrated center wavelength set by the spectrometer. Data obtained while the mid-IR beam path was purged with dry air.
Figure 2.6. Intensity of mid-IR pulse measured on selected pixels used for spectral calibration: pixel 0 (black, right), pixel 15 (red), pixel 16 (green), and pixel 31 (blue, left) as a function of the uncalibrated center wavelength set by the spectrometer. Data obtained while the mid-IR beam path was purged with dry air.
Figure 2.7. Intensity of mid-IR pulse measured on selected pixels used for spectral calibration: pixel 0 (black, right), pixel 15 (red), pixel 16 (green), and pixel 31 (blue, left) as a function of the uncalibrated center wavelength set by the spectrometer. Data obtained while the mid-IR beam path was not purged with dry air.
Figure 2.8. Intensity of mid-IR pulse measured on pixels 0 (black, bottom) and 31 (blue, top). The spectral features are separated by 181 nm, the spectral range of the MCT array at the wavelength and with a 100 mm$^{-1}$ grooved grating and a 320 mm focal length imaging spectrometer.
Figure 2.9. Spectrum of the mid-IR pulse under purged (black, top) and unpurged (red, middle) conditions. Water vapor absorption spectrum (blue, bottom) obtained according to Equation 5.1.
Figure 2.10. Mid-IR spectrum of water vapor measured with a calibrated Perkin-Elmer Spectrum 2000 FTIR spectrometer (black, solid) and the mid-IR pulse (red, line). The wavelengths of the pulse spectrum have been linearly scaled so as to overlap the two spectra, providing a calibration offset parameter of -444.5 nm and a scaling factor of 1.
Figure 2.11. Schematic layout of DFG setup during normal use (top) and during HeNe alignment (bottom). Numerically labeled items are described in Table 2.4.
CHAPTER 3

VIBRATIONAL COOLING

3.1. Introduction

Many photochemical and photophysical processes lead to molecules with excess vibrational energy; these molecules are referred to as vibrationally “hot.” In solution, a vibrationally hot molecule returns to thermal equilibrium with its surroundings through a process called vibrational cooling (VC). Vibrational cooling has of interest for decades and a number of excellent reviews have been published on the subject.1-4

The reason for this significant interest is due to the important role that vibrational cooling plays in chemical, photochemical and photophysical dynamics. Reaction pathways are generally thought to follow the minimum energy pathway (MEP) along the potential energy surface (PES). However, a reactive system is never truly at this minimum energy due to vibrational energy. Depending on the amount of vibrational energy, the system may or may not be able to overcome barriers on the PES that lead to different products than the MEP. The most obvious example is thermally-activated reactions. In these cases the amount of excess vibrational energy, and therefore the likelihood of crossing the reaction barrier, is simply determined by the temperature of the reactants.
Excess vibrational energy can also be generated through photoexcitation, photophysical and photochemical process, as well as exothermic chemical reactions. In these cases, the probability of crossing a particular barrier on the PES depends on both the initial amount of excess vibrational energy and the rate at which the systems undergoes vibrational relaxation. This is shown schematically in Figure 3.1. Here, vibrational relaxation is used as a general term for both relaxation of a single vibration or a set of vibrations. If vibrational relaxation is fast then the system will follow the MEP directly toward product A but if vibrational relaxation is slow then the system may retain vibrational energy long enough to overcome a barrier and reach product B. Thus, the fate of excited molecules depends on the rate of vibrational relaxation.

Despite its importance, vibrational cooling is still poorly understood, particularly in molecules as large as DNA bases, and is still an important subject of research. For this reason we carried out femtosecond transient absorption studies on the vibrational cooling of DNA base monomers in solution. Base monomers are excellent models for studying vibrational cooling for three reasons: (1) The excited-state lifetimes of base monomers are short (<1 ps). (2) The transition energy between $1\pi\pi^*$ and $S_0$ is large (> 30 000 cm$^{-1}$) leading to a highly vibrationally-excited ground state. (3) The compounds have multiple solute-solvent hydrogen-bonding sites leading to strong interactions with the solvent.

N9-substituted adenine derivatives were chosen for most of our studies because their excited-state lifetimes ($\tau \approx 300$ fs) are among the shortest of all bases and because, unlike pyrimidine bases (see Chapter 4), internal conversion occurs with nearly 100% efficiency. The bases used in these studies are shown in Figure 3.2. Our results demon-
strate that vibrational cooling in base monomers is strongly sensitive to the solvent and particularly solute-solvent hydrogen bonding. Furthermore, the vibrational cooling rate depends on the isotopic composition of the solvent. We show that the solvent-isotope effect suggests that a significant amount of vibrational energy is flowing between high-frequency modes of the solute and solvent. The important role of high-frequency vibrational energy transfer to the solvent may suggest that intramolecular vibrational redistribution is incomplete during the vibrational cooling.

3.2. Results

3.2.1 Solvent-Dependent UV Absorption Spectra

Ground-state UV absorption spectra of 9MA in H$_2$O, methanol and acetonitrile are shown in Figure 3.3. The spectra have only a modest dependence on solvent. The band maximum shifts from 261 nm in both H$_2$O and methanol to 258 nm in acetonitrile. The full-width at half-maximum of the band is 31 nm in acetonitrile, 30 nm in methanol and 29 nm in H$_2$O.

In Figure 3.4 the ground-state UV absorption bands of 9MA in H$_2$O and D$_2$O are compared. The spectra have the same shape within our sensitivity but the spectrum of D$_2$O is shifted by 0.5 nm with respect to H$_2$O.

3.2.2 Vibrational Dynamics in H$_2$O

Shown in Figure 3.5 are transient absorption signals for 9MA in H$_2$O at probe wavelengths of 570, 285 and 255 nm. The signal at 570 nm is instrument limited and
decays with a time constant of 0.36 ps. This is in good agreement with the time constant of 0.22 ps report by Cohen et al.\textsuperscript{5}

A negative signal is observed at 255 nm. Negative signals occur when, at the probe wavelength, the extinction coefficient of the ground state is greater than that of any populated transient states, leading to an overall loss of absorption due to depopulation of the ground state (bleaching). The kinetics of bleaching signals monitor the return of population to $S_0$. It is not surprising to observe a bleach signal at this probe wavelength because of its proximity to the ground-state absorption band maximum, 261 nm (see Figure 3.3). The bleach signal at 255 nm recovers completely to the baseline with a single, 2.0 ps time constant.

Transient absorption at 285 nm rises with a 0.47 ps time constant, only slightly longer than the excited-state decay observed at 570 nm. As seen in Figure 3.3, the ground state of 9MA has negligible absorption at this wavelength. This, along with the lack of a 0.47 ps component in the bleach signal at 255 nm, rules out the possibility that the rise results from a fast recovery of $S_0$. The signal at 285 nm reaches a maximum at $\sim$ 1ps followed by a 2.11 ps decay to zero, in good agreement with the bleach recovery time observed at 255 nm.

