A Tale of Coupled Vibrations in Solution Told by Coherent Two-Dimensional Infrared Spectroscopy

by

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B.A. Colgate University, 1998

Submitted to the Department of Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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Abstract

Coherent two-dimensional infrared (2D IR) spectroscopy is used as a tool for investigating the molecular structure and dynamics of coupled vibrations in solution on a picosecond timescale. The strongly coupled asymmetric and symmetric carbonyl stretches of Rh(CO)$_2$C$_8$H$_7$O$_2$ (RDC) dissolved in hexane serve as a convenient model system. Fourier transform 2D IR spectra are obtained from heterodyne-detected third-order nonlinear signals using a sequence of broad bandwidth femtosecond IR pulses. A 2D IR correlation spectrum with absorptive lineshapes results from the addition of 2D rephasing and non-rephasing spectra, which sample conjugate frequencies in the evolution time period. The 2D IR correlation spectrum contains peaks with different positions, signs, amplitudes and lineshapes. The positions of the peaks map the transition frequencies between the ground, singly, and doubly excited states of the system, and thus describe the anharmonic vibrational potential. Peak amplitudes reflect the relative magnitudes and orientations of the transition dipole moments in the molecular frame, the electrical anharmonicity of the system, and the vibrational relaxation dynamics. The 2D line shapes are sensitive to the complicated system-bath interactions in solution. 2D IR spectra taken with varying polarization conditions and as a function of a variable waiting time can be used to isolate and quantify these spectroscopic observables. The polarization-selective 2D IR spectra of RDC in hexane are analyzed in terms of two coupled local coordinates to obtain their mutual orientation and the magnitude of the coupling between them. Evidence of vibrational coherence transfer between close-lying transition frequencies is indicated by the presence of extra relaxation induced peaks in 2D IR rephasing spectra. The data is modeled by using Redfield theory to account for coherence transfer, vibrational dephasing and population relaxation in a multilevel vibrational system. Building on the studies of the RDC model system, 2D IR spectroscopy is used to study the thermal denaturation of RNase A by characterizing the temperature-dependent Amide I band. A nonlinear IR probe is used to study the early events in the laser temperature-jump initiated denaturation of RNase A.

Thesis Supervisor: Andrei Tokmakoff
Title: Associate Professor of Chemistry
To Ma, Baba and Huzefa
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Chapter 1

Introduction

1.1 Solution phase chemistry – a microscopic viewpoint

From living cells to test tubes, a vast number of important chemical reactions occur in solution. From a microscopic viewpoint, understanding a chemical reaction in solution requires knowledge of the many forces within and between molecules, which dictate the time-evolution of molecular species from reactants to products. In other words, it is ultimately the altering microscopic environment of the solute and the surrounding solvent that determines the course of a chemical reaction. The experimental visualization of molecular dynamics in solution requires both high time and spatial resolution. Additionally, the experimentalist should be able to relate the experimental observables to meaningful microscopic parameters allowing the translation of the experimental data into motion pictures of molecules.

This thesis reports on the development of coherent two-dimensional infrared (2D IR) spectroscopy – an experimental technique, which has the potential to follow time-evolving structures in solution. Experiments on a model system of two coupled vibrations show how 2D IR spectroscopy can directly probe vibrational couplings, transition dipole orientations and vibrational relaxation processes. A theoretical framework is provided within which the spectroscopic observables are related to anharmonicities in the nuclear potential, angles between local molecular coordinates and timescales of solute-solvent interactions. These efforts are then extended to understand protein folding – a biologically significant chemical reaction.
1.2 Assembling a new spectroscopic toolkit

Effective experimental probes of molecular dynamics in solution have to be sensitive to structural changes on picosecond \( (10^{12} \text{ s}) \) and longer timescales. This is because the timescale for nuclear motions in small molecules is on the order of \( 10^{13} - 10^{12} \text{ s} \). It is misleading to think of a single molecular structure for a species in solution. For example, the solute molecules may simultaneously lie in multiple different solvent environments, which are constantly changing. This adds the additional constraint that the experimental probe should be sensitive to structural variation within an ensemble and the time-evolution of the structural variance.

1.2.1 Accessing a second dimension in infrared spectroscopy

One-dimensional (1D) vibrational spectroscopies in the time or frequency domain have the intrinsic time-resolution required for such studies and have been used to study molecular dynamics in solution. Vibrational frequencies of a molecule are determined by its chemical structure (bond-strengths and masses of the vibrating atoms) and its surroundings. This means that the positions and amplitudes in a vibrational spectrum will be extremely sensitive to the bond-lengths, bond-strengths, bond angles and conformation. In addition to the positions and intensities, the vibrational line-widths are a sensitive measure of the structural heterogeneity or conformational freedom present in the molecular sample. Since the molecule interacts with its surroundings the above-mentioned parameters will be affected by solvent-induced local electric fields and hydrogen bonding effects. The major disadvantage of 1D spectroscopies is that it projects the ensemble-averaged response of multiple nuclear coordinates onto one frequency or time axis. Additionally, the vibrational spectra of molecules in solution often contain broad and featureless lineshapes. This makes the interpretation of 1D spectra ambiguous because a single set of experimental observables is mutually affected by multiple molecular coordinates, making it virtually impossible to disentangle all the underlying molecular information.

Multidimensional, nonlinear coherent spectroscopies have been developed to address this problem by disentangling the underlying molecular interactions that are obscured in traditional 1D spectra. Such techniques use interactions with a sequence of radiation fields to monitor a system as a function of multiple time periods or frequency variables. In this manner, coherent nonlinear vibrational spectroscopic techniques can study the correlation of nuclear motions as a function of the multiple experimental time periods yielding information about time-dependent vibrational couplings and relative orientations between interacting chemical moieties.
Two-dimensional (2D) methods have been used for some time in nuclear magnetic resonance (NMR) as a sensitive tool for determining solution phase structures. In a 2D NMR spectrum, spectral information is spread over two frequency axes, and cross peaks between the resonances on the diagonal axis indicate coupling between spins experiencing different chemical environments.\textsuperscript{1,5} Knowledge of the spin coupling mechanism allows information on connectivity or separation of nuclei to be established.

Inspired by the success of NMR and the development of ultrafast solid-state laser technology, multi-dimensional optical and infrared (IR) techniques based on coherent nonlinear spectroscopy have been proposed and experimentally realized in the last decade.\textsuperscript{2-4,6-14} These methods include Fourier transform time-domain experiments, in which the evolution of coherences in two time-periods is probed, and quasi-frequency-domain experiments, in which tunable narrow band excitation is followed by broad band probing. 2D Raman experiments have been employed to study the nature of low-frequency collective liquid motions.\textsuperscript{15-17} 2D electronic correlation spectroscopy with phase-sensitive detection of third-order nonlinear signals has been demonstrated and applied to the study of polar and non-polar solvation dynamics by the Jonas group.\textsuperscript{18-21} A number of related 2D optical techniques based on the photon echo have been used to understand couplings between electronic degrees of freedom, to study solvation dynamics, and to quantify the effects of solvent induced disorder.\textsuperscript{22-25} Mixed IR-Raman 2D spectroscopies are being proposed and implemented.\textsuperscript{8} The coherent doubly vibrationally enhanced (DOVE) method is being used to study vibrational couplings and mixtures of liquids by Wright and co-workers,\textsuperscript{26,27} and 2D vibrational spectroscopy using IR excitation and spontaneous Raman probing is being implemented by Dlott and co-workers to reveal vibrational relaxation pathways and spectral diffusion.\textsuperscript{28,29} This thesis focuses on the resonant experimental technique of 2D IR vibrational spectroscopy, which has been used to study transient structure, conformational fluctuations, solvation and relaxation dynamics by the Hochstrasser, Hamm, Fayer and Tokmakoff groups.\textsuperscript{10,30-32}

\subsection*{1.2.2 Fourier Transform 2D IR spectroscopy}

Fourier transform 2D IR spectroscopy is a third-order nonlinear spectroscopy derived from IR vibrational echoes,\textsuperscript{33-36} in which three femtosecond IR pulses interact with a multilevel vibrational system. The experiment is characterized by three experimental time delays: the evolution ($\tau_1$), waiting ($\tau_2$), and detection ($\tau_3$) periods, which follow the three successive input pulses as shown in Figure 1-1(a). The first pulse prepares the system in a particular vibrational coherence, which
oscillates during the evolution period and is also known as the free induction decay (FID). The second pulse acts on the system to (i) create a population in the ground or excited state (ii) to further excite the same vibrational coordinate, or (iii) to excite another coordinate. The last interaction creates a vibrational coherence whose evolution is followed during the detection period. The last two panels of Figure 1-1, (b) and (c), depict interaction sequences, where the final vibrational coherence involves a two-quantum and one-quantum excited state respectively. In a 2D experiment, the propagation during the evolution and detection periods is observed, by varying $\tau_1$ and characterizing the amplitude and phase of a nonlinear signal field radiated during $\tau_3$ with a heterodyne detection scheme.\textsuperscript{19,20,37,38} The data are represented through a 2D Fourier transform as a 2D spectrum that describes the vibrational transitions sampled during $\tau_1$ and $\tau_3$.

![Figure 1-1](image)

**Figure 1-1.** (Color) (a) Pulse sequence used in coherent Fourier transform 2D IR spectroscopy. The pulse envelopes represent the three femtosecond IR fields; the oscillatory functions are the linear and nonlinear free induction decays (FIDs) detected in the experiment (via a heterodyne detection scheme) as a function of the evolution ($\tau_1$) and detection ($\tau_2$) periods for a particular waiting period ($\tau_3$). The 2D IR spectrum is obtained by Fourier transforming the FID signals. Level diagrams depicting sequences where the last interaction excites a two-quantum (b) or a fundamental (c) state of a multilevel vibrational system. The numerals 0, 1, and 2 represent the ground and the vibrational states in the one- and two-quantum manifolds respectively.
Figure 1-2 displays a cartoon of a 2D spectrum of two coupled vibrations. The corresponding 1D spectrum is plotted on the top panel and illustrates the two vibrations designated $\omega_a$ and $\omega_b$. From the 1D spectrum, it is hard to tell to see whether the two vibrations are coupled and if so what is the magnitude of the relative coupling and orientation between the transition dipole moments of the coupled modes. The 2D spectrum is plotted as a function of $\omega_1$ and $\omega_3$, which are the Fourier transform pairs of the experimental time variables $\tau_1$ and $\tau_3$ in Figure 1-1(a). The resonances in the $\omega_1$ dimension lie along $\omega_a$ and $\omega_b$, reflecting the dipoles excited by the interaction of the first IR field with the sample. Interactions with the remaining two successive IR pulses can result in the signal field oscillating at the fundamental frequencies ($\omega_a$ and $\omega_b$) or at the difference in frequencies between the fundamental and overtone bands ($\omega_a-\Delta_a$, $\omega_b-\Delta_b$, $\omega_a-\Delta_{ab}$ and $\omega_b-\Delta_{ab}$). This leads to the resonances in Figure 1-2 lying along six different frequencies in the $\omega_3$ dimension. There are peaks lying along the diagonal axis (drawn in the figure) and the off-diagonal axes. The positions and amplitudes of the cross peaks are a direct measure of the couplings and orientations between the vibrations $\omega_a$ and $\omega_b$. The signs of the peaks indicate whether the final field-matter interaction involves a fundamental (positive, red) or an overtone or combination band (negative, green) vibration.

![Figure 1-2.](Image)

**Figure 1-2.** (Color) A cartoon of the 1D (top) and 2D (bottom) spectrum of two-coupled vibrations designated as $\omega_a$ and $\omega_b$. The red and green color in the 2D spectrum represents positive and negative going peaks. The amplitudes of the cross-peaks are sensitive to the angle ($\Theta$) between the transition dipole moments ($\mu_a$ and $\mu_b$) of the coupled vibrations. The positions of the eight resonances map out the anharmonic potential of the two-coupled modes as depicted by the energy level diagram on the left. In particular, the peak splittings are a direct measure of the vibrational anharmonicities: $\Delta_a$, $\Delta_b$ and $\Delta_{ab}$.
Besides the position and amplitude of the peaks in a 2D IR spectrum, the 2D lineshapes provide important clues into the nature of the interactions between the coupled vibrational modes with their surroundings. Figure 1-3 illustrates the 1D and 2D vibrational lineshapes for the case of homogenous (fast) and inhomogenous (static) dynamics with respect to the experimental timescale \((\tau_1 + \tau_2 + \tau_3)\). While these two different mechanisms are indistinguishable in the measurement of the 1D lineshape, the 2D lineshapes are completely different. This tells us that 2D spectroscopy can separate the underlying molecular contributions of broad featureless 1D lineshapes providing a sensitive measure of conformational heterogeneity. Additionally, the observables in a 2D IR spectrum can prove to be stringent tests of theoretical models and computer simulations describing molecular dynamics in solution. The time scale of the experiment is typically in the picosecond range, offering a short window to sample the structure and dynamics of the system. 2D IR spectra collected as a function of \(\tau_2\) (a “relaxation experiment”), contain signatures of coherent and incoherent vibrational relaxation processes, spectral diffusion phenomena, and fluctuations in vibrational couplings and angles.\(^{21,29,30,39-41}\) This information forms the basis for describing structural changes and intermolecular interactions in the condensed phase.

**Figure 1-3.** (Color) A cartoon of the 1D (top) and 2D (bottom) vibrational lineshapes in the limit of homogenous and inhomogenous solute-solvent interactions. A description of 2D line shapes using the simple Bloch model shows that homogeneous and inhomogeneous line broadening mechanisms are discerned by the degree of ellipticity of the 2D line shapes.

Based on the above discussion, the case can be made that a careful analysis of 2D IR spectra taken at various time points along a chemical reaction can provide a wealth of information regarding the time-evolution of vibrational couplings, bond angles and changing local and collective environments. The field of 2D IR spectroscopy is heading in that direction with efforts in the Hamm
and Tokmakoff groups to apply this technique in understanding the phenomena of protein and peptide folding/unfolding. The last chapter of my thesis reports on the preliminary results in the Tokmakoff group to study the thermal denaturation of a small globular protein RNase A using 2D IR spectroscopy.

### 1.3 Thesis outline

The body of work presented in this thesis describes experiments designed to obtain 2D IR spectra with absorptive features, where the signs and positions of close-lying resonances are resolved and presents an approach towards searching for an accurate and physically intuitive picture of molecular interactions in solution that reproduces the positions, amplitudes and lineshapes in experimental spectra. This thesis describes experiments, which probe the carbonyl stretches of two very different molecular systems illustrated in Figure 1-4. The coupled asymmetric and symmetric C≡O stretches of Rh(CO)$_2$C$_5$H$_7$O$_2$ (top left) dissolved in hexane serve as an ideal model system of two coupled vibrations and we use them to learn the nuts and bolts of 2D IR spectroscopy. This promising new technique is then applied to study the changing characteristics of the Amide I band of RNase A upon thermal denaturation of the protein.

![Figure 1-4](image.png)

*Figure 1-4.* (Color) The two molecular systems studied by 2D IR experiments in this thesis. The system on the left is a metal carbonyl compound: Rh(CO)$_2$C$_5$H$_7$O$_2$. The two terminal asymmetric and symmetric C≡O stretching vibrations at 2015 cm$^{-1}$ and 2084 cm$^{-1}$ respectively are probed via 2D IR spectroscopy. The thermal denaturation of RNase A (right) was studied by probing the broad Amide I band (see FTIR spectrum) using 2D IR spectroscopy.
Figure 1-5. (Color) A flowchart describing how we search for an accurate and physically intuitive picture of molecular interactions in solution that reproduce the positions, amplitudes and lineshapes in experimental 2D IR correlation spectra. We start by selecting a complete set of generalized coordinates \( \mathbf{Q} \) that yield the most intuitive physical picture. The Hamiltonian \( \hat{H}_S(\mathbf{Q}) \) describes the couplings between the local coordinates and depends on the local structure(s). Appropriate unitary transformation results in the system vibrational eigenstates \( \{ \hat{H}_S \} \), which interact with the external IR fields used in the experiment. The effects of the bath on the system are incorporated in the system–bath Hamiltonian \( \hat{H}_{SB} \), which is written in the basis of the vibrational eigenstates. The resultant parameters of the bath-renormalized eigenstates are used to calculate the third-order response function \( \hat{R} \) and to simulate the experimental 2D spectrum. The decoupling of molecular structure from its bath-induced dephasing dynamics results from using a local basis to describe the equilibrium structure and an eigenbasis to describe the effects of the bath on the system.

The flowchart in Figure 1-5 outlines the approach used in this thesis to obtain microscopic information from 2D IR spectroscopy. The starting point is a complete set of generalized coordinates that best describe the time-evolving molecular system under study. These local coordinates \( \mathbf{Q} \) are picked to yield the most intuitive physical picture and do not necessarily have to be associated with a specific nuclear coordinate, a normal mode or/and a local mode. The properties of the system are contained in the Hamiltonian \( \hat{H}_S(\mathbf{Q}) \), which describes the couplings between the local coordinates. The coupling parameters may yield direct structural information depending on the coupling mechanism. If the system undergoes conformational fluctuations on the timescale of the experiment, then the time-dependence of the fluctuations in the couplings between the local coordinates needs to
be taken into account. An appropriate unitary (local-to-eigenstate) transformation of the above Hamiltonian results in the vibrational system eigenstates. These are the eigenstates, which interact with the external radiation fields during the experiment. In solution, the local coordinates are constantly interacting with a surrounding bath. The effects of the bath on the system are incorporated in the system–bath Hamiltonian \( H_{SB} \), which is written in the basis of the vibrational eigenstates. The interaction with the bath can cause fluctuations and shifts in the energies of the system eigenstates. It is important to realize that the experimentally observed peak positions, amplitudes and lineshapes in 2D IR spectra depend on the properties of the system, the bath and the system-bath interactions. A 2D IR spectrum can be simulated using the calculated eigenenergies and lineshape functions. Fitting the simulated and experimentally determined spectra reveal the set of local coordinates and their couplings, which best describe the molecular system under study.

I start with a theoretical investigation of the signatures of vibrational anharmonicities in a 2D IR spectrum of two coupled normal modes in Chapter 2. The next chapter describes our model system of Rh(CO)\(_2\)C\(_5\)H\(_7\)O\(_2\) dissolved in hexane and interprets our first results on this system by revealing the vibrational anharmonicities governing the nuclear potential of the coupled carbonyl stretches. Chapter 4 presents a theoretical framework for the description of 2D IR spectroscopy, providing an approach for simulating polarization selective 2D IR spectra starting from a local description and incorporating the effects of the system-bath interactions. A detailed description of the experiments needed to obtain intuitive 2D IR correlation spectra is presented in Chapter 5. The analysis of the 2D IR spectrum in terms of the positions, amplitudes and lineshapes of the various resonances provides an intuitive description of the system eigenstates and system-bath interactions in Chapter 6. Chapter 7 interprets the polarization-selective 2D IR spectra in terms of local bond-stretch coordinates. Bath-induced vibrational relaxation processes including coherence and population transfer and their manifestations in 2D IR correlation spectra are described in Chapter 8. Finally, Chapter 9 applies 2D IR spectroscopy to study the thermal denaturation of RNase A as observed by the changes in the Amide I resonance.
References


Chapter 2

Signatures of vibrational interactions in 2D IR spectra

The work presented in this chapter has been published in the following paper:


The first two-dimensional infrared (2D IR) spectra obtained using the double resonance two-pulse technique was reported in the literature in 1998. The study aimed to understand the vibrational coupling mechanisms in the Amide I bands of small globular proteins. Since then, there has been considerable interest among the experimentalists as well as theorists to unearth the microscopic information embedded in these IR spectra of coupled vibrations. With the rapid development of the 2D IR technique, it becomes necessary to understand the vibrational interactions that give rise to a 2D vibrational spectrum. The varying ways in which the vibrational modes of a system can interact raises several questions. How do the different vibrational couplings manifest themselves in a 2D spectrum? Do these mechanisms have distinct signatures in a 2D map? And perhaps, more importantly, can we identify a microscopic basis for their existence? The aim of this chapter is to elucidate the vibrational interactions that give rise to coherent 2D IR signals in general, and, in particular to describe these effects in a 2D heterodyne-detected rephasing or echo spectrum using a model of anharmonically-coupled oscillators. Inhomogeneous broadening and orientational effects are not considered here.

It is well known that for a particular fundamental vibration to be infrared active and reveal itself in a linear 1D spectrum, its vibrational motion must cause a change in the permanent dipole moment $\partial \mu / \partial q \neq 0$. This condition forms a selection rule for linear spectroscopy. To observe a signal from nonlinear vibrational spectroscopic techniques, such as the 2D experiments discussed
here, other considerations also apply. It is well understood that the nonlinear optical response from a
set of harmonic oscillators vanishes due to destructive interference between coherences involving
adjacent vibrational levels. Thus observing a nonlinear signal requires a deviation from harmonic
behavior. In particular, we will show that one of the following conditions must be fulfilled to observe
a 2D spectrum: (1) anharmonicity in the ground state potential, (2) nonlinear dependence of the
transition dipole on vibrational coordinates, or (3) level-dependent dephasing dynamics. This set of
conditions represents three manners of deviation from purely harmonic behavior, and thereby forms a
set of selection (or propensity) rules for 2D IR spectroscopy. It is shown that these three vibrational
interactions affect the positions, amplitudes and shapes of the various peaks in a 2D spectrum,
respectively. In the limit of slow damping of coherences, the nonlinear exciton model for the optical
response of two coupled vibrations predicts the existence of ten peaks arising from transitions
involving the one- and two-exciton states. Here we discuss the source of these peaks using
perturbation theory described in terms of the eigenstates of the system. The existence of these ten
peaks has been experimentally observed in the 2D IR rephasing spectrum of the coupled CO stretches
of Rh(CO)₂(C₅H₇O₂) in hexane and will be discussed in the following chapter.

The chapter is organized as follows. Section 2.1 describes the anharmonically coupled
oscillator model, along with the calculation of the nonlinear response for such a system. Section 2.3
describes each of the three vibrational interactions forming the set of selection rules, in an attempt to
catalogue their effects in a 2D spectrum. For a strongly coupled system in the weak damping limit,
each type of interaction has a clear manifestation in the 2D absolute value spectrum. Finally, section
2.4 will discuss the implications of these calculations in understanding 2D spectra of complex
systems and suggest rules for analyzing experimental data sets.

2.2 Nonlinear Response of Coupled Oscillators

2.2.1 Cubic Anharmonic Oscillators

To describe the 2D IR spectroscopy of coupled vibrations, we use a system of two oscillators
with fundamental frequencies \( \omega_a \) and \( \omega_b \) and expand the potential to include cubic anharmonic terms
in both coordinates:

\[
V(Q_a, Q_b) = \frac{1}{2} \hbar \omega_a^0 Q_a^2 + \frac{1}{2} \hbar \omega_b^0 Q_b^2 + \frac{1}{6} \left( g_{aaa} Q_a^3 + g_{bbb} Q_b^3 + 3g_{aab} Q_a^2 Q_b + 3g_{abb} Q_a Q_b^2 \right)
\]  

(2.1)

Here \( Q_a \) and \( Q_b \) are the reduced vibrational coordinates of the normal coordinates, \( q_a \) and \( q_b \).
\[ Q_i = \sqrt{\frac{2m_i \omega_i^0}{\hbar}} q_i \quad (2.2) \]

where \( m_i \) represents the reduced mass of the \( i^{th} \) vibrational coordinate. The expansion coefficients, \( g_{ijk} \) are the third-order derivatives of the potential surface with respect to its coordinates evaluated at the equilibrium position.

\[ g_{ijk} = \left( \frac{\partial^3 V}{\partial Q_i \partial Q_j \partial Q_k} \right)_{Q_e} \quad (2.3) \]

Coupling terms are taken to be invariant to the interchange of indices \( i, j \) and \( k \), implying that \( g_{aab} = g_{aba} = g_{baa} \) and \( g_{baa} = g_{bab} = g_{abb} \). The energies of the various vibrational levels can be calculated by perturbation theory to second-order in the coupling terms as shown below:\(^6\)\(^-\)^\(^8\)

\[
\frac{E_{nk}}{\hbar c} = X_a \left( n + \frac{1}{2} \right) + X_b \left( k + \frac{1}{2} \right) + X_{aa} \left( n + \frac{1}{2} \right)^2 + X_{bb} \left( k + \frac{1}{2} \right)^2 + X_{ab} \left( n + \frac{1}{2} \right) \left( k + \frac{1}{2} \right) \quad (2.4)
\]

In the above equation, \( n \) and \( k \) are the vibrational quantum numbers of the anharmonically-coupled vibrations \( \nu_a \) and \( \nu_b \). The parameters \( X_i \) and \( X_{ij} \) are related to the anharmonic parameters in the nuclear potential in the following way:

\[ \hbar c X_a = \hbar \omega_a^0 \quad (2.5 \ a) \]

\[ \hbar c X_b = \hbar \omega_b^0 \quad (2.5 \ b) \]

\[ \hbar c X_{aa} = \frac{g_{aab}^2}{2\hbar} \left( \frac{\left( \omega_b^0 \right)^2 - 8 \left( \omega_a^0 \right)^2}{\left( \omega_a^0 \right)^2 - 4 \left( \omega_a^0 \right)^2} \right) - \frac{5}{6 \hbar \omega_a^0 g_{aaa}^2} \quad (2.5 \ c) \]

\[ \hbar c X_{bb} = \frac{g_{abb}^2}{2\hbar} \left( \frac{\left( \omega_a^0 \right)^2 - 8 \left( \omega_b^0 \right)^2}{\left( \omega_a^0 \right)^2 - 4 \left( \omega_b^0 \right)^2} \right) - \frac{5}{6 \hbar \omega_b^0 g_{bbb}^2} \quad (2.5 \ d) \]

\[ \hbar c X_{ab} = \frac{g_{aab}^2}{\hbar} \left( \frac{4 \omega_b^0}{\left( \omega_a^0 \right)^2 - 4 \left( \omega_b^0 \right)^2} \right) + \frac{g_{aab}^2}{\hbar} \left( \frac{4 \omega_a^0}{\left( \omega_b^0 \right)^2 - 4 \left( \omega_a^0 \right)^2} \right) \quad (2.5 \ e) \]

\[ \hbar c X_0 = -\frac{g_{abb}^2}{8\hbar} \left( \frac{3 \omega_a^0}{\left( \omega_a^0 \right)^2 - 4 \left( \omega_b^0 \right)^2} \right) + \frac{g_{aab}^2}{8\hbar} \left( \frac{3 \omega_b^0}{4 \left( \omega_a^0 \right)^2 - \left( \omega_b^0 \right)^2} \right) \quad (2.5 \ f) \]

\[ -\frac{7}{72 \hbar \omega_a^0 g_{aaa}^2} - \frac{7}{72 \hbar \omega_b^0 g_{bbb}^2} \]
For the purpose of calculating transition dipole moments, the anharmonic eigenstates are obtained by including the first–order corrections to the harmonic wave functions. Please note that the units for the frequencies and the expansion coefficients are Hertz and Joules respectively.

2.2.2 Calculating a 2D IR rephasing (echo) spectrum

Fourier transform 2D IR spectroscopy observes the creation and evolution of coherences involving one or more vibrational states, and represents them as a 2D spectrum whose frequency axes describe the evolution and detection period. In a resonant 2D IR rephasing or echo experiment, three time-ordered fields generate a nonlinear signal field in the wave-vector direction, $k_s = -k_1 + k_2 + k_3$. The first pulse excites vibrational coherences for all transitions that lie within the bandwidth of the pulse. During an experimentally controlled evolution time, $\tau_1$, coherences evolve and relax through pure dephasing, population relaxation, and orientational motion. After $\tau_1$, a pair of pulses with wave-vectors $k_2$ and $k_3$ resonant with the fundamental and higher lying transitions, acts on this evolving coherence, inducing – among other things – coherence transfer to other vibrational states and rephasing processes. The time-evolution of the system in this final coherent state during time $\tau_3$, is described by the third-order polarization, and can be observed by characterizing the amplitude and phase of the radiated nonlinear signal field. A third-order non-linear experiment measures the convolution of the tensorial third-order response function, with the three input electric fields. In a heterodyne-detected experiment, the signal field generated from the third-order nonlinear polarization is mixed with a pulsed local oscillator (LO) field to characterize the phase as well as the amplitude of the signal of interest. If the pulses are considered to be in the delta function limit the signal becomes proportional to the third-order response function. Generally for a system of coupled vibrations, we must consider both the dynamics of the vibrational interactions and relaxation, and the orientation of the dipoles associated with these coordinates in describing the material response. The vibronic part contribution is isotropic in nature and describes the vibrational dynamics for a particular sequence of excitations. The orientational response function accounts for the relative orientation of the dipoles of the system, their interactions with the arbitrarily polarized input light fields, and the orientational relaxation processes. In this chapter we focus only on the isotropic vibronic contribution from the two anharmonically-coupled oscillators. A more thorough description of the theoretical background for 2D IR spectroscopy is reserved for Chapter 4.
Figure 2-1. Energy level structure for two anharmonically coupled vibrations designated as a and b. Shown are the various eigenstates $|n,k\rangle$ where n and k are the respective vibrational quantum numbers for modes a and b. The eigenstates in the two-quantum manifold are red shifted due to the anharmonicity in the nuclear potential by splittings $\Delta_a$, $\Delta_b$ and $\Delta_{ab}$. The straight lines show the allowed one-quantum transitions for a harmonic system. The dotted lines show 3-quantum transitions that are allowed due to the anharmonicity in the nuclear potential.

Figure 2-1 shows an energy level diagram of the six vibrational levels for the two anharmonically-coupled oscillators that are relevant to the experiment. In addition to the ground state, the eigenstates $|0,1\rangle$ and $|1,0\rangle$ form the one-quantum manifold, and the three states $|0,2\rangle$, $|2,0\rangle$ and $|1,1\rangle$ constitute the two-quantum manifold. The transition frequencies, $\omega_{fi}$, are defined as:

$$\omega_{fi} = \frac{E_f - E_i}{\hbar}$$

The two frequencies, $\omega_{a,0}$ and $\omega_{b,0}$, represent the two fundamental transitions of the coupled modes. These frequencies are shifted from their unperturbed values, $\omega_{a,0}^0$ and $\omega_{b,0}^0$ because of the anharmonicity in the potential. The anharmonicity also affects the energies of the states in the two-quantum manifold indicated by the energy splittings, $\Delta_a$, $\Delta_b$ and $\Delta_{ab}$. Using eq. 2.4, these anharmonic splittings can be related to the anharmonic coupling constants, $g_{ijk}$, through

$$\Delta_a = 2E_{i0} - E_{20} - E_{00} = -2X_{aa}$$

$$\Delta_b = 2E_{01} - E_{02} - E_{00} = -2X_{bb}$$

$$\Delta_{ab} = E_{10} + E_{01} - E_{11} - E_{00} = -X_{ab}.$$  

The incident fields are short pulses with broad bandwidth that spans both fundamental transitions, and is also resonant with transitions between the one- and two-quantum manifold. The coherences initially excited and observed during $\tau_1$ involve superpositions of ground and one-quantum states. After the remaining excitation fields, the system can evolve during $\tau_3$ by oscillating at either a fundamental frequency, $\omega_{a,0}$ and $\omega_{b,0}$, or it can oscillate in a coherence involving superpositions of the
one- and two-quantum states. These are the remaining transitions pictured in Figure 2-1, with the following six frequencies: $\omega_{2a,a} = \omega_{a,0} - \Delta_a$, $\omega_{2b,b} = \omega_{b,0} - \Delta_b$, $\omega_{ab,a} = \omega_{b,0} - \Delta_{ab}$, $\omega_{ab,b} = \omega_{a,0} - \Delta_{ab}$, $\omega_{2a,b} = 2\omega_{a,0} - \omega_{b,0} - \Delta_a$ and $\omega_{2b,a} = 2\omega_{b,0} - \omega_{a,0} - \Delta_b$. These frequencies represent transitions from singly excited states to overtones, to the combination band, or otherwise forbidden three quantum transitions.

Figure 2-2. The 20 different vibronic pathways resulting from three Feynman diagrams following the rotating wave approximation, which contribute to the vibronic part of the third-order response function. Diagrams I and II show correlations between one-quantum transitions in both the time periods. Ladder diagrams corresponding to Feynman diagram III, show correlations between the one-quantum and the two-quantum states.

For the IR rephasing experiment on vibrational systems, there are three general interfering Feynman diagrams that describe the propagation of coherences in Liouville space $^{10,11}$. When extended to consider all six-levels, there are 20 possible vibronic pathways that contribute to the vibrational part of the response function. These are represented as 20 different ladder diagrams in
Figure 2-2. Diagrams I(a-d) show the system oscillating at either of the fundamental frequencies during both the coherence periods. Diagrams II(a-d) also concern themselves with dynamics in the one-quantum manifold except that the system is in an excited state population during $\tau_2$. Diagrams III (a-l) show the system oscillating at one of the fundamental frequencies during $\tau_1$, and oscillating in a coherence involving a two-quantum state during $\tau_3$. Diagrams III (f, h-l) involve three quantum transitions for the third interaction, and would not exist in the harmonic approximation. These transitions become allowed because of the mixing of the harmonic oscillator eigenfunctions.

The response functions for each of the twenty pathways are calculated using a Bloch model with a phenomenological damping constant $\Gamma_{ij}$. The response function can be Fourier transformed along the time coordinates $\tau_1$ and $\tau_3$ to yield a 2D spectrum. The spectrum displays the 2D data intuitively as a map of frequencies, $\omega_1$ and $\omega_3$, sampled by the system during each of the experimentally controlled time periods. In a 2D experiment, $\tau_2 = 0$, and is therefore neglected. For the Bloch model, the Fourier transformed echo signal can be written as:

$$S(\omega_1, \omega_3) = \frac{2\mu_{a,0}}{i(\omega_1 + \omega_{a,0}) + \Gamma_{a,0}} \frac{2\mu_{b,0}}{i(\omega_1 - \omega_{b,0}) + \Gamma_{b,0}} + \frac{2\mu_{b,0}}{i(\omega_1 + \omega_{b,0}) + \Gamma_{b,0}} \frac{2\mu_{a,0}}{i(\omega_1 - \omega_{a,0}) + \Gamma_{a,0}}$$

$$+ \frac{\mu_{ab,a}}{i(\omega_1 + \omega_{a,0}) + \Gamma_{a,0}} \frac{\mu_{ab,b}}{i(\omega_1 - \omega_{b,0} - \Delta_{ab}) + \Gamma_{ab,a}} + \frac{\mu_{ab,b}}{i(\omega_1 + \omega_{b,0}) + \Gamma_{b,0}} \frac{\mu_{ab,a}}{i(\omega_1 - \omega_{a,0} - \Delta_{ab}) + \Gamma_{ab,b}}$$

$$- \frac{\mu_{2a,a}}{i(\omega_1 + \omega_{a,0}) + \Gamma_{a,0}} \frac{\mu_{2a,b}}{i(\omega_1 - 2\omega_{b,0} - \omega_{a,0} - \Delta_b) + \Gamma_{2a,a}} - \frac{\mu_{2b,b}}{i(\omega_1 + \omega_{b,0}) + \Gamma_{b,0}} \frac{\mu_{2b,a}}{i(\omega_1 - 2\omega_{a,0} - \omega_{b,0} - \Delta_a) + \Gamma_{2b,b}}$$

$$+ \frac{\mu_{2a,b}}{i(\omega_1 + \omega_{b,0}) + \Gamma_{b,0}} \frac{\mu_{2b,a}}{i(\omega_1 - 2\omega_{a,0} - \omega_{b,0} - \Delta_b) + \Gamma_{2b,a}}$$

$$- \frac{\mu_{2b,a}}{i(\omega_1 + \omega_{a,0}) + \Gamma_{a,0}} \frac{\mu_{2b,b}}{i(\omega_1 - 2\omega_{a,0} - \omega_{b,0} - \Delta_a) + \Gamma_{2b,b}}$$

(2.8)

Here $\mu_{ij}$ represents the transition dipole matrix element $\langle i|\tilde{\mu}|j\rangle$, with $\tilde{\mu}$ being the dipole operator. The first two terms on the right hand side of eq. 2.8 arise from diagrams I (a,c) and II (a,c). The second two terms arise from I (b,d) and II(b,d). Similarly, diagrams III (a-l) give rise to terms 5-10 respectively.

Before discussing specific vibrational interactions giving rise to 2D spectra, first consider the form of eq. 2.8 for a purely harmonic system. The absence of anharmonicity implies that $\Delta_a=\Delta_b=\Delta_{ab}=0$. For a linear transition dipole moment, the dipole matrix elements follow the harmonic
approximation, $\mu_{n,n-1}=\sqrt{n}\mu_{1,0}$, such that $\mu_{2a,a}=\sqrt{2}\mu_{a,0}$, $\mu_{2b,b}=\sqrt{2}\mu_{b,0}$, $\mu_{ab,a}=\mu_{a,0}$, $\mu_{ab,b}=\mu_{b,0}$ and $\mu_{2a,b}=\mu_{2b,a}=0$. We also assume that the dephasing constants for a particular fundamental frequency are the same: $\Gamma_{2a,a}=\Gamma_{ab,a}=\Gamma_{a,0}$ and $\Gamma_{2b,b}=\Gamma_{ab,b}=\Gamma_{b,0}$. These constraints lead to destructive interferences that cause the nonlinear response to vanish. In this limit, eq. 2.8 vanishes due to cancellations between the first four terms, which arise from purely one-quantum coherences, and the last six terms, which involve transitions into the two-quantum manifold. It becomes evident that in order to observe a 2D IR signal, one or more of the following conditions must be met: (1) ground state anharmonicity, (2) non-linearity in the transition dipole moment, and (3) level-dependent dephasing dynamics. In the following section, it is shown that these conditions lead to distinct signatures in the 2D IR rephasing spectrum.

2.3 Vibrational Interactions in 2D IR experiments

2.3.1. Anharmonicity in the ground state potential surface

Anharmonicity in the nuclear potential leads to frequency shifts between fundamental transitions and transitions to two-quantum manifold. These anharmonic splittings, described by eq. 2.7, lead to shifts of the various resonances in eq. 2.8, and to the appearance of ten peaks in the 2D spectrum. Thus anharmonicity of the system can be investigated by measuring peak splittings in the $\omega_3$ dimension.

We demonstrate this effect using parameters that are used to reproduce the 2D IR-rephasing spectrum of Rhodium (I) dicarbonylacetylacetonate (Rh(CO)$_2$(C$_5$H$_7$O$_2$), or RDC) in hexane. The fundamental transition frequencies, $\omega_{a,0}$ and $\omega_{b,0}$, taken from the absorption peaks of the symmetric and asymmetric CO stretches in the FTIR spectrum, are 2085 cm$^{-1}$ and 2014 cm$^{-1}$ respectively. The values of the anharmonic splittings $\Delta_a$, $\Delta_b$, and $\Delta_{ab}$, were taken to be 10.6 cm$^{-1}$, 12 cm$^{-1}$, and 25 cm$^{-1}$, respectively. These values correspond closely to what has been observed in transient pump-probe and photon echo experiments on this compound. From eq. 2.5(a-f), the anharmonic parameters for the nuclear potential are calculated to be $g_{aa}=32$ cm$^{-1}$, $g_{bb}=32$ cm$^{-1}$, and $g_{ab}=g_{ba}=22$ cm$^{-1}$. The amplitudes of the ten peaks are determined by the transition dipole moments, $\mu_{ij}$. Here, the dipole moments have been expanded linearly in each of the vibrational coordinates. The matrix elements for each of the transitions are calculated using the harmonic oscillator eigenfunctions corrected to first-order. This results in the following transition dipole moments scaling relationships: $\mu_{b,0}=1.3\mu_{a,0}$, $\mu_{2a,a}=\mu_{2b,b}=1.3\mu_{a,0}$, $\mu_{ab,a}=\mu_{ab,b}=0.9\mu_{a,0}$, $\mu_{2a,b}=\mu_{2b,a}=-0.04\mu_{a,0}$, which vary modestly from the harmonic
approximation: \( \mu_{2a,a} = \sqrt{2} \mu_{a,0} \), \( \mu_{ab,a} = \mu_{a,0} \); and \( \mu_{2b,a} = 0 \). All the dephasing constants, \( \Gamma_{ij} \), are taken to be constant and equal to 1 cm\(^{-1}\).

Table 2-1: Positions of the various peaks for a system of two anharmonically coupled vibrational modes, \( \nu_a \) and \( \nu_b \) on a 2D IR-rephasing spectrum as a function of \( \Omega_1 \) and \( \Omega_3 \) along with the corresponding ladder diagram from Fig 2 giving rise to the resonances.

<table>
<thead>
<tr>
<th>Peaks</th>
<th>Position</th>
<th>Ladder diagram</th>
<th>Peaks</th>
<th>Position</th>
<th>Ladder diagram</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \omega_1 )</td>
<td>( \omega_3 )</td>
<td>I (a), II(a)</td>
<td>( \omega_1 )</td>
<td>( \omega_3 )</td>
<td>I(c), II(c)</td>
</tr>
<tr>
<td>1</td>
<td>-( \omega_{b,0} )</td>
<td>( \omega_{b,0} )</td>
<td></td>
<td>1’</td>
<td>-( \omega_{a,0} )</td>
</tr>
<tr>
<td>2</td>
<td>-( \omega_{b,0} )</td>
<td>( \omega_{a,0} )</td>
<td>I(b), II(b)</td>
<td>2’</td>
<td>-( \omega_{a,0} )</td>
</tr>
<tr>
<td>3</td>
<td>-( \omega_{b,0} )</td>
<td>( \omega_{2b,b} )</td>
<td>III(e,f)</td>
<td>3’</td>
<td>-( \omega_{a,0} )</td>
</tr>
<tr>
<td>4</td>
<td>-( \omega_{b,0} )</td>
<td>( \omega_{ab,b} )</td>
<td>III(a,b)</td>
<td>4’</td>
<td>-( \omega_{a,0} )</td>
</tr>
<tr>
<td>5</td>
<td>-( \omega_{b,0} )</td>
<td>( \omega_{2a,b} )</td>
<td>III(i,j)</td>
<td>5’</td>
<td>-( \omega_{a,0} )</td>
</tr>
</tbody>
</table>

Incorporating all the values of the anharmonic splittings and the transition dipole moments given above in eq. 2.8 results in an absolute value 2D IR-rephasing spectrum shown in Fig. 2-3(a). Ten peaks are observed and they can be associated with each of the 20 vibronic pathways shown in Figure 2-2. Table 2-1 labels each of the ten peaks with their corresponding positions and the ladder diagrams that created them. All resonances in the \( \omega_1 \) dimension lie at one of the two fundamental frequencies, reflecting the two possible coherences excited during \( \tau_1 \). (These two possibilities are distinguished by numbering the peaks with or without a prime). The features on the diagonal axis and the cross-peaks each consist of a pair of closely spaced peaks split along \( \omega_3 \), corresponding to coherences amongst adjacent levels. For example, peaks 1 and 3 represent the system evolving during \( \tau_3 \) in \( |01\rangle\langle 00| \) and \( |02\rangle\langle 01| \) coherences respectively. These frequencies are split by \( \Delta_b \). Peaks 2 and 4 represent the system evolving in \( |10\rangle\langle 00| \) and \( |11\rangle\langle 01| \) coherences respectively during \( \tau_3 \). These frequencies are split by \( \Delta_{ab} \). Similar arguments can be made for peaks 1’-4’. In addition to these eight peaks, the presence of the anharmonicity leads to two otherwise forbidden transitions at 5 and 5’. These peaks involve three-quantum transitions during the final field interaction involving changes of quantum number for both states subject to \( (\Delta n + \Delta k) = \pm 1 \). The amplitude of peak 5 has to be multiplied by a factor of 50 for its contours to be at the same order of magnitude as the other peaks. Harmonically forbidden transitions also contribute to the amplitude of peaks 3 and 3’, through level
diagrams, III(f) and III(h). However the contribution of these diagrams is just 3% of the total amplitude of these peaks.

**Figure 2-3 a.** The absolute value 2D IR-rephasing spectrum of two anharmonically coupled vibrations. The values in the nuclear potential are: \( \omega_{a,0} = 2101 \text{ cm}^{-1}, \omega_{b,0} = 2034 \text{ cm}^{-1}, g_{ab} = g_{bb} = 22 \text{ cm}^{-1}, g_{aa} = 32 \text{ cm}^{-1}, g_{bb} = 32 \text{ cm}^{-1} \) resulting in \( \Delta_a = 10.6 \text{ cm}^{-1}, \Delta_b = 12 \text{ cm}^{-1} \) and \( \Delta_{ab} = 25 \text{ cm}^{-1} \). The dipole moment is expanded linearly in the vibrational coordinates with \( \mu_{a,0} = 1 \). The various transition dipole moments in terms of \( \mu_{a,0} \) are: \( \mu_{b,0} = \mu_{a,0} \), \( \mu_{2a,a} = \mu_{2b,b} = 1.3 \mu_{a,0} \), \( \mu_{ab,a} = \mu_{ab,b} = 0.9 \mu_{a,0} \), \( \mu_{2a,b} = \mu_{2b,a} = -0.04 \mu_{a,0} \). All the dephasing constants, \( \Gamma \) are taken to be constant and equal to 1 cm\(^{-1}\). **(b)** The real part of the spectrum showing a slice along \( \omega_1 = -\omega_{b,0} \) showing the relative signs of the coherences oscillating during \( \tau_3 \).

For a system of two anharmonically-coupled vibrations, the ten peaks lie on specific diagonal or anti-diagonal axes. The diagonal axis is defined as

\[-\omega_1 = \omega_3 \tag{2.9}\]

and anti-diagonal axes are defined as

\[-\omega_1 + \omega_3 = \text{constant}. \tag{2.10}\]

Two peaks along the diagonal axis (1 and 1’) represent the individual fundamental vibrations of the system, which arise from the coherences between the ground and one-quantum states during \( \tau_1 \) and \( \tau_3 \). The remaining eight peaks lie along anti-diagonal axis. The cross-peaks (peaks 2 and 2’) lie on the anti-diagonal axis defined as: \( -\omega_1 + \omega_3 = \omega_{a,0} + \omega_{b,0} \). These peaks result from the system oscillating in coherences involving the ground state and different one-quantum manifold states during \( \tau_1 \) and \( \tau_3 \). Peaks 4 and 4’ arise from the system oscillating at one of the fundamental frequencies during \( \tau_1 \) and
in a coherence involving the $|1,1\rangle$ state during $\tau_3$. These peaks also lie along the anti-diagonal, 
\[ -\omega_1 + \omega_3 = \omega_{a,0} + \omega_{b,0} - \Delta_{ab}, \]
parallel to the cross-peaks, and separated by the anharmonic splitting, $\Delta_{ab}$. Peaks 3 and 5 also lie on an anti-diagonal defined by $-\omega_1 + \omega_3 = 2\omega_{b,0} - \Delta_b$. Similarly peaks 5 and 3 lie along $-\omega_1 + \omega_3 = 2\omega_{a,0} - \Delta_a$. These rules can aid in describing congested multilevel spectra with arbitrary anharmonic splittings: $\Delta_a$, $\Delta_b$ and $\Delta_{ab}$.

To illustrate the relative signs of the five peaks, Fig. 2-3 b shows a slice of the real part of the 2D spectrum along $\omega_3$ for $\omega_1 = -\omega_{b,0}$. Peaks 1 and 2, which arise only from transitions between the ground and one-quantum manifold, are of opposite sign from peaks 3 and 4, which involve transitions to the two-quantum states. Absence of the anharmonic splitting ($\Delta_b = 0$) would lead to the resonances for peaks 1 and 3 at the same value of $\Omega_3$, and destructive interference between these contributions would lead to zero signal along the diagonal axis. A similar argument can be made for the cross peaks 2 and 4, which are separated by $\Delta_{ab}$. The peak splitting between peaks 2 and 4 would disappear in the limit of zero off-diagonal anharmonicity ($g_{aab} = g_{abb} = 0$). Figure 2-3 demonstrates that the presence of ground state anharmonicity results in ten peaks in the 2D spectrum described by energy differences $\Delta_a$, $\Delta_b$ and $\Delta_{ab}$, and unequal amplitudes of the peak pairs 1, 3 and 2, 4. The position of the various peaks allows us to map out the six-level energy structure shown in Figure 2-1 in a very intuitive fashion. The experimentally observed energy splittings, $\Delta_a$, $\Delta_b$ and $\Delta_{ab}$ can be further related to the diagonal and off-diagonal anharmonic coupling parameters, $g_{ijk}$, if we take all off-diagonal elements to be equal, i.e. $g_{aab} = g_{abb}$.

### 2.3.2. Nonlinear transition dipole moment

Generally, the dipole moment of a molecule, expressed as a function of its vibrational coordinates can be expanded in a power series about the equilibrium configuration $Q_0$: \(^{12}\)

\[
\tilde{\mu} = \tilde{\mu}(Q_0) + \sum_i \left( \frac{\partial \tilde{\mu}}{\partial Q_i} \right) Q_i + \frac{1}{2} \sum_{ij} \left( \frac{\partial^2 \tilde{\mu}}{\partial Q_i \partial Q_j} \right) Q_i Q_j + \frac{1}{6} \sum_{ijk} \left( \frac{\partial^3 \tilde{\mu}}{\partial Q_i \partial Q_j \partial Q_k} \right) Q_i Q_j Q_k + ... \\
= \mu^{(0)} + \sum_i \mu^{(1)}_i Q_i + \frac{1}{2} \sum_{ij} \mu^{(2)}_{ij} Q_i Q_j + \frac{1}{6} \sum_{ijk} \mu^{(3)}_{ijk} Q_i Q_j Q_k + ... 
\]

(2.11)

The magnitude and sign of the expansion coefficients $\mu^{(1)}$ and $\mu^{(2)}$ represent the tangent and curvature of the dipole moment at equilibrium. When molecules interact with electromagnetic radiation through the transition dipole moment, which represents the change of charge distribution through the field-
induced displacement of the normal coordinates. The selection rule for linear spectroscopy in the dipole approximation considers only the terms linear in the vibrational coordinates. Thus, for a particular mode, i to be infrared active, \( \mu_i^{(1)} \neq 0 \), and the selection rule of \( \Delta n = \pm 1 \) is obtained. As illustrated in eq. 2.8, using the dipole approximation in a harmonic system leads to a zero 2D signal. The destructive interference between quantum pathways can also be broken by considering the nonlinear dependence of the transition dipole moment on the vibrational coordinates, i.e. electrical anharmonicity. The effects of electrical anharmonicity have been studied experimentally\(^1\) by observing the intensities of fundamental and overtone bands as well as theoretically using \textit{ab initio} methods to calculate dipole moment surfaces.\(^{14,15}\) The results of Fair \textit{et al.} indicate that the electrical anharmonicity causes the transition dipole moment vector of the OH stretch to rotate counter clockwise from the OH bond by almost 20° going from \( \nu_{\text{OH}} \) to \( 4\nu_{\text{OH}} \). The 2D dipole moment surface calculated for the isolated CH chromophore in CHCl\(_3\) using \textit{ab initio} methods by Lin \textit{et al.} show that values of \( \mu^{(2)} \) range from 20% - 200% of \( \mu^{(1)} \).

To investigate the nonlinear dipole, let us first consider a harmonic system of two uncoupled normal modes with fundamental frequencies \( \omega_{a,0} \) and \( \omega_{b,0} \). Following eq. 2.11, the dipole moment for this system is expanded in the vibrational coordinates (\( Q_a \) and \( Q_b \)), and the nonlinear expansion terms are considered to be invariant to the interchange of indices. For the harmonic system, the expansion must be continued to third-order, since second-order dipole matrix elements (\( \Delta n + \Delta k = 0, \pm 2 \)), do not contribute to the one-quantum transitions observed in this experiment (\( \Delta n + \Delta k = \pm 1 \)). Setting \( \mu^{(3)}_{ijk} = 0.08 \mu_i^{(1)} \), the various transition dipole moments are calculated to be \( \mu_{b,0} = \mu_{a,0} = 1; \mu_{2a,a} = \mu_{2b,b} = 1.5 \mu_{a,0}; \mu_{ab,a} = \mu_{ab,b} = 1.07 \mu_{a,0}; \mu_{2b,a} = \mu_{2a,b} = 0.05 \mu_{a,0} \). Expansion of the dipole moment to third-order in the vibrational coordinates makes harmonically forbidden transitions such as, \( |1,0\rangle \rightarrow |0,2\rangle \) possible. The 2D IR signal can be calculated using these dipole matrix elements, together with the previous fundamental frequencies and damping constants, and is plotted in Figure 2-4a. Note that in the absence of any anharmonicity in the ground state potential, \( \Delta_a = \Delta_b = \Delta_{ab} = 0 \).

The 2D spectrum in Fig. 2-4a shows two features along the diagonal and two-cross-peaks along the anti-diagonal. Due to the lack of anharmonicity, coherences involving adjacent vibrational levels oscillate at the same frequency. This results in peaks 3 and 4 being at the same position as peaks 1 and 2 respectively. Peak 5(5*) results from the system evolving in the \( |20\rangle \langle 01| \) ( \( |02\rangle \langle 10| \) ) coherence during \( \tau_3 \). Relative to Fig. 2-3a, it is shifted in position by \( \Delta_3(D_3) \), as there is no anharmonicity present in this simulation. Peaks 1 and 2 are of unequal amplitude and the magnitude of peak 5 is 1/3 that of peak 1. This is seen more clearly in the real part of the spectrum plotted in Fig. 2-4b. The solid line in Figure 2-4b shows a slice of the real part of the 2D spectrum along \( \omega_3 \) for
ω₁ = −ω₀ showing that all peaks have the same sign. Changing the sign of μᵢⱼᵏ⁽³⁾ to negative changes the sign of the peaks, although this results in a smaller overall amplitude. When vibrational anharmonicity is included with the nonlinear dipole expansion, both effects can be observed and separated in 2D spectra. As in Fig. 2-3a, ten peaks are observed at positions dictated by the anharmonic parameters. The inclusion of the non-linear transition dipole reveals itself as a difference in the amplitude of different peak pairs that interfere in the harmonic limit. In general, peaks 1, 2 and 5 will have opposite sign from peaks 3 and 4. The difference in amplitude between peak pair 1 and 3 and peak pair 2 and 4 reflects the magnitude of the correction to harmonic scaling due to electrical anharmonicity.

**Figure 2-4.** Nonlinear dependence of the transition dipole moment on the vibrational coordinates. (a) The absolute value 2D IR-rephasing spectrum for two harmonic modes where the dipole moment has been expanded to third order in the vibrational coordinates. In this simulation the value of μᵢ⁽¹⁾ = 1 and all μᵢⱼᵏ⁽³⁾ = 0.08 μᵢ⁽¹⁾. The values of the dipole parameters are: μ₀,b₀=μ₀,a₁=1, μ₂,a₂ = μ₂,b₂ = 1.5μ₀,a₁, μ₂,b₁ = μ₀,b₁ = 1.07μ₀,a₁, μ₂,a₂ = μ₂,b₂ = 0.05μ₀,a₁. (b) The real part of the spectrum showing a slice along ω₁ = −ω₀ for the absolute value 2D spectrum in (a). The straight and dotted lines show the 2D echo signal for μᵢⱼᵏ⁽³⁾ = 0.08 μᵢ⁽¹⁾ and for μᵢⱼᵏ⁽³⁾ = -0.08 μᵢ⁽¹⁾ respectively.

### 2.3.3 Quantum number dependence of dephasing

Imperfect cancellation between destructively interfering quantum pathways can also occur if the dynamics for the interfering levels are different. This third selection rule implies that conditions leading to Γ₀,a₀ ≠ Γ₂,a₂ and Γ₀,a₀ ≠ Γ₀,b₀, i.e. quantum-number dependent dephasing, will lead to a 2D signal. These types of dynamics will arise from coupling either between the system states, or
between the system and bath. Vibrational relaxation of a multi-level system can occur through pure dephasing, population relaxation, coherence transfer and population transfer. The Redfield equations of the density matrix account for these relaxation pathways through a superoperator $\Gamma_{ij'j}$, which couples the various elements of the density matrix. The secular approximation, which is used in the Bloch equations, considers only the $\Gamma_{ij}ij'$ elements of the relaxation operator. The effects of coherence transfer and population transfer are ignored in this approximation resulting in the familiar representation of the decay rate:

$$\Gamma_{ij} = \frac{1}{2}(\Gamma_{ii} + \Gamma_{jj}) + \gamma_{ij}$$ (2.12)

where $\Gamma_{ii}$ and $\Gamma_{jj}$ are the population decay rates out of states i and j and $\gamma_{ij}$ is the pure dephasing rate.

To illustrate possible quantum number scaling relationships for vibrational dephasing, population and phase relaxation of our two coordinate system is considered using a model of a harmonic system coupled to a quantum mechanical bath consisting of linearly coupled harmonic oscillators. This model has been used to study the non-linear signals obtained in third and fifth-order Raman scattering experiments, relaxation of product species following photolysis and two-pulse photon echoes for harmonic as well as anharmonic systems. The population relaxation term is obtained by considering linear coupling of the harmonic system oscillators to each of the bath oscillators and the pure dephasing rate arises from a quadratic coupling between system and bath. This is a direct approach to obtain level dependent dephasing dynamics.

For two harmonic oscillators linearly coupled to a quantum bath, the downward rate of population relaxation from a state with quantum numbers n and k is given by:

$$\Gamma_{nknk} \propto \left|\langle n-1,k-1|Q_a + Q_b|n,k\rangle\right|^2$$

$$\propto n + k$$ (2.13)

The downward rate of population relaxation scales with the quantum number of the initial state.

Using the above equation along with eq. 2.12 and assuming that $\Gamma_{a,0} = \Gamma_{b,0}$, it can be shown that $\Gamma_{2a,a} = \Gamma_{2b,b} = \Gamma_{ab,a} = \Gamma_{ab,b} = 3\Gamma_{a,0}$. Note that pure dephasing ($\gamma_{ij}$) has been neglected, and the dephasing rates are given exclusively by population relaxation.

Neglecting population relaxation, the pure dephasing rate can be obtained by the quadratic coupling of the system to the bath. It is written below only in terms of the system coordinates:

$$\gamma_{nkn'k} \propto \left|\langle n,k|Q_a^2 + Q_b^2|n,k\rangle - \langle n',k'|Q_a^2 + Q_b^2|n',k'\rangle\right|^2$$

$$\propto 4(n - n' + k - k')^2$$ (2.14)
For our six-level system, the pure dephasing rate for coherent superpositions of one- and two-quantum states will be identical to those coherences between the one-quantum and the ground states. To this approximation, all $\gamma_{ij}$ will be the same.

**Figure 2-5.** Level-dependent population relaxation. (a) The absolute value 2D IR-rephasing spectrum for a harmonic oscillator coupled linearly to a bath. The values for the dephasing constants are: $\Gamma_{a,0} = \Gamma_{b,0} = 1$ cm$^{-1}$ and $\Gamma_{2a,a} = \Gamma_{2b,b} = \Gamma_{ab,a} = \Gamma_{ab,b} = 3\Gamma_{a,0}$. The dipole moments follow harmonic scaling. (b) Anharmonically coupled oscillators coupled linearly to a bath of harmonic oscillators. The values for the transition dipole moments and the anharmonicity parameters are the same as those in Fig. 2-3(a). (c) Dependence of peak heights as a function of $\Gamma_2/\Gamma_1$. $\Gamma_2$ represents $\Gamma_{2b,b}$, $\Gamma_{ab,b}$ and $\Gamma_{2a,b}$, and $\Gamma_1$ represents $\Gamma_{b,0}$.

We consider the effect of level-dependent dephasing dynamics in 2D spectra by calculating the case of vibrational dephasing dominated by lifetime broadening, $\gamma_{ij}=0$. We use two harmonic oscillators with fundamental frequencies $\omega_{a,0}$ and $\omega_{b,0}$, $\Delta_a=\Delta_b=\Delta_{ab}=0$, and harmonic scaling of the transition dipole moment. The dephasing rates reflecting only the population relaxation are $\Gamma_{a,0}=\Gamma_{b,0}$.
= 1 cm\(^{-1}\), and \(\Gamma_{2a,a} = \Gamma_{2b,b} = \Gamma_{ab,a} = \Gamma_{ab,b} = 3\) cm\(^{-1}\). The level-dependent dephasing parameters result in the imperfect cancellation of response functions involving the one-quantum and two-quantum states giving rise to a 2D IR signal. The absolute value 2D spectrum is plotted in Figure 2-5(a). Four peaks are observed, two diagonal and two cross peaks. These appear in the same position at peaks 1-4 in Fig. 2-4a, although now the shape of the peaks is elongated along the \(\omega_3\) direction.

Level-dependent dephasing reveals itself in the asymmetric shape of the peaks in the 2D IR spectrum. Since the dephasing is faster during \(\tau_3\), it is expected that peaks would be broadened in the \(\omega_3\) direction, but the opposite is observed. This counter-intuitive result occurs because of the different lineshapes associated with the destructively interfering pathways contributing to these peaks. The 2D lineshapes arising from coherences between the one-quantum manifold and ground state during \(\tau_3\), have a symmetric diamond shape, since they dephase at the same rate during both the time periods. The 2D lineshapes arising from coherences between the one and two-quantum manifold during \(\tau_3\) are broadened along the \(\omega_3\) direction, as they dephase three times as fast during the second time period. The interference between these contributions lead to the lineshape observed in Fig. 2-5a.

The interfering contributions are resolved in a system where anharmonicity in the ground state potential is included with lifetime broadening. Figure 2-5b shows the characteristic asymmetric broadening of peaks 3 and 4 along the \(\Omega_3\) direction. A comparison of Fig. 2-5b with Fig. 2-3a also shows that the change in the dephasing parameters results in a lower amplitude of peaks 3, 4, and 5, while peaks 1 and 2 remain unaffected. Thus level-dependent dephasing affects the shape and amplitude of the 2D lineshape.

Dephasing and population relaxation measurements of fundamental and overtone transitions have resulted in various quantum number scaling relationships for the relaxation rate\(^{22,24}\). Fig. 2-5c plots the effects of arbitrary quantum number dependent dephasing of \(\Gamma_{2b,b}, \Gamma_{ab,b}\) and \(\Gamma_{2a,b}\) relative to \(\Gamma_{b,0}\). The amplitude of all peaks are plotted for the ratio of the dephasing rates, \(\Gamma_2/\Gamma_1\). The heights of peaks 3, 4 and 5 drop rapidly, reflecting the increased dephasing rates along \(\omega_3\), while the heights of peaks 1 and 2 are roughly constant.

The effect of anharmonicity on the level-dependent dephasing has been ignored here. Vibrational relaxation of an anharmonic potential can be modeled using a vibrational master equation approach to solve for the population relaxation accounting for both the upward and downward population relaxation in a quantum mechanical bath\(^{22}\). Alternatively, Redfield relaxation theory would allow population and coherence transfer to be addressed along with population relaxation and pure dephasing\(^{25}\). Pure dephasing has also been neglected, since quantum number dependent dynamics are not expected for a harmonic system coupled quadratically to a quantum bath. This result
agrees with that obtained from Redfield theory for a one-dimensional harmonic oscillator coupled to a
thermal bath at zero temperature. However, once anharmonicity is included the pure dephasing rate
will become quantum number dependent.

2.4 Discussion and Conclusions

We have considered three interaction mechanisms between vibrational coordinates, which
can be observed through distinct signatures in the 2D IR-rephasing spectrum. These interactions may
be considered a set of selection (or propensity) rules that lead to a nonlinear optical response from the
system. These include any interactions that lead to breaking of the purely harmonic behavior for
which the 2D signal vanishes.

In practice, a distinct separation between these vibrational nonlinearities will not always be
observed. Indeed, vibrational anharmonicity can influence all of them. Anharmonicity of the system
coordinates leads to splitting of diagonal and cross peaks in such a spectrum, and influences their
amplitudes through the nonlinearity of the transition dipole. Anharmonicity involving the system or
bath coordinates can lead to relaxation behavior that is quantum number dependent, which also
effects lineshapes and amplitude.

The 2D IR spectrum allows the simultaneous influence of these effects to be observed. For
the weak damping case (\(\Gamma \ll \Delta\)) described here, each of these effects can be separated and quantified
in the absolute value spectrum. This is illustrated in Fig. 2-6a, which demonstrates the simultaneous
effects of anharmonicity in the ground state potential, nonlinear dependence of the transition dipole
moment on the vibrational coordinates, and level-dependent dephasing. We have used the parameters
from previous examples, along with nonlinear dipole terms up to third-order. The vibrational
anharmonicity is measured through the energy splittings \(\Delta_a, \Delta_s \) and \(\Delta_{ab}\). The nonlinear dependence of
the transition dipole moment on the vibrational coordinates results in unequal amplitudes of the
diagonal (1,3) or cross-peak (2,4) pairs. The change in amplitude of peaks 3 and 4 is also due to the
inclusion of quantum-number-dependent vibrational relaxation, which affects the amplitude of the
resonances, along with elongation of two-quantum resonances in the \(\omega_3\) dimension. A slice of the
real part of the 2D spectrum at \(\omega_1 = -\omega_{b0}\) is shown in Fig. 2-6b. Again of the five peaks seen, peaks 3
and 4 have opposite sign than the rest, and their amplitudes differ from peaks 1 and 2. Also, the
broader linewidth of peaks 3, 4, and 5 reflect the quantum-number-dependent population relaxation.
Figure 2-6  (a) Combined effects of the anharmonicity of the ground state potential, expansion of the transition dipole moment in a nonlinear fashion on the coordinates and the quantum number dependence of the lifetime. The values of the anharmonicity parameters are those used in Fig. (3). The values of the transition dipole moments used to calculate Fig. 6 are: $\mu_i^{(1)} = 1$, $\mu_{ij}^{(2)} = 0.8 \mu_i^{(1)}$ and $\mu_{ijk}^{(3)} = 0.08 \mu_i^{(1)}$. This leads to: $\mu_{b,0} = \mu_{a,0}$, $\mu_{2a,a} = \mu_{2b,b} = 1.7\mu_{a,0}$, $\mu_{ab,a} = \mu_{ab,b} = 1.2\mu_{a,0}$, $\mu_{2b,a} = -0.09\mu_{a,0}$ and $\mu_{2b,b} = -0.08\mu_{a,0}$.  (b) The real part of the spectrum showing a slice along $\Omega_1 = -\omega_{b,0}$ with sensitivity to the relative signs of the coherences oscillating during $\tau_3$.