### 3.2.3 Probe Wavelength Dependence

Figure 3.6 compares the bleach recovery signals for 9MA in H$_2$O observed at 250 nm and 255 nm. As discussed in Section 3.2.2, the recovery at 255 nm is monoexponential with a 2.0 ps time constant. On the other hand, the recovery at 250 nm is biexponential with time constants of 0.5 and 2.4 ps. The latter component agrees well with the
recovery observed at 250 nm and the recovery observed at 255 nm and the data in Figure 3.6 have been scaled to emphasize this agreement.

Similar behavior is observed at 250 nm in both methanol and acetonitrile. In all cases, the positive amplitude component decays with a time constant slower than the decay measured in the visible (see Table 3.1 and Figure 3.7). This is particularly true for methanol and acetonitrile which differ by factors of 2.3 and 5.1, respectively.

### 3.2.4 Dynamics in Other Solvents

As shown in Figure 3.7, the dynamics of 9-methyladenine observed at 570 nm are nearly independent of solvent. The decay time constants observed in H₂O, D₂O, methanol and acetonitrile are 0.36, 0.34, 0.43, and 0.410 ps, respectively.

In contrast, signals at UV probe wavelengths depend sensitively on solvent. At 250 nm, the positive-amplitude component decays with a lifetime of 0.5, 0.3, 1.0 and 2.10 ps in H₂O, D₂O, methanol and acetonitrile, respectively. The bleach recovery at 250 nm recovers with a time constant of 2.4, 4.2, 4.5 and 13.1 ps, in H₂O, D₂O, methanol and acetonitrile, respectively. A similar difference between H₂O and D₂O is observed for the bleach recovery observed at 255 nm and the decay component observed at 285 nm (see Table 3.1).

### 3.3. Discussion

#### 3.3.1 Internal Conversion to the Ground State

Nucleobases absorb strongly in the UV because of strongly allowed \(^1\pi\pi^*\) transitions. The favorable Frank-Condon overlaps for this strong absorption should also be
favorable for strong emission, but the fluorescence quantum yields of bases are extremely low, ranging between $0.45 \times 10^{-4}$ for uracil and $3.0 \times 10^{-4}$ for guanine. The lack of strong fluorescence from the bright $\pi\pi^*$ state suggests that it is quenched by rapid nonradiative decay.

Following previous studies, we assign the sub-ps decays observed at visible probe wavelengths to excited-state absorption of the $\pi\pi^*$ state. Cohen et al. demonstrated that the $\pi\pi^*$ lifetime of 9MA only differed modestly between H$_2$O and acetonitrile. That result is extended here to include methanol and D$_2$O. Identical $\pi\pi^*$ decays in H$_2$O and D$_2$O were previously observed for adenine.

The observation of rapid, single-exponential bleach recovery of 9MA at 255 nm suggests that the $\pi\pi^*$ state decays directly to the ground state with, for this base, near 100% yield. However the time constant for ground-state recovery in the UV is roughly 6-fold greater than the $\pi\pi^*$ lifetime measured in the visible. This is because internal conversion from $\pi\pi^*$ directly populates a highly-vibrationally excited (“hot”) ground state and return to the pre-excitation, thermalized ground-state is rate-limited by vibrational cooling, as described in the next section.

### 3.3.2 Hot Band Evolution

Studies have shown that excess vibrational energy has a large effect on the ground-state absorption band of chromophores. Hot chromophores have broadened spectral widths and absorption maxima strongly shifted to longer wavelengths, compared with their room-temperature spectra. This is shown schematically in Figure 3.8. As a hot molecule returns to thermal equilibrium with the room-temperature solvent molecules
through vibrational cooling, the hot absorption band evolves in a manner to recover the room-temperature absorption band. Thus, the vibrational cooling of the molecules can be monitored by probing at wavelengths where either the hot ground state or the thermalized ground state absorption dominates. Figure 3.8 shows that at 285 nm there is negligible contribution from room-temperature absorption.

Most studies have monitored vibrational cooling through hot-band absorption, \(^{13,16,18,19}\) although vibrational-cooling studies have also been conducted using ground-state bleach recovery signals.\(^{17}\) Here we have used both methods and show that, with the proper choice of probe wavelengths, the results agree well. Probe wavelength selection is critical because vibrational-cooling signals are highly probe-wavelength dependent. This is because the hot-band spectrum undergoes a continuous spectral evolution beginning, presumably, within the time resolution of the measurement and continuing until thermal equilibrium with the solvent is reached. This evolution consists of band-narrowing and blue-shifting.\(^{17}\) Thus, faster decays are seen generally observed at longer probe wavelengths.\(^{13,14,19,20}\) A particularly-relevant example is the transient absorption study of adenosine in H\(_2\)O by Pecourt et al.\(^{21,22}\) Using probe wavelengths extending from the visible through the near UV, they observed the signal decay with time constants increasing from 0.4 ps at 340 to 2.0 ps at 270 nm. Recent broadband transient absorption measurements by Kwok et al. have since shown that there is a second excited-state absorption band with a maximum at 350 nm,\(^{23}\) however the probe-wavelength dependence of near-UV signals still primarily result from vibrational cooling.
3.3.3 Phenomenological Vibrational Cooling Rate

The strong probe-wavelength dependence of vibrational cooling signals complicates a definitive characterization of the vibrational cooling rate. In some cases it has been possible to model the temperature dependence of the ground-state absorption, allowing the decay rate of the microcanonical temperature or excess vibrational energy to be determined. However, because these analyses involve many assumptions and are difficult to verify we followed a simpler procedure.

It is clear that vibrational cooling signals will not persist after thermalization is complete. Thus, the longest observed time constant attributable to vibrational cooling provides a lower limit on the vibrational cooling rate. For 9MA and other adenine derivatives, the longest time constants are observed at probe wavelengths of roughly 250-255 nm and 285 nm. Vibrational-cooling rates obtained from these different probe wavelengths generally agree within ~15%, close to the relative uncertainty of the time constants themselves. Furthermore, the focus of this study is on the solvent dependence of the vibrational-cooling rate which is highly consistent among these probe wavelengths. We have therefore chosen the value of the long time constant at 250 nm as a phenomenological vibrational cooling time.

In previous cases of weak solute-solvent interactions, biexponential decays associated with vibrational cooling were found, suggesting a two-step mechanism. Energy is first transferred to molecules in the first solvent shell through vibrational energy transfer (VET) and then through the bulk solvent through thermal diffusion. In support of this mechanism, the longer time constant of the biexponential cooling rates were strongly
correlated with the thermal diffusivity of the solvent. The model has been used to provide
a rough estimate of the number of solvent molecules in the first solvent shell around
specific solutes.\textsuperscript{17}

Here, however, the decays associated with vibrational cooling are all single exponen-
tial. This suggests that one of the two VC steps dominates. Given the multiple hydro-
gen-bonding sites available for DNA bases, it seems likely that the dominant step would
be the initial vibrational energy transfer. However, further insight is provided by studying
the solvent dependence of the VC rates.