What is evident from the simulations of two anharmonically-coupled oscillators is that a thorough analysis of 2D-IR spectra can reveal a wealth of information regarding the strength and time course of vibrational interactions. We have shown that anharmonicity in the ground state potential, nonlinear dependence of the transition dipole moment on its vibrational coordinates and the quantum number dependent dephasing are revealed in the positions, amplitudes and shape of the various peaks in the spectrum. The next chapters will explain in detail how to simulate, collect and interpret a 2D IR spectrum with absorptive features enabling us to test the capabilities of this promising new technique.
References

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Chapter 3

Initial experiments on a model system of two coupled vibrations

The work presented in this chapter has been published in the following papers:


The initial demonstration of 2D IR spectroscopy as a viable tool for understanding molecular structure and dynamics in solution rests on the choice of an appropriate model system. We started our efforts with a system of two-coupled vibrations to learn how to do the experiment and model the microscopic information present in the experimental spectra. This chapter reports on our model system and the initial heterodyne-detected experimental 2D IR spectrum. The experimental results are discussed in terms of the vibrational anharmonicities giving rise to the nonlinear IR signal by using the ideas presented in the previous chapter.

3.1 Model system of two strongly coupled vibrations

The generalized multilevel vibrational system probed by third-order IR spectroscopies consists of a set of $n$ fundamental vibrational states and $n(n+1)/2$ two-quantum states. The transitions into the two-quantum states are referred to as combination bands and overtones, whose energy shifts relative to the fundamentals reflect the coupling and anharmonicity for the system. We
use the asymmetric and symmetric carbonyl stretches of dicarbonylacetylacetonato rhodium (I) (RDC) dissolved in hexane and chloroform to study the 2D IR spectroscopy of two coupled vibrations. RDC (Rh(CO)₂C₅H₇O₂) pictured in Figure 3-1, is a square planar $d^8$ compound with two chemically equivalent terminal carbonyl groups and a bidentate acac (OC(CH₃)CHC(CH₃)O) ligand coordinated to the rhodium metal center. The same figure depicts the six-lowest lying vibrational eigenstates for this particular model system. These six states have been labeled according to the quanta of energy present in the symmetric and asymmetric stretches. They include a common ground vibrational state $|0\rangle$, the two one-quantum states $|a,0\rangle$ and $|s,0\rangle$ and the three two-quantum states consisting of the overtones $|2a,a\rangle$ and $|2s,s\rangle$ and the combination band $|as,a\rangle$.

**Figure 3-1.** (a) The molecular structure of our model system RDC and the vibrational energy level diagram for two anharmonically coupled symmetric and asymmetric C = O vibrations designated as $a$ and $s$. The six lowest eigenstates are shown as $|a,s\rangle$ where, $a$ and $s$ are the respective vibrational quantum numbers for the asymmetric and symmetric stretches. Solid and dashed arrows respectively indicate the harmonically allowed and harmonically forbidden transitions amongst the eigenstates. The actual values of the transition frequencies measured for RDC dissolved in hexane are: $\omega_{a,0} = 2015$ cm$^{-1}$, $\omega_{s,0} = 2084$ cm$^{-1}$, $\omega_{2a,a} = 2001$ cm$^{-1}$, $\omega_{2s,s} = 2073$ cm$^{-1}$, $\omega_{as,a} = 2058$ cm$^{-1}$, $\omega_{as,s} = 1989$ cm$^{-1}$, $\omega_{2a,s} = 1932$ cm$^{-1}$ and $\omega_{2s,a} = 2142$ cm$^{-1}$. (b) The linear FTIR spectrum of RDC in hexane (solid) and in chloroform (squares) showing the two fundamental asymmetric and symmetric transition frequencies at 2015 and 2084 cm$^{-1}$. The full width half maxima of the asymmetric and symmetric vibrational lines are 2.6 cm$^{-1}$ in hexane, and 14.6 cm$^{-1}$ and 9.3 cm$^{-1}$ in chloroform respectively.
The fundamental transitions are observed at 2084 (2085) and 2015 (2014) cm\(^{-1}\) from the FTIR spectra of RDC dissolved in hexane (chloroform) and are assigned to the symmetric and asymmetric combinations of the –C≡O stretches, respectively.\(^1\) The anharmonicity of the nuclear potential, governing the –C≡O stretching motions, leads to the anharmonic frequency shifts of the overtone spectrum. These shifts are measured from the 2D IR correlation spectrum of RDC in hexane at \(\Delta_a = 11\) cm\(^{-1}\) and \(\Delta_a = 14\) cm\(^{-1}\) for the symmetric and asymmetric vibrations. The combination band, which reflects the coupling between the carbonyls, is red shifted by \(\Delta_{as} = 26\) cm\(^{-1}\) with respect to the sum of the fundamental frequencies.

The vibrational dynamics of the carbonyl transitions for RDC in different solvents have been previously characterized with IR pump-probe and two pulse IR echo spectroscopy.\(^1\)\(^-\)\(^3\) These studies demonstrated that the narrow line shapes observed for the –C≡O stretches of RDC and other metal carbonyls in hexane and 2-methylpentane are well described by the motionally narrowed or homogenous limit. The full width at half maximum of the asymmetric and symmetric –C≡O stretches of RDC in hexane from the FTIR spectrum (Fig. 3-1b) is measured to be \(\sim 2.6\) cm\(^{-1}\) – significantly narrower than the anharmonic frequency shifts. This allows us to resolve all the possible resonances in a 2D nonlinear experiment and we are able to model the structure of RDC on a \(\sim 20\) ps timescale through an analysis of the peaks positions and amplitudes of polarization-selective 2D spectra of the molecule dissolved in hexane.\(^4\) The dynamics of the same molecule are completely different in chloroform where the linewidths of the individual asymmetric and symmetric –C≡O stretches are 14.6 cm\(^{-1}\) and 9.3 cm\(^{-1}\) respectively, while the anharmonicities remain approximately the same. 2D IR experiments of RDC in chloroform investigate the underlying mechanism of this dramatic change in the 1D absorption linewidths and characterize the magnitude and timescales of the correlations in the transition energy fluctuations of the coupled asymmetric and symmetric vibrational modes.\(^5\)\(^-\)\(^7\)

### 3.2 First experiments on RDC

Our first 2D IR experiments were performed on RDC, where we measured the heterodyne-detected absolute value rephasing spectrum. Figure 3-2 displays the experimental absolute value of the 2D IR rephasing spectrum of RDC in hexane in the all-parallel geometry. The spectrum looks very similar to the simulations presented in the previous chapter. The resonances in the \(\omega_1\) dimension represent the vibrations excited in the evolution period, \(\tau_1\) and lie along the fundamental frequencies, \(\omega_{a0}\) and \(\omega_{s0}\). The second frequency dimension – \(\omega_3\) reveals the final state of the initially excited
transitions and we see that the signal field contains frequencies at all the one and two-quantum transitions shown in Figure 3-1(a).

The 2D spectrum contains diagonal and off-diagonal features lying along the axes $-\omega_1 = \omega_3$ and $-\omega_1 + \omega_3 = \text{constant}$. As discussed in the previous chapter, each diagonal feature arises from processes occurring within one vibrational manifold, consists of two spectral peaks of similar intensity separated along the $\omega_3$ axis by 11 cm$^{-1}$ and 14 cm$^{-1}$ for the symmetric and asymmetric vibrations, respectively. The off-diagonal contributions to the 2D spectrum, or cross-peaks, arise from processes involving two different vibrational coordinates and contain information about the vibrational coupling between the corresponding vibrations. Similar to the diagonal features, each of the off-diagonal features is composed of two spectral peaks, which are separated along the $\omega_3$ axis by 26 cm$^{-1}$. The amplitude of the off-diagonal features is $\sim 1/3$ of the amplitude of the diagonal contributions. In addition to the eight relatively strong diagonal and off-diagonal peaks, two single weak peaks were observed at $(\omega_1, \omega_3) =$ (2015 cm$^{-1}$, 2148 cm$^{-1}$) and (2084 cm$^{-1}$, 1928 cm$^{-1}$).

![Figure 3-2. Absolute value 2D IR rephasing spectrum of RDC in hexane. The signal in the upper and lower panels is magnified by a factor of 100 to emphasize the weak spectral peaks at $(\omega_1, \omega_3) =$ (2015 cm$^{-1}$, 2148 cm$^{-1}$) and (2084 cm$^{-1}$, 1928 cm$^{-1}$). Dashed lines indicate the frequencies of the fundamental transitions.](image)

We will use the experimental spectrum in Fig 3-2 to test out the ideas presented in Chapter 2. We can see that the peak positions in the 2D spectrum contain information about the vibrational anharmonicity present in our model system of two coupled carbonyls in RDC dissolved in hexane. The anharmonic frequency shifts, $\Delta_\text{s} = 11$ cm$^{-1}$ for the symmetric and $\Delta_\text{a} = 14$ cm$^{-1}$ for the asymmetric
stretch, are measured directly from the separation of the spectral peaks constituting the corresponding diagonal features. The splitting of the peaks in the off-diagonal features ($\Delta_{sa} = 26 \text{ cm}^{-1}$) is related to the strength of the coupling between the symmetric and asymmetric C-O vibrational modes. The sensitivity of 2D IR spectroscopy to the anharmonicities of the nuclear potential is further revealed by the observation of the weak spectral features at ($\omega_1, \omega_3$) = (2015 cm$^{-1}$, 2148 cm$^{-1}$) and (2084 cm$^{-1}$, 1928 cm$^{-1}$). These peaks involve three-quantum transitions between different vibrational manifolds, which are strictly forbidden in a harmonic system.

The characteristics of the nuclear potential $V$ can be deduced from the experimental data by using the model of coupled anharmonic oscillators presented in Chapter 2. The nuclear potential is expanded in the reduced normal coordinates $Q_s$ and $Q_a$:

$$ V(Q_s, Q_a) \approx \frac{1}{2} \hbar \omega_s^0 Q_s^2 + \frac{1}{2} \hbar \omega_a^0 Q_a^2 + \frac{1}{6} \left( g_{sss} Q_s^3 + g_{asa} Q_a^3 + 3 g_{ssa} Q_s^2 Q_a + 3 g_{saa} Q_s Q_a^2 \right) $$

Here $\omega_i^0$ represents the harmonic frequency of mode $i$. The anharmonicity is described by $g_{sss}$, $g_{asa}$, $g_{ssa}$, and $g_{saa}$, the third-order derivatives of the nuclear potential in the equilibrium configuration. In particular, the off-diagonal anharmonicities $g_{saa}$ and $g_{ssa}$ describe the coupling between the $Q_s$ and $Q_a$ normal coordinates. The experimental values of $\Delta_s$, $\Delta_a$, and $\Delta_{sa}$ can be related to the parameters describing the shape of the nuclear potential in a straightforward manner as shown in the previous chapter. For RDC in hexane the corresponding values are $\omega_s^0 = 2108 \text{ cm}^{-1}$, $\omega_a^0 = 2038 \text{ cm}^{-1}$, $g_{sss} = 32 \text{ cm}^{-1}$, $g_{saa} = 32 \text{ cm}^{-1}$, and $g_{ssa} = g_{saa} = 22 \text{ cm}^{-1}$. A closer look at the relative amplitudes of the split diagonal and off diagonal peaks reveals a small effect of electrical anharmonicity in this experimentally measured spectrum. Also at first glance, the 2D lineshapes seems to be homogenous, and do not show a variation in linewidth in the two frequency dimensions, indicating that there is no effect of quantum-number dependent dephasing dynamics in this system.

Using the vibrational anharmonic parameters described above and the recipe presented in Chapter 2 we can simulate the experimental absolute value rephasing spectrum of RDC in hexane. Figure 3-3 presents a simulated absolute value 2D spectrum of RDC for the all-parallel polarization geometry. The orientational contributions to the response function were derived by projecting the polarizations of the incident fields onto the directions of the molecular transition dipoles, averaging over all molecular orientations, and accounting for orientational diffusion between interactions with the external fields. The calculation assumes that the transition dipole moments of the symmetric and asymmetric vibrations are perpendicular. The transition dipole moment strengths of the fundamental transitions of the symmetric and asymmetric vibrations were assumed to be equal. The dipole moments for the transitions into higher lying vibrational states were determined by first
calculating the wave functions of the anharmonic two-dimensional oscillator to the first order of perturbation theory, and then evaluating the dipole moment matrix element in the perturbed wave functions. The remarkable similarities in the observed and calculated spectra clearly demonstrates the ability of 2D IR nonlinear spectroscopy in determining the vibrational anharmonicities and the relative orientation of the interacting dipoles.

**Figure 3-3.** Simulated absolute value 2D IR rephasing spectrum of RDC in hexane. Dashed lines indicate the frequencies of the fundamental transitions. The ladder diagrams demonstrate the incomplete destructive interference of nonlinear responses involving one and two-quantum states due to the presence of vibrational anharmonicity in this model system. For example, we see the off-diagonal feature because the value of $\Delta_s \neq 0$. Similarly we see the weak peaks at $(\omega_1, \omega_3) = (2015 \text{ cm}^{-1}, 2148 \text{ cm}^{-1})$ and $(2084 \text{ cm}^{-1}, 1928 \text{ cm}^{-1})$ because $\mu_{2s,a}$ and $\mu_{2a,s} \neq 0$ respectively.

These early experiments presented in this chapter form the basis for the remaining experiments described in this thesis. Using the experimental 2D spectrum of Figure 3-2 we have been able to validate the selection rules for 2D IR spectroscopy presented in Chapter 2 and successfully reveal the anharmonicities in the nuclear potential with a model of two anharmonically coupled normal modes to yield the experimentally observed eigenstates. Our data also shows the limitations in our first-generation experimental set-up and theoretical framework. To be able to extract structural information from these spectra we need to model the data not in terms of the delocalized eigenstates, but rather the local carbonyl stretches. In addition, we want to obtain spectra that are sensitive to the signs of the resonances and do not suffer from the experimental artifacts of asymmetric lineshapes such as those shown in Figure 3-2. In conclusion, to be able to extract all the microscopic
information present in the nonlinear signal field we have to be able to collect and model 2D IR spectra with absorptive lineshapes revealing accurately the positions, signs, lineshapes and amplitudes of the various spectral features. The following chapters describe our efforts at pursuing these goals using RDC as a model system.
References


Chapter 4

Calculating a 2D IR correlation spectrum for a multilevel system

The work presented in this chapter has been published in the following papers:


Two-dimensional IR correlation spectroscopy belongs to the general class of time-resolved four-wave mixing experiments where three femtosecond IR fields with well-defined wave-vectors interact with the sample to generate a nonlinear signal field that contains the microscopic information of interest. This chapter provides the theoretical background for a semi-classical description of 2D IR spectroscopy of multi-level vibrational systems. The goal is to provide the reader with an approach for simulating polarization-selective 2D IR correlation spectra for a system of coupled vibrations, starting from a local or site description of the system of interest and incorporating the influence of the surroundings. We draw on the numerous descriptions of third-order nonlinear spectroscopy present in the literature, but the emphasis here is on the less commonly described response of a multilevel system, for which we follow the treatment of Sung and Silbey.\textsuperscript{1,2} Section 4.1 defines a model material Hamiltonian probed by classical electric fields. The third order nonlinear polarization decomposed into a nonlinear vibrational and orientational response is the subject of Section 4.2. Section 4.3 discusses the radiated nonlinear signal fields for the wave-vector geometry used in our experiments and the last section explains how to obtain intuitive 2D lineshapes using a linear combination of two conjugate signal fields. The Appendix for this chapter provides the details for calculating a 2D IR correlation spectrum of our model system RDC described in Chapter 3.
4.1 Material and Interaction Hamiltonians

Theoretical descriptions of nonlinear spectroscopy begin with a quantum mechanical material Hamiltonian \( H_M \) containing information about the system under study and an interaction Hamiltonian \( H_{int} \) describing the coupling of the system to classical external radiation fields.

\[
H = H_M + H_{int}
\]  

(4.1)

\( H_{int} \) will be treated as a perturbation. The total material Hamiltonian is generally written as a sum of the Hamiltonian for the system, bath and the system-bath interactions,

\[
H_M = H_S + H_B + H_{SB}
\]

(4.2)

For the vibrational systems of interest here, \( H_S \) contains an arbitrary number of coupled vibrational coordinates \( \mathbf{Q} \), which are interrogated by the applied radiation. The system-bath interaction leads to the nontrivial dynamics of the system states, including fluctuations in the vibrational transition frequencies, vibrational relaxation processes, and reorientational dynamics. The system Hamiltonian can be expressed in a basis of the local modes, normal modes, or eigenstates, depending on the information of interest. Constructing \( H_S \) in a local mode representation allows for an intuitive representation of the coordinates of the molecular structure of interest.

The system-bath interaction leads to the fluctuations and shifts in the vibrational transition frequencies, vibrational relaxation processes, and reorientational dynamics. For the purpose of introducing the interaction with a bath, we diagonalize \( H_S \) and couple the system eigenstates to a harmonic bath. The Hamiltonian \( H_{SB} \), contains information about the system-bath coupling and is linear in the bath coordinates \( q_\nu \). Following Sung and Silbey, we can represent the total material Hamiltonian as

\[
H_S = \sum_a |a\rangle \langle E^a_0 | \langle a|
\]

(4.3)

\[
H_B = \frac{1}{2} \sum_\nu \left( p_\nu^2 + \omega_\nu^2 q_\nu^2 \right)
\]

(4.4)

\[
H_{SB} = \sum_a |a\rangle \left( \sum_\nu \chi^a_\nu q_\nu \right) \langle a| + \sum_{a\neq b} |a\rangle \left( \sum_\nu \chi^{ab}_\nu q_\nu \right) \langle b|
\]

(4.5)

where \( a \) and \( b \) are the indices for the system eigenstates, and \( \chi^a_\nu \) is the matrix element coupling the \( a \) state of the system to the \( \nu \)th bath oscillator. In eq 4.5, the first term is diagonal with respect to the system eigenstates and represents the fluctuations of the system energies as a result of the system-bath coupling. The second term is off-diagonal in the system eigenstates and contains information about
relaxation processes amongst the system eigenstates induced by the bath. Generally, for ground state vibrational systems both of these terms are significant and it is of interest to treat both of them.

The interaction Hamiltonian describing the interaction of the system with the external radiation fields $\mathbf{E}$ is,

$$H_{int} = -\mathbf{M}(\mathbf{Q}) \cdot \mathbf{E} = -\sum_{a,b} |a\rangle \mu^{a,b} \cdot \mathbf{E} \langle b|$$ \quad (4.6)

where $\mathbf{M}(\mathbf{Q})$ represents the dipole operator and $\mu^{a,b} = \langle a|\mathbf{M}(\mathbf{Q})|b\rangle$ are the transition dipole matrix elements. Equation 4.6 expresses the interaction in terms of the system eigenstates $(a,b)$, which are described by a set of vibrational quantum numbers. The dipole operator, expressed as a function of the system vibrational coordinates, can be expanded in a power series around the equilibrium position.\textsuperscript{13,18}

$$\mathbf{M}(\mathbf{Q}) = \mu^{(0)} + \sum_i \mu^{(1)}_i \mathbf{Q}_i + \frac{1}{2} \sum_{ij} \mu^{(2)}_{ij} \mathbf{Q}_i \mathbf{Q}_j + \cdots.$$ \quad (4.7)

In the above expression, $\mu^{(0)}$ represents the permanent dipole moment of the system. The linear expansion coefficient $\mu^{(1)}_i = (\partial \mathbf{M}/\partial \mathbf{Q}_i)_{Q_0}$ is the transition dipole moment reflecting the change of the charge distribution through the field-induced displacement of the $i^{th}$ vibrational coordinate. The dipole approximation only accounts for the linear term in the above expansion, leading to the selection rule for linear IR spectroscopy of $\Delta n = \pm 1$ for the vibrational quantum number, $n$. The nonlinear dependence of the dipole operator on the vibrational coordinates, or electrical anharmonicity, is indicated by the presence of non-zero values of higher-order expansion coefficients $\mu^{(n)}$, which relax the above-mentioned selection rule and affect the intensity ratios of fundamentals to their corresponding overtone bands as shown in chapter 2.

The electric field $\mathbf{E}$ in eq 4.6, is a real quantity expressed as a linearly polarized plane wave

$$\mathbf{E}(k, \nu, t) = \sum_j \mathbf{J}_j \mathbf{e}_j(t) \cos(2\pi \nu t + \varphi(t) - k \cdot r).$$ \quad (4.8)

where $k$ is the incident wave vector, $\nu$ is the carrier frequency, $\varphi(t)$ is the time-dependent phase and $\mathbf{e}(t)$ is the time-dependent electric field envelope. The polarization direction of the electric field vector is given by the unit vector $\mathbf{J}$ expressed in the Cartesian coordinates of the laboratory fixed frame, $\mathbf{J} \in \{X,Y,Z\}$. 

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4.2 Third-order Nonlinear Polarization

Figure 4-1a (Color) The pulse sequence and time variables for a 2D IR experiment. The field matter interactions occur at times $t_n$ within the envelope of the three input pulses, $E_n$, to radiate a third-order nonlinear polarization, $P^{(3)}$ at time $t$ following the final pulse. The variables $\tau_n$ represent the experimentally controlled delays between successive input fields measured with respect to the center of the pulses, $t_n$. The variables $\tau'_n$ represent the time interval between the field-matter interactions, $(t_{n+1} - t_n)$. The amplitude and phase of signal field radiated from the sample is obtained by mixing it with a well-characterized local oscillator ($E_{LO}$) field, which is delayed by $\tau_s$ with respect to the final input pulse. The experimental delays $\tau_1$, $\tau_2$ and $\tau_s$ are known as the evolution, waiting and detection periods respectively. (4-1b) The noncollinear “box-cars” phase-matching geometry for the three input fields in 2D IR spectroscopy. The top panel shows the three beams $E_{\alpha}$, $E_{\beta}$ and $E_{\chi}$, entering the sample in a box-car geometry and the signal being emitted in the phase-matched direction: $k_s = -k_{\alpha} + k_{\beta} + k_{\chi}$.

Two-dimensional IR spectroscopy measures the third-order nonlinear polarization $P^{(3)}$ induced by the interaction of the material with three IR fields. The formal expression for $P^{(3)}$ written in the interaction picture after a perturbative expansion of the interaction Hamiltonian, is given by
\[ P^{(3)}(k_1, t, t_2, t_1) = \int_0^\infty \int_0^\infty R\left(t_3', t_2', t_1'\right) E_3\left(k_1, V_3, t - t_3\right) E_2\left(k_2, V_2, t + t_2 - t_3' - t_2'\right) \times E_1\left(k_1, V_1, t + t_2 + t_1 - t_3' - t_2' - t_1'\right) d\tau_1' d\tau_2' d\tau_3'. \] (4.9)

\( \tilde{R}\left(t_3', t_2', t_1'\right) \) is the material response function and the various time variables in eq 4.9 are illustrated in Figure 4-1. The maximum of the field envelopes representing their relative positions are denoted by \( t_n \) and the times at which the successive field-matter interactions occur within the field envelope are represented by \( t'_n \). The delays between the successive input pulses and the successive field-matter interactions are given by \( \tau_n = t_{n+1} - t_n \) and \( \tau'_n = t'_{n+1} - t'_n \) respectively. The experimental delays \( \tau_1, \tau_2 \) and \( \tau_3 \) are known as the evolution, waiting and detection time periods respectively.

\[ \sum \begin{array}{cccc}
R_1 & R_2 & R_3 & R_4 \\
ket-bra-bra & bra-ket-bra & bra-bra-ket & ket-ket-ket \\
| dc & bc & bc & ba \\
db & bd & ac & ca \\
da & ad & ad & da \\
da & a & aa & aa \\
\end{array} 
\]

\[ \begin{array}{cccc}
(R_1)^* & (R_2)^* & (R_3)^* & (R_4)^* \\
br-ket-ket & ket-bra-ket & ket-ket-bra & bra-bra-bra \\
c & cb & cb & ab \\
db & db & ca & ac \\
d & da & da & ad \\
a & a & aa & aa \\
\end{array} \]

Figure 4-2. Evolution of the density matrix for each of the eight terms resulting from the expansion of the total resonant third-order material response function given in eq 4-10. The indices abcd represent the eigenstates of the system under study.

The material response for a third-order resonant experiment is expressed as a four-point correlation function of the dipole operator and contains information about the time-evolution under the material Hamiltonian,\(^19\)

\[ \tilde{R}\left(t_3', t_2', t_1'\right) = \left(\frac{\hbar}{i}\right)^3 \left\{ \left[\left[ M(t_3' + t_2' + t_1') \right. \right. \right. \right. \left. \left. \left. M(t_2' + t_1') \right] M(t_1') \right. \right. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right. \left. \right. \right
These represent the set of Liouville pathways pictured in Figure 4-2 describing the evolution of the system during \( \tau_1, \tau_2, \) and \( \tau_3 \) following interaction with the light fields at \( \tau'_1, \tau'_2, \) and \( \tau'_3 \). The response function is a fourth rank tensor quantity that contains not only the information relevant to vibrational dynamics, but also the relative orientation and orientational dynamics of the dipoles interrogated by the polarized electric fields. Most descriptions of the nonlinear response assume an isotropic material Hamiltonian. This assumption is of limited use for multi-level vibrational systems where the relative orientations of coupled transition dipoles will affect the material response function probed by polarized light fields.\(^{20-22}\)

Tensorial descriptions of the nonlinear response generally begin with the simplifying assumption that the vibronic and rotational degrees of freedom are separable,\(^{17,20,23}\)

\[
H_S = H^V_S + H^R_S
\]  

This separation of variables also allows the transition moments to be written as a product of a unit vector \( \hat{\mu}^{a,b} \) along the coordinate(s) that couples eigenstates \( a \) and \( b \) and the magnitude \( \mu^{a,b} \) of the transition dipole matrix element

\[
\mu^{a,b} = \hat{\mu}^{a,b} \mu^{a,b}
\]  

Following this argument, each of the tensorial responses in eq 4.11 can be written as a product of an isotropic nonlinear vibrational response function \( R^{a,b,c,d}_n \), which describes the vibrational dynamics, and a tensorial nonlinear orientational response function \( (Y_n)_{ijkl}^{a,b,c,d} \), which describes the influence of dipole orientation and orientational dynamics:\(^{20}\)

\[
\hat{R}_n (\tau'_3, \tau'_2, \tau'_1) = \sum_{ijkl} \sum_{abcd} (Y_n)_{ijkl}^{a,b,c,d} \left( \tau'_3, \tau'_2, \tau'_1 \right) R^{a,b,c,d}_n \left( \tau'_3, \tau'_2, \tau'_1 \right).
\]  

The indices for the orientational response \( (I, J, K, L) \) refer to a permutation over the laboratory frame indices \( \{X, Y, Z\} \). The evaluation of the sum over orientational indices in eq. 4.14 is greatly simplified when considering the symmetry relationships for isotropic media, which result in four non-vanishing tensor components \( Y_{ZZZZ}, Y_{ZYYZ}, Y_{ZYZZ}, \) and \( Y_{ZYZZ} \), three of which are independent: \( Y_{ZZZZ} = Y_{ZYYZ} + Y_{ZYZZ} + Y_{ZYYZ} \).\(^{24}\)
4.2.1 Nonlinear Vibrational Response

Using the Liouville-pathways illustrated in Figure 4-2, we can write the four vibrational response functions as,

\[ R_{1}^{a,b,c,d}(r_{3}', r_{2}', r_{1}') = P_{a} \mu_{c,d}^{b,c} \mu_{b,a}^{d,a} \exp \left( -i\omega_{d,e}^{0} r_{3}' - i\omega_{d,b}^{0} r_{2}' - i\omega_{d,a}^{0} r_{1}' \right) F_{1}^{a,b,c,d}(r_{3}', r_{2}', r_{1}') \]

\[ R_{2}^{a,b,c,d}(r_{3}', r_{2}', r_{1}') = P_{a} \mu_{c,d}^{b,c} \mu_{b,a}^{d,a} \exp \left( -i\omega_{b,e}^{0} r_{3}' + i\omega_{d,b}^{0} r_{2}' + i\omega_{d,a}^{0} r_{1}' \right) F_{2}^{a,b,c,d}(r_{3}', r_{2}', r_{1}') \]

\[ R_{3}^{a,b,c,d}(r_{3}', r_{2}', r_{1}') = P_{a} \mu_{c,d}^{b,c} \mu_{b,a}^{d,a} \exp \left( -i\omega_{b,e}^{0} r_{3}' + i\omega_{c,a}^{0} r_{2}' + i\omega_{d,a}^{0} r_{1}' \right) F_{3}^{a,b,c,d}(r_{3}', r_{2}', r_{1}') \]

\[ R_{4}^{a,b,c,d}(r_{3}', r_{2}', r_{1}') = P_{a} \mu_{c,d}^{b,c} \mu_{b,a}^{d,a} \exp \left( -i\omega_{b,a}^{0} r_{3}' - i\omega_{c,a}^{0} r_{2}' - i\omega_{d,a}^{0} r_{1}' \right) F_{4}^{a,b,c,d}(r_{3}', r_{2}', r_{1}') . \]

(4.15 a-d)

In the above equations, \( P_{a} \) reflects the probability of occupying the initial state \( a \). Equation 4.15 shows that the nonlinear vibrational response function is a product of the magnitudes of the four interacting dipoles \( \left( \mu^{p,q} \right) \), an exponential oscillating at the system eigen frequencies

\[ \left( \omega_{p,q}^{0} \right) = \left( E_{p}^{0} - E_{q}^{0} \right) / h \] sampled during \( r_{1}' \), \( r_{2}' \) and \( r_{3}' \), and a nonlinear dephasing function \( \left( F^{a,b,c,d}_{n} \right) \).

Starting with the material Hamiltonian that treats the bilinear coupling of the system to a harmonic bath and assuming Gaussian statistics, the fluctuation dynamics of the system-bath interactions enter into the nonlinear response through the nonlinear dephasing functions \( F^{a,b,c,d}_{n} \).