3.3.4 Solvent Dependence of Vibrational Cooling

As shown in Figure 3.1, the vibrational cooling of 9MA is strongly solvent de-
pendent. Solvent-dependent VC rates have observed for many solute molecules.\textsuperscript{15,17,25-28}
In some cases the VC rate was found to be proportional to the thermal diffusivity of the
solvents.\textsuperscript{17,26-28} This can be understood if the energy transfer to the bulk solvent via diffu-
sion is the rate-limiting step. However, as shown in Table 3.2, the VC rates of 9MA do
d not show a good correlation with the thermal diffusivities of the solvents studied here.
This together with the observation of single exponential VC dynamics suggests that the
initial vibrational energy transfer from hot 9MA to first solvent shell is the rate limiting
step.

The significantly faster VC observed in protic vs. aprotic solvents suggests that
hydrogen bonding strongly accelerates vibrational cooling. Previous studies have also
suggested this.\textsuperscript{15,17,25} More specifically, considering our above arguments, this suggests
that solute-solvent hydrogen bonding accelerates VET to the first solvent shell. This
effect has also been observed by Kovalenko et al.\textsuperscript{17} They studied the vibrational cooling of \textit{p}-nitroaniline (PNA) and (dimethlyamino)-\textit{p}-nitroaniline using broadband UV-visible transient absorption. The dimethylated nitroaniline has fewer solute-solvent hydrogen bonding sites and underwent slower vibrational cooling in protic solvents, while the two molecules exhibited similar vibrational cooling rates in acetonitrile.

Previous studies have correlated VC rates in protic solvents with the number density of solvent OH groups. Our measured VC rates are best correlated with this metric, except however for the different VC rates observed in H\textsubscript{2}O and D\textsubscript{2}O.

### 3.3.5 Solvent Isotope Effect on Vibrational Cooling

As shown in Figure 3.9, vibrational cooling is 1.7 times slower in D\textsubscript{2}O than in H\textsubscript{2}O. These solvents have the same OH/OD bond densities. Additionally, the thermal diffusivity and other macroscopic solvent properties differ by \(\leq 10\%\) between the two solvents. In particular, the hydrogen bonding is generally thought to be 10\% stronger in D\textsubscript{2}O which, bases on our previous arguments for the effect of hydrogen bonding, should lead to accelerated VC in D\textsubscript{2}O, opposite of the observed effect. None of these metrics adequately explain the observed solvent isotope effect.

A similar isotope effect of 1.3 was observed for 1-cyclohexyluracil in D\textsubscript{2}O vs. H\textsubscript{2}O.\textsuperscript{29} It has also been observed for specific vibrational modes of some triatomic molecules.\textsuperscript{30} A solvent isotope effect for vibrational cooling has also been observed for PNA.\textsuperscript{17} Of the cases in which an isotope effect has been observed, PNA is the most similar to 9MA. Both undergo internal conversion to the ground state in \(\sim 0.3\) ps in aqueous solution (H\textsubscript{2}O or D\textsubscript{2}O). Both have \(\geq 4\) solute-solvent hydrogen bonding sites. Additional-
ly, the two molecules have similar vibrational degrees of freedom (42 for PNA and 48 for 9MA).

One significant difference between PNA and 9MA is that biexponential VC dynamics were observed for PNA. Therefore in order to facilitate comparison with 9MA, we calculated amplitude-averaged time constants, see Equation 5.1, from the data provided in Table 1 of Ref. 17.

\[
\langle \tau \rangle = \sum_{i=1}^{2} A_i \tau_i
\]

3.1

The resulting time constants for vibrational cooling of PNA in H2O and D2O are 1.5 and 2.6 ps, respectively. The ~50% faster vibrational cooling time for PNA than 9MA may result from the smaller excess vibrational energy in the hot PNA ground state in these experiments. However, the solvent isotope effect from these values – 1.8 – is in excellent agreement with the 1.7 value we have measured for 9MA. To explain the solvent isotope effect for PNA, Kovalenko et al. proposed that there 30% less strongly-coupled solvent molecules in the first solvent shell.17 Because the assumption that the structure of the first solvent shell would be largely different between H2O and D2O is unreasonable, we have sought a different explanation.

### 3.3.6 Microscopic Description of Vibrational Cooling

The conventional picture of vibrational cooling in large molecules the size of 9MA suggests that direct energy transfer to the solvent (VET) occurs primarily between the lowest frequency modes of the solute and solvent. This can largely be argued based on an understanding of the density of states in the solute and the vibrational friction of the
solvent, depicted schematically in Figure 3.10. The vibrational density of states of the solute increases rapidly with frequency because of combination and overtone states. Because of this high density of states, any localized vibrational excitation at high frequency can rapidly relax to populate lower frequency vibrations (IVR). This process is further accelerated by a solvent in two ways. (1) The high density of low-frequency phonon-like solvent bath modes can accept small amounts of energy to achieve energy matching between solute modes.  

(2) Dynamic solvent motion produce a fluctuation electrostatic environment which can tune solute modes into resonance. The processes lead to solvent-assisted IVR. IVR rates on the range of 10-400 fs have been suggested in previous studies. This suggests that the lowest frequency modes of the solute are rapidly the only modes significantly populated.

In contrast, the magnitude of the solvent-induced vibrational friction decreases with increasing frequency because of the large number of translational and rotational modes present at low frequency. Higher frequency motions of the solvent primarily consist of a smaller number of intramolecular vibrations. Vibrational energy transfer to solvent modes results from the fluctuations of the solvent molecules and the forces they apply on the solute. Vibrational friction from these solvent motions dampens the oscillations of the solute and induces energy transfer from solute to solvent. The rate of vibrational energy transfer is proportional to magnitude of the force-force autocorrelation function at the frequency of the vibration. Specifically, it is the solvent forces that are fluctuating at the same frequency and along the same coordinate at the solute vibration that induce the energy transfer. In analogy to the solvent density of states, the solvent
vibrational friction is greatest at low frequencies and decreases sharply with increasing frequency except at frequencies coincident with intramolecular vibrations of the solvent which produce sharp peaks in the friction spectrum.

Because of the high vibrational friction and high solute-mode populations at low frequency, it is generally thought that vibrational energy transfer takes place predominantly between low frequency solute and solvent modes. However, as we will now discuss, the solvent isotope effect for vibrational cooling suggests that this is not that case.

3.3.7 Source of the Solvent Isotope Effect

We have described how the solvent dependence of vibrational cooling suggests that VC rates are determined by the rate of solute-solvent vibrational energy transfer (Section 3.3.4) and that the rate of vibrational energy transfer is proportional to the magnitude of the vibrational friction from the solvent (Section 3.3.6). We will now discuss the possibility that the solvent isotope effect for vibrational cooling can be understood in terms of the vibrational friction spectrum of H₂O vs. D₂O. Currently, the only way to obtain complete friction spectra is through molecular dynamics simulations. Vibrational friction spectra for both H₂O and D₂O have been simulated using the solutes CN⁻, ClO⁻, and OCIO. In principal, the friction spectrum depends on the solute and can be different for these small molecules compared to 9MA. In practice, the three evaluated friction spectra are nearly identical and, in particular, here we are interested in comparing the qualitative difference between friction spectra of H₂O and D₂O which are the same for all three
solutes. Thus, these friction spectra provide a reasonable, qualitative, approximation to the friction spectra of 9MA.

In all cases studied, the friction spectra of H$_2$O and D$_2$O are nearly identical at frequencies below 700 cm$^{-1}$. Additionally, diatomic molecules whose sole vibrational frequency lies near or below 700 cm$^{-1}$, ClO$^-$ (713 cm$^{-1}$) and I$_2^-$ (113 cm$^{-1}$), show identical vibrational relaxation rates in H$_2$O vs. D$_2$O and ethanol vs. ethanol-$d_1$, respectively. On the other hand, the vibrational friction spectra of H$_2$O and D$_2$O differ significantly at higher frequencies. Accordingly, vibrational relaxation of CN$^-$, which has a frequency near 2000 cm$^{-1}$, does exhibit a solvent isotope effect in water. Other small molecules with frequencies above 700 cm$^{-1}$ undergo slower vibrational relaxation in D$_2$O than H$_2$O: N$_3^-$ and a series of pseudo-halide anions XCN$^-$ (X=O, S, Se).

These trends are reasonable considering the types of motions that constitute the solvent vibrations below and above 700 cm$^{-1}$. The low frequency motions are due to hindered translations. The frequencies of these translations differ insignificantly between H$_2$O and D$_2$O because they depend on the total molecular masses and the magnitude of the intermolecular forces, both of which differ by $\leq 10\%$. On the other hand, higher frequency motions near and above 700 cm$^{-1}$ arise from hindered rotational motions, also called librations. Librational frequencies in H$_2$O vs. D$_2$O differ by a factor equal to the square root of the moments of inertia, $(I_{H_2O}/I_{D_2O})^{1/2}$. This quantity is different for different rotational axes of the water molecule but varies between 0.71 and 0.75, a much larger effect than for translations. At even higher frequencies, intramolecular vibra-
tional frequencies shift by a similar factor because of the different reduced mass of H$_2$O vs. D$_2$O.

Based on this analysis we propose that the solvent isotope effect observed for vibrational cooling of 9MA results from different rates of energy transfer between solute and solvent vibrations with frequencies above $\sim$700 cm$^{-1}$. Librational modes between roughly 700 and 1000 cm$^{-1}$ are particularly likely to be important. These are the lowest frequency modes for which there is a significant friction difference between H$_2$O and D$_2$O. Furthermore, these librations lie at such high frequencies due to the strong solvent-solvent hydrogen bonding in these solvents. Thus high-frequency librations may be linked to the ability of hydrogen-bonding solvents to accelerate vibrational cooling. The first overtone of these librational modes has good energy matching to the many vibrations of 9MA that lie between about 1400 and 1800 cm$^{-1}$ and therefore could efficiently accept energy from these modes.

### 3.4. Conclusions

UV transient absorption in a variety of solvents has presented a number of insights in the ground-state vibrational cooling of 9MA. The solvent dependence of the VC rates cannot be accounted for by macroscopic properties of the solvent and suggests that vibrational cooling is strongly accelerated by solute-solvent hydrogen bonding. This, together with the solvent isotope effect, suggests that the limiting factor in determining the VC rate is the rate of vibrational energy transfer to the first solvent shell. The solvent isotope effect furthermore suggests that a significant fraction of the excess vibrational
energy in the hot ground state exits the molecules through unexpectedly high-frequency vibrations.

It is not clear how these aspects of DNA base vibrational cooling will survive in single- or double-stranded DNA. Base stacking in DNA strands limits base exposure to the solvent. However, both base stacking and base pairing introduce vibrational couplings between bases,\textsuperscript{49,50} perhaps providing new pathways for vibrational relaxation. Further studies are required to explore these questions.

3.5. References


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<th>τ2 (ps)</th>
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<td>2.0 ± 0.2</td>
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Table 3.1. Best fit parameters for transient absorption of 9-methyladenine.
<table>
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<th>D₂O</th>
<th>methanol</th>
<th>acetonitrile</th>
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<td>Thermal Diffusivity (10&lt;sup&gt;8&lt;/sup&gt; m&lt;sup&gt;2&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>14.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.7&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Thermal Conductivity&lt;sup&gt;d&lt;/sup&gt; (W m&lt;sup&gt;-1&lt;/sup&gt; K&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.61</td>
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<td>1.00</td>
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<td>0.11</td>
<td>0.11</td>
<td>0.03</td>
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<sup>a</sup> Calculated using τ<sub>2</sub> from Table 1 at 250 nm.
<sup>b</sup> from Ref. 51.
<sup>c</sup> from Ref. 52.
<sup>d</sup> data for D₂O from Ref. 53, others from Ref. 54.
<sup>e</sup> calculated as in Ref. 25.

Table 3.2. Comparison of vibrational cooling rates for 9MA and macroscopic solvent properties
Figure 3.1. Vibrational relaxation rate modulate reaction pathways.
Figure 3.2. Structure of 9-methyladenine.
Figure 3.3. Ground-state UV absorption spectra of 9-methyladenine in H$_2$O (blue, solid), methanol (green, dotted) and acetonitrile (red, dashed).
Figure 3.4. Ground-state UV absorption spectra of 9-methyladenine in H\textsubscript{2}O (blue, solid) and D\textsubscript{2}O (green, dashed). The D\textsubscript{2}O spectrum is also shown shifted by 0.5 nm (red, dashed).
Figure 3.5. Normalized transient absorption signals for 9-methyladenine in H₂O. Excitation was at 270 nm and probe wavelengths were 255 nm (blue squares), 285 nm (red circles) and 570 nm (green crosses). Solid lines are fits to the data.
Figure 3.6. Transient absorption of 9-methyladenine in H$_2$O at probe wavelengths of 250 nm (blue squares) and 255 nm (red circles). The data has been scaled to agree at delay times $> 3$ ps. Solid lines are fits to the data.
Figure 3.7. Transient absorption of 9-methyladenine at 570 nm in H₂O (blue circles), methanol (green squares), and acetonitrile (red triangles), following excitation at 267 nm.
Figure 3.8. Schematic depiction of the hot ground-state absorption spectrum (red) of 9MA with respect to the room-temperature absorption spectrum (blue). Select probe wavelengths used in this study are noted.
Figure 3.9. Transient absorption at 255 nm (negative $\Delta A$) and 285 nm (positive $\Delta A$) of 9MA in H$_2$O (blue circles) and D$_2$O (red squares).
Figure 3.10. Schematic depiction of the density of states in the solute and the vibrational friction from the solvent bath.
CHAPTER 4