Analytical solutions for the dephasing functions are obtained by approximating the system-bath interaction Hamiltonian to be diagonal in the system eigenstates, which describes transition energy fluctuations induced by the system-bath interactions. Following ref 1, they can be written in terms of the lineshape functions \( h_{pq}(t) \) as

\[ -\ln F_{1}^{a,b,c,d}(r_{3}', r_{2}', r_{1}') = h_{cc}(r_{3}') + h_{bb}(r_{2}') + h_{dd}(r_{1}' + r_{2}' + r_{3}') + h_{bc}(-r_{3}' - r_{2}') - h_{bc}(-r_{3}') \]

\[ -h_{bc}(-r_{2}') + h_{cd}(r_{1}' + r_{2}') - h_{cd}(r_{1}' + r_{2}' + r_{3}') - h_{cd}(-r_{3}') \]

\[ +h_{bd}(r_{1}') - h_{bd}(-r_{3}' - r_{2}') - h_{bd}(r_{1}' + r_{2}') + h_{bd}(-r_{3}') \]

(4.16 a)

\[ -\ln F_{2}^{a,b,c,d}(r_{3}', r_{2}', r_{1}') = h_{cc}(r_{3}') + h_{dd}(r_{1}' + r_{2}') + h_{bb}(-r_{2}' - r_{3}') + h_{bc}(-r_{2}') - h_{bc}(r_{3}') \]

\[ -h_{bc}(-r_{2}' - r_{3}') + h_{cd}(r_{1}' + r_{2}' + r_{3}') - h_{cd}(r_{1}' + r_{2}') - h_{cd}(r_{3}') \]

\[ +h_{bd}(r_{1}') - h_{bd}(-r_{3}' + r_{1} + r_{2}') - h_{bd}(-r_{2}') + h_{bd}(r_{3}') \]

(4.16 b)
The time dependent fluctuations of the vibrational transition frequency $\omega_{p,q}$ can be expressed as the bath-induced frequency shifts $\delta \omega_{p,q}$ about the ensemble-averaged value $\omega_{p,q}^0$,

$$\omega_{p,q}(t) = \omega_{p,q}^0 + \delta \omega_{p,q}(t).$$  

(4.17)

The time scales of these fluctuations for the different vibrational transitions are expressed in terms of the energy gap auto- ($\xi_{pp}$) and cross- ($\xi_{pq}$) correlation functions \(^1\)

$$\xi_{pq}(t) = \langle \delta \omega_{p,a}(t) \delta \omega_{q,a}(0) \rangle = \frac{1}{\hbar^2} \langle \delta H_{pa}(t) \delta H_{qa}(0) \rangle_b$$

(4.18)

$\zeta$ correlates the fluctuations in the $\omega_{q,a}$ transition energy gap with the $\omega_{p,a}$ energy gap over a time period $t$, where $a$ is the initial state of the density matrix. The term

$$\delta H_{pa} = \sum_{\nu} \left( \chi_{\nu}^p - \chi_{\nu}^a \right) \left( q_{\nu} + \frac{\chi_{\nu}^a}{\omega_{\nu}^2} \right)$$

reflects the change in the system energies as a result of coupling with the $\nu^{th}$ bath oscillator. The lineshape function $h_{pq}(t)$ is related to the energy gap correlation function by

$$h_{pq}(t) = \int_0^t ds_2 \int_0^{s_2} ds_1 \xi_{pq}(s_2 - s_1)$$

(4.19)

Formulating the nonlinear dephasing functions in terms of both auto-and cross- correlation functions demonstrates that 2D vibrational spectroscopies are sensitive to the correlations between the energy gap fluctuations of different vibrational coordinates. \(^11,12,25\) We include the effects of population relaxation with a phenomenological exponential damping constant. The above approximation works well for molecular systems where the dominant relaxation processes are bath-induced vibrational dephasing and reorientational dynamics, such as the metal carbonyls studied here. \(^26-28\) In this formalism, arbitrary time scales can be used to describe the system-bath interactions. Often it is adequate or simpler to model experimental data in the limits of extremely fast (homogenous) or extremely slow (inhomogenous) time scales for the system-bath interactions.
For the fast modulation limit, the timescale of the energy gap correlation function is much faster than the experimental time windows and can be described as \( \zeta_{pq}(t) \approx \Gamma_{pq} \delta(t) \), where \( \Gamma_{pq} \) is the damping constant, giving the time scale of homogeneous system-bath interactions. The corresponding homogeneous lineshape function then becomes linear in time: \( h_{pq}(t) \approx \Gamma_{pq} t \). The nonlinear dephasing functions in this limit simplify to:

\[
- \ln F_1^{a,b,c,d}(\tau_3, \tau_2, \tau_1) = (\Gamma_{dd} + \Gamma_{cc} - 2 \Gamma_{dc}) \tau_3 + (\Gamma_{dd} + \Gamma_{bb} - 2 \Gamma_{db}) \tau_2 + \Gamma_{dd} \tau_1
\]

\[
- \ln F_2^{a,b,c,d}(\tau_3, \tau_2, \tau_1) = (\Gamma_{bb} + \Gamma_{cc} - 2 \Gamma_{bc}) \tau_3 + (\Gamma_{dd} + \Gamma_{bb} - 2 \Gamma_{db}) \tau_2 + \Gamma_{dd} \tau_1
\]

\[
- \ln F_3^{a,b,c,d}(\tau_3, \tau_2, \tau_1) = (\Gamma_{bb} + \Gamma_{cc} - 2 \Gamma_{bc}) \tau_3 + \Gamma_{cc} \tau_2 + \Gamma_{dd} \tau_1
\]

\[
- \ln F_4^{a,b,c,d}(\tau_3, \tau_2, \tau_1) = \Gamma_{bb} \tau_3 + \Gamma_{cc} \tau_2 + \Gamma_{dd} \tau_1
\]

In the above expressions, \( \Gamma_{xy} = \Gamma_{yx} \) and when \( x \) or \( y \) is the ground state \( a \), \( \Gamma_{xy} = 0 \) implying that \( \Gamma_{xa} = \Gamma_{aa} = 0 \).

In the inhomogeneous limit, the energy gap correlation function is static on the timescale of the experiment and is described as \( \zeta_{pq}(t) = \rho_{pq} \sigma_{pp} \sigma_{qq} \) and the lineshape function is given by

\[ h_{pq}(t) = \rho_{pq} \sigma_{pp} \sigma_{qq} t^2 / 2 \].

The variable \( \rho_{pq} \) is the dimensionless correlation coefficient defined as

\[ \rho_{pq} = \frac{\langle \delta \omega_{p,a} \delta \omega_{q,a} \rangle_B}{\sigma_{pp} \sigma_{qq}} \]

and \( \sigma_{pp} \langle \sigma_{qq} \rangle \) is proportional to the magnitude of the static distribution of the transition frequencies of the system eigenstate \( p(q) \) around its central transition frequency. For two coupled vibrations \( p \) and \( q \), the nature of the microscopic system-bath interactions and the way in which they affect the coupling may lead to a statistical interdependence between the individual transition shifts \( \delta \omega_{p,a} \) and \( \delta \omega_{q,a} \). This effect is characterized by the value of the correlation coefficient varying from –1 for an anti-correlated to +1 for a completely correlated system respectively.\textsuperscript{29-31} The nonlinear dephasing functions in the inhomogenous limit are Gaussian functions of \( \tau_1', \tau_2' \), and \( \tau_3' \):

\[
- \ln F_1^{a,b,c,d}(\tau_3', \tau_2', \tau_1') = \left[ \sigma_{bb}^2 \tau_3' + \sigma_{cc}^2 \tau_2' + \sigma_{dd}^2 (\tau_1' + \tau_2')^2 \right] / 2 - \rho_{bc} \sigma_{bb} \sigma_{cc} \tau_3' (\tau_2' + \tau_3')
\]

\[
+ \rho_{bd} \sigma_{bb} \sigma_{dd} (\tau_2' + \tau_3') (\tau_1' + \tau_2') + \rho_{cd} \sigma_{cc} \sigma_{dd} (\tau_3' + \tau_2')^2
\]

\[
- \ln F_2^{a,b,c,d}(\tau_3', \tau_2', \tau_1') = \left[ \sigma_{bb}^2 \tau_3' + \sigma_{cc}^2 \tau_2' + \sigma_{dd}^2 \tau_1' \right] / 2 + \rho_{bc} \sigma_{bb} \sigma_{cc} \tau_2' \tau_3'
\]

\[
+ \rho_{cd} \sigma_{cc} \sigma_{dd} (\tau_3' + \tau_2')^2 + \rho_{bd} \sigma_{bb} \sigma_{dd} (\tau_1' + \tau_3')^2
\]
\[ -\ln F_{3}^{a,b,c,d}(t_3, t_2, t_1) = \left[ \sigma_{bb}^2 \tau_3^2 + \sigma_{cc}^2 (t_2 + t_3)^2 + \sigma_{dd}^2 \tau_1^2 \right]^{1/2} - \rho_{bc} \sigma_{bb} \sigma_{cc} \tau_3 (t_2 + t_3) \]

\[ -\rho_{bd} \sigma_{bb} \sigma_{dd} \tau_2 (t_1 + t_2) + \rho_{cd} \sigma_{cc} \sigma_{dd} \tau_1 (t_2 + t_3) \]

\[ -\ln F_{4}^{a,b,c,d}(t_3, t_2, t_1) = \left[ \sigma_{bb}^2 \tau_2^2 + \sigma_{cc}^2 \tau_3^2 + \sigma_{dd}^2 (t_1 + t_2 + t_3)^2 \right]^{1/2} + \rho_{bc} \sigma_{bb} \sigma_{cc} \tau_2 \tau_3 \]

\[ -\rho_{bd} \sigma_{bb} \sigma_{dd} \tau_2 (t_1 + t_2 + t_3) - \rho_{cd} \sigma_{cc} \sigma_{dd} \tau_3 (t_1 + t_2 + t_3) \]

(4.21 a-d)

For a system with a well-defined separation of time scales the system-bath interactions can be divided into fast and slow components, which are treated in the homogeneous and inhomogeneous limits respectively. The nonlinear dephasing functions for this model are obtained from the product of the nonlinear dephasing functions for the homogeneous and inhomogeneous limits.\textsuperscript{29}

For a multilevel vibrational system, the nonlinear dephasing function is expressed in terms of energy gap correlation functions involving the ground, singly and doubly excited vibrational states.\textsuperscript{32-36} In the case of weakly anharmonic systems, this large set of correlation functions can be reduced by using the harmonic approximation to express the frequency fluctuations between the one- and two-quantum states in terms of the frequency fluctuations between the ground and one-quantum states.

Using the six-level system of RDC (\(0, a, s, 2a, as, 2s\)) as an example (see Fig 3-1), we find that

\[ \zeta_{a,2a} = 2\zeta_{aa}; \quad \zeta_{s,2s} = 2\zeta_{ss} \] \hspace{1cm} (4.22 a)

\[ \zeta_{2a,2a} = 4\zeta_{aa}; \quad \zeta_{2s,2s} = 4\zeta_{ss} \] \hspace{1cm} (4.22 b)

\[ \zeta_{a,as} = \zeta_{aa} + \zeta_{as}; \quad \zeta_{s,as} = \zeta_{ss} + \zeta_{as} \] \hspace{1cm} (4.22 c)

\[ \zeta_{as,as} = \zeta_{aa} + \zeta_{ss} + 2\zeta_{as} \] \hspace{1cm} (4.22 d)

The above approximation works when the fluctuations in the anharmonicities are negligible and the energy-gap correlation functions are real quantities.\textsuperscript{37}

### 4.2.2 Nonlinear Orientational Response

We now turn our attention to the calculation of the orientational response function, which has been described in detail in refs 20 and 1 within the model of orientational diffusion. This response reflects the sequential projection of the electric fields in the laboratory frame onto the molecular transition dipole moments for a particular sequence of electric field interactions, allowing the rigid molecules to orientationally diffuse between successive field-matter interactions. Starting from an isotropic distribution, each field interaction projects out a subset of molecules from the evolving orientational distribution. The orientational response is evaluated by (1) expressing the orientation of
the four time-ordered transition dipole operators in a molecular body-fixed frame \((i,j,k,l \in x,y,z)\) and

(2) transforming the motion in the molecular frame into the laboratory frame through an orientational average. The four factors \(Y_n\) differ only in the sequence of interactions with the electric field:

\[
(Y_1)_{ijkl}^{a,b,c,d} (\tau_3, \tau_2, \tau_1) = \sum_{ijkl} \tilde{Y}_{ijkl} \left( \tau_3, \tau_2, \tau_1 \right) \left[ \hat{\mu}^a \cdot \hat{i} \right] \left[ \hat{\mu}^b \cdot \hat{j} \right] \left[ \hat{\mu}^c \cdot \hat{k} \right] \left[ \hat{\mu}^d \cdot \hat{i} \right] \quad (4.23a)
\]

\[
(Y_2)_{ijkl}^{a,b,c,d} (\tau_3, \tau_2, \tau_1) = \sum_{ijkl} \tilde{Y}_{ijkl} \left( \tau_3, \tau_2, \tau_1 \right) \left[ \hat{\mu}^c \cdot \hat{i} \right] \left[ \hat{\mu}^b \cdot \hat{j} \right] \left[ \hat{\mu}^d \cdot \hat{k} \right] \left[ \hat{\mu}^a \cdot \hat{i} \right] \quad (4.23b)
\]

\[
(Y_3)_{ijkl}^{a,b,c,d} (\tau_3, \tau_2, \tau_1) = \sum_{ijkl} \tilde{Y}_{ijkl} \left( \tau_3, \tau_2, \tau_1 \right) \left[ \hat{\mu}^b \cdot \hat{i} \right] \left[ \hat{\mu}^d \cdot \hat{j} \right] \left[ \hat{\mu}^a \cdot \hat{k} \right] \left[ \hat{\mu}^c \cdot \hat{i} \right] \quad (4.23c)
\]

\[
(Y_4)_{ijkl}^{a,b,c,d} (\tau_3, \tau_2, \tau_1) = \sum_{ijkl} \tilde{Y}_{ijkl} \left( \tau_3, \tau_2, \tau_1 \right) \left[ \hat{\mu}^a \cdot \hat{i} \right] \left[ \hat{\mu}^b \cdot \hat{j} \right] \left[ \hat{\mu}^c \cdot \hat{k} \right] \left[ \hat{\mu}^d \cdot \hat{i} \right] \quad (4.23d)
\]

The transformation of the diffusive orientational motion of the molecular frame into the laboratory frame is treated classically, and is expressed as a four-point joint probability function,

\[
\tilde{Y}_{ijkl} \left( \tau_3, \tau_2, \tau_1 \right) = \int d\Omega_0 \int d\Omega_2 \int d\Omega_1 \int d\Omega_0 \left[ \hat{i} (\Omega_0) \cdot \hat{i} \right] G \left( \Omega_0, \tau_3 | \Omega_2 \right) \\
\times \left[ \hat{j} (\Omega_2) \cdot \hat{j} \right] G \left( \Omega_2, \tau_2 | \Omega_1 \right) \left[ \hat{k} (\Omega_1) \cdot \hat{k} \right] \\
\times G \left( \Omega_1, \tau_1 | \Omega_0 \right) \left[ \hat{l} (\Omega_0) \cdot \hat{l} \right] P(\Omega_0) \quad (4.24)
\]

In the above expression, \(\Omega\) represents the Euler angles that transform the microscopic frame into the laboratory fixed frame, and \(P(\Omega_0) = 1/8\pi^2\) is the initial isotropic orientation of molecules. Note that the time-ordering of the polarization indices are read from right to left. The term \(G(\Omega_{n+1}, \tau_{n+1} | \Omega_n, 0)\) is a conditional probability that relates the initial orientation of a molecule \(\Omega_n\) to an orientation \(\Omega_{n+1}\) after a time \(\tau_{n+1}\) assuming that \(\Omega(t)\) is a Markovian process on the experimental timescale. These conditional probability functions have been described for solutions to various orientational diffusion equations.38,39

To calculate the analytical expressions for the orientational averages \(\tilde{Y}_{ijkl}^{a,b,c,d}\) described by eq 4.24 we need to describe the conditional probability distribution function \(G(\Omega_{n+1}, \tau_{n+1} | \Omega_n, 0)\) for an ensemble of symmetric diffusers. This is expressed in terms of Wigner rotation matrices, \(D^J_{K,M}(\Omega)\) as shown below and explained in refs 38 and 40,

\[
G(\Omega_{n+1}, \tau_{n+1} | \Omega_n, 0) = \sum_{J,K,M} \frac{2J+1}{8\pi^2} C^J_{K,M}(\tau_{n+1}) D^J_{K,M}(\Omega_n) D^J_{K,M}^*(\Omega_{n+1}) \quad (4.25)
\]

The integrals in equation 4.24 can be simplified by use of the orthogonality properties of the Wigner rotation matrices as shown below.40
\[
\int D_{K_1, M_1}^{J_1} \left( \Omega \right) D_{K_2, M_2}^{J_2} \left( \Omega \right) d\Omega = \frac{2\pi^2}{2J_1 + 1} \delta_{K_1, K_2} \delta_{M_1, M_2} \delta_{J_1, J_2}
\]

\[
\int D_{K_3, M_3}^{J_3} \left( \Omega \right) D_{K_2, M_2}^{J_2} \left( \Omega \right) D_{K_1, M_1}^{J_1} \left( \Omega \right) d\Omega = \frac{2}{2J_3 + 1} \delta_{K_1 + K_2, K_3} \delta_{M_1 + M_2, M_3}
\]

\[C(J_1; J_2; J_3; K_1; K_2) C(J_1; J_2; J_3; M_1; M_2)\]  \tag{4.26}

In the above expressions, \(C\) represents the Clebsch-Gordon coefficients, which are tabulated in books of angular momentum such as ref 40. The time dependence in eq 4.25 is carried by the expansion coefficients,

\[
c_{K,M}^J (\tau_{n+1}) = \exp \left[ J(J+1)D_{or}^\perp \tau_{n+1} + M^2 \left( D_{or}^\perp - D_{or}^\parallel \right) \tau_{n+1} \right]
\]  \tag{4.27}

The above expression is written for the symmetric diffuser where \(D_{or}^\perp\) and \(D_{or}^\parallel\) are the diffusion coefficients for rotation perpendicular and parallel to the unique axis respectively.\(^{22}\) The expression simplifies for the case of the spherical diffuser where, \(D_{or}^\perp = D_{or}^\parallel\) and the expansion coefficients depend only on the \(J\) quantum number,

\[
c_{K,M}^J (\tau_{n+1}) = c_J (\tau_{n+1}) = \exp \left[ J(J+1)D_{or} \tau_{n+1} \right]
\]  \tag{4.28}

Equation 4.24 can be solved using the above equations and given the orientations of the transition dipoles in the molecular body fixed frame and the polarization of the input light fields.

The analytical expressions for \(\tilde{\gamma}_ijkl^L_{LKL}\) calculated by evaluating eq. 12 for the response from parallel or two orthogonal dipole moments are tabulated in Table 4-1. The expressions for the orientational response for four arbitrary dipoles probed with arbitrary polarization can be written as a linear superposition of these functions weighted by the projection of the molecular transition dipoles onto an axis system in the molecular body-fixed frame as shown in eqs 4.23 a-d in the main text. In practice, the summation over microscopic frame indices in eq 4.23 is greatly simplified by using the existing symmetry relations regarding the interchange of the laboratory and microscopic frame indices.\(^{20}\) A judicious choice of polarizations for the incident fields or a combination of experiments with varying polarization can be used to reveal the relative orientation of the system transition dipole moments and to separate the contributions of the vibrational and orientational responses in the measured signal.\(^{4,41,42}\)
Table 4-1 Non-vanishing tensor components of the orientational part of the response function for interactions involving parallel (zzzz) and two orthogonal transition dipole moments z and y in the molecular body fixed frame. In the table below, $c_1(\tau^*) = \exp[-2D_{zz}\tau^*]$ and $c_2(\tau^*) = \exp[-6D_{zz}\tau^*]$.

<table>
<thead>
<tr>
<th>Tensor element</th>
<th>Orientational part of the response function</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_{zzzz}$</td>
<td>$\frac{1}{9} c_1(\tau_1)c_1(\tau_1)\left(1+\frac{4}{5} c_2(\tau_2)\right)$</td>
</tr>
<tr>
<td>$Y_{yyzz}$</td>
<td>$\frac{1}{9} c_1(\tau_1)c_1(\tau_1)\left(1-\frac{2}{5} c_2(\tau_2)\right)$</td>
</tr>
<tr>
<td>$Y_{yzzz} = Y_{ivz}$</td>
<td>$\frac{1}{15} c_1(\tau_1)c_1(\tau_3)c_2(\tau_2)$</td>
</tr>
<tr>
<td>$Y_{zyzy}$</td>
<td>$\frac{1}{9} c_1(\tau_1)c_1(\tau_1)\left(1-\frac{2}{5} c_2(\tau_2)\right)$</td>
</tr>
<tr>
<td>$Y_{iyzy} = Y_{ivzy}$</td>
<td>$\frac{1}{15} c_1(\tau_1)c_1(\tau_3)c_2(\tau_2)$</td>
</tr>
<tr>
<td>$Y_{zyzy} = Y_{evzy}$</td>
<td>$\frac{1}{15} c_1(\tau_1)c_1(\tau_3)c_2(\tau_2)$</td>
</tr>
<tr>
<td>$Y_{zyzy} = Y_{iyzy}$</td>
<td>$-\frac{1}{30} c_1(\tau_1)c_1(\tau_3)c_2(\tau_2)$</td>
</tr>
<tr>
<td>$Y_{zyzy} = Y_{evzy}$</td>
<td>$\frac{1}{20} c_1(\tau_1)c_1(\tau_3)c_2(\tau_2) - \frac{1}{12} c_1(\tau_1)c_1(\tau_3)c_1(\tau_2)$</td>
</tr>
</tbody>
</table>

4.3 Phase Matching and Pulse Time-Ordering

Using the theory outlined above and explained in further detail in ref 1 we can obtain an expression for the third-order nonlinear polarization given (1) a system Hamiltonian, (2) an energy gap correlation function, and (3) polarization of input fields. The eight contributions to the material response shown in Figure 4-2 can be written specifically for any combination of input wave-vectors, frequencies and time-orderings. Eight possible permutations of interactions with the three input
electric fields results in 48 possible components to the response function. Each of these 48 contributions consists of multiple possible Liouville pathways based on the number of eigenstates of a specific system. In practice, only a subset of the 48 particular components of the third-order nonlinear polarization vector are measured for a choice of frequency, wave-vectors, time-orderings and polarizations of the input pulses and detecting the signal field in a specific phase-matched direction. Under perfect phase matching conditions, assuming that the electric field is a plane wave and the slowly varying envelope approximation holds, the signal field for the particular input conditions is given as,

\[
E_{\text{sig}}(k_s,t,\tau_2,\tau_1) = i\omega_s \frac{2\pi\ell}{n(\omega_s)c} P^{(3)}(k_s,t,\tau_2,\tau_1) \tag{4.29}
\]

In the above expression, \(\omega_s\) is the signal frequency, \(n\) is the index of refraction, \(c\) is the speed of light, and \(\ell\) is the interaction length.

For the resonant 2D IR experiments described in this paper, we consider three input fields \(E_\alpha, E_\beta\) and \(E_\chi\), each with a unique incident wave-vector \(k_\alpha, k_\beta\) and \(k_\chi\). The polarization vectors of the input fields \(\alpha, \beta\) and \(\chi\) are represented by the indices \(L, K\) and \(J\) respectively. These pulses cross in the sample to generate a third-order nonlinear polarization, which radiates a signal field into the phase-matched direction (Fig 4-1). For this phase-matching condition and assuming the rotating wave approximation, there are sixteen double-sided Feynman diagrams relevant to a multi-level vibrational system derived from the eight Liouville pathways shown in Figure 4-2. These sixteen diagrams, illustrated in Figure 4-3, describe the evolution of the density matrix for all the possible time-orderings (1-2-3) of the wave-vectors \(\alpha, \beta\) and \(\chi\). Each diagram, in turn, has a number of components depending on the specific Liouville pathway for the six eigenstates of our system: the ground state \(0\), the one-quantum states \(\{a, s\}\) and the two-quantum states \(\{2a, 2s\}\). The diagrams are broadly separated into two main categories of rephasing (\(S_I\)) and nonrephasing (\(S_{II}\) and \(S_{III}\)) third-order response functions. They are further classified by the time-orderings of pulses along the incident wave-vectors as, \(S_I = -k_1 + k_2 + k_3\), \(S_{II} = +k_1 - k_2 + k_3\) and \(S_{III} = +k_1 + k_2 - k_3\). In a rephasing (or echo) experiment, the phase acquired by coherences during the evolution period, \(e^{i\omega_p r_1}\), is the conjugate of that for the detection period \(e^{-i\omega_p r_3}\). The nonrephasing diagrams of \(S_{II}\) and \(S_{III}\) evolve with the same frequency during \(r_1\) and \(r_3\) and cannot rephase macroscopically. Note that the contributions from \(S_{III}\) are
unique to multi-level systems, where it is possible to create a vibrational coherence between the ground and two-quantum state after the first two interactions.

Figure 4-3. The double sided Feynman diagrams contributing to the rephasing and nonrephasing Liouville-space pathways for all possible time ordering of the input fields $E_\alpha$, $E_\beta$ and $E_\chi$ given the phase-matching condition: $s_\alpha s_\beta s_\chi = -k_1 + k_2 + k_3$. The numbering 0, 1 and 2 represent the ground, first and second excited states of a multi-level system. For our model system RDC, $\{1,1\} \in \{a,s\}$ and $2 \in \{2a,as,2s\}$.

For the purposes of simulating the experimental signals, we assume that the pulse length in our experiment is much shorter than any of the vibrational dynamics of interest, making the
evaluation of the triple convolution integral in eq 4.9 trivial. In this limit, we take the observed time-domain signal to be

$$S(k_s, \tau_3, \tau_2, \tau_1) \propto \text{Re} \left[ \hat{R}(\tau_3, \tau_2, \tau_1) \right].$$

(4.30)

The data is represented as a correlation map of frequencies through a double Fourier transform (FT)

$$\tilde{S}(k_s, \omega_3, \tau_2, \omega_1) = \text{Re} \left[ \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} S(k_s, \tau_3, \tau_2, \tau_1) e^{i\omega_1 \tau_1} e^{i\omega_3 \tau_3} d\tau_1 d\tau_3 \right].$$

(4.31)

where $\omega_1$ and $\omega_3$ are Fourier transform pairs of the experimental delays, $\tau_1$ and $\tau_3$.

### 4.4 Absorptive Lineshapes in 2D FT spectroscopy

The information about the material response discussed in the earlier sections of this chapter is contained in the positions, amplitudes and line shapes of the resonances in a 2D spectrum. Figure 4-4 illustrates the commonly encountered absolute value, phase-twisted and absorptive lineshapes encountered in 2D Fourier transform spectroscopy. For well-separated resonances, an absolute value 2D spectrum is sufficient to reveal the spectroscopic observables necessary to obtain information about molecular structure and dynamics. In congested absolute value 2D spectra with close-lying resonances, the broad wings and missing sign of the peaks hinder the analysis (Fig 4-4, left panel). Even the 2D cosine Fourier transform spectrum of a particular phase-matched signal (i.e. rephasing or nonrephasing) is not ideal because it contains “phase-twisted” peaks, with inherently mixed absorptive and dispersive character (Fig 4-4, middle panel). For an intuitive interpretation and simplified modeling of 2D spectra, purely absorptive features (Fig 4-4, right panel) free of distorting dispersive components are desired.

![Figure 4-4](image)

**Figure 4-4.** (Color) Commonly encountered line shapes in 2D Fourier transform spectroscopy.
As observed originally in 2D NMR, the line shapes obtained from any 2D FT spectroscopy are of a mixed-phase character.\textsuperscript{49} Absorptive line shapes in 2D spin correlation spectra are obtained by phase-cycling techniques where two signals oscillating with conjugate frequencies during the evolution period are added to remove the dispersive character of the phase-twisted peaks.\textsuperscript{50,51} In the case of nonlinear optical and IR spectroscopy, the use of a non-collinear excitation geometry where the signal is heterodyne-detected in a specific phase-matched direction substitutes for phase-cycling used in NMR.\textsuperscript{17,46,47,52,53} The phase relationship between the vibrational frequencies excited during the initial and final time periods is dictated by the time ordering of the input pulses for a particular phase-matching geometry. A 2D IR correlation spectrum with absorptive line shapes is obtained by the summation of 2D spectra measured by complementary rephasing and nonrephasing experiments.\textsuperscript{54} These experiments sampling conjugate frequencies in the initial time period are performed in a fixed phase-matched direction by exchanging the sequence of the first two pulses along the incident wave-vectors.

The time sequence for the three input pulses (1-2-3) entering the sample for the rephasing and nonrephasing experiments are $\alpha - \beta - \chi$ and $\beta - \alpha - \chi$. When $\tau_2 = 0$ and the last two pulses are time-coincident on the sample, the pulse orderings for the rephasing sequence, $\alpha - \beta - \chi$ and $\alpha - \chi - \beta$ are indistinguishable. Similarly, contributions from the pulse orderings $\beta - \alpha - \chi$ and $\beta - \chi - \alpha$ are present in the nonrephasing experiment when $\tau_2 = 0$. The Liouville pathways sampled by the rephasing experiments at $\tau_2 = 0$ are illustrated in the first two rows of Figure 4-3, while those sampled in the nonrephasing experiment are shown in the fourth and sixth row of the same figure. The presence of the $S_{III}$ contribution at $\tau_2 = 0$ in the nonrephasing response for multilevel systems avoids the discontinuity at $\tau_1 = 0$ which has been predicted and observed for two-level systems in 2D electronic spectroscopy.\textsuperscript{53,55}

Figure 4-5 offers a pictorial representation of how the sum of 2D spectra obtained from conjugate rephasing and nonrephasing experiments gives rise to absorptive features. The response functions $R_R$ and $R_{NR}$ for rephasing and nonrephasing experiments on a two-level system are illustrated in Figure 4-5a, showing that the system evolves in conjugate frequencies during the evolution period. The signal for each of these experiments, written using the procedure outlined in Section I for infinitely short input pulses, is

$$S_R(k_x, \tau_3, \tau_2, \tau_1) \propto (Y_2)^{0,a,0,a}_{ZZZZ} \mu_{0,a} \mu^{a,0} \mu^{a,0} \mu_{0,a} \exp(-i\omega^{0}_{a,b} \tau_3 + i\sigma^{0}_{a,b} \tau_1) F_2^{0,a,0,a} (\tau_3, \tau_2, \tau_1) + c.c. \quad (4.32)$$
\[ S_{NR}(k_s, \tau_3, \tau_2, \tau_1) \propto \left(Y_{ZZZZ}^{0,a,0,a} \mu^{0,a} \mu^{a,0} \mu^{a,0} \mu^{0,a} \exp(-i\omega_{a,0}^0 \tau_3 - i\omega_{a,0}^0 \tau_1) \right) F_{1}^{0,a,0,a}(\tau_3, \tau_2, \tau_1) + c.c. \]

(4.33)

In the above expression, the polarization of the input fields are aligned along the \( \hat{Z} \) axis in the laboratory fixed frame. We assume that the dynamics of the system can be described in the motionally narrowed limit, for which \( F_{2}^{0,a,0,a} = F_{1}^{0,a,0,a} = \exp\left[-\left(\Gamma_{aa}^\tau \tau_3 + \Gamma_{aa}^\tau \tau_1 \right)\right] \) at \( \tau_2 = 0 \). The timescale of the system-bath interactions resulting in the fluctuations of the transition energies of state \( a \) with respect to the ground state \( 0 \) is given by \( \Gamma_{aa}^{-1} \). The only difference in the two signals, \( S_R \) and \( S_{NR} \) is the oscillation of the system in conjugate frequencies during the evolution period. The 2D spectra are obtained after a double Fourier transform of the response functions following eq 4.31 and setting \( \tau_2 = 0 \) in the above equations.

**Figure 4-5.** (Color) (a) The Feynman diagrams for a particular rephasing and nonrephasing pathway for a two-level system consisting of a ground (0) and an excited state (a). (b) The corresponding 2D spectra (\( \tilde{S}_R \) and \( \tilde{S}_{NR} \)) showing phase-twisted features plotted in the \((\pm \omega_1, \pm \omega_3)\) and \((\pm \omega_1, \pm \omega_3)\) quadrants of the Fourier plane, respectively. (c) Mirror image (\( \tilde{S}_R \)) of the 2D rephasing spectrum plotted in the \((\pm \omega_1, \pm \omega_3)\) quadrants. (d) Purely absorptive 2D correlation spectrum obtained from the addition of the nonrephasing (\( \tilde{S}_{NR} \)) and rephasing (\( \tilde{S}_R \)) 2D spectra in (b) and (c).
The rephasing and nonrephasing 2D spectra are plotted below their respective response functions in Figure 4-5b. We see that the spectral representation of the rephasing and nonrephasing signals $\tilde{S}_R$ and $\tilde{S}_{NR}$ appear in the $((\pm \omega_1, \pm \omega_3))$ and $(\pm \omega_1, \pm \omega_3)$ quadrants of the Fourier plane, respectively. The conjugate symmetry for $\tilde{S}_R$ and $\tilde{S}_{NR}$ results from the oscillation of the system in conjugate frequencies during $\tau_1$ in the two experiments. Figure 4-5c shows the mirror image of the rephasing spectrum in the $(\pm \omega_1, \pm \omega_3)$ quadrants. We will refer to this mirror image as the rephasing spectrum $\tilde{S}_R$ for the rest of the paper and it will be plotted in the $(\omega_1, \omega_3)$ quadrant of the Fourier plane.

The rephasing and nonrephasing 2D spectra plotted in the $(\omega_1, \omega_3)$ quadrant show phase-twisted line shapes elongated along the diagonal and off-diagonal axes. This phase-twisted line shape is composed of absorptive and dispersive features. Only a slice taken exactly along the resonance in either frequency dimension yields an absorptive feature. The line shape becomes distorted for a slice taken slightly off-resonance along any frequency dimension. The addition of the two spectra, shown in Figure 4-5d, yields a purely absorptive feature, by canceling the dispersive components of the individual spectra.

The purely absorptive line shape shown in Figure 4-5d results from the addition of equally weighted rephasing and nonrephasing Liouville pathways. Figure 4-6 illustrates line shapes obtained in 2D IR correlation spectra from the summation of unequally weighted rephasing and nonrephasing pathways. These 2D line shapes have some mixed-phase character reflected by their varying tilts from the $\omega_1$ axis. The degree of phase-twist can be quantified by an angle $\Psi$ given by

$$\tan(\Psi) = (A_R - A_{NR})/(A_R + A_{NR})$$

(4.34)

where $A_R$ and $A_{NR}$ represent the amplitudes of the rephasing and nonrephasing signals respectively. The above definition implies that when $A_{NR} < A_R$ the 2D line shape will be tilted along the diagonal axis and $0 < \Psi < \pi/4$. Alternatively, the line shape will be tilted along the off-diagonal axis when $A_R < A_{NR}$ and $\pi/2 < \Psi < 3\pi/4$.