EXCITED-STATE DYNAMICS OF PYRIMIDINE BASES

4.1. Introduction

The majority of photoexcited nucleic acid bases return to their ground state ($S_0$) via internal conversion, a nonradiative process that converts chemically-useful electronic energy into comparatively-inert vibrational energy. The initially-excited $^1\pi\pi^*$ states of all five canonical nucleosides decay with a lifetime of 1 ps or less in solution.\textsuperscript{1,2} This ultra-fast excited-state deactivation has been speculated as the basis for DNA’s photostability.\textsuperscript{1,4}

Recent work, however, has shed new light on this picture. For example, 2006 marked the discovery, in pyrimidine bases, of slower excited-state decay pathways through $^1\pi\pi^*$ and $^3\pi\pi^*$ states.\textsuperscript{5,6} The $^1\pi\pi^*$, $^1\pi\pi^*$, $^3\pi\pi^*$ and $S_0$ states of single pyrimidine bases have strongly-overlapping electronic absorption spectra which complicates the study of their dynamics.

Vibrational absorption spectra, more so than electronic absorption spectra, are unique to a given species or electronic state. We therefore seek unique vibrational marker bands for the relevant electronic states of pyrimidine bases using femtosecond time-
resolved IR spectroscopy (TRIR). Marker bands allow for the dynamics of different states to be followed independently, when their electronic spectra may significantly overlap. TRIR techniques have been fruitfully applied to the study cyclobutane dimer formation in thymine oligonucleotides.\textsuperscript{7}

Here we report TRIR studies of the excited-state dynamics of the pyrimidine bases shown in Figure 4.1, thymine and 1-cyclohexyluracil, in the carbonyl stretch region (1500-1800 cm\textsuperscript{-1}). Acetonitrile was chosen as a solvent because the $^1\text{n}\pi^*$ and $^3\pi\pi^*$ states of pyrimidine bases are significantly populated in organic solvents.\textsuperscript{5} Measurements in methanol-$d_1$ were also made for comparison.

We identify unique marker bands for both the $^1\text{n}\pi^*$ and $^3\pi\pi^*$ states. The $^1\text{n}\pi^*$ marker band maximum lies at higher frequency than nearby ground-state vibrations and is independent of solvent. We suggest that this vibration involves stretching of the C4-C5 bond. Triplet marker bands are consistent with recent nanosecond TRIR measurements as well as calculations.\textsuperscript{8} The dynamics of the $^3\pi\pi^*$ band is consistent with recent conclusions about the intersystem crossing rate in these systems.\textsuperscript{5}

4.2. Results

4.2.1 Ground-State Spectra

The steady-state IR spectra of CHU in methanol-$d_1$ (black), and acetonitrile (light grey) as well as thymine in acetonitrile-$d_3$ (dark grey), in the 1575 – 1800 cm\textsuperscript{-1} region, are shown in Figure 4.2. The steady-state spectra are assumed to contain contributions from only the ground state. All spectra contain at least two strong bands due to C=O stretch
vibrations. For thymine in acetonitrile-$d_3$, there are two strong bands at 1683 and 1724 cm$^{-1}$ which are assigned to C4=O and C2=O stretching vibrations (see Figure 4.1 for numbering), respectively, following Hare et al.$^8$ and others.$^{9-13}$

For CHU in acetonitrile these peaks shift to 1691 and 1709 cm$^{-1}$. Similar shifts have been observed when comparing thymine and thymidine.$^8$ In methanol-$d_1$, the two most intense bands in the CHU spectrum shift to 1655 and 1674 cm$^{-1}$. Hydrogen bond donating solvents are known to shift carbonyl stretching frequencies by as much as 50 cm$^{-1}$. Weaker bands in the spectra are due to carbon-carbon stretches and other skeletal vibrations.$^{9-13}$

4.2.2 Mid-IR Transient Absorption Spectra of CHU

Transient spectra obtained for CHU in acetonitrile are shown in Figure 4.3. The most prominent features are negative ground-state bleach bands at 1688 and 1713 cm$^{-1}$. The bleach signals decay with two components, one with a lifetime of 16 ± 2 ps and another with a lifetime of 3-6 ns. Also present is a weak positive band at 1760 cm$^{-1}$ with a FWHM of 33 cm$^{-1}$. The latter band decays with a single lifetime of 3.1 ± 0.8 ns.

Transient spectra obtained for CHU in methanol-$d_1$ are shown in Figure 4.4. The results are qualitatively similar to those in acetonitrile but the decay of all transient features is accelerated and residual offsets are not seen at long time delays. Bleach bands are observed at 1705 and 1673 cm$^{-1}$, respectively, and decay with 10.8 ± 1.6 ps and 0.52 ± 0.06 ns lifetimes. The positive band at 1760 cm$^{-1}$ decays with a single lifetime of 0.51 ± 0.09 ns.
4.2.3 Mid-IR Transient Absorption Spectra of Thymine

Figure 4.5a shows a transient spectrum obtained for thymine in actonitrile-d$_3$. To improve signal-to-noise, the spectrum was generated by averaging spectra obtained at time delays between 0.03 and 3 ns. Negative ground-state bleach bands are observed at 1683 and 1726 cm$^{-1}$. Positive bands are observed at 1603 and 1714 cm$^{-1}$. The latter band is partially obscured by the nearby bleach bands. This spectrum is in excellent agreement with the nanosecond TRIR and calculated transient spectra present in Figure 4.5b and Figure 4.5c, respectively.

Transient kinetic decays at 1602 cm$^{-1}$ and 1628 cm$^{-1}$ are shown in Figure 4.6. Signals occurring just before and at time zero are due to perturbed free-induction decay$^{15}$ and the optical Kerr effect,$^{16}$ respectively. The latter signal provides a measure of the instrument response time, which we estimate to be 450 fs (FWHM). At delays between 0 and 10 ps, the signal is dominated by a broad positive absorption band initially centered at ~1640 cm$^{-1}$. This band subsequently narrows, shifts to higher frequencies and decays by 30 ps. Due to these complex spectral dynamics, the transient absorption signal at 1628 cm$^{-1}$ rises to a maximum at 5 ps and decays to zero by 30 ps, while the signal measured at 1602 cm$^{-1}$ rapidly decays from its initial amplitude to a small offset by 10 ps. This offset is due to the presence of another much weaker band centered at 1600 cm$^{-1}$. This band shows no change in intensity or spectral shape, within experimental uncertainty, from 10 to 2800 ps.
4.3. Discussion

4.3.1 Ultrafast Decay Channel

The major decay channel for single bases following 270 nm excitation to the $^1\pi\pi^*$ state is rapid internal conversion.\cite{1,2} The $^1\pi\pi^*$ lifetime for CHU in methanol and acetonitrile is 0.14 and 0.20 ps, respectively.\cite{5} Because our instrument response is 0.45 ps, we could not detect any positive features assignable to the $^1\pi\pi^*$ state. However, evidence for this ultrafast decay channel is provided by the bleach recovery signals. At 1688 cm$^{-1}$ the bleach of CHU in acetonitrile recovers with two time constants, 16 ps and $\sim$4 ns. A recovery component matching the $^1\pi\pi^*$ lifetime is not observed because, for this decay channel, return to the ground-state minimum is rate-limited by vibrational cooling within the ground-state vibrational manifold.\cite{17} The vibrational cooling time for thymidine in acetonitrile measured with UV transient absorption is 9.1 ps,\cite{5} in good agreement with the fast bleach-recovery component. The fast recovery component for CHU in methanol-$d_1$ is 11 ps. The vibrational cooling time for CHU in methanol measured with UV transient absorption is 3.7 ps. Vibrational cooling of DNA bases is strongly solvent dependent and is accelerated in protic solvents (see Chapter 3). The presence of the $\sim$4 and 0.52 ns recovery components in acetonitrile and methanol-$d_1$, respectively, is evidence of additional decay channels through intermediate, longer-lived excited states.

4.3.2 Marker Band for the $^1n\pi^*$ State

The most notable feature in the transient spectra of CHU is the positive band centered at 1760 cm$^{-1}$ in acetonitrile and methanol-$d_1$. The band appears within the instru-
ment response time of 0.45 ps and decays with a 3.1 ns lifetime. The lifetime is in excellent agreement with the 3.2 ns lifetime of the $^1n\pi^*$ state in acetonitrile measured via UV-visible transient absorption. The band decays in methanol-$d_1$ with a 0.51 ns lifetime, while the lifetime measured in methanol with UV-visible transient absorption is 0.110 ns. The origin of this difference is under investigation. However, given the agreement in acetonitrile and the consistent trend with solvent, we assign this band to the $^1n\pi^*$ state.

Promotion of an electron from a nonbonding orbital to an anti-bonding orbital should, in general, weaken bonds and shift excited-state vibrations to lower frequencies. Indeed, this holds true in many cases. However, there are no significant ground-state vibrations whose frequency could reasonably be expected to shift down to 1760 cm$^{-1}$. The $^1n\pi^*$ band must therefore arise from a lower-frequency ground-state vibration. The two most likely candidates are C=O and carbon-carbon stretch vibrations.

Carbonyl stretching frequencies can shift to higher frequencies as a result of solute-solvent hydrogen-bond breaking. This explanation can be ruled out because the $^1n\pi^*$ band is observed at the same frequency in both acetonitrile and methanol-$d_1$. High-level quantum calculations on pyrimidine-base excited states predict that the C4-C5 bond contracts by 0.075 Å in the $^1n\pi^*$ state. This suggests that the 1760 cm$^{-1}$ band belongs to a vibration involving the C4-C5 stretching coordinate. High-quality theoretical calculations of the $^1n\pi^*$ normal modes would aide in a definitive assignment.

4.3.3 Marker Band for the $^3\pi\pi^*$ State

High-quality normal mode calculations have already been performed for the lowest-energy $^3\pi\pi^*$ state. Together with nanosecond TRIR measurements, the calculations
allow for definitive assignment of the positive bands seen in Fig. 5. The band observed at 1603 cm⁻¹ is assigned to the C4=O stretch. The band observed at ~1714 cm⁻¹ is assigned to the C2=O stretch. This latter band is also apparent in the transient spectra for CHU in acetonitrile at long delay times, see Figure 4.3.

Unfortunately, the rise of the triplet bands is obscured by larger amplitude bands at delay times below 10 ps, making it impossible to precisely determine the intersystem crossing rate. The larger amplitude bands narrow, shift to higher frequency, and decay by 30 ps. This behavior is a well-known signature of vibrational relaxation and these bands are assigned to vibrationally-excited, ground-state C4=O stretch, resulting from internal conversion to S₀ from the ¹ππ* state.

The IR experiments also show that the triplet state is fully formed on a timescale significantly faster than the ¹nπ* lifetime. This result suggests that intersystem crossing from the ¹nπ* state to the ³ππ* state occurs before the excited population reaches the minimum on the ¹nπ* potential surface, supporting previous conclusions from UV-visible transient absorption measurements.

4.4. Conclusions

These studies demonstrate the advantages of TRIR for elucidating the nature and dynamics of pyrimidine-base excited states. The identification of unique marker bands for the ¹nπ* and ³ππ* states will allow for further studies on these systems. This will be particularly important for future studies on DNA oligomers where the presence of additional excited states results in even greater spectral congestion. Because vibrational frequencies are sensitive to the electronic and structural properties of the molecule, these
spectra additionally provide specific criteria for evaluating the accuracy of the high-level quantum mechanical calculations performed on pyrimidine bases.
4.5. References


Figure 4.1. Structures and numbering for thymine, CHU and CHU-$d_1$. 

Thymine: $R1 = CH_3$, $R2 = H$, $R3 = H$

CHU: $R1 = H$, $R2 = C_1C_2$, $R3 = H$

CHU-$d_1$: $R1 = H$, $R2 = C_1C_2$, $R3 = D$
Figure 4.2. Steady-state IR spectra of CHU\textsubscript{-d$_1$} in methanol\textsubscript{-d$_1$} (blue), CHU in acetonitrile (red) and thymine in acetonitrile\textsubscript{-d$_3$} (green).
Figure 4.3. Transient mid-IR spectra of CHU in acetonitrile following excitation at 270 nm. Spectra are labeled with their respective time delays.
Figure 4.4. Transient mid-IR spectra of CHU in methanol-$d_1$ following excitation at 270 nm. Spectra are labeled with their respective time delays.
Figure 4.5. Transient mid-IR spectra of thymine in acetonitrile-$d_3$. Top: fs-TRIR spectrum averaged over 0.03 - 3 ns. Middle: ns-TRIR spectrum averaged over 0 - 1 μs. Bottom: Simulated transient spectrum calculated at the B3LYP/6-31++G** level. Figure adapted from Ref. 8.
Figure 4.6. Transient kinetics of thymine in acetonitrile-$d_3$ at 1602 cm$^{-1}$ (solid blue circles) and 1628 cm$^{-1}$ (open red circles). Delay times after 30 ps are shown on a logarithmic scale. Solid lines are provided to guide the eye.
CHAPTER 5

EXCITED-STATE DYNAMICS OF POLY(A)

5.1. Introduction

Initial studies of DNA photophysics focused on individual bases as these are the fundamental building blocks of DNA. Of course, cellular DNA consists of billions of individual bases strung together along a sugar-phosphate backbone. Naturally, the field of DNA photophysics has consistently moved toward more complex systems involving more than one base, hereafter referred to as base multimers.