There are a number of effects that give rise to differing amplitudes in the rephasing and nonrephasing signals for a particular peak in a 2D IR correlation spectrum. The first is an unequal sampling of rephasing and nonrephasing pathways to form the same spectral feature in the 2D correlation spectrum. For all multi-level vibrational systems there exists an inherent asymmetry in the number of rephasing and nonrephasing pathways contributing to the formation of some of the features in a 2D correlation spectrum (see Table 4A-13 in the Appendix 4A). This results in the cross-peaks
being tilted with respect to the diagonal peaks in the 2D IR correlation spectrum of RDC in hexane.\textsuperscript{54} Other than a discrepancy in the number of pathways the relative amplitudes of the rephasing and nonrephasing signals are also affected by microscopic factors contained in the material response of the system probed during the two different experiments. These include (i) the effect of different dephasing dynamics sampled in rephasing and non-rephasing experiments, (ii) inhomogeneity in the system and the degree of correlation of inhomogeneity between the coupled modes\textsuperscript{25,30,57} (iii) the amplitude of the dipole products for particular peaks in the two different signals and (iv) the different orientational contributions to the rephasing and non-rephasing spectra depending on which tensor component of the nonlinear signal is measured\textsuperscript{42}. 2D electronic correlation spectra indicate that the presence of a Stokes shift would also lead to a phase-twist in the 2D electronic correlation spectrum\textsuperscript{52,57}. In general, the angle $\Psi$ is also affected by the timing and optical phase of the fs pulses used to excite and detect the third-order nonlinear polarization. With respect to the signals, $\tilde{S}_R$ and $\tilde{S}_{NR}$ given in eqs 4.32 and 4.33, it would imply that the two nonlinear dephasing functions are different and as a result $F_2^{0,a,0,a} \neq F_1^{0,a,0,a}$. The effect of inhomogeneity present in the system and the degree of correlation of inhomogeneity between coupled modes are observed experimentally in the 2D IR correlation spectrum of RDC in chloroform.\textsuperscript{30,37} Different orientational contributions, $Y_{ijkl}^{LK}$ to the rephasing and nonrephasing spectrum also result in phase-twisted line shapes as seen in the polarization-selective 2D IR correlation spectra of RDC dissolved in hexane (See chapters 6 and 7).

**Figure 4-6.** (Color) Phase-twisted line shapes in 2D correlation spectra resulting from the addition of unequally weighted rephasing and nonrephasing signals. In this figure, $A_R$ and $A_{NR}$ represent the amplitudes of the rephasing and nonrephasing signals respectively. For the five cases illustrated $A_R = 0$, $A_R = \frac{1}{2} A_{NR}$, $A_R = A_{NR}$, $A_R = 2A_{NR}$ and $A_{NR} = 0$, the phase twist angle $\Psi = 3\pi/4$, $3\pi/5$, $0$, $\pi/10$ and $\pi/4$. 

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Appendix 4.A

In this Appendix we will use the theory presented in chapter 4 to calculate the 2D IR correlation spectrum of a multi-level system. Our example will be the model system RDC described in chapter 3. Tables 4A1-12 represent all the doubles sided Feynman diagrams giving rise to the various peaks in a 2D IR spectrum. There will be 12 peaks labeled 1(1')- 6 (6') as shown in Figure 6-1 of Chapter 6. These diagrams reveal the inherent imbalance of the rephasing and non-rephasing pathways present in the 2D vibrational correlation spectrum of a multi-level system, which is tabulated in Table 4A-13.

Using the harmonic approximation for the nonlinear dephasing functions, they can be further simplified as shown in Table 4A-14.

Tables 4A15-17 give the explicit expressions for the orientational response functions containing all the angles between the transition dipoles of the eigenstates affecting the amplitudes of the ten peaks in the 2D IR correlation spectrum of RDC. The angle between $\hat{\mu}^{s,0}$ and $\hat{\mu}^{s,0}$ is known as $\Theta_1$, while the other angles are defined below:

\[ \Theta_2 : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{s,a} \]
\[ \Theta_3 : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{2a,s} \]
\[ \Theta_4 : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{s,a} \]
\[ \Theta_5 : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{a,s} \]
\[ \Theta_6 : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{a,a} \]
\[ \Theta_7 : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{a,s} \]
\[ \Theta_8 : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{2a,s} \]
\[ \Theta_9 : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{2s,a} \]
\[ \Theta_{10} : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{2s,a} \]
\[ \Theta_{11} : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{2a,a} \]
\[ \Theta_{12} : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{2s,a} \]
\[ \Theta_{13} : \text{Angle between} \quad \hat{\mu}^{s,0} \quad \text{and} \quad \hat{\mu}^{2s,a} \]

The tensor components for two dipoles aligned along the z and y directions of the molecular body frame have been given in Table 4-1. To calculate the orientational factors for the experimental polarization selective spectra, the indices IJKL have to be replaced by the appropriate indices of the laboratory frame and using the Table 4-1.
Figure 4A-1. All the possible vibrational transitions for our model six-level system, where 0 represents the ground state and a and s represent one quantum of energy in the asymmetric and symmetric stretches respectively. Harmonic oscillator selection rules are employed, which allow the vibrational quantum number of the asymmetric (a) stretch to change by $\pm 1$, while the quantum number of the symmetric (s) stretch is held constant, and vice versa. The forbidden three-quantum transitions, where the vibrational quantum number of the asymmetric stretch change by $\pm 1$ and simultaneously the quantum number of the symmetric stretch changes by $\mp 2$, and vice versa are indicated by dashed lines connecting a to 2s and s to 2a.
### Table 4A-1. Double-sided Feynman diagrams resulting in the formation of diagonal peak 1 at \((\omega_A, \omega_3) = (\omega_{A1}, \omega_{A2})\)

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasing</th>
<th>Non-rephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(S_I)</td>
<td>(S_{II})</td>
</tr>
<tr>
<td></td>
<td>(\alpha - \beta - \chi)</td>
<td>(\alpha - \chi - \beta)</td>
</tr>
<tr>
<td>(K^0_{3},0,0,s) ((Y_3))_{JKL}</td>
<td>(s\ 0)</td>
<td>(0\ s)</td>
</tr>
<tr>
<td>(K^0_{2},0,0,s) ((Y_2))_{JKL}</td>
<td>(s\ 0)</td>
<td>(0\ s)</td>
</tr>
<tr>
<td>(K^0_{4},0,0,s) ((Y_4))_{JKL}</td>
<td>(s\ 0)</td>
<td>(0\ s)</td>
</tr>
<tr>
<td>(K^0_{1},0,0,s) ((Y_1))_{JKL}</td>
<td>(s\ 0)</td>
<td>(0\ s)</td>
</tr>
<tr>
<td>(K^0_{4},0,0,s) ((Y_4))_{JKL}</td>
<td>(s\ 0)</td>
<td>(0\ s)</td>
</tr>
<tr>
<td>(K^0_{4},0,0,s) ((Y_4))_{JKL}</td>
<td>(s\ 0)</td>
<td>(0\ s)</td>
</tr>
<tr>
<td>(K^0_{4},0,0,s) ((Y_4))_{JKL}</td>
<td>(s\ 0)</td>
<td>(0\ s)</td>
</tr>
<tr>
<td>(K^0_{4},0,0,s) ((Y_4))_{JKL}</td>
<td>(s\ 0)</td>
<td>(0\ s)</td>
</tr>
<tr>
<td>(K^0_{4},0,0,s) ((Y_4))_{JKL}</td>
<td>(s\ 0)</td>
<td>(0\ s)</td>
</tr>
</tbody>
</table>
Table 4A-2. Double-sided Feynman diagrams resulting in the formation of diagonal peak 3 at \((\omega_1, \omega_2) = (\omega_{3,0}, \omega_{2,3})\)

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasing</th>
<th>Non-rephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S_i)</td>
<td>(S_i)</td>
<td>(S_{III})</td>
</tr>
<tr>
<td>(\alpha - \beta - \chi)</td>
<td>(\alpha - \chi - \beta)</td>
<td>(\beta - \alpha - \chi)</td>
</tr>
<tr>
<td>(\beta - \chi - \alpha)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| \((R_1^{0,2,3})^*\) \((Y_1)_{U,K,L}^{0,2,3,5}\) | 2s s s s | 2s s s s |
| \((R_2^{0,2,3})^*\) \((Y_2)_{U,K,L}^{0,2,3,5}\) | 0 s 0 s | 0 s 0 s |
| \((R_3^{0,2,3})^*\) \((Y_3)_{U,K,L}^{0,2,3,5}\) | 2s s s s | 2s s s s |

Table 4A-3. Double-sided Feynman diagrams resulting in the formation of peak 5 at \((\omega_1, \omega_2) = (\omega_{3,0}, \omega_{2,3})\). Note that there are no rephasing pathways for this peak.

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasing</th>
<th>Non-rephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S_i)</td>
<td>(S_i)</td>
<td>(S_{III})</td>
</tr>
<tr>
<td>(\alpha - \beta - \chi)</td>
<td>(\alpha - \chi - \beta)</td>
<td>(\beta - \alpha - \chi)</td>
</tr>
<tr>
<td>(\beta - \chi - \alpha)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| \((R_1^{0,2,3,5})^*\) \((Y_1)_{U,K,L}^{0,2,3,5}\) | 2s s s s | 2s s s s |
| \((R_2^{0,2,3,5})^*\) \((Y_2)_{U,K,L}^{0,2,3,5}\) | 0 s 0 s | 0 s 0 s |
| \((R_3^{0,2,3,5})^*\) \((Y_3)_{U,K,L}^{0,2,3,5}\) | as a s a | as a s a |
| \(\alpha - s a\)                                      | \(s a\)   | \(s a\)       |
Table 4A-4. Double-sided Feynman diagrams resulting in the formation of peak 2 at \( (\omega_1, \omega_2) = (\omega_{\alpha, \beta}, \omega_{\beta, \alpha}) \).

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasering</th>
<th>Non-rephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( S_I )</td>
<td>( S_{II} )</td>
</tr>
<tr>
<td></td>
<td>( \alpha - \beta - \chi )</td>
<td>( \alpha - \chi - \beta )</td>
</tr>
<tr>
<td>( R_3^{0,a,0,s} ) ( (Y_3)^{0,a,0,s}_{UJKL} )</td>
<td>( a ) ( 0 ) ( 0 ) ( 0 )</td>
<td>( a ) ( 0 ) ( 0 ) ( 0 )</td>
</tr>
<tr>
<td>( R_2^{0,a,0,s} ) ( (Y_2)^{0,a,0,s}_{UJKL} )</td>
<td>( a ) ( 0 ) ( 0 ) ( 0 )</td>
<td>( a ) ( 0 ) ( 0 ) ( 0 )</td>
</tr>
<tr>
<td>( R_4^{0,a,0,a} ) ( (Y_4)^{0,a,0,a}_{UJKL} )</td>
<td>( a ) ( 0 ) ( 0 ) ( 0 )</td>
<td>( a ) ( 0 ) ( 0 ) ( 0 )</td>
</tr>
</tbody>
</table>

Table 4A-5. Double-sided Feynman diagrams resulting in the formation of peak 6 at \( (\omega_1, \omega_2) = (\omega_{\alpha, \beta}, \omega_{\beta, \alpha}) \). Note that there are no contributions from the rephasing pathways for this particular peak and the non-rephasing pathways involve forbidden transitions. The weak nature of the forbidden transitions make these peaks extremely weak and invisible on the contour plot of Figure 6-1. The peak amplitudes change as population transfer amongst the symmetric and asymmetric vibrations comes into play as a function of \( \tau_2 \).

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasering</th>
<th>Non-rephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( S_I )</td>
<td>( S_{II} )</td>
</tr>
<tr>
<td></td>
<td>( \alpha - \beta - \chi )</td>
<td>( \alpha - \chi - \beta )</td>
</tr>
<tr>
<td>( R_2^{0,a,2a,a} ) ( (Y_2)^{0,a,2a,a}_{UJKL} )</td>
<td>( 2a ) ( a ) ( s ) ( a ) ( s )</td>
<td>( 2a ) ( a ) ( s ) ( a ) ( s )</td>
</tr>
<tr>
<td>( R_3^{0,a,2a,a} ) ( (Y_3)^{0,a,2a,a}_{UJKL} )</td>
<td>( 2a ) ( a ) ( s ) ( a ) ( s )</td>
<td>( 2a ) ( a ) ( s ) ( a ) ( s )</td>
</tr>
</tbody>
</table>
Table 4A-6. Double-sided Feynman diagrams resulting in the formation of peak 4 at $(\omega_1, \omega_2) = (\omega_{\alpha,0}, \omega_{\beta,0})$.

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasing</th>
<th>Non-rephasing</th>
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<tbody>
<tr>
<td></td>
<td>$S_I$</td>
<td>$S_I$</td>
</tr>
<tr>
<td></td>
<td>$\alpha - \beta - \chi$</td>
<td>$\alpha - \chi - \beta$</td>
</tr>
<tr>
<td>$(R_{1}^{0,\alpha,\beta,s})^{a}$</td>
<td>as s</td>
<td>as s</td>
</tr>
<tr>
<td>$(Y_{1})_{NLKL}^{0,\alpha,\beta,s}$</td>
<td>as s</td>
<td>as s</td>
</tr>
<tr>
<td></td>
<td>0 s</td>
<td>0 s</td>
</tr>
<tr>
<td></td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>$(R_{2}^{0,\alpha,\beta,s})^{a}$</td>
<td>as s</td>
<td>as s</td>
</tr>
<tr>
<td>$(Y_{2})_{NLKL}^{0,\alpha,\beta,s}$</td>
<td>as s</td>
<td>as s</td>
</tr>
<tr>
<td></td>
<td>0 s</td>
<td>0 s</td>
</tr>
<tr>
<td></td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>
Table 4A-7. Double-sided Feynman diagrams resulting in the formation of diagonal peak 1’ at $(\omega_1, \omega_2) = (\omega_{a,0}, \omega_{a,0})$

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasing</th>
<th>Non-rephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S_I$</td>
<td>$S_{II}$</td>
</tr>
<tr>
<td></td>
<td>$\alpha - \beta - \chi$</td>
<td>$\alpha - \chi - \beta$</td>
</tr>
<tr>
<td>$R_0^{0,a,0,a}$, $Y_0^{0,a,0,a}$, $Y_{1K}^{0,a,0,a}$</td>
<td>a 0</td>
<td>a 0</td>
</tr>
<tr>
<td></td>
<td>0 0 a</td>
<td>0 0 a</td>
</tr>
<tr>
<td>$R_2^{0,a,0,a}$, $Y_2^{0,a,0,a}$, $Y_{1K}^{0,a,0,a}$</td>
<td>a 0</td>
<td>a 0</td>
</tr>
<tr>
<td></td>
<td>a a 0</td>
<td>a a 0</td>
</tr>
<tr>
<td>$R_4^{0,a,0,a}$, $Y_4^{0,a,0,a}$, $Y_{1K}^{0,a,0,a}$</td>
<td>a 0</td>
<td>a 0</td>
</tr>
<tr>
<td></td>
<td>a a a 0</td>
<td>a a a 0</td>
</tr>
<tr>
<td>$R_4^{0,a,0,a}$, $Y_4^{0,a,0,a}$, $Y_{1K}^{0,a,0,a}$</td>
<td>a 0</td>
<td>a 0</td>
</tr>
<tr>
<td></td>
<td>a a a a 0</td>
<td>a a a a 0</td>
</tr>
<tr>
<td>$R_4^{0,a,0,a}$, $Y_4^{0,a,0,a}$, $Y_{1K}^{0,a,0,a}$</td>
<td>a 0</td>
<td>a 0</td>
</tr>
<tr>
<td></td>
<td>a a a a a 0</td>
<td>a a a a a 0</td>
</tr>
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<td></td>
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</tbody>
</table>
**Table 4A-8.** Double-sided Feynman diagrams resulting in the formation of diagonal peak 3’ at \((\omega_1, \omega_2) = (\omega_{a,0}, \omega_{2,a,0})\)

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasing</th>
<th>Non-rephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(S_I) (\alpha - \beta - \chi)</td>
<td>(S_{II}) (\beta - \alpha - \chi)</td>
</tr>
<tr>
<td></td>
<td>(S_I) (\alpha - \chi - \beta)</td>
<td>(S_{III}) (\beta - \chi - \alpha)</td>
</tr>
<tr>
<td>((R_1^{0,a,2,a,0})^*) ((Y_1)_{U,2,a,0})</td>
<td>2a a</td>
<td>2a a</td>
</tr>
<tr>
<td></td>
<td>a a a a</td>
<td>a a a a a</td>
</tr>
<tr>
<td></td>
<td>0 a a</td>
<td>0 a a a a a</td>
</tr>
<tr>
<td></td>
<td>0 0 0 0</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>((R_2^{0,a,2,a,0} \alpha)) ((Y_2)_{U,2,a,0})</td>
<td></td>
<td>2a a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a a a a a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>((R_3^{0,a,2,a,0})^*) ((Y_3)_{U,2,a,0})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>((R_4^{0,a,2,a,0})^*) ((Y_4)_{U,2,a,0})</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Table 4A-9.** Double-sided Feynman diagrams resulting in the formation of diagonal peak 5’ at \((\omega_1, \omega_2) = (\omega_{a,0}, \omega_{2,a,0})\). Note that there are no rephasing contributions for this peak.

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasing</th>
<th>Non-rephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(S_I) (\alpha - \beta - \chi)</td>
<td>(S_{II}) (\beta - \alpha - \chi)</td>
</tr>
<tr>
<td></td>
<td>(S_I) (\alpha - \chi - \beta)</td>
<td>(S_{III}) (\beta - \chi - \alpha)</td>
</tr>
<tr>
<td>((R_2^{0,a,2,a,0} \alpha)) ((Y_2)_{U,2,a,0})</td>
<td></td>
<td>as s a a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a 0 0 0 0</td>
</tr>
<tr>
<td>((R_3^{0,a,2,a,0})^*) ((Y_3)_{U,2,a,0})</td>
<td></td>
<td>as s a a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4A-10. Double-sided Feynman diagrams resulting in the formation of diagonal peak 2' at \( (\omega_1, \omega_2) = (\omega_{a,0}, \omega_{a,0}) \)

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasing</th>
<th>Non-rephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( S_I )</td>
<td>( S_I )</td>
</tr>
<tr>
<td></td>
<td>( \alpha - \beta - \chi )</td>
<td>( \alpha - \chi - \beta )</td>
</tr>
<tr>
<td></td>
<td>( S_{II} )</td>
<td>( S_{III} )</td>
</tr>
<tr>
<td></td>
<td>( \beta - \alpha - \chi )</td>
<td>( \beta - \chi - \alpha )</td>
</tr>
</tbody>
</table>

\[ R_3^{0,0,0,a} \]
\( (Y_3)^{0,0,0,a}_{UKL} \)

\[ R_2^{0,0,0,a} \]
\( (Y_2)^{0,0,0,a}_{UKL} \)

\[ R_4^{0,0,0,a} \]
\( (Y_4)^{0,0,0,a}_{UKL} \)

\[ R_4^{0,0,0,a} \]
\( (Y_4)^{0,0,0,a}_{UKL} \)

\[ R_4^{0,0,0,a} \]
\( (Y_4)^{0,0,0,a}_{UKL} \)

Table 4A-11. Double-sided Feynman diagrams resulting in the formation of diagonal peak 6' at \( (\omega_1, \omega_2) = (\omega_{a,0}, \omega_{2s,a}) \). Note that there are no contributions from the rephasing pathways for this particular peak and the non-rephasing pathways involve forbidden transitions. The weak nature of the forbidden transitions make these peaks extremely weak and invisible on the contour plot of Figure 6-1. The peak amplitudes change as population transfer amongst the symmetric and asymmetric vibrations comes into play as a function of \( \tau_2 \).

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasing</th>
<th>Non-rephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( S_I )</td>
<td>( S_I )</td>
</tr>
<tr>
<td></td>
<td>( \alpha - \beta - \chi )</td>
<td>( \alpha - \chi - \beta )</td>
</tr>
<tr>
<td></td>
<td>( S_{II} )</td>
<td>( S_{III} )</td>
</tr>
<tr>
<td></td>
<td>( \beta - \alpha - \chi )</td>
<td>( \beta - \chi - \alpha )</td>
</tr>
</tbody>
</table>

\[ (R_2^{0,0,2s,a})^* \]
\( (Y_2)^{0,0,2s,a}_{UKL} \)

\[ (R_3^{0,0,2s,a})^* \]
\( (Y_3)^{0,0,2s,a}_{UKL} \)

\[ (R_3^{0,0,2s,a})^* \]
\( (Y_3)^{0,0,2s,a}_{UKL} \)

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Table 4A-12. Double-sided Feynman diagrams resulting in the formation of diagonal peak 4' at $(\omega_4, \omega_5) = (\omega_{a_0}, \omega_{a_{0,0}})$

<table>
<thead>
<tr>
<th>Vibrational and orientational nonlinear response functions</th>
<th>Rephasing</th>
<th>Non-rephasing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S_I$</td>
<td>$S_I$</td>
</tr>
<tr>
<td></td>
<td>$\alpha - \beta - \chi$</td>
<td>$\alpha - \chi - \beta$</td>
</tr>
<tr>
<td></td>
<td>$S_{II}$</td>
<td>$S_{III}$</td>
</tr>
<tr>
<td></td>
<td>$\beta - \alpha - \chi$</td>
<td>$\beta - \chi - \alpha$</td>
</tr>
<tr>
<td>$(R_{0}^{a_{0,0,0,0}})^{\ast}$</td>
<td>as a</td>
<td>as a</td>
</tr>
<tr>
<td>$(Y_{0}^{a_{0,0,0,0}})_{UKL}$</td>
<td>a a</td>
<td>a a</td>
</tr>
<tr>
<td></td>
<td>0 a</td>
<td>0 a</td>
</tr>
<tr>
<td></td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>$(R_{1}^{a_{0,0,0,0}})^{\ast}$</td>
<td>as a</td>
<td>as a</td>
</tr>
<tr>
<td>$(Y_{1}^{a_{0,0,0,0}})_{UKL}$</td>
<td>a s</td>
<td>a s</td>
</tr>
<tr>
<td></td>
<td>0 a</td>
<td>0 a</td>
</tr>
<tr>
<td></td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>$(R_{2}^{a_{0,0,0,0}})^{\ast}$</td>
<td>as a</td>
<td>as a</td>
</tr>
<tr>
<td>$(Y_{2}^{a_{0,0,0,0}})_{UKL}$</td>
<td>a a</td>
<td>a 0</td>
</tr>
<tr>
<td></td>
<td>a 0</td>
<td>0 0</td>
</tr>
<tr>
<td>$(R_{3}^{a_{0,0,0,0}})^{\ast}$</td>
<td>as a</td>
<td>as a</td>
</tr>
<tr>
<td>$(Y_{3}^{a_{0,0,0,0}})_{UKL}$</td>
<td>a a</td>
<td>a 0</td>
</tr>
<tr>
<td></td>
<td>a 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>
Table 4A-13. The number of rephasing and non-rephasing Liouville-pathways contributing to the formation of the various peaks in the 2D IR correlation spectra of RDC. The pathways involving harmonically forbidden transitions are contained in the parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>$\tau_2 = 0$</th>
<th></th>
<th>$\tau_2 &gt; 0$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rephasing</td>
<td>Non-Rephasing</td>
<td>Rephasing</td>
</tr>
<tr>
<td></td>
<td>$S_I$</td>
<td>$S_I$</td>
<td>$S_{II}$</td>
</tr>
<tr>
<td>Peak 1</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Peak 2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Peak 3</td>
<td>1 (1)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Peak 4</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Peak 5</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Peak 6</td>
<td>0</td>
<td>0</td>
<td>0 (1)</td>
</tr>
</tbody>
</table>

Table 4A-14. Reduction of dephasing functions using harmonic scaling relationships for the six level system of RDC.

<table>
<thead>
<tr>
<th></th>
<th>$S_I$</th>
<th></th>
<th>$S_{II}$</th>
<th></th>
<th>$S_{III}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_3^{0,a,0,a}$</td>
<td>$F_2^{0,a,0,a}$ = $F_1^{0,a,2a,a}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_3^{0,s,0,s}$</td>
<td>$F_2^{0,s,0,s}$ = $F_1^{0,s,2s,s}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_2^{0,s,0,a}$</td>
<td>$F_1^{0,a,as,a}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_2^{0,a,0,s}$</td>
<td>$F_1^{0,s,as,s}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_2^{0,s,0,s}$</td>
<td>$F_1^{0,a,as,s}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$F_2^{0,a,0,a}$</td>
<td>$F_1^{0,a,as,a}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4A-15. Expressions for the nonlinear orientational response functions in the $S_I$ rephasing pathways.

<table>
<thead>
<tr>
<th>Orientational response function</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(Y_1)^{0,0,a,s}_{LKL}$</td>
<td>$Y_{LKL}^{0,0,a,s}(\tau_3,\tau_2,\tau_1)$</td>
</tr>
<tr>
<td>$(Y_2)^{0,0,0,a}_{LKL}$</td>
<td>$Y_{LKL}^{0,0,0,a}(\tau_3,\tau_2,\tau_1)\cos(\Theta_1)^2 + Y_{LKL}^{0,0,0,a}(\tau_3,\tau_2,\tau_1)\sin(\Theta_1)^2$</td>
</tr>
<tr>
<td>$(Y_3)^{0,0,a,0}_{LKL}$</td>
<td>$Y_{LKL}^{0,0,a,0}(\tau_3,\tau_2,\tau_1)\cos(\Theta_1)^2 + Y_{LKL}^{0,0,a,0}(\tau_3,\tau_2,\tau_1)\sin(\Theta_1)^2$</td>
</tr>
<tr>
<td>$(Y_2)^{0,2,a,0}_{LKL}$</td>
<td>$Y_{LKL}^{0,2,a,0}(\tau_3,\tau_2,\tau_1)\cos(\Theta_1)^2 + Y_{LKL}^{0,2,a,0}(\tau_3,\tau_2,\tau_1)\sin(\Theta_1)^2$</td>
</tr>
<tr>
<td>$(Y_1)^{0,a,2,a}_{LKL}$</td>
<td>$Y_{LKL}^{0,a,2,a}(\tau_3,\tau_2,\tau_1)\cos(\Theta_2)^2 + Y_{LKL}^{0,a,2,a}(\tau_3,\tau_2,\tau_1)\sin(\Theta_2)^2$</td>
</tr>
<tr>
<td>$(Y_1)^{0,a,a,s,a}_{LKL}$</td>
<td>$Y_{LKL}^{0,a,a,s,a}(\tau_3,\tau_2,\tau_1)\cos(\Theta_1)^2 + Y_{LKL}^{0,a,a,s,a}(\tau_3,\tau_2,\tau_1)\sin(\Theta_1)^2$</td>
</tr>
<tr>
<td>$(Y_1)^{0,a,a,a,s}_{LKL}$</td>
<td>$Y_{LKL}^{0,a,a,a,s}(\tau_3,\tau_2,\tau_1)\cos(\Theta_1)^2 + Y_{LKL}^{0,a,a,a,s}(\tau_3,\tau_2,\tau_1)\sin(\Theta_1)^2$</td>
</tr>
<tr>
<td>$(Y_1)^{0,a,2,a,s}_{LKL}$</td>
<td>$Y_{LKL}^{0,a,2,a,s}(\tau_3,\tau_2,\tau_1)\cos(\Theta_1)^2 + Y_{LKL}^{0,a,2,a,s}(\tau_3,\tau_2,\tau_1)\sin(\Theta_1)^2$</td>
</tr>
<tr>
<td>$(Y_1)^{0,a,2,a,s}_{LKL}$</td>
<td>$Y_{LKL}^{0,a,2,a,s}(\tau_3,\tau_2,\tau_1)\cos(\Theta_1)^2 + Y_{LKL}^{0,a,2,a,s}(\tau_3,\tau_2,\tau_1)\sin(\Theta_1)^2$</td>
</tr>
</tbody>
</table>

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Table 4A-16. Expressions for the nonlinear orientational response functions in the $S_{1g}$ non-rephasing pathways.

<table>
<thead>
<tr>
<th>Orientational response function</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(Y_1)_{0,0,0}^{0,0,0} _{UKL}$</td>
<td>$Y_{UKL}^{0,0,0} (\tau_3, \tau_2, \tau_1)$</td>
</tr>
<tr>
<td>$(Y_4)_{0,0,0}^{0,0,0} _{UKL}$</td>
<td>$Y_{UKL}^{0,0,0} (\tau_3, \tau_2, \tau_1) \cos (\Theta_3)^2 + Y_{UKL}^{0,0,0} (\tau_3, \tau_2, \tau_1) \sin (\Theta_3)^2$</td>
</tr>
<tr>
<td>$(Y_2)_{0,0,0}^{0,0,0} _{UKL}$</td>
<td>$Y_{UKL}^{0,0,0} (\tau_3, \tau_2, \tau_1) \cos (\Theta_2)^2 + Y_{UKL}^{0,0,0} (\tau_3, \tau_2, \tau_1) \sin (\Theta_2)^2$</td>
</tr>
<tr>
<td>$(Y_2)_{0,0,0}^{0,0,0} _{UKL}$</td>
<td>$Y_{UKL}^{0,0,0} (\tau_3, \tau_2, \tau_1) \cos (\Theta_2)^2 + Y_{UKL}^{0,0,0} (\tau_3, \tau_2, \tau_1) \sin (\Theta_2)^2$</td>
</tr>
<tr>
<td>$(Y_2)_{0,0,0}^{0,0,0} _{UKL}$</td>
<td>$Y_{UKL}^{0,0,0} (\tau_3, \tau_2, \tau_1) \cos (\Theta_2)^2 + Y_{UKL}^{0,0,0} (\tau_3, \tau_2, \tau_1) \sin (\Theta_2)^2$</td>
</tr>
<tr>
<td>$(Y_2)_{0,0,0}^{0,0,0} _{UKL}$</td>
<td>$Y_{UKL}^{0,0,0} (\tau_3, \tau_2, \tau_1) \cos (\Theta_2)^2 + Y_{UKL}^{0,0,0} (\tau_3, \tau_2, \tau_1) \sin (\Theta_2)^2$</td>
</tr>
</tbody>
</table>
Table 4A-17. Expressions for the nonlinear orientational response functions in the $S_{nt}$ non-rephasing pathways.

<table>
<thead>
<tr>
<th>Orientation</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(Y_{4}^{Q_{1},a,a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_6 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_6 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_2 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_2 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_2 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_2 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_2 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_2 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_2 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_2 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_2 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_2 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},a,a})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_2 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
<tr>
<td>$(Y_{4}^{Q_{1},s,s})_{LKL}$</td>
<td>$Y_{LKL}^{IIIS} \left( \tau_1, \tau_2, \tau_1 \right) \cos \left( \Theta_3 \right)^2 + Y_{LKL}^{III} \left( \tau_1, \tau_2, \tau_1 \right) \sin \left( \Theta_7 \right)^2$</td>
</tr>
</tbody>
</table>
References

Chapter 5

Collecting a 2D IR correlation spectrum

The work presented in this chapter has been published in the following papers:


5.1 Five pulse mid-infrared interferometer and heterodyne detection

Coherent two-dimensional IR experiments on multi-level vibrational systems are performed using short pulses with enough spectral bandwidth to excite all the vibrational transitions of interest. The experimental technique to obtain 2D IR correlation spectra involves the production of short, transform-limited mid IR pulses and the careful propagation and manipulation of these pulses through a five-beam interferometer to measure the third-order nonlinear rephasing and nonrephasing signals. Broadband mid-IR pulses (λ=4.9 μm; ν=2050 cm⁻¹) are obtained by difference frequency mixing of the signal and idler outputs of a near-IR BBO optical parametric amplifier pumped by a 1 kHz Ti: Sapphire regenerative amplifier. The signal and idler are focussed onto a 1 mm AgGaS₂ crystal with a 20 cm fused silica lens. The difference frequency radiation was collected and partially collimated by a 25 cm BaF₂ lens. The remaining near-IR light was filtered out with a 1 mm AR-coated Ge
window placed normal to the incident light beam. To improve the polarization quality of the mid-IR radiation the beam passed through a 3 mm thick Ge Brewster window, which was also used to overlap the IR beam with a He-Ne tracer beam. Following a polarization-flipping periscope the mid-IR beam was expanded and collimated in an all-reflective 1:2 telescope consisting of curved gold mirrors with effective focal lengths of 25 cm and 50 cm.

To simplify the alignment of the multi-pulse IR interferometer used in the 2D VE experiments it was imperative to trace the mid-IR beam by overlapping and mode-matching it with a visible beam. A He-Ne laser beam was reflected off the back side of the Ge Brewster window and overlapped with the IR beam with the aid of two alignment irises separated by 100 cm and placed before the first mirror of the 1:2 telescope. Alignment of the mid-IR beam through the irises was monitored with pyroelectric detectors placed immediately after the irises. To best mode-match the IR and the tracer, the size and divergence of the He-Ne beam were controlled by changing the length and the exit lens in a Galilean telescope inserted in the visible beam before the reflection from the Ge window. Since the multi-pulse interferometer utilizes all reflective focusing optics, careful mode-matching of the IR and the tracer beams insured that both beams would focus identically simplifying the task of re-crossing of the IR beams in the sample.