Based on other multichromophoric systems, e.g. light-harvesting complexes, dendrimers, polymers and self-assembled systems, it could be expected that the close proximity between DNA base chromophores in DNA strands would lead to interbase electronic interactions and therefore different states and dynamics. Early steady-state fluorescent studies suggested that this was the case, as multimers exhibit a second red-shifted emission band not seen from monomers (see Ref. 1). Later, time-resolved measurements showed that the dynamics of multimers can, in fact, be orders of magnitude slower than monomers.2-6

The long-lived states observed in multimers have long been described by the term excimer because of their red-shifted emission. Excimers have historically referred to a
molecular complex between two identical molecules in which only one is initially excited. It is not clear that excitations in base monomers are initially localized on a single base, however, the IUPAC definition of the term is vague enough to allow its use, with the above caveat regarding its historical interpretation noted.

An ever-growing number of studies have been conducted on base multimers with generally consistent results.\textsuperscript{2-6} However, the interpretation of these results have been the subject of much controversy. Some groups have suggested that the long-lived states observed in multimers are excitonic in nature and are delocalized over many bases, varying between 2.5-10.\textsuperscript{6-10} Growing evidence suggests, however, that these excited states are highly localized and are excimer-like in nature.\textsuperscript{2}

Because vibrational spectra are particularly sensitive to the bonding within a molecule which is determined by the electronic nature of the state it is in. Mid-IR characterization of the excimer state could therefore provide a useful metric for understanding the nature of the long-lived state in multimers. Here we describe UV and mid-IR probe transient absorption measurements on poly(A) in D\textsubscript{2}O solution. For comparison we also present UV and mid-IR transient absorption measurements on AMP in D\textsubscript{2}O. This comparison allows for an estimate of the quantum yield for formation of the long-lived state. Comparison of the transient and ground-state absorption spectra for poly(A) suggests that the IR band near 1630 cm\textsuperscript{-1} lies at slightly lower frequency in the excimer state than in the ground-state.
5.2. Results

5.2.1 Mid-IR transient absorption spectra

Mid-IR transient absorption spectra of AMP in D₂O following 270 nm excitation are shown in Figure 5.1 at ten time delays ranging from 1 to 100 ps. At 1 ps there are three main spectral features. The most prominent feature is a large bleach band centered at 1623 cm⁻¹. Another, weaker bleach band is present at 1573 cm⁻¹ but is largely offset by a broad positive absorption feature with a maximum at 1597 cm⁻¹. The two bleach bands are close in frequency to ground-state absorption bands of AMP. The maximum of the broad positive band both shifts to higher frequency, narrows in width and decays in amplitude within 10 ps. The maximum of the large bleach band at 1623 cm⁻¹ remains nearly independent of time delay but its amplitude decays with a 5.1 ± 0.4 ps time constant to a small offset (see Figure 5.2). The amplitude of this offset is 4% of the maximum bleach amplitude.

Mid-IR transient absorption spectra of poly(A) in D₂O following 270 nm excitation are shown in Figure 5.3 at eleven time delays ranging from 1 to 2900 ps. The spectral features in Figure 5.3 are similar to those observed for AMP (see Figure 5.1) although the frequencies are different. A strong bleach is observed at 1629 cm⁻¹ and a weaker bleach at 1571 cm⁻¹. The broad positive absorption observed for AMP is also present for poly(A) and, at 1 ps, is nearly flat from 1610 to 1585 cm⁻¹. At earlier delay times the dynamics of poly(A) are also similar to AMP. The broad positive feature narrows, shifts to higher frequency and decays within 20 ps. Also like AMP, the maximum of the large bleach band at 1629 cm⁻¹ remains nearly independent of time delay.
The most significant difference between AMP and poly(A) is that the bleach of the latter does not decay with a single time constant like in the former system, as shown in Figure 5.4. The transient at 1629 cm\(^{-1}\) is best fit with the sum of two exponential terms with time constants of 5.1 ± 0.3 and 195 ± 18 ps, respectively, and an offset. As can be seen from the 2900 ps spectrum in Figure 5.3, the long-lived offset has two contributions. One is a broad bleach that covers the entire spectral range of the measurement, as seen for AMP, and another, comparatively narrow, bleach band similar in shape and frequency as the strong bleach band at 1 ps. The amplitude of the former bleach at 50-100 ps is 2.5% the maximum bleach amplitude.

5.2.2 UV transient absorption

The transient absorption at 250 nm of AMP and poly(A) in buffer solution is shown in Figure 5.5. Like in the mid-IR the ground-state bleach signals recover with a single time constant in AMP and with two time constants in poly(A). AMP recovers with a 2.2 ± 0.6 ps time constant while poly(A) recovers with time constants of 3.5 ± 0.5 ps and 0.20 ± 0.07 ns. The fast component of the poly(A) signal comprises 79 ± 3% of the total signal amplitude with the slow component comprising the remaining 21 ± 3%.

The transient absorption at 250 nm of AMP and poly(A) in D\(_2\)O solution are shown in Figure 5.6. The results are very similar to those obtained in buffer solution although the fast time constants are slower. In D\(_2\)O, AMP recovers with a time constant of 2.9 ± 0.2 ps while poly(A) recovers with time constants of 4.9 ± 0.9 ps and 0.34 ± 0.14 ns. The fast component of the poly(A) signal comprises 78 ± 3% of the total signal amplitude with the slow component comprising the remaining 21 ± 3%.
5.3. Discussion

5.3.1 Ultrafast internal conversion to the ground state and vibrational cooling.

The mid-IR spectral dynamics observed for both AMP and poly(A) during the first 10-20 ps have been seen for other systems\textsuperscript{11-13} and are highly indicative of vibrational cooling. Furthermore, the 5 ps bleach recovery constant is consistent with the vibrational cooling dynamics of these systems measured in the UV. The excited-state lifetime of AMP is well known to be under 1 ps. We are unable to directly detect this time-constant in our measurements because of an instrument response function of 450 fs and the spike observed at zero time delay. However, the vibrational cooling signals are evidence of this subpicosecond decay to the ground state.

The time constants for bleach recovery of AMP in D\textsubscript{2}O at 1623 cm\textsuperscript{-1} and 250 nm, 5.1 and 2.9 ps, respectively, are similar to the vibrational cooling time observed for 9MA in D\textsubscript{2}O, 4.2 ps, supporting assignment of these dynamics to vibrational cooling. The shorter vibrational cooling times observed for AMP in H\textsubscript{2}O buffer solution at 250 nm, 2.2 ps, reflect the solvent isotope effect for vibrational cooling discussed in Chapter 3. The different vibrational cooling times observed in the mid-IR and UV suggests that there are mode-specific vibrational dynamics during vibrational cooling.