Figure 5-1. (Color) A typical pulse spectrum centered at 2050 cm\(^{-1}\) with a FWHM of 160 cm\(^{-1}\). Underneath the spectral envelope is the linear FTIR spectrum of RDC in hexane (red) and in chloroform (blue) showing the two fundamental asymmetric and symmetric transition frequencies at 2015 and 2084 cm\(^{-1}\). The full width half maxima of the asymmetric and symmetric vibrational lines are 2.6 cm\(^{-1}\) in hexane, and 14.6 cm\(^{-1}\) and 9.3 cm\(^{-1}\) in chloroform respectively. The inset shows an interferometric autocorrelation of a nearly-transform limited 90 fs mid-IR pulse.

Mid-IR pulses were compressed by compensating for the second-order dispersion of the optical elements in the beam path.\(^{1}\) This was accomplished by balancing the amount of material with positive and negative second-order dispersion. In practice the shortest pulses were obtained by inserting CaF\(_2\).
(negative dispersion) windows of varying thickness into the mid-IR beam largely to compensate for
the positive dispersion of the Ge widows used to filter out the near-IR radiation and overlap the He-
Ne tracer beam with mid-IR radiation. Fig. 5-1 shows the interferometric autocorrelation and
spectrum of typical pulses used in the experiment. On average, the pulses were 90 fs long with a
bandwidth of FWHM =160 cm\(^{-1}\). The figure also shows a FTIR spectrum of RDC in hexane. The
pulses have enough bandwidth to cover the fundamental transitions as well as those involving the one
and two quantum vibrational manifolds.

Figure 5-2. (Color) Experimental layout of the five-beam IR interferometer showing the three input
pulses \(\alpha, \beta,\) and \(\chi\), the tracer (T) and the LO pulse. M: gold-coated mirror, BS: 50-50, 4 mm thick
ZnSe beam splitter, C: 4 mm thick ZnSe compensation plate, RR: 2” cube retroreflectors, PM: 10
cm focal length parabolic mirrors, WP: 2mm thick MgF\(_2\) wave-plates, P: wire-grid ZnSe polarizers,
S: sample, BS’: 4 mm thick ZnSe plate (AR coated on one side, uncoated on the other), A: analyzer,
Mo: monochromator and D: single channel HgCdTe detector.

The resultant near transform-limited pulses entering the multi-beam interferometer illustrated
in Figure 5-2 are vertically polarized and 12 mm in diameter. It is important to note that for
experiments with optimally compressed pulses at the sample position requires the design of an
interferometer that effectively nulls the dispersion experienced by the IR fields to second order. The
incoming beam is split into three input beams \(E_\alpha, E_\beta,\) and \(E_\chi\) of equal intensity using 50-50 4 mm
thick ZnSe beam splitters (Rocky Mountain Instrument Co.). The remaining fourth beam is split into
a local oscillator and a tracer beam. The tracer, which is used for rough alignment and pump-probe
experiments, follows the path of the signal and is blocked during collection of the 2D data. The
intensity and polarization of each of these five beams is controlled using a zero-order 2 mm thick
MgF\(_2\) half-wave-plate (Karl Lambrecht Corp.) followed by a 2 mm thick ZnSe wire-grid polarizer
(Molectron Detector, Inc.). The optical material in each arm of the interferometer is balanced by
using 4 mm ZnSe compensation plates (Rocky Mountain Instrument Co.). The three input beams, each with \( \sim 50 \) nJ of energy are incident on the sample in a box geometry and focused to 150 \( \mu \)m spot sizes using a 100 mm focal length off-axis parabolic mirror (Janos Technology Inc.). To ensure optimal overlap at the sample the three beams are put through a pinhole placed at the focus of the beams. Zero timing between the three input and tracer beams is set to within \( \pm 5 \) fs by taking background free intensity autocorrelations of each pulse-pair in a 0.5 mm long Type I AgGaS\(_2\) crystal (Eksma Co.) placed at the focal plane. The relative timing between the input pulses is controlled by 2 inch cube retroreflectors (PLX Inc.) mounted on a stepper-motor-driven linear stages (Newport) with 0.1 \( \mu \)m (0.667 fs) resolution and a repeatability of 1.5 \( \mu \)m (10 fs). For the rephasing experiments, \( \mathbf{E}_\alpha \) is followed by \( \mathbf{E}_\beta \) and then \( \mathbf{E}_\chi \), while in the nonrephasing experiments, \( \mathbf{E}_\beta \) enters the sample initially followed by \( \mathbf{E}_\alpha \) and \( \mathbf{E}_\chi \). Before entering the interferometer, the IR beam is polarized in the vertical direction \((Z)\) of the laboratory frame. Polarization-selective 2D IR spectra are collected at two polarization geometries \( ZZZZ \) (parallel) and \( ZZYY \) (crossed) by changing the input polarization of beams \( \mathbf{E}_\alpha \) and \( \mathbf{E}_\beta \). The waveplates in these two arms are adjusted to ensure that the same input energy reaches the sample in the two polarization geometries.

The third order nonlinear rephasing and nonrephasing signals are generated in the phase-matched direction, \( k_3 \), as shown in Figure 4-1. To completely characterize the nonlinear signal field, it is overlapped temporally and spatially with a local oscillator (LO). After passing through an analyzing polarizer (A), the two fields are dispersed in a monochromator, Mo 1 (Spex Industries Inc.) equipped with a liquid nitrogen cooled 64 element MCT array detector (IR Associates, Inc.; each pixel is 0.1 mm wide with a height of 1 mm). The initial spatial and temporal overlap is performed using the tracer (which follows the signal path) and the LO. The LO is spatially overlapped with the tracer on a 4 mm ZnSe window (BS') using two irises separated by a distance of 1 m and monitoring the intensity of IR light detected by a single channel liquid nitrogen cooled MCT detector (Electro-Optical Systems Inc.). The timing between the LO and the tracer is set by monitoring their interference on a single channel MCT detector placed before the monochromator. It is noticed that the tracer beam does not always follow the same path as the emitted signal field and adjustments need to be made in the signal and LO arms to ensure complete spatial overlap at the focal plane of the array detector. This is done by introducing a 1-2 ps delay \( \tau_3 \) between the LO and the signal fields with the help of a retroreflector mounted on a computer-controlled delay stage. The spectral interferogram of the signal and LO detected at the focal plane of the monochromator displays interference fringes whose frequency spacing reflect the value of the delay \( \tau_3 \). The spatial overlap of the local oscillator
and signal fields is adjusted using the mirror pairs M1, M2 and M3, M4 respectively to increase the depth of the interference fringes in the spectral interferogram. Once optimal spatial overlap is achieved, the position of the LO is moved to set \( \tau_3 = 0 \) as previously determined from the interference between the tracer and LO fields in an earlier step. We are able to set the \( \tau_3 \) delay to within \( \pm 25 \) fs using this method. The spectral resolution in the \( \omega_3 \) dimension is dictated by the entrance slit width of the monochromator (150 \( \mu m \)), the linear dispersion at the focal plane determined by the groove density of the grating, and the pixel width on the array detector (100 \( \mu m \)). Two different gratings with groove densities of 150 lines/mm and 90 lines/mm, corresponding to spectral resolution of \( \sim 1.3 \) \( cm^{-1} \) and \( \sim 3 \) \( cm^{-1} \) are used for experiments of RDC dissolved in hexane and chloroform respectively.

The MCT array detector is accompanied by a high-speed signal acquisition system and data acquisition software (Infrared Systems Development Corporation) which samples the data from the array detector at the 1 kHz pulse repetition rate. A LabView routine is used to collect arrays of spectrally dispersed heterodyne detected rephasing and nonrephasing signals as a function of \( \tau_1 \) by moving the computer controlled translation stages for \( \mathbf{E}_\alpha \) and \( \mathbf{E}_\beta \). The data for RDC in hexane is collected by stepping the time-delay \( \tau_1 \) in steps of \( \sim 2 \) fs up to \( \tau_1 = 8 \) ps. A mechanical chopper operating at 500 Hz chops beam \( \mathbf{E}_\alpha \), and differential detection of the spectral data at the chopping frequency allows us to isolate the heterodyned signal of interest. Our experimental signal-to-noise ratio is determined by the noise on our LO. We adjust the intensity of the LO to be ten times the signal intensity for the heterodyne detection scheme described above. For a particular value of \( \tau_2 \), we collect the rephasing and nonrephasing signals followed by a dispersed pump-probe using the tracer as the probe and the beam \( \mathbf{E}_\alpha \) as the pump-beam. The room temperature \( 1 \times 10^{-3} \) M samples of RDC dissolved in hexane are held in a stationary 200 \( \mu m \) thick CaF\(_2\) cell with 4 mm thick windows corresponding to a peak optical density of 0.25.

In our earlier data shown in Figure 3-2 of chapter 3, we noticed distorted line shapes and incorrect determination of resonant frequencies sampled during the evolution period which hindered the interpretation of the resultant 2D spectra.\(^2,3\) These effects result from an improper calibration of the \( \tau_1 \) axis due to the limitations of the stepper-motor-driven linear stages. To remedy this problem, the \( \tau_1 \) timing was determined externally to within \( \pm 1 \) fs by overlapping pulses \( \mathbf{E}_\alpha \) and \( \mathbf{E}_\beta \) after the sample and dispersing them in a \( 1/4 \) m home-built monochromator (Mo2) with 100 \( \mu m \) wide entrance and exit slits and a 300 lines/mm grating. Interference fringes collected at 1975 \( cm^{-1} \) as a function of the \( \tau_1 \) delay are used to calibrate the \( \tau_1 \) axis. We have tested our calibration method by
performing a linear FID measurement of RDC dissolved in chloroform and compared the resultant Fourier transformed line shapes with an absorption spectrum from a commercial FTIR spectrometer. Using the calibration technique described above, the line shapes obtained after a Fourier transform of the FID data matched the FTIR spectrum within experimental noise.

5.2 Treatment of the raw data

The detection monochromator effectively performs a cosine transform of the overlapped signal and local oscillator fields and we detect the interference term of interest by chopping one of the input beams as described in the previous section. In the limit where \( |E_{LO}|^2 \gg |E_{sig}|^2 \), the cosine transform of the interference term can be written as

\[
O(k_s, \omega_1, \tau_3, \tau_2, \tau_1) = \int_0^\infty E_{sig}(k_s, t, \tau_2, \tau_1) E_{LO}(k_s, t - \tau_3) \cos(\omega_1 t) dt. \quad (5.1)
\]

In the above expression, \( \omega_1 \) is the frequency of the dispersed signal and local oscillator fields. Note that the desired interference term as written in eq 5.1 is not what the detector sees. Instead the array detector measures the intensity of the light fields integrated over the width of the pixel \( (2\Delta\omega) \).

Furthermore, the intensity of the detected light field is a convolution of the desired signal with an instrument response function, \( W(\omega_1) \) as shown below,

\[
S(k_s, \omega_3, \tau_2, \tau_1) = \int_{\omega_1 - \Delta\omega}^{\omega_1 + \Delta\omega} O(k_s, \omega_1, \tau_3, \tau_2, \tau_1) \otimes W(\omega_1) d\omega_1 + c.c. \quad (5.2)
\]

The instrument response function depends on the characteristics of the monochromator such as entrance-slit width, focal length and diffraction grating. As a result, the data is sampled as an array of discrete frequencies, \( \omega_3 \) for a particular \( \tau_1 \) delay. It is important to note that this array is equally spaced in wavelength and not in frequency. The issues of spectral resolution and unequal sampling in spectral interferometry have been the subject of a recent publication by Dorrer et. al.\(^4\)

The signal expressed by eq. 5.2 and shown in Fig 5-3 is depicted as a function of \( \omega_1 \) and \( \omega_3 \) after a Fourier cosine transform of the data along the evolution time period,

\[
\tilde{S}(k_s, \omega_3, \tau_2, \omega_1) = 2 \int_0^\infty S(k_s, \omega_3, \tau_2, \tau_1^*) \cos(\omega_1 \tau_1^*) d\tau_1^* \quad (5.3)
\]
Before performing the Fourier transform on the data, the original $\tau_1$ axis is calibrated, and interpolated to equally spaced time points represented by $\tau_1^*$ in the above equation. The interpolated data is multiplied by a triangular apodization function of the same length. This ensures that data set decays smoothly to zero and that there are no spectral artifacts present after performing the Fourier transform. Finally, the data is zero padded to a length of $2^{15}$ elements and then a Fourier transform is performed, resulting in a spectral resolution of 1 cm$^{-1}$ in the $\omega_1$ dimension. We notice that using the apodization function has a slight effect on the 2D line shapes. To nullify this effect, we multiply our calculated signals used to fit the experimental data with the same apodization function to extract correct linewidth parameters. Since the monochromator performs the Fourier cosine transform along $\tau_3$, the 2D rephasing and nonrephasing spectra obtained after the transform along $\tau_1$ appear in the $(\pm \omega_1, + \omega_3)$ and $(\pm \omega_1, + \omega_3)$ quadrants.

![Figure 5-3. Heterodyne detected rephasing signal as a function of $\tau_1$ with $\omega_3 = \omega_a,0$. The top panel shows the signal with fast oscillations at the fundamental frequencies and slower oscillations at 70 cm$^{-1}$. The lower panel shows the signal trace with the signal decaying by 8 ps along $\tau_1$. The $\tau_1$ axis has been calibrated as described in the text.](image)

The resultant 2D rephasing $\hat{S}_R$ and nonrephasing spectra $\hat{S}_{NR}$ are added to produce a 2D correlation spectrum $\hat{S}_C$. It is important to note that we do not know the absolute phase of the signal represented by this 2D correlation spectrum. This is a result of our inability to determine the absolute zero timing between the input pulses ($\tau_1 = \tau_2 = 0$), the error in our ability to set $\tau_3 = 0$ and phase errors introduced by slight optical imbalances in the interferometer arms. To correct for the non-zero timings we need to “phase” the 2D correlation spectrum in the $\omega_1$ and $\omega_3$ dimensions. It has been
shown using the projection-slice theorem for 2D Fourier transforms that the projection of a 2D IR correlation spectrum at a particular $\tau_2$ delay onto the $\omega_1 = 0$ axis is equal to the dispersed pump-probe signal measured for the same $\tau_2$ value.\(^5\) We make use of this fact to determine the correct phase of our nonlinear signal. This is done by multiplying the rephasing and nonrephasing spectra by a factor of $\exp[-i\omega_1 \Delta \tau_1 - i\omega_3 \Delta \tau_3 + i\phi]$ to obtain absorptive spectral features for the following slices: $\omega_3 = \omega_{s,0}$ and $\omega_3 = \omega_{s,0}$ and to fit the projection of the 2D IR correlation spectrum to the particular dispersed pump-probe measurement. Note that this phasing procedure accounts for timing errors in $\tau_3$ when making use of the projection slice theorem. The phasing in the $\omega_1$ dimension to correct for timing errors in $\tau_1$ assumes that the 1D line shapes along the fundamental frequencies are absorptive, which is not strictly true. We notice that factors of $\pm 4$ fs are generally needed to correctly phase 2D spectra, accounting for the timing errors ($\Delta \tau_1$ and $\Delta \tau_3$).

### 5.3 Experimental Data

The rephasing and non-rephasing 2D spectra and their sum are shown in Figure 5-4 for $\tau_2 = 0$ fs (5-4a) and $\tau_2 = 470$ fs (5-4b). The time sequence for the three input pulses (1-2-3) entering the sample for the rephasing and nonrephasing experiments are $\alpha - \beta - \chi$ and $\beta - \alpha - \chi$. When $\tau_2 = 0$ and the last two pulses are time-coincident on the sample, the pulse orderings for the rephasing sequence, $\alpha - \beta - \chi$ and $\alpha - \chi - \beta$ are indistinguishable. Referring back to Figure 4-3 in Chapter 4, we can see that both these contributions are classified as $S_I$ signals. Similarly, contributions from the pulse orderings $\beta - \alpha - \chi$ and $\beta - \chi - \alpha$ are present in the nonrephasing experiment when $\tau_2 = 0$. These contributions are classified as $S_{II}$ and $S_{III}$ signals respectively. The Liouville pathways sampled by the rephasing experiments at $\tau_2 = 0$ are illustrated in the first two rows of Figure 4-3, while those sampled in the nonrephasing experiment are shown in the fourth and sixth row of the same figure. When $\tau_2 = 470$ fs, the pulse orderings $\alpha - \beta - \chi$, $\beta - \alpha - \chi$ and $\beta - \chi - \alpha$ corresponding to $S_I$, $S_{II}$ and $S_{III}$ are distinguishable from each other because the two pulses are no longer time-coincident on the sample.
Figure 5-4 (Color) (a) The 2D IR rephasing spectrum, 2D IR nonrephasing spectrum and their sum, the 2D IR correlation spectrum for RDC in hexane at $\tau_2 = 0$. These spectra were obtained in the all-parallel geometry. Fifteen equally spaced contour levels from minimum to the maximum value are drawn for each 2D plot. (b) The $S_i$, $S_{ii}$ and $S_{iii}$ contributions for $\tau_2 = 470$ fs. The last panel shows the sum of the $S_i$ and $S_{ii}$ contributions resulting in the 2D IR correlation spectrum for $\tau_2 = 470$ fs. The spectrum displaying the sum $S_i + S_{ii} + S_{iii}$ shows phase-twisted diagonal peaks tilted towards the off-diagonal axes resulting from the excess nonrephasing contribution.
Looking at Figure 5-4a, we see that the first two panels representing the rephasing and non-rephasing pathways show phase-twisted lineshapes elongated along the diagonal and the off-diagonal axes. Their sum, plotted on the last panel, is the absorptive 2D IR correlation spectrum, where the dispersive lobes are cancelled from the addition of the 2D rephasing and non-rephasing spectra. The 2D IR correlation spectrum shows ten peaks, which arise as a result of the system sampling the Liouville-space pathways shown in Figure 4-3 and drawn out explicitly in Appendix 4A. The positions, amplitudes and lineshapes of the resonances are related to the anharmonicity in the nuclear potential, relative orientations and the system-bath interactions of the coupled carbonyl stretches with the solvent. The positive contributions involve processes in which the system evolves on the fundamental transitions during $\tau_3$, whereas the negative features arise from coherences involving the doubly excited states.

To facilitate a comparison of the rephasing and non-rephasing 2D spectra, the five resonances lying along $\omega_1 = \omega_{s,0}$ have been labeled. We observe that the diagonal peaks (1 and 3) have nearly similar amplitudes, but the cross peak (2 and 4) amplitudes are unequal in the two different measurements. Furthermore, the resonance labeled as 5 is only present in the non-rephasing measurement. This arises from the inherent imbalance of interaction pathways leading to the formation of the various peaks in the rephasing and non-rephasing spectra for this multi-level system as tabulated in Table 4A-13 of Appendix 4A. For example, there are twice the number of diagrams giving rise to peak 2 in the rephasing than in the non-rephasing experiment for any $\tau_2$. Peak 5, which involves four different dipole interactions, is sampled only by the non-rephasing experiment for all $\tau_2$ delays.

The unequal contribution of Liouville pathways sampled in the formation of a particular peak in the rephasing and non-rephasing spectra results in that peak being tilted along the diagonal or off-diagonal axes in the correlation spectra by the angle $\psi$ defined in chapter 4. For the case of $\tau_2 = 0$, the cross-peaks in the correlation spectra are tilted along the diagonal axes as more pathways are sampled by the rephasing spectrum. This is the same case for the correlation spectrum resulting from the sum of $S_I$ and $S_{II}$ at $\tau_2 = 470$ fs. The $S_{III}$ spectrum at $\tau_2 = 470$ fs is identical to the $S_{II}$ spectrum in terms of peak positions, amplitudes and lineshapes. While the peak positions will always be the same by virtue of studying the identical system, the amplitudes and lineshapes will in general be different. This is because all $S_{III}$ contributions oscillate in a two-quantum coherence during $\tau_2$ as seen in Figure 4-3 of the previous chapter. The rephasing and non-rephasing spectra have similar amplitude and lineshapes, because the system-bath dynamics can be described in the homogenous limit for our model system RDC dissolved in hexane. The spectra corresponding to the sum of $S_I + S_{II} + S_{III}$ at
$\tau_2 = 470$ fs shows phase-twisted diagonal peaks tilted towards the off-diagonal axes resulting from the excess nonrephasing contribution ($S_{ii} + S_{iii}$). But the cross-peaks in this case, appear to have less of a phase-twist resulting from an almost equal addition of the rephasing and nonrephasing amplitudes. The next chapter describes the features of the 2D IR correlation spectrum in further detail.
References

Chapter 6

Characteristics of a 2D IR correlation spectrum

The work presented in this chapter has been published in the following papers:


Any nonlinear response for a purely harmonic system is zero. As discussed in Chapter 2, this implies that the very existence of a third-order material response requires vibrational interactions, which include: (1) anharmonicity in the ground state potential, (2) nonlinear dependence of the transition dipole moment on the vibrational coordinates, or (3) nonlinear coupling in the system-bath interactions. These effects are reflected in the positions, amplitudes and line shapes of 2D IR correlation spectrum. The positions of the peaks describe the transition frequencies of the vibrational eigenstates of the system. The peak amplitudes reflect the relative magnitudes and orientations of the transition dipole moments in the microscopic frame. The 2D line shapes are determined by the details of the system-bath interactions, which could result in the statistical variation of the eigenenergies in systems with coupled transition dipoles. These three observables functionally give a detailed characterization of the system eigenstates and the system-bath interactions, which will form the basis for modeling transient molecular structure and solvation dynamics. Here we will use 2D IR correlation spectra of RDC dissolved in hexane to characterize the energies and transition dipoles of the system eigenstates. The interaction of RDC with hexane is weak allowing the solvation dynamics to be treated in the homogeneous limit. This is not the case for RDC dissolved in chloroform and its 2D correlation spectra describe the effects of solvation dynamics on 2D line shapes. Figure 6-1 shows the 2D IR correlation spectrum of RDC in hexane depicting ten resonances with varying signs, amplitudes and tilts. We will refer to the different peaks in the 2D IR correlation spectrum as lying
along the diagonal and off-diagonal axes according to their numbering scheme displayed in Figure 6-1. In particular, peaks 1(1’) and 2(2’) are referred to as the diagonal and cross-peaks respectively.

**Figure 6-1.** (Color) The 2D IR correlation spectrum for RDC in hexane at \( r_2 = 0 \). This spectrum was obtained in the all-parallel geometry. Fifteen equally spaced contour levels from minimum to the maximum value are drawn for each 2D plot.

### 6.1 Position and Sign of Resonances

The position of resonances in the 2D spectrum of RDC is dictated by the consecutive interactions of the multilevel vibrational system with a sequence of three electric fields. The \( \omega_1 \) axis represents the frequency of the vibrational coherences excited after the first field-matter interaction and therefore all the peaks in this dimension lie along the fundamental transitions: \( \omega_1 = \omega_{a,0} \) and \( \omega_1 = \omega_{s,0} \). The \( \omega_3 \) axis indicates the state of the system after the third interaction and we observe six possible resonance frequencies, corresponding to the six transitions shown in the vibrational energy level diagram of RDC in Figure 3-1. The diagonal peaks 1 and 1’ in Figure 6-1 represent four field interactions with the fundamental transitions, whereas cross-peaks 2(2’) involve the transfer of coherence from one fundamental transition to the other. The remaining, oppositely signed peaks involve signals radiated from coherences involving the two-quantum states. For example, peak 3
(shifted below the diagonal peak) arises from a transition between the symmetric overtone \((2s)\) and its fundamental \((s)\), whereas peak 4 (shifted below the cross peak 2) arises from a transition involving the combination band \((as)\) and the symmetric fundamental \((s)\). The specific time-ordered interaction sequences contributing to the formation of each of these ten peaks can be obtained by expanding the double-sided Feynman diagrams in Figure 4-3 in terms of the six eigenstates of RDC. This expansion leads to a total of 66 diagrams, when considering all the possible transitions resonant with the mid-infrared fields including the harmonically allowed one-quantum, and the harmonically forbidden three-quantum transitions. (These diagrams are presented in the appendix to Chapter 4).

The positions of the peaks characterize the anharmonic nuclear potential of the coupled asymmetric and symmetric carbonyl stretches. The splittings between the labeled peaks correspond to anharmonic frequency shifts of the overtones \(\Delta_a = 14 \text{ cm}^{-1}, \Delta_s = 12 \text{ cm}^{-1}\) and the combination band \(\Delta_{as} = 26 \text{ cm}^{-1}\). The signs of the peaks indicate whether the system evolves in a superposition of the fundamental and the ground state (positive) or in a superposition of the fundamental and doubly excited states (negative) during the detection period. The observed peak splittings in the 2D spectrum are related to the anharmonic expansion terms in the nuclear potential describing the coupled carbonyl stretches using normal or local vibrational coordinates.\(^1\)\(^,\)\(^8\) In the limit of a harmonic nuclear potential \(\Delta_a = \Delta_s = \Delta_{as} = 0\), and the oppositely signed resonances peaks 3 and 5 would lie on top of peak 1, and peak 4 on top of peak 2. These peaks would destructively interfere, leading to a decrease or disappearance of the peaks in the 2D spectrum. This explains the appearance of cross peaks for coupled vibrations. For two uncoupled vibrations, the off-diagonal anharmonicity \(\Delta_{as} = 0\), and the cross peak vanishes due to destructive interference between pathways that lead to peaks 2 and 4. The presence of 10 distinct peaks in the 2D IR correlation spectrum of hexane indicates that there is a nonlinear dependence (cubic and higher) of the nuclear potential on the vibrational coordinates and satisfies the first selection rule for 2D IR spectroscopy.

### 6.2 Peak amplitudes

While the positions of the peaks in a 2D IR correlation spectrum are dictated by the transition frequencies between system eigenstates probed by the sequence of external fs fields, their amplitudes are determined by the relative strength and direction of the four interacting transition dipoles. Analysis of the relative peak amplitudes reveals (1) information about the projection angle between transition dipoles of the eigenstates and (2) the presence of electrical anharmonicity in the system or
the nonlinear dependence of the transition dipole moments on the vibrational coordinates. Such an analysis requires separating the nonlinear vibrational and orientational response functions as they contain complementary information about the amplitudes and relative angles of the transition dipoles respectively.

To separate out these two contributions, we measure the 2D IR correlation spectra in two different polarization geometries, which sample response functions differing only in the orientational factors, $Y_{ijkl}$. For a particular resonance, the ratio of its amplitude from two different geometries yields information about the angle(s) between transition dipoles involved in its formation. In the case of cross-peaks, this gives direct information on the projection angle between two coupled transition dipole moments.\(^9\)

![Figure 6-2](image.png)

Figure 6-2. (Color) Experimental 2D correlation spectra obtained in the ZZZZ and ZYYZ polarization geometries for RDC in hexane at $\tau_2 = 0$. The bottom row shows the experimentally collected 2D correlation IR spectra. Twenty-one equally spaced contour levels from minimum to the maximum value are drawn for each 2D plot. The top row depicts the slices along $\omega_1 = \omega_5 = 0$.

The effects of electrical anharmonicity are revealed by comparing the ratio of the amplitudes of oppositely signed peak pairs in 2D IR correlation spectra collected in two different polarization geometries. For a linear dependence of the dipole moment on vibrational coordinate, the transition dipole moment amplitudes connecting the zero (0)- and one (1)- quantum states $\mu_{1,0}$ to those connecting the one- and two (2) - quantum states $\mu_{2,1}$, follow harmonic scaling and $\mu_{2,1} = \sqrt{2} \mu_{1,0}$.\(^1\)
In this limit, the amplitudes of the oppositely signed peak pairs, 1 and 3, or 2 and 4 would be equal. A nonlinear dependence of the transition dipole vectors on the vibrational coordinates results in the variation of the amplitude in these peak pairs.

The second row in Figure 6-2 shows polarization-selective 2D IR correlation spectra of RDC in hexane collected in the Z-Z-Z-Z and Z-Z-Y-Y polarization geometries (α−β−χ−sig). We refer to these as the parallel $\tilde{S}_||$ and crossed $\tilde{S}_\perp$ 2D IR correlation spectra. Since these spectra are collected for $\tau_2 = 0$, the time-ordering $(1−2−3)$ of pulses $\alpha − \beta − \chi$ and $\alpha − \chi − \beta$ are indistinguishable for the rephasing experiment. This implies that the rephasing experiment in the crossed polarization geometry samples both the $Y_{ZZYY}$ and $Y_{ZYZY}$ tensor elements of the $S_I$ response function. Similarly, the pulse sequences $\beta − \alpha − \chi$ and $\beta − \chi − \alpha$ cannot be separated in the nonrephasing experiment for $\tau_2 = 0$ and the nonrephasing experiment samples the $Y_{ZZYY}$ and $Y_{ZYZY}$ tensor components of the $S_{II}$ and $S_{III}$ response, respectively.

The peak positions in each of the polarization-selective spectra are identical, however the relative amplitudes and tilts of the various peaks are different. The ratios of the amplitudes of the cross-peaks to the diagonal peaks are approximately $\sim 1/4$ and $\sim 1$ in the 2D spectra obtained in the parallel and crossed polarization geometry respectively. Additionally, the diagonal amplitude of the crossed-polarized 2D spectra is $\sim 1/4$ that of the spectrum obtained in the all-parallel geometry.

![Figure 6-3](image-url) (Color) The ratio of the diagonal and cross peak amplitudes ($A_\perp/A_||$) in the 2D IR correlation spectra obtained in the parallel and crossed polarization geometries as a function of the angle $\Theta$ between the transition dipoles of two coupled vibrational modes. The plot is shown for the case when $\tau_2 > 0$, but it also holds at $\tau_2 = 0$ provided that the transition dipole vectors $\mu^{1,0}$ and $\mu^{2,1}$ are parallel to each other.
The dependence of the amplitude ratios for a particular peak in the two experiments \((A_{\perp}/A_{||})\) can be related to the projection angle between the two fundamental transitions, \(\Theta\), using the orientational response functions contributing to a particular peak.\(^9,10\) Figure 12 shows a plot of \(A_{\perp}/A_{||}\) as a function of \(\Theta\) for the diagonal and cross peak amplitudes in the 2D IR correlation spectra between the transition dipoles of two vibrational modes. As the angle between the dipole varies from parallel to orthogonal, the cross-peak ratio varies from 1/3 to 7/6 and the diagonal peak ratio varies from 1/3 to 6/23. The calculation in Fig 6-3 is for the case \(\tau_2 > 0\), but it also holds at \(\tau_2 = 0\) provided that the transition dipole vectors \(\hat{\mu}^{1,0}\) and \(\hat{\mu}^{2,1}\) are parallel to each other. The latter assumption is nearly true for our present case due to the intrinsic symmetry of the molecule. A comparison of the amplitudes of the cross and diagonal peaks from the slices along \(\omega_3 = \omega_{s,0}\) plotted on the first row of Figure 6-2 reveal ratios of \(\sim 1\) and \(\sim 1/4\) respectively indicating that the transition dipole vectors of the coupled asymmetric and symmetric stretches are orthogonal to each other.

In general, it is not straightforward to directly measure the angle \(\Theta\) between the two-coupled modes by comparing the amplitudes of cross-peaks from polarization-selective 2D IR correlation spectra containing rephasing and non-rephasing signals at \(\tau_2 = 0\). This is because the cross-peaks resulting from the \(S_{III}\) diagrams in the non-rephasing signal require interactions from four different transition dipole moments as shown in Appendix 4A. For example, the \(S_{III}\) diagram leading to the formation of peak 2 involves interacting with the following four transition dipoles: \(\mu^{s,0}_{s,a}, \mu^{a,s}_{s,a}, \mu^{a,s}_{a,s}, \mu^{s,0}_{a,a}\). Therefore, the ratio of the amplitude peak 2 from 2D IR correlation spectra in the parallel and crossed polarization geometry contain contributions from three different angles \(\Theta\) (angle between \(\mu^{a,0}_{s,a}\) and \(\mu^{s,0}_{s,a}\), \(\Theta_1\) (angle between \(\mu^{s,0}_{s,a}\) and \(\mu^{a,s}_{s,a}\)) and \(\Theta_3\) (angle between \(\mu^{s,0}_{a,a}\) and \(\mu^{a,s}_{a,a}\)). However, if we assume that the angles between the transition dipole moments from the zero- to one- quantum states \((\mu^{1,0})\) are parallel to those from the one- to two- quantum states \((\mu^{2,1})\), the ratio of the cross-peak amplitudes from polarization-selective measurement will be sensitive to only the angle \(\Theta\) between the two-coupled vibrations. For the case mentioned above, such an assumption would result in \(\Theta_3 = 0\) and \(\Theta_7 = \Theta\). The ambiguity in the angle determination is also solved by making measurements at a finite value of \(\tau_2\) which avoids the \(S_{III}\) contributions.