Given that vibrational cooling contributes significantly to the overall poly(A) signal, it is clear that a large percentage of excitation event in poly(A) results in ultrafast internal conversion to the ground state. The structure of poly(A) in solution is disordered\textsuperscript{14,15} and these monomer-like decay pathways likely result from bases which are not well stacked with neighboring bases along the strand. Assuming a simple two-state model
for stacked and unstacked bases in poly(A), the relative amplitudes of the vibrational cooling component in AMP and poly(A) can provide an estimate of the fraction of stacked bases using Equation 5.1.2

\[ f = 1 - \frac{\Delta A_{\text{poly(A)},1}}{\Delta A_{\text{AMP}}} (1 - h) \]  

where \( f \) is the fraction of stacked bases, \( \Delta A_{\text{poly(A)},1} \) is the amplitude of the fast component in the poly(A) signal at 250 nm, \( \Delta A_{\text{AMP}} \) is the amplitude of the AMP signal at 250 nm and \( h \) is the hypochromism of the ground-state absorption coefficient at the pump wavelength.

\[ h = 1 - \frac{\varepsilon_{\text{poly(A)}}}{\varepsilon_{\text{AMP}}} \]  

Using a hypochromism of 0.367 for poly(A)\(^{16}\) and signal amplitude obtained for nonlinear least-square fitting, a stacking fraction of ~54% is obtained.

### 5.3.2 Local heating of the solvent

Vibrational cooling of DNA bases following ultrafast internal conversion to the ground state deposits > 30 000 cm\(^{-1}\) of energy into the surrounding solvent (see Chapter 3). This leads to a temperature increase in the D\(_2\)O solvent within the laser focus. As previously observed by Schreier et. al\(^{17}\) this local heating affects the IR spectrum of D\(_2\)O and produces a weak but broad bleach signal extending from roughly 1500 to 1800 cm\(^{-1}\). We therefore assign the broad bleach observed for AMP and poly(A) at time delays greater than 50 ps to hot D\(_2\)O solvent.
The bleach induced by local heating contributes to 2.5% and 4% of the overall
time-zero bleach amplitude in poly(A) and AMP, respectively. The different amplitudes
reflect the different quantum yields for ultrafast internal conversion in these two systems.
While nearly 100% for AMP (see Section 3.3.1), the quantum yield for poly(A) is lower
due to the presence of additional decay pathways through long-lived intermediate states.
As shown by Schreier et. al, the magnitude of the bleach is roughly proportional to the
temperature increase of the solvent, at least for the relatively small temperature range
relevant here ($\Delta T < 20$ K).\cite{17} We furthermore suggest that the temperature increase is
proportional to the number of excitations that lead to ultrafast internal conversion to the
ground state. Under these assumptions, comparison of the relative amplitude of the hot
D$_2$O signal in AMP and poly(A) provides an estimate of the quantum yield for ultrafast
internal conversion to the ground state in poly(A). This estimate places the quantum yield
at ~63%. This is in excellent agreement with the stacking fraction of 54% obtained from
analysis of transient absorption signals at 250 nm (see Section 5.3.1).

5.3.3 Excimer state lifetime and spectrum

The long-lived transients observed in the mid-IR and UV transient absorption sig-
nals of poly(A) are consistent with those previously attributed to excimer-like states.\cite{2-4,18}
The lifetime of this state observed at 1629 cm$^{-1}$ in D$_2$O, 0.195 ns, is in excellent agree-
ment with the lifetime obtained at 250 nm in buffer, 0.20 ns. The lifetime obtained from
fitting the 250 nm signal in D$_2$O is slightly longer, 0.34 ns, but there is a large uncertainty
in this value (41%) and we do not feel this difference is significant. The lifetime of the
excimer state measured here is also in reasonable agreement with lifetimes previously observed in homoadenine oligomers which ranged from 126-182 ps.\textsuperscript{3,4,18}

It is of great interest to determine marker bands for the excimer state in DNA oligomers. As shown in Figure 5.4, no positive signals are observed at time delays greater than 50 ps when only excimer signals remain. This does not mean that no excimer bands exist in this region, merely that the extinction coefficient is greater in the ground-state than in the excimer state at all frequencies in this region. Direct comparison of the long time delay transient spectra and the inverted ground-state absorption spectrum in Figure 5.7 makes clear that the signal neat 1630 cm\textsuperscript{-1} is not a pure bleach. This suggests that there is a positive absorption feature from the excimer state between 1630 and 1615 cm\textsuperscript{-1}.

5.3.4 Conclusions

Femtosecond mid-IR transient absorption spectroscopy has been used to investigate the excited state dynamics of poly(A) and AMP. These studies, in general, support the results of previous studies that probed in the UV and visible regions. We have proposed a method of estimating the quantum yield for ultrafast internal conversion in DNA multimers. The method takes advantage of the temperature-dependent IR spectrum of water and the local heating of the solvent that results from vibrational cooling. The quantum yields estimated in the manner are consistent with a previously method that used UV probe transient absorption signals. While an IR marker band for the excimer was not directly resolved, comparison of the bleach signal at long time delay with the ground-state absorption band suggests that an excimer band exists near 1620 cm\textsuperscript{-1}. These results set the basis for future IR studies that can explore these questions more thoroughly.
5.4. References


Figure 5.1. Mid-IR transient absorption spectra of AMP in D₂O following 270 nm excitation. The legend indicates the time delays at which the spectra were taken.
Figure 5.2. Mid-IR transient absorption of AMP in D₂O following excitation at 270 nm. Kinetic trace at 1623 cm⁻¹ (crosses) and best fit curve (solid line).
Figure 5.3. Mid-IR transient absorption spectra of poly(A) in D₂O following 270 nm excitation. The legend indicates the time delays at which the spectra were taken.
Figure 5.4. Mid-IR transient absorption of poly(A) in D$_2$O following excitation at 270 nm. Kinetic trace at 1629 cm$^{-1}$ (crosses) and best fit curve (solid line). The time delay axis is presented on a linear scale up to 15 ps and a logarithmic scale thereafter.
Figure 5.5. Transient absorption at 250 nm of AMP (red squares) and poly(A) (green circles) in buffer solution following 267 nm excitation. The time delay axis is presented on a linear scale up to 15 ps and a logarithmic scale thereafter. Solid lines are best fit curves.
Figure 5.6. Transient absorption at 250 nm of AMP (red squares) and poly(A) (green circles) in D$_2$O solution following 267 nm excitation. The time delay axis in presented on a linear scale up to 15 ps and a logarithmic scale thereafter. Solid lines are best fit curves.
Figure 5.7. Normalized transient mid-IR spectra of poly(A) in D$_2$O at 100, 250 and 500 ps (red, green and blue, respectively). The ground-state IR absorption of poly(A) is also shown inverted and scaled for comparison (thick black line).

(2) *From DNA Photolesions to Mutations, Skin Cancer and Cell Death*; Evelyne Sage; Régen Drouin; Rouabhia, M., Eds.; Royal Society of Chemistry, 2005.