In addition to revealing the angle between the coupled carbonyl stretches, a comparison of the polarization-selective spectra indicates the presence of electrical anharmonicity. In the experimental 2D spectra of Figure 6-1 we notice that the amplitude of peak 1 is greater than that of the negatively signed peak 3, and this ratio of amplitudes is unchanged with different polarization geometries.
Similarly, the amplitude of peak 2 is greater than that of peak 4. These observations imply that, \( \mu^{2s,s} \neq \sqrt{2}\mu^{s,0} \) and \( \mu^{as,a} \neq \mu^{s,0} \). The effects of electrical anharmonicity on the peak amplitudes of 2D IR spectra have been treated in detail in Chapter 2. The presence of electrical anharmonicity for a mechanically harmonic system \( (\Delta_x = \Delta_y = \Delta_{as} = 0) \) ensures that the interference between pathways involving only the fundamental states and those involving the two-quantum states will be incomplete resulting in the non-zero amplitude of the 2D IR correlation spectrum. Generally both electrical and nuclear anharmonicity result in a deviation from harmonic scaling for the amplitudes for \( \mu^{1,0} \) and \( \mu^{2,1} \), and also make these two transition dipoles non-collinear with respect to each other. It should be pointed out that we had not observed the effects of electrical anharmonicity in our earlier experiments where the amplitudes of peak pairs 1,3 and 2,4 were nearly identical (see Figure 3-2).\(^{11,12}\) We believe that this occurred because the spectra were taken with a high peak optical density of 0.7 leading to an increase in the amplitude of the peaks involving the two-quantum transitions with respect to those involving one-quantum transitions and smearing out the effects of electrical anharmonicity.

It is important to note that the amplitude of peaks in the 2D IR spectrum are affected by relaxation processes during all three time variables. Coherent and incoherent population relaxation or exchange processes lead to a change of peak amplitudes both through changes in amplitude in the density matrix elements for the system arising from these relaxation processes,\(^{13}\) and also as changes in the orientational projections of the fields onto the dynamically evolving system. These effects are discussed in Chapter 8.

6.3 2D IR Line shapes

The line shape of a given resonance in a 2D IR spectrum, reflects the effects of the bath on the four interacting transition dipoles. Here, we will restrict ourselves to exploring only the effects of vibrational dephasing on the 2D IR line shape based on a linear interaction between the system and bath coordinates. Within this model, the 2D line shape allows us to quantitatively describe the time scales of the transition frequency fluctuations by characterizing the system-bath correlation functions \( \zeta \).\(^{14,15}\) This can be illustrated through calculations of the 2D line shape for a two-level system using a stochastic model for the frequency fluctuations. In this model, the transition frequency fluctuates through an amplitude \( \sigma \) with a characteristic correlation time \( \tau_c \) and \( \zeta(\tau) = \sigma^2 \exp(-\tau/\tau_c) \). This
model can be continuously varied between the homogeneous \((\sigma \tau_c \ll 1)\) and inhomogeneous \((\sigma \tau_c \gg 1)\) limits of the system-bath dynamics.\(^{16}\)

![Figure 6-4. (Color) The characteristic 2D line shapes for a two level system undergoing stochastic modulation of the transition frequency \(\omega_{a0}\) whose correlation function defined as \(\langle \delta \omega_{a0}(t) \delta \omega_{a0}(0) \rangle = \sigma^2 e^{-t/\tau_c}\) with fluctuation amplitude \(\sigma\) and correlation time \(\tau_c\). The spectral line shapes describe the limits of fast fluctuations, or low amplitude regime (homogeneous, \(\sigma \tau_c = 0.1\)) (a, d, and g), intermediate regime (\(\sigma \tau_c = 1\)) (b, e, and h) and the slow fluctuations (inhomogeneous, \(\sigma \tau_c = 10\)) regime (c, f, and i). The labels \(\tilde{S}_C\), \(\tilde{S}_R\), and \(\tilde{S}_{NR}\) correspond to 2D correlation, rephasing, nonrephasing spectra, respectively. Note that as the system-bath interaction dynamics moves from fast to slow limit, the 2D line shape goes from being symmetric (a) to being diagonally elongated (b, c). Also, the relative amplitudes of the rephasing spectra (d, e and f) increase with respect to nonrephasing spectra (g, h, and i). The contours are plotted at 8% intervals.

Figure 6-4 shows simulations of the 2D line shape for a two level system within this stochastic model for different values of \(\sigma \tau_c\) varying from homogeneous to inhomogeneous. For \(\sigma \tau_c = 0.1\) (Fig. 6-4a), the 2D correlation spectrum shows a homogeneously broadened, symmetric diamond line shape. The homogenous limit implies that the time scale of the energy gap fluctuations are fast compared to the experimental time scales \(\tau_1\), \(\tau_2\), and \(\tau_3\). This results in exponential dynamics during \(\tau_1\) and \(\tau_3\), which are reflected by the diamond-shaped line.\(^{17,18}\) When the system-bath dynamics are homogenous, the rephasing (Figure 13d) and nonrephasing (Figure 13g) 2D spectra have the same amplitude and show the characteristic phase twisted line shapes aligned along
the diagonal and anti-diagonal axes respectively. This is seen in Figure 5-4 in the 2D rephasing and nonrephasing spectra of RDC dissolved in hexane at $\tau_2 = 0$ and at $\tau_2 = 470$ fs. The degree of elongation of the line shape along the diagonal axis increases with increasing $\sigma \tau_c$.

Figure 6-4c shows the 2D correlation spectrum in the inhomogeneous limit ($\sigma \tau_c = 10$), where the experimental time scales are much shorter than the time scale of the transition fluctuations. For this static limit, the ensemble can be described as a static distribution of homogeneous line shapes summed along the diagonal. The diagonal slice is representative of the width of the distribution ($\sigma$), whereas a slice along the anti-diagonal is a measure of the homogeneous linewidth for the ensemble ($\sigma^2 / \tau_c$). $S_R$ and $S_{NR}$ in this limit are shown in Figs 6-4f and 6-4i, respectively. Because the individual homogenous line shapes in the rephasing and nonrephasing 2D spectra lie along the diagonal and off-diagonal axes respectively, the summation of contributions from the ensemble along the diagonal leads to constructive interference in the former case and destructive interference in the latter. This explains the much higher intensity and diagonal elongation of the rephasing experiment relative to the nonrephasing experiment. The inhomogeneity of the system can therefore be indirectly related to the relative amplitude of the rephasing ($A_R$) and nonrephasing spectra ($A_{NR}$).

As defined in chapter 4, this ratio can be expressed as an angle $\Psi$ given by $\tan(\Psi) = (A_R - A_{NR}) / (A_R + A_{NR})$. These observations can also be explained from the dynamics of the ensemble in the time-domain. In a rephasing experiment, the dephasing experienced by the static ensemble during $\tau_1$ is rephased during $\tau_3$, due to the conjugate nature of the phase acquired by the coherences in the two periods. This results in the formation of a strong echo when $\tau_1 = \tau_3$, the Fourier transform of which directly corresponds to the diagonally elongated 2D line shape in the rephasing spectrum. In the nonrephasing experiment, the decrease in signal level during $\tau_1$ due to inhomogeneous dephasing continues uninterrupted during $\tau_3$, reflecting the low amplitude yet roughly symmetric line shape in Figure 6-4i. It should be noted that even though the rephasing and nonrephasing spectra in the inhomogeneous limit are of different amplitudes, the dispersive components of their phase-twisted line shapes are still properly cancelled in the 2D correlation spectrum (Figure 6-4c).

Following the above model calculations, the diamond shaped peaks observed in 2D correlation spectra of RDC in hexane are indicative of a homogeneously broadened system where the rephasing and nonrephasing experiments sample the same dynamics. However, all the peaks in the 2D IR correlation spectrum of Fig 6-1 do not have purely absorptive features, with some of the peaks retaining a partially phase-twisted character. We have shown in Appendix 4A that in a multi-level
vibrational system, there is an inherent asymmetry in the rephasing and nonrephasing Liouville pathways contributing to the formation of the ten resonances. For example, in the case of peak 2(2’) there are twice as many rephasing pathways as nonrephasing pathways. Also, only nonrephasing pathways contribute to the formation of peak 5(5’). Using this information we would predict that the 2D IR correlation spectrum would show peaks 2(2’) and 5(5’) tilted along the diagonal and off-diagonal axes respectively as indeed is the case. We see that the line shapes of peaks 2 (2’) and 4 (4’) are tilted by approximately $\pi/10$ from the $\omega$ axis. On the other hand, peaks 1(1’) and 3(3’) are symmetric about the $\omega$ axis and purely absorptive in nature suggesting that they have equal amplitudes in the rephasing and nonrephasing 2D spectra. A comparison of the polarization-selective 2D IR spectra of RDC in hexane reveals how microscopic factors such as the tensor components measured in the rephasing and nonrephasing experiment affect the tilts of the various features in a 2D IR correlation spectrum for a homogeneously broadened system. In particular we see that cross-peaks in $\hat{S}_{\perp}$ are almost symmetric about the $\omega$ axis, while those in $\hat{S}_{\parallel}$ are phase-twisted and tilted towards the diagonal axis.
References

Chapter 7

Obtaining structural information from polarization-selective 2D IR spectroscopy

The work presented in this chapter has been published in the following papers:


The analysis of the positions, amplitudes and line shapes of the various peaks in the 2D IR correlation spectra of RDC characterizes the system eigenstates and the specific system-bath interactions of our six-level vibrational system as described in the previous chapter. These observables allow us to model structure, understand the variation of molecular conformations, and follow the relaxation and conformational dynamics of molecules in the condensed phase on the time scale of our experiment. In the particular case of RDC dissolved in hexane where the interaction with the solvent is particularly weak and the individual linewidths of the transitions are much narrower than the anharmonic frequency shifts, polarization-selective 2D IR spectra provide an intuitive mapping of the system eigenstates and reveal the orthogonal projection angle between the two transition dipole vectors of the symmetric and asymmetric states. These eigenstates are delocalized over the molecule; therefore their characterization does not provide an immediate and obvious connection to local transient structure. In Chapter 3, we used two non-degenerate anharmonically coupled delocalized normal modes to model the eigenstates in an absolute value rephasing spectrum.
In this chapter, we will use a local mode description and relate the eigenstate parameters of polarization-selective 2D IR spectra of RDC in hexane to the coupling strength and angle between the C≡O bonds.

### 7.1 Structural Analysis

![Diagram of local molecular coordinates](image)

**Figure 7-1.** (Color) An illustration depicting the intuitive local molecular coordinates coupled together to yield the delocalized experimentally observed eigenstates. See text for details.

The 2D correlation spectra of RDC are modeled in terms of local carbonyl bond stretches with reduced vibrational coordinates $Q_m$ and $Q_n$. Their transition dipole vectors, $\mu_m$ and $\mu_n$, lie along the C≡O bond and are aligned at an angle $\theta$ with respect to each other. The bond stretch coordinates are represented as cubic anharmonic oscillators coupled through a bilinear interaction.

The local vibrational system Hamiltonian $\tilde{H}_S^V$ is given below:

$$\tilde{H}_S^V (Q_m, Q_n) = \frac{1}{2} \hbar \omega_m Q_m^2 + \frac{1}{2} \hbar \omega_n Q_n^2 + V_{mn} Q_m Q_n + \frac{1}{6} (g_{mmn} Q_m^3 + g_{mnn} Q_n^3) + P_{m}^2 / 2m + P_{n}^2 / 2m$$

(7.1)

In the above expression, the bilinear coupling constant is given by $V_{ij}$ and the cubic anharmonic coefficients are represented by $g_{iii}$. Equation 7.1 assumes symmetry with respect to the order of indices $m$ and $n$ in the definition of the bilinear coupling constant. The local vibrational coordinates
in eq 7.1 can be written in terms of raising and lowering operators, which are commonly used to describe the nuclear potential of interest. In particular it can be observed that adding the bilinear coupling term results in the coupling of states with the same total number of quanta and coupling between manifolds with total number of quanta differing by two. The addition of cubic anharmonic terms to the harmonic Hamiltonian leads to the mixing of states in manifolds with total number of quanta differing by 1 or 3. The matrix in eq 7.2 writes the elements of the local Hamiltonian explicitly in the uncoupled basis set of the bond-stretch coordinates,

\[
\begin{pmatrix}
0 & \frac{1}{2} g_{nn} & \frac{1}{2} g_{nn} & V_{nn} \\
\frac{1}{2} g_{mm} & \omega_m^0 & \sqrt{2} g_{mm} & \frac{1}{2} g_{nn} \\
\frac{1}{2} g_{nn} & V_{nn} & \omega_n^0 & \frac{1}{2} g_{mm} & \sqrt{2} g_{nn} \\
V_{nn} & \frac{1}{2} g_{nn} & \sqrt{2} g_{nn} & \omega_m^0 + \omega_n^0 & \sqrt{2} V_{nn} \\
\frac{1}{2} g_{mm} & \sqrt{2} g_{nn} & \sqrt{2} V_{nn} & 2 \omega_m^0 & 2 \omega_n^0 \\
\vdots & \vdots & \vdots & \vdots & \ddots
\end{pmatrix}
\]  (7.2)

Since the bilinear and anharmonic terms lead to the coupling of manifolds with different total number of quanta, the Hamiltonian matrix generally cannot be block-diagonalized. Instead, to determine the eigenstates of the total Hamiltonian, the eigenvalues and eigenvectors of the full (infinite) matrix should be found. In practice, however, the matrix size is chosen to achieve numerical convergence of the lowest lying six eigenvalues and corresponding eigenvectors of the matrix. To determine the experimentally observed eigenstates the local Hamiltonian is numerically diagonalized. The unitary transformation between the local basis set and the eigenstate basis is defined by the matrix \( T \),

\[
H_S = T^{-1} \tilde{H}_S^V T
\]  (7.3)

where \( H_S \) is the Hamiltonian matrix in the eigenstate basis set. Eigenvectors, constituting the columns of the transformation matrix \( T \), represent the contributions of the states in the local basis to the eigenstates of the total Hamiltonian. The lowest six energy levels of the resultant Hamiltonian in the eigenbasis correspond to the zero, one and two quantum states of the symmetric and asymmetric carbonyl stretches.

The transition dipole vectors among the eigenstates are obtained by writing the dipole operator in terms of the local coordinates and performing the appropriate transformation to the eigenbasis. To account for the electrical anharmonicity observed in the experimental 2D correlation
spectra, the dipole operator in the local basis $\mathbf{M}$, is expanded to the third order in each of the local vibrational coordinates as shown below,

$$\mathbf{M}(Q_m, Q_n) = \mu_m^{(1)} Q_m + \frac{1}{6} \mu_m^{(3)} Q_m^3 + \mu_n^{(1)} Q_n + \frac{1}{6} \mu_n^{(3)} Q_n^3$$  \hspace{1cm} (7.4)

In eq 7.4, $\mu_m^{(1)}$ and $\mu_m^{(3)}$ are the first and third order expansion coefficients of the dipole vector $\mu_m$ evaluated at the equilibrium configuration. The second-order expansion is ignored, as those dipole matrix elements do not contribute to the resonant transitions observed in this experiment. The transition dipole moments between the eigenstates of the system Hamiltonian $H_S$ are obtained by performing the appropriate unitary transformation,

$$\mathbf{M} = T^{-1} \mathbf{M} T$$  \hspace{1cm} (7.5)

where $\mathbf{M}$ is the transition dipole operator in the eigenstate basis. The transition dipole moments for the transitions among the eigenstates are given by the linear combinations of the local transition dipole moments. The weighting coefficients in the linear combination are generally determined by the harmonic frequencies of the local modes, the bilinear coupling coefficient, the cubic anharmonicities and the first and third-order expansion coefficients of the local transition dipole moments. The discussion above outlines a general recipe that may be used to make a local to eigenstate transformation for a system of two coupled vibrations.

Let us consider a special case of a symmetry degenerate system where the local vibrational coordinates are equivalent imposing the following conditions on eqs 7.1 and 7.4: $\omega_m^0 = \omega_n^0$, $g_{mmn} = g_{nnm}$, $\mu_m^{(1)} = \mu_n^{(1)}$ and $\mu_m^{(3)} = \mu_n^{(3)} = 0$. Symmetry degenerate systems have several important characteristics. In the harmonic limit when $g_{ik}=0$, the eigenstate basis containing the normal modes will be formed by a symmetric ($Q_s$) and asymmetric ($Q_a$) combination of the local coordinates:

$$Q_s = \frac{1}{\sqrt{2}} (Q_m + Q_n)$$  \hspace{1cm} (7.6a)

$$Q_a = \frac{1}{\sqrt{2}} (Q_m - Q_n)$$  \hspace{1cm} (7.6b)

The transition dipole moments for the fundamental transitions from the ground state to the asymmetric $\mu^{s,0}$ and symmetric $\mu^{s,0}$ normal modes are given by:

$$\mu^{s,0} = a(\mu_m + \mu_n)$$  \hspace{1cm} (7.7a)

$$\mu^{a,0} = b(\mu_m - \mu_n)$$  \hspace{1cm} (7.7b)

where the coefficients $a$ and $b$ depend on the local frequencies, the bilinear coupling constant, and the angle between the local coordinates. If the cubic anharmonic terms are included in the Hamiltonian,
the eigenstates will generally have contributions from all states of the local basis, although they will retain the symmetric and antisymmetric character with respect to the interchange of the local coordinates $Q_m$ and $Q_n$. The transition dipole moments between the states of a symmetry degenerate Hamiltonian are also symmetric with respect to interchange of the transition dipole moments for the local vibrations. Since the absolute value of $\mu_m$ and $\mu_n$ are equal and the two local frequencies are the same, the transition dipoles of the fundamental transitions of a symmetry degenerate Hamiltonian must be orthogonal to each other, independent of the mutual orientation of the transition dipoles of local vibrations. The symmetry degenerate case will always be described in the strong coupling limit of vibrational interactions, which is dictated by the strength of interaction relative to the energy splitting between the uncoupled local basis states.

The parameters in the Hamiltonian $\hat{H}_S$ describing the two local vibrations of our model system RDC are found by fitting the experimental 2D correlation spectra $\hat{S}_||$ and $\hat{S}_\perp$. We obtain analytical expressions for the observed signals following the procedure outlined in Chapter 4 and assuming infinitely short input pulses. The fitting routine involves writing out the system Hamiltonian and dipole operator in the local basis using eqs 7.1 and 7.4 and accounting for vibrational states with ten quanta of energy. The matrices are then transformed to obtain the eigenenergies, eigenvectors and transition dipole vectors of the six lowest lying vibrational states. Using the obtained eigenstate parameters, appropriate line shape functions characteristic of a homogenously broadened system, and the correct orientational factors, the 2D correlation spectra for the parallel and crossed polarization geometries are calculated. We assume that the parameters in the local Hamiltonian are invariant to the indices $m$ and $n$, due to the intrinsic symmetry of the molecule in which the two carbonyl stretches are chemically equivalent. All the dephasing constants are set to be the same ($\Gamma_{pq} = \Gamma$) as indicated by the identical linewidths of all the resonances in the 2D correlation spectra in both the frequency dimensions. A total of six parameters are floated including, $\omega_m^0 = \omega_n^0$, $V_{mn}$, $g_{mmm} = g_{nnn}$, $\theta$, $\mu^{(3)}_{mmm} / \mu^{(1)}_m = \mu^{(3)}_{nnn} / \mu^{(1)}_n$ and $\Gamma$. These parameters are refined to obtain the best fit to the experimental 2D spectra. In the above analysis, there are no assumptions made regarding the relative orientations of all six transition dipoles.

The nonlinear least squares fitting of the experimental data resulted in the following values for the best-fit parameters: $\omega_m^0 = \omega_n^0 = 2074 \pm 1$ cm$^{-1}$, $V_{mn} = 35 \pm 0.5$ cm$^{-1}$, $g_{mmm} = g_{nnn} = 172 \pm 1$ cm$^{-1}$, $\theta = 91 \pm 1^\circ$, $\mu^{(3)}_{mmm} / \mu^{(1)}_m = \mu^{(3)}_{nnn} / \mu^{(1)}_n = -0.4$ and $\Gamma = 2 \pm 1$ cm$^{-1}$. The 2D correlation spectra shown in the last row of Figure 7-2 are simulated using the results of the best fit and are successfully able to reproduce the positions, relative amplitudes and line shapes of all the resonances in the experimental
spectra. All the parameters of the local Hamiltonian and the eigenstates are tabulated in Table 7-1. The bilinear coupling constant, frequencies and cubic anharmonicities of the local stretches determine the accurate positions of the peaks in the 2D IR spectrum. The angle between the local stretches and the magnitude of the third-order coefficients of the dipole operator determine the relative amplitude of the ten resonances in the 2D IR spectrum, while the value of $\Gamma$ reflects the width of the 2D line shape in both dimensions.

![Figure 7-2.](Color) Experimental and calculated 2D correlation spectra obtained in the ZZZZ and ZYYZ polarization geometries for RDC in hexane at $\tau_z = 0$. The top row shows the experimentally collected 2D correlation spectra. The bottom row depicts the calculated 2D spectra using the eigenstate parameters obtained from the best-fit results. Twenty-one equally spaced contour levels from minimum to the maximum value are drawn for each 2D plot.

The analysis reveals the accurate positions of the fundamental frequencies at $\omega_{a,0} = 2015$ cm$^{-1}$ and $\omega_{s,0} = 2084$ cm$^{-1}$ and the corresponding anharmonic splittings of the higher lying states, $\Delta_a$, $\Delta_s$ and $\Delta_{as}$ are 14, 11 and 26 cm$^{-1}$ respectively. The value of $\Gamma$ reflects the linewidth of the fundamental transitions obtained from linear FTIR measurements. The value of the angle, $\theta$ between
the transition dipoles of the two local modes was found to be $91 \pm 1^\circ$ which affects the amplitudes of the transition dipoles between the fundamental eigenstates found to be $\mu^{s,0} = 0.9 \mu^{a,0}$. This result is consistent with previously measured 2D IR rephasing spectra and with data obtained from x-ray crystallography.\textsuperscript{2,3} It should be noted here that due to the intrinsic symmetry of the molecule any value of $\theta$ would result in the two fundamental transition dipoles being mutually orthogonal.

The electrical anharmonicity of the local mode transition dipoles results in the non-harmonic scaling of the amplitudes of the transition dipoles among the six eigenstates. For example, we note that $\mu^{2a,a} = \mu^{2s,s} = 1.2 \mu^{a,0}$ and $\mu^{as,a} = \mu^{as,s} = 0.7 \mu^{a,0}$ as reflected in the unequal amplitudes of the peak pair 1(1') and 3(3') and the pair 2(2') and 4(4') in the experimental 2D spectra. The fact that the observed amplitudes of the transition dipoles are less than their harmonic values is indicative of the negative value of $\mu^{(3)}_{mnm}$ and $\mu^{(3)}_{nnn}$. The amplitudes of the transition dipole moments between the eigenstates are also affected by the diagonal and off-diagonal cubic anharmonic terms, although to a lesser extent than the electrical anharmonicity. For instance, if we set $\mu^{(3)} = 0$ leaving the other parameters the same, the transition dipoles deviate from harmonic scaling by a maximum of 4%.

**Table 7-1:** The best-fit values of the parameters in the local Hamiltonian (left column), which reproduce the parameters characterizing the eigenstates (right column). The angles $\Theta_1$ to $\Theta_{13}$ have been defined in Appendix 4A.

<table>
<thead>
<tr>
<th>Parameters of the local Hamiltonian</th>
<th>Characteristics of the eigenstates obtained from the best fit results</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\omega_m^0 = \omega_n^0 = 2074 \pm 1 \text{ cm}^{-1}$</td>
<td>$\omega_{a,0} = 2015 \text{ cm}^{-1}$</td>
</tr>
<tr>
<td>$v_{mn} = 35 \pm 0.5 \text{ cm}^{-1}$</td>
<td>$\omega_{s,0} = 2084 \text{ cm}^{-1}$</td>
</tr>
<tr>
<td>$g_{mmn} = g_{nmm} = 172 \pm 1 \text{ cm}^{-1}$</td>
<td>$\Delta_a = 14 \text{ cm}^{-1}$, $\Delta_s = 11 \text{ cm}^{-1}$, $\Delta_s = 26 \text{ cm}^{-1}$</td>
</tr>
<tr>
<td>$</td>
<td>\hat{\mu}_1</td>
</tr>
<tr>
<td>$\mu^{(3)}<em>{mnm} / \mu^{(3)}</em>{m} = \mu^{(3)}<em>{nnn} / \mu^{(3)}</em>{n} = -0.4 \pm 0.05$</td>
<td>$\mu^{2a,a} = \mu^{2s,s} = 1.2 \mu^{a,0}$, $\mu^{as,a} = \mu^{as,s} = 0.7 \mu^{a,0}$</td>
</tr>
<tr>
<td>$\theta = 91 \pm 1^\circ$</td>
<td>$\mu^{2a,a} = 0.3 \mu^{a,0}$, $\mu^{2s,s} = 0.1 \mu^{a,0}$</td>
</tr>
<tr>
<td>$\Gamma = 2 \pm 1 \text{ cm}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\Theta_1 = 90^\circ$</td>
<td>$\Theta_8 = 0.7^\circ$</td>
</tr>
<tr>
<td>$\Theta_2 = 0.2^\circ$</td>
<td>$\Theta_9 = 2^\circ$</td>
</tr>
<tr>
<td>$\Theta_3 = 0.2^\circ$</td>
<td>$\Theta_{10} = 89.3^\circ$</td>
</tr>
<tr>
<td>$\Theta_4 = 0.5^\circ$</td>
<td>$\Theta_{11} = 88^\circ$</td>
</tr>
<tr>
<td>$\Theta_5 = 0.6^\circ$</td>
<td>$\Theta_{12} = 89.8^\circ$</td>
</tr>
<tr>
<td>$\Theta_6 = 89.4^\circ$</td>
<td>$\Theta_{13} = 89.8^\circ$</td>
</tr>
<tr>
<td>$\Theta_7 = 89.5^\circ$</td>
<td></td>
</tr>
</tbody>
</table>
The above analysis also reveals the angles between the transition dipoles of the eigenstates connecting the zero- to one- quantum states \( \( \mu^{1,0} \) \) and those connecting the one- to two- quantum states \( \( \mu^{2,1} \) \) and the as noted in the above table. For a harmonic system, we would expect the two angles to be parallel to each other. Although we have an anharmonic system, we notice that the angles deviate very slightly from harmonic behavior. The slight discrepancy arises from the presence of the electrical anharmonicity.

7.2 Discussion

In this chapter, we have presented experimental and theoretical methods using polarization-selectivity to obtain information about angles between coupled transition dipoles of the eigenstates and fitted the experimental data to extract parameters related to the local structure. We have been able to model transient structural elements on a \(~20\) ps timescale. The general recipe for structure determination can be applied to any multi-level vibrational system consisting of two or more coupled vibrations. In particular, including electrical anharmonicity in the local modes and writing the orientational factors for the response functions without making any assumptions about \( \mu^{2,1} \) being parallel to \( \mu^{1,0} \) will have a significant impact on the analysis of 2D correlation spectra of molecules with no intrinsic symmetry with respect to the local vibrations.

Microscopically, the bilinear interaction between the carbonyls arises from mutual covalent interactions with the rhodium metal center. The Rh mediates the interactions between the two carbonyls largely through the shift of electron density from Rh to the respective C=O arising from \( d_{\pi-\pi^*} \) back-bonding. Oscillation of one of the carbonyl dipoles modulates the electron density on the Rh, which in turn will be coupled through \( d_{\pi-\pi^*} \) interactions to the other carbonyl. This picture is borne out by our recent studies of the correlation of vibrational frequency fluctuations for the two C=O stretches of RDC in CHCl₃.⁴⁻⁶ We have shown that the fluctuations of the two transition frequencies were highly correlated, suggesting that solvent-induced modulation of the electron density on the Rh center from the axial direction modulates the strength of the \( d_{\pi-\pi^*} \) bonding between Rh and C=O groups. Such an interaction would symmetrically influence the individual C=O anharmonicities, leading to correlated transition-frequency fluctuations. Transition dipole-dipole interactions between the C=O s can be neglected in describing their couplings. If dipole-dipole couplings did contribute, it is expected that the splitting between symmetric and asymmetric modes would show a strong solvent dependence, yet the splitting (\( \approx 70 \) cm\(^{-1}\)) is virtually unchanged in
various nonpolar and polar (protic and aprotic) solvents. The splitting in the gas phase is measured to be 64.4 cm\(^{-1}\) which increases when RDC is put into solution and appears to be solvent-independent.\(^7\)

Our approach to the structure inversion problem differs from those used by traditional gas phase spectroscopists to fit line positions and intensities of vibrational spectra and the approach used by other 2D IR spectroscopists to study the structure of small peptides in solutions.\(^8\)-\(^11\) Both these approaches use a local basis set of anharmonic Morse oscillators coupled through a bilinear interaction. This results in a system Hamiltonian, which is approximated to be block-diagonal for the one- and two-quantum states. Another equivalent approach utilizes couplings amongst normal mode coordinates to simulate the eigenstate parameters. This is similar to our approached in Chapters 2 and 3 where we used a model of two non-degenerate normal modes coupled through cubic anharmonicity of the nuclear potential to calculate the energies of the eigenstates using second-order perturbation theory.

It is important to note that the validity of a particular approach depends on the system being studied. If the vibrational eigenstates are delocalized as is the case in the present study, a normal mode approach would not reveal information about local transient structure. On the other hand, in weakly coupled systems such as the Amide I modes of peptides and small proteins the term normal and local mode may be used interchangeably as the excitations are localized to the carbonyl region of the amide group. To compare our model system with a model peptide such as alanine tripeptide, we obtain eigenstate parameters in terms of the anharmonicity \(\Delta\), and bilinear coupling \(\beta\) for two Morse oscillators. Strong coupling is characterized by \(\beta/\Delta \gg 1\) and weak coupling by \(\beta/\Delta \ll 1\). We obtain values of \(\Delta = 13\ \text{cm}^{-1}\) and \(\beta = 35\ \text{cm}^{-1}\) while a similar analysis for the tripeptide reveals \(\Delta = 16\ \text{cm}^{-1}\) and \(\beta = 6\ \text{cm}^{-1}\). It is clear from this analysis that the two carbonyl groups are strongly coupled to each other in our model system, compared to the two Amide I oscillators of alanine tripeptide.

A more detailed picture of molecular structure from the above analysis requires an understanding of the vibrational coupling mechanism. The extent to which this is understood directly determines the type of structural information that can be accessed through 2D IR spectroscopy. Generally speaking, “through-bond” and “through-space” interaction between two vibrational coordinates will affect the magnitude of their coupling. For “through bond” covalent interactions, couplings can be related to connectivity. The electrostatic “through-space” interactions have well-defined distance scaling relationships and can potentially relate couplings to intra- and inter-molecular distances. For the particular case of the strongly coupled vibrations in RDC, the coupling is thought to arise from covalent interactions of the carbonyl groups with the Rh metal center.
primarily through $d_\pi - \pi^*$ back bonding effects.\textsuperscript{12} In the case of weakly coupled Amide I vibrations in small peptides, the coupling has been modeled in terms of through–space Coulombic interactions.\textsuperscript{13-15} Recent \textit{ab initio} studies on the coupling between the Amide I modes of a glycine dipeptide analog reveal that one needs to account for through-bond effects characterizing the anharmonicity of the nuclear potential along with couplings between a distribution of transition charges to obtain quantitative agreement with experimental results.\textsuperscript{11,16,17}

As seen from our analysis, the ability to obtain accurate structural information on the coupling strengths and orientation of coupled dipoles from 2D IR VE spectroscopy depends on fitting the amplitudes and shapes of the various features in a 2D spectrum. A number of experimental factors, particularly the excitation pulse bandwidth and intensity, and the frequency dependent optical density (OD) of the sample, influence these observables. The spectral profile of the IR pulses and the detuning of the resonances from the center frequency affect the relative amplitude of peaks in an observed 2D IR spectrum. The calculation and correction of such effects can be treated in a straightforward manner by direct time-domain calculations of the nonlinear polarization with the exact optical excitation fields. In the present experiments, the broad spectral profiles of the fs IR pulses were centered on the six primary resonance frequencies of the sample leading to small variation of the field amplitudes at those frequencies, and therefore not affecting the data analysis of the 2D spectra.

Our analysis of the 2D IR spectrum to reveal transient structure has focused on fitting the positions and amplitudes of the ten resonances in the polarization-selective spectra of RDC dissolved in hexane. The very narrow homogeneously broadened 2D lineshapes have allowed us to ignore the effects of the solvent on the transition energies of the system providing a convenient model system to test our method of spectral inversion from the local to eigenstate. In general, the system-bath interactions cannot be ignored and the effects of fluctuations of transition energies have to be taken into account as demonstrated by the 2D IR correlation spectra of RDC dissolved in chloroform and the 2D IR spectra of proteins shown in the last chapter.

The focus of the next chapter will be the discussion of 2D IR spectra of RDC in hexane collected as a function of the waiting period, $\tau_2$. These spectra provide evidence of coherence transfer and incoherent population transfer amongst the two-coupled symmetric and asymmetric eigenstates. We will show how these relaxation processes affect the determination of angles between the two coupled vibrations, the parameters in the local Hamiltonian as well as the electrical anharmonicity of the local transition dipole operator.
References

Chapter 8

Vibrational coherence transfer characterized with Fourier-transform 2D IR spectroscopy

8.1 Introduction

Vibrational relaxation processes of polyatomic molecules in solution involve the redistribution and relaxation of vibrational energy and phase resulting from the complex interactions among the intra and intermolecular vibrational coordinates of the solute and solvent. The study of vibrational relaxation continues to be a subject of considerable interest to experimentalists and theorists alike for two main reasons. It has long been recognized that a deeper understanding of vibrational energy and phase relaxation on excited electronic surfaces will help in elucidating chemical reaction mechanisms. Secondly, a detailed knowledge of vibrational relaxation is crucial in developing vibrational spectroscopy to be a true local probe of structure and dynamics in liquids where experimental observables can be related to microscopic molecular parameters.

A substantial literature exists describing the origin of vibrational relaxation of a single vibrational degree of freedom in solution through inter- and intra-molecular interactions with its unobserved surroundings, the bath. Vibrational population relaxation (or $T_1$) processes and vibrational pure-dephasing processes ($T_2^*$) are commonly expressed in terms of correlation functions involving the Hamiltonian that describes the interaction of the vibrational coordinate with the bath. For a rapidly fluctuating bath interacting with a two-level system, the dephasing time is related to the pure dephasing ($T_2^*$) and population relaxation ($T_1$) times as

$$\frac{1}{T_2} = \frac{1}{2T_1} + \frac{1}{T_2^*}. \quad (8.1)$$
This equation is inadequate for studying multiple vibrational degrees of freedom interacting with one another and a common bath, since it becomes necessary to include the effects of population and coherence transfer among the system eigenstates.\textsuperscript{11-13} For polyatomic molecules cascaded population transfer between multiple vibrations is observed,\textsuperscript{14,15} and can be described with a master equation for the kinetics of population relaxation between levels.\textsuperscript{16,17} Coherence transfer is an additional relaxation pathway, in which an oscillating vibrational superposition state is transferred other states without loss of phase memory. This process arises from the mutual interaction of two vibrational coordinates with a state in the bath, and for the case of weak coupling to the bath, can be described by Redfield theory.\textsuperscript{16-19} In this chapter, I experimentally characterize all of the above relaxation processes in a model system of two coupled vibrations, focusing primarily on the experimental signatures and consequences of vibrational coherence transfer.

For a general description of spectroscopic investigations of condensed phase vibrational relaxation, we can write an effective Hamiltonian of the form,

\begin{equation}
H = H_S + H_B + H_{SB} + H_{int}.
\end{equation}

Here the molecular degrees of freedom have been partitioned into a system ($H_S$), bath ($H_B$), and a system-bath ($H_{SB}$) Hamiltonian. The system is defined as the set of coordinates ($Q_a$) that interact directly with the electromagnetic fields as described by the interaction Hamiltonian, $H_{int}$. The multiple vibrations of the system can be expressed as coupled local coordinates or anharmonically coupled normal modes depending on the information of interest and the nature and magnitude of the vibrational couplings present. Either representation can be suitably transformed to yield the vibrational system eigenstates, and $H_S$ will be diagonal when written in the system eigenstate basis. The bath includes all other inter- and intra-molecular degrees of freedom ($q_\alpha$) that act through $H_{SB}$ to “dress” the system eigenstates. The interaction of the “dark” bath states with the system eigenstates results in the bath-renormalized eigenstates whose transition frequencies are detected in the experimental spectrum. The system-bath interaction describes the vibrational relaxation processes in solution including population relaxation, population transfer, dephasing and coherence transfer among the system eigenstates. When $H_{SB}$ is written in the basis of the system eigenstates, assuming a linear interaction with the bath coordinates, the linear terms ($Q_a q_\alpha$) describe the vibrational pure dephasing and coherence transfer ($\langle Q_a + Q_b \rangle q_\alpha$), while the higher-order terms describe the population relaxation of individual system coordinates (i.e, $q_a^2 q_\alpha$) and population relaxation ($Q_a Q_b q_\alpha$) between vibrations.\textsuperscript{11,12,20}

As the terms population and coherence transfer are often used in vastly different contexts, we illustrate these processes for the purposes of this paper in Figure 8-1. The double-headed arrows and
the curved arrows represent the traditional vibrational dephasing and population relaxation processes between the ground and excited states respectively. The curved arrows connecting states \( a \) and \( s \) indicate vibrational population exchange between the excited vibrational states. Finally, the squiggly arrows show coherence transfer among the two close-lying vibrational transitions. For example, an interaction with an external light-field creates a superposition of two system eigenstates \( 0 \) and \( s \), oscillating at frequencies \( \omega_{0,s} \). Interaction with a bath mode (intra- or intermolecular in nature) may lead to a coherent oscillatory exchange of amplitude to the close-lying transition, \( \omega_{a,0} \). The frequency of the bath mode, must be equal to the difference in frequency of the two close-lying excited states, \( \omega_{a,a} = \omega_{s,0} - \omega_{a,0} \). Spectroscopically, this represents the fact that vibrational eigenstates of the molecule can involve strong mutual mixing to coordinates that are not observed or dark to the experiment. Figure 8-1 also illustrates motional narrowing between the excited vibrational states, which is a consequence of coherence transfer. This implies that the experimentally observed peak positions involve transitions to a new set of bath-renormalized states \((A,S)\), which are different from the energies of the system-eigenstates \((a,s)\) because of the bath-induced coherence transfer processes.

The phenomenon of coherence transfer has been a subject of experimental and theoretical studies since the 1970s. The role of vibrational coherence in understanding the yield of products in photochemical reactions in liquids has been an area of long-standing interest. These reactions range from elementary studies of the photodissociation of triatomic molecules to complex photobiological processes in light harvesting compounds. The effects of coherence transfer have
also been observed in the temperature-dependent spontaneous Raman spectra of high-frequency C-H vibrations and in microwave rotational spectra.\textsuperscript{35-40}

Previous investigations that have studied coherence transfer in the condensed phase have often been limited by insensitive or inconclusive experimental techniques. This is because it has been difficult to separate the effects of coherence transfer on the positions, amplitudes and lineshapes of vibrational spectra from other relaxation processes. The advent of time-domain Fourier transform two-dimensional infrared spectroscopy offers a uniquely sensitive probe of coherence transfer, by virtue of its ability to directly visualize how vibrational excitation of a given transition migrates among system states due to the various relaxation processes.\textsuperscript{41-43} Cross peaks reveal couplings and orientations between vibrations and the surrounding bath. The time scale of the experiment is typically in the picosecond range, offering a short window to sample the structure and dynamics of the system. 2D IR spectra collected as a function of a variable waiting time (a “relaxation experiment”), contain signatures of coherent and incoherent vibrational relaxation processes, spectral diffusion phenomena, and fluctuations in vibrational couplings and angles. This information forms the basis for describing structural changes and intermolecular interactions in the condensed phase.\textsuperscript{44}

A recent theoretical paper describing the 2D IR spectroscopy of the Amide I modes of a dipeptide, Mukamel and co-workers pointed out that the presence of a peak in a 2D IR spectrum was solely due to coherence transfer processes.\textsuperscript{45} Their results indicate that coherence transfer will play a role in understanding the structure and dynamics of molecules in solution in the ground electronic state. This chapter reports on the experimental observation of extra resonances in 2D IR spectra solely due to coherence transfer processes and investigations into all of the coherent and incoherent vibrational relaxation pathways for a model system of two coupled vibrations. The two terminal carbonyl stretches of Rh(CO)\textsubscript{2}acac in hexane serve as a model system in which the intramolecular couplings are strong and interactions to the solvent are weak, offering a system in which sharp vibrational resonances allow various relaxation channels to be cleanly separated. By monitoring the change of peak amplitudes and growth of new peaks in 2D IR relaxation experiments, we can visualize the vibrational dephasing, population relaxation, population exchange, and coherence transfer in this system of coupled vibrations. We describe a diagrammatic approach to incorporating such processes into the description of nonlinear experiments, concluding that observation of coherence transfer in 2D experiments require broadband Fourier transform experiments, as opposed to narrow band pump-probe (or double resonance) experiments.\textsuperscript{44} In an effort to understand the consequences of vibrational coherence transfer as manifested in 2D IR spectroscopy, we use Redfield theory to model the various relaxation processes occurring as a result of the system-bath interactions during the experiment. The accompanying orientational response function is also calculated. These
Rh(CO)$_2$acac (RDC) is a widely studied model system for investigations into solution phase vibrational dynamics. IR Pump-probe experiments of the symmetric and asymmetric stretches have been used to characterize vibrational population transfer and relaxation, and IR vibrational echo experiments have been used to study the temperature dependent vibrational dephasing.\textsuperscript{3,46-48} More recently, RDC has been used to demonstrate how 2D IR spectroscopy reveals the structure and dephasing dynamics of molecules in solution.\textsuperscript{49-53} The work presented here builds on a series of investigations of the femtosecond IR spectroscopy of this system, for which experimental details are presented elsewhere.\textsuperscript{43,54} Third-order nonlinear 2D IR experiments of the symmetric and asymmetric terminal carbonyl stretching vibrations of RDC are sensitive to the transitions amongst six experimentally observed eigenstates. These include the two fundamental transitions and four transitions between the singly excited states (S and A) and the doubly excited states: two overtones (2S and 2A) and a combination band (AS). The actual values of the transition frequencies measured for RDC dissolved in hexane are: $\omega_{\text{A},0} = 2015 \text{ cm}^{-1}$, $\omega_{\text{S},0} = 2084 \text{ cm}^{-1}$, $\omega_{2\text{A},\text{A}} = 2001 \text{ cm}^{-1}$, $\omega_{2\text{S},\text{S}} = 2073 \text{ cm}^{-1}$, $\omega_{\text{AS},\text{A}} = 2058 \text{ cm}^{-1}$, $\omega_{\text{AS},\text{S}} = 1989 \text{ cm}^{-1}$, $\omega_{2\text{A},\text{S}} = 1932 \text{ cm}^{-1}$ and $\omega_{2\text{S},\text{A}} = 2142 \text{ cm}^{-1}$.

These eigenstates result from the interaction of the system states with a bath. To distinguish between the two basis sets, we refer to the eigenstates of the system Hamiltonian as \{0,a,s,2a,as and s\} and the experimentally observed, bath-renormalized eigenstates as \{0,A,S,2A,AS and S\}. Note that previously we have treated the system-bath interaction in a phenomenological manner, which resulted in the bath renormalized eigenstates being equal to the system eigenstates.[Golonzka, 2001 #1547; Khalil, 2003 #1860]

A detailed description of the methods used to obtain 2D IR spectra has been given elsewhere.\textsuperscript{43} Here, we briefly outline the method. The Fourier transform 2D IR experiment is performed using a sequence of three broadband femtosecond IR fields and is characterized by three experimental time delays: the evolution ($\tau_1$), waiting ($\tau_2$), and detection ($\tau_3$) periods, which follow the three successive input pulses. The three femtosecond pulses are crossed in the sample, each with a unique incident wavevector $k_i$. The short pulses have sufficient bandwidth that they are resonant both with the fundamental ($v=0\rightarrow 1$) amide I transitions, as well as the transitions between the anharmonically shifted $v=1\rightarrow 2$ transitions. These pulses sequentially drive absorption and stimulated
emission processes between these three levels. After each field, the system evolves freely during three sequential time-periods, $\tau_i$. The third field induces the radiation of a background-free nonlinear signal field during $\tau_3$ into a wave-vector matched direction $k_s$. We characterize the coherent evolution of the system during $\tau_1$ and $\tau_3$. The coherent states sampled during the two time periods following the initial excitation and the radiation of the signal are represented as a 2D spectrum in the Fourier transform variables $\omega_1$ and $\omega_3$.

Generally speaking, a 2D IR correlation spectrum with properly signed absorptive peaks is obtained by summing two complementary spectra with phase-twisted lineshapes, the rephasing and nonrephasing spectra. The names derive from the phase relationship with which vibrational coherences evolve during $\tau_1$ and $\tau_3$. The rephasing signal is derived from a stimulated photon-echo pulse sequence with the phase matching condition, $k_R = -k_1 + k_2 + k_3$ in which the phase acquired by coherences during the evolution period, $e^{i\Omega i\tau_1}$, is the conjugate of that for the detection period, $e^{-i\Omega i\tau_3}$. Coherences in nonrephasing measurements, scattered into the $k_{NR} = +k_1 - k_2 + k_3$ phase matching direction, evolve with the same phase during both $\tau_1$ ($e^{-i\Omega i\tau_1}$) and $\tau_3$ ($e^{-i\Omega i\tau_3}$). The rephasing and nonrephasing spectra also differ in the allowed excitation and relaxation pathways observed in the spectra, and thus we analyze them separately here, focusing primarily on absolute value 2D rephasing spectra.

Femtosecond IR experiments were performed with three identical 100 nJ, 90 fs pulses at a wavelength of ~5 µm with a spectral bandwidth of 160 cm$^{-1}$. This bandwidth is broad enough to excite all the transitions between the ground and first excited vibrational states and between the first and second excited states. The three independently timed pulses were focused into the sample with incident wave vectors $k_a$, $k_b$ and $k_c$ to generate the signal field along the phase-matched direction $k_s = -k_1 + k_2 + k_3$. Rephasing and nonrephasing signals were generated by interchanging pulses a and b as the first and second pulses. The generated nonlinear signal field was heterodyne detected by spatially and temporally overlapping it with a local oscillator field, and spectrally dispersing the fields with a 190-mm monochromator onto a 64-channel array detector. Arrays of data were collected for each value of $\tau_1$ in steps of 2 fs for a delay up to 6 ps. A 2D IR spectrum was obtained by Fourier transforming the data along the $\tau_1$ dimension. A detailed description of the treatment of the raw data has been provided elsewhere. Rephasing and non-rephasing 2D IR spectra were obtained for $\tau_2 = 0$ fs, 470 fs, 705 fs, 940 fs, 1175 fs, 4.7 ps, 7.05 ps and 11.75 ps in the ZZZZ (all-parallel) polarization geometry. In addition, we also performed broadband dispersed pump-probe experiments, crossing a pump pulse with a time-delayed probe pulse in the sample, spectrally dispersing the transmitted probe.
in a monochromator, and detecting the transmittance change on the probe as a function of delay \( \tau_2 \).

Experiments were performed on a room-temperature sample of \( 1 \times 10^{-3} \) M RDC in hexane held in a 200 \( \mu \)m thick CaF\(_2\) cell corresponding to a peak optical density of 0.25.

### 8.3 Results

**Figure 8-2**: Absolute value 2D IR rephasing spectra of RDC in hexane for waiting times of \( \tau_2 = \) (a) 0 fs, (b) 470 fs, (c) 705 fs, and (d) 11.75 ps. Note that the overall amplitude of the spectra decreases with \( \tau_2 \), so that the amplitudes of (c) and (d) have been scaled as noted. Twenty equally spaced contours from the minimum to the maximum value have been drawn for each plot. The polarization condition of all the input fields is the same (ZZZZ).

Figure 8-2 shows absolute value 2D IR rephasing spectra for waiting times, \( \tau_2 = 0 \) fs, 470 fs, 705 fs and 11.75 ps. We see that the 2D spectrum at \( \tau_2 = 0 \) fs is characterized by eight dominant peaks consisting of four pairs of peaks with roughly equal amplitude. The peaks that lie along the diagonal axis result from interactions of the light fields with only the asymmetric (\( a \)) or symmetric (\( s \)) manifolds in the experimental time periods. The corresponding peak pair at a slightly lower frequency along the \( \omega_3 \) axis represents interactions with the anharmonically shifted overtone peaks in the \( \tau_3 \) time period. The cross peaks lying along the off-diagonal axis represent interactions with the two different vibrational manifolds during the evolution and detection time periods. Their corresponding peak pair involves interactions with the anharmonically shifted combination band. As we have noted before, the positions of the spectral features lying along the diagonal and off-diagonal axes measure the diagonal anharmonicity (\( \Delta_A \) and \( \Delta_S \)) of the individual vibrations and the mixed-mode or off-diagonal anharmonicity (\( \Delta_{AS} \)) in the nuclear potential nuclear potential of our strongly coupled system. The relative amplitudes of the cross-peaks which is roughly 1/3 indicates that the
transition dipole moments for the \( a \) and \( s \) transitions are mutually orthogonal. The diamond shape of the 2D resonances is indicative of the homogenous nature of the system-bath interactions, where the timescale of the bath fluctuations is much faster than our experimental timescale. All the resonances along \( \omega_l = \omega_{S,0} \) have been labeled to allow for an easier discussion of the results. The diagonal peak and its anharmonically-shifted overtone are numbered 1 and 3, while the cross peak and its anharmonically-shifted combination band is indicated as peaks 2 and 4. From the relative splitting of the peak pairs, we observe that the off-diagonal anharmonicity is approximately twice the value of the diagonal anharmonicity.

The presence of the eight diagonal and cross-peaks in a 2D IR rephasing spectrum of two coupled vibrations as described above can be predicted by writing out all the Feynman level diagrams describing the Liouville-pathways for a six-level system interrogated in a third-order nonlinear experiment. However, the presence of the peaks indicated by the arrows at \((\omega_1,\omega_3) = (\omega_{A,0},\omega_{AS,S})\) and \((\omega_{S,0},\omega_{AS,A})\) in the 2D IR rephasing spectrum at \( \tau_2 = 0 \) cannot be explained simply through standard interactions of the electric fields with a six level vibrational system. As we will see in the next section, the presence of these two peaks in the rephasing 2D IR spectrum is a clear indication of coherence transfer processes occurring between close-lying vibrational levels during \( \tau_1 \) and \( \tau_3 \).

A number of features change with increased waiting time \( \tau_2 \) as seen in the remaining 2D IR spectra of Figure 8-2. We observe for shorter delays \( \tau_2 < 2 \) ps that the cross peaks disappear and then reappear, the relative amplitude of the diagonal and cross peaks equalizes, new peaks grow in, with each peak doublet evolving gradually into a triplet and the overall amplitude of the peaks decrease with time. These effects can be related to the dynamics observed in a broad-band dispersed pump-probe experiment, and example of which is shown in Figure 8-3(a-b). The short time modulation of the cross peak reflects the quantum beats that arise from the \( A,S \) superpositions prepared during \( \tau_2 \). For rephasing spectra, the third-order interaction pathways that involve these coherences lead to only to cross peaks, whereas for nonrephasing spectra such pathways lead only to diagonal peaks. The result of this observation is illustrated in Fig. 8-3, where we show the amplitudes of the various peaks in absolute value rephasing and nonrephasing spectra as a function of \( \tau_2 \), by plotting slices through them for \( \omega_l = \omega_{S,0} \). The waiting times for these spectra is chosen to coincide with the minimum or maximum of the 470 fs quantum beats observed during \( \tau_2 \) in the pump-probe experiment. From this figure it is clear that for rephasing spectra, the cross-peaks are modulated at the 470 fs period and diagonal peaks remain roughly constant, whereas diagonal peaks are modulated in the nonrephasing spectrum and the cross peaks are roughly constant. We see that the intensity of the cross-peak reaches the maximum in Fig. 8-3(b) when \( \tau_2 = n\pi/2\omega_{AS} \) where \( n \) is an even integer and its minimum value
when \( n \) is an odd integer. The frequency, \( \omega_{AS} \) is equal to the frequency splitting between the symmetric and asymmetric carbonyl stretches. This is also true for the diagonal peaks in the slices from non-rephasing 2D IR spectra shown in Figure 8-3(c). It is important to note that the pump-probe experiment is a measure of the rephasing and non-rephasing pathways and therefore, the quantum beats reflect beating of both cross and diagonal peaks. These effects can be cleanly separated in the 2D IR experiments.

**Figure 8-3**: (a) A broad-band dispersed pump-probe trace at \( \omega_1 = \omega_{S,0} \) showing the long time population decay. (b) A blow-up of the pump-probe transient shown above, emphasizing the quantum beats with a period of 470 fs that arise from superpositions of the \( A \) and \( S \) states prepared during \( \tau_2 \). The amplitudes of peaks 1-6 in the rephasing and non-rephasing absolute value spectrum are shown in (c) and (d) by taking slices for \( \omega_1 = 2084 \text{ cm}^{-1} \). Slices are taken for \( \tau_2 = 470, 705, 940, \) and 1175 fs, which, as shown, correspond to maxima and minima of the \( \alpha, \beta \) quantum beats in the pump-probe signal.

To allow for a comparison of the timescales for the various relaxation processes, the amplitudes of six characteristic peaks in the rephasing spectrum of the two coupled vibrations are plotted in the left panel of Fig. 8-4 as a function of the waiting period. These six peaks, chosen for \( \omega_1 \)
$\omega_{S,0}$, are the diagonal peaks, cross peaks, and relaxation-induced peaks (5 and 6). The diagonal peaks decay monotonically on two time-scales: a fast $\sim$3 ps decay followed by a longer 60 ps. The cross peaks are strongly modulated at the 470 fs period as noted in Figure 8-3b, but they rise with the $\sim$3 ps time scale and then decay with the long 60 ps time scale. Additionally, the relaxation-induced peaks have small but non-zero amplitude for $\tau_2 = 0$ due to the effect of vibrational coherence transfer. Both these peaks rise and decay with the timescales associated with the cross-peaks. Peak 6 is seen to beat slightly at short delays, but this effect is essentially arises from interference with the cross peaks 2 and 4.

![Figure 8-4: Integrated amplitudes of the diagonal peaks (1 and 3), cross-peaks (2 and 4), and relaxation-induced peaks (5 and 6) for $\omega_1 = \omega_{S,0}$ in the absolute value 2D IR rephasing spectrum. Experimental results are shown on the left, and the results of the model are on the right. Note that the scale of the panels for the different types of peaks varies. The amplitudes are plotted over an integration area of $\pm 3$ cm$^{-1}$ in the $\omega_1$ and $\omega_3$ dimension from the center of each of the resonances.](image)

In summary, the results presented in Figures 8-2-8-4 provide evidence for the following relaxation processes in our model system of two strongly coupled carbonyl stretches: (i) coherence transfer between the two fundamental vibrations affecting the amplitude of all the peaks and in particular, leading to the presence of peaks at $(\omega_1, \omega_3) = (\omega_{A,0}, \omega_{AS,S})$ and $(\omega_{S,0}, \omega_{AS,A})$ in the 2D IR rephasing spectrum at $\tau_2 = 0$, (ii) coherent dynamics of the $AS$ superposition during $\tau_2$ reflected by the
modulation of cross-and diagonal peak amplitudes in the rephasing and nonrephasing 2D spectra respectively and the presence of quantum beats in a dispersed pump-probe experiment with a time period corresponding to the splitting of 70 cm\(^{-1}\) between the two fundamental vibrations, (iii) Fast population exchange (~3 ps) during \(\tau_2\) between the symmetric and asymmetric manifolds, leading to the growth of relaxation-induced peaks (peaks 5 and 6) and a decrease in the ratio of the amplitudes of the diagonal (peak 1) to the cross-peaks (peak 2) and (iv) population relaxation to the ground state on a 60 ps timescale leading to the simultaneous decay of all the twelve resonances in the 2D IR spectra and the long time decay of the pump-probe transient. All of these effects will be taken into account while modeling the data in terms as described in the next two sections.

### 8.4 Vibrational Relaxation Modeling

To describe all of the vibrational relaxation processes observed in this system for the three time intervals sampled in the 2D IR experiments, we need to consider the population relaxation, dephasing, and coherence transfer processes amongst the six system eigenstates and their surroundings. One of the commonly used approaches to study quantum dynamics in the condensed phase is the reduced density matrix theory developed by Redfield.\(^{16,18,19}\) We start with the Hamiltonian described in equation 8.2 and assume that (1) the bath dynamics are much faster than the timescales of the system relaxation (Markovian approximation) and (2) the system is weakly coupled to the bath allowing us to treat the system-bath interactions in a perturbative manner. The Redfield equation is obtained by using second-order perturbation theory to treat the system-bath interactions and the reduced density matrix is propagated by,

\[
\frac{\partial \rho_{ab}}{\partial t} = -i \omega_{ab} \rho_{ab} + \sum_{cd} \Gamma_{ab,cd} \rho_{cd}
\]

(8.3)

In the above equation, \(\rho\) is the reduced density matrix, \(\Gamma\) is the Redfield relaxation tensor written in the basis set of the eigenstates of the system Hamiltonian \((a,b,c,d)\) and \(\omega_{ab}\) is the transition frequency between the system eigenstates \(a\) and \(b\). The Redfield tensor describes the rate of transfer of amplitude between elements of the reduced density matrix in the system eigenstates. In this tetradic notation, the population relaxation is reflected by the \(\Gamma_{aa,aa}\) elements, population cross-relaxation is reflected by the \(\Gamma_{aa,bb}\) elements, dephasing of coherences is expressed as \(\Gamma_{ab,ab}\) and coherence transfer through \(\Gamma_{ab,cb}\) and \(\Gamma_{ab,cd}\). Detailed balance is satisfied in this formalism by ensuring that the following relationship holds for the elements of the Redfield tensor, \(\Gamma_{aa,bb}/\Gamma_{bb,aa} = \exp[-\hbar \omega_{ab}/kT]\). The elements of the Redfield tensor can be formally expressed in terms of Fourier transforms of
correlation functions in the system-bath interaction \((H_{SB})\) evaluated at the energy gaps between system states.\(^{16,17,19}\) When the system-bath interactions are treated as Markovian, as in this paper, we can replace elements of \(\Gamma\) with exponential rate constants \(\gamma\) leading to a set of kinetic equations linking the elements of the density matrix.

Two-dimensional IR spectroscopy measures the third-order nonlinear polarization \(\mathbf{P}^{(3)}\) induced by the interaction of the material with three femtosecond IR fields. The non-linear polarization is given by the convolution of the material response function with each of the three input fields. The material response for a third-order resonant experiment in the interaction picture, is expressed as a four-point correlation function of the dipole operator \((\mathbf{M})\),

\[
\mathbf{R}\left(\tau_3, \tau_2, \tau_1\right) = \left(\frac{i}{\hbar}\right)^3 \left[ \left[ \left[ \mathbf{M}\left(\tau_3 + \tau_2 + \tau_1\right)\mathbf{M}\left(\tau_2 + \tau_1\right)\mathbf{M}\left(\tau_1\right)\mathbf{M}\left(0\right) \right] \rho_0 \right] \right]. \tag{8.4}
\]

Here \(\rho_0\) is the equilibrium reduced density matrix for the system eigenstates and \(\tau_n\) represent the delays between the successive field-matter interactions occurring within the field envelope. The evaluation of the commutator in the above equation is simplified by invoking the rotating wave approximation and using the diagrammatic density matrix approach. This gives rise to eight rephasing and non-rephasing Liouville pathways shown in chapter 4. The response function is a fourth rank tensor quantity that contains not only the information relevant to vibrational dynamics, but also the relative orientation and orientational dynamics of the dipoles interrogated by the polarized electric fields.\(^{57}\)

The three double sided Feynmann diagrams contributing to the 2D IR rephasing signals generated in the phase matched direction, \(\mathbf{k}_R = -\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3\) are illustrated in Figure 8-5A. In the limit of impulse excitation, the measured signals are proportional to the material response function and can be written as a function of the experimental delays \(\tau_1, \tau_2, \) and \(\tau_3\). Using the assumption that the vibrational and orientational degrees of freedom are separable, the \(R_2\) response illustrated in the first panel of Figure 8-5A can be written as: \(^{58}\)

\[
R_2 = (i/\hbar)^3 \sum_{ijkl} \sum_{01} \sum_{ij} \left\{ \tilde{Y}^{ijkl}_{ijkl}(\tau_3, \tau_2, \tau_1) \left[ \hat{\mu}^{01} \cdot \hat{j} \left[ \hat{\mu}^{10} \cdot \hat{k} \left[ \hat{\mu}^{00} \cdot \hat{i} \right] \right] \right] \right\} \tag{8.5}
\]

The first term in the above equation is the nonlinear orientational response reflecting the sequential projection of the electric fields in the laboratory frame onto the molecular transition dipole moments \((0,1,1' \in a,b,c,d)\) for a particular sequence of electric field interactions, allowing the rigid molecules to orientationally diffuse between successive field-matter interactions. Starting from an isotropic distribution, each field interaction projects out a subset of molecules from the evolving orientational
distribution. In the above expression, the orientation of the four time-ordered transition dipole operators is written in a molecular body-fixed frame \( (i,j,k,l \in x,y,z) \) and the motion in the molecular frame is transformed into the laboratory frame through an orientational average, \( \hat{\Gamma}_{ijkl} (\tau_1, \tau_2, \tau_3) \). The analytical expressions for \( \hat{\Gamma}_{ijkl} \) are tabulated in Chapter 4.\(^{43,59}\) The indices \( \{L,J,K,L\} \) refer to the permutation over the laboratory frame indices \( \{X,Y,Z\} \). We see that the dipole product for each of the response functions in Fig 8-5A can be rearranged to have a cyclic set of superscripts of the form \( \mu^{ab} \mu^{bc} \mu^{cd} \mu^{da} \).\(^{60}\)

![Diagram](image)

**Figure 8-5.** Double-sided Feynmann diagrams for the wavevector matching condition \( k_R = -k_1 + k_2 + k_3 \). (A) The three processes without any effects of relaxation transfer processes. (B) Pathways for the response function \( R_2 \) including include vibrational population and coherence transfer indicated as a dashed line during the time-intervals \( \tau \). The first row includes diagrams that allow for coherence transfer processes during \( \tau_1 \) and \( \tau_3 \) (left), \( \tau_2 \) (middle), and during all time intervals (right). The second row includes the effects of population transfer during \( \tau_2 \) (left) and coherence transfer during \( \tau_1 \) and \( \tau_3 \) and population transfer during \( \tau_2 \) (right).
The second term in eq 8.5 is the nonlinear isotropic vibrational response where the variables \( G(\tau) \) are the solution to the Redfield equation described in eq 8.4, describing the propagation of the density matrix during each experimental time period. In the Bloch limit, when the effects of population and coherence transfer are neglected, the solution to the Redfield equation becomes trivial and

\[
G_{ij}(\tau) = \exp\left[-i\omega_{ij} \tau - \gamma_{ij} \tau \right],
\]

where \( \gamma_{ij} \) is the relaxation rate.

From the above discussion we can see that accounting for vibrational dephasing, population relaxation, and coherence transfer processes present in a multilevel vibrational system increases the number of possible interaction sequences with the three fields considerably and it is no longer possible to obtain analytical expressions for the vibrational response function. Modifying the Feynmann diagrams as shown in Figure 8-5B can include these relaxation transfer processes in the existing formalism. The figure illustrates modified Feynmann diagrams for the \( R_2 \) response illustrated in the first panel of Figure 8-5A. The traditional diagram with no transfer processes represents the state of the density matrix and the associated dephasing dynamics after each of three field interactions. For a given sequence, a product of four transition dipole operators with a cyclic set of indices can be written to describe the four field interactions of the field with the states. When coherence transfer is also considered, three other types of diagrams can be written. These allow the transfer during both \( \tau_1 \) and \( \tau_3 \) coherence evolution time periods, during \( \tau_2 \), or during all three time periods. All other types of diagrams, such as those that involving a single coherence transfer event during \( \tau_1 \) or \( \tau_3 \) do not contribute to the final trace over the density matrix. With the inclusion of coherence transfer, the cyclic symmetry of the transition dipole product vanishes. In other words, the dipole product for each of the response functions in the first row of Fig 8-5B cannot be rearranged to have a cyclic set of superscripts.

The inclusion of population transfer amongst the singly excited vibrational states during \( \tau_2 \) adds two more diagrams shown in the second row of Figure 8-5B. In the first case, there is only population exchange during \( \tau_2 \). The influence of such processes on the nonlinear response has recently been considered for coupled electronic states and reactive systems. In the second case there is coherence transfer during \( \tau_1 \) and \( \tau_3 \) in addition to population transfer during \( \tau_2 \). Note that, the presence of population transfer enforces the cyclic symmetry of the transition dipole product. A close inspection of all these Feynman diagrams reveals that coherence transfer processes can be observed only when the first two field-matter interactions are resonant with two different vibrational transitions. Thus, in 2D vibrational experiments, coherence transfer events will only be observed in broadband Fourier transform 2D IR spectroscopy and not in narrow band double-resonance 2D IR experiments.
With the large number of interaction pathways that must be considered for a six level system with these vibrational relaxation processes, it is not practical to model an experiment by expressing and evaluating all possible permutations of these diagrams over the system eigenstates as was shown for the traditional diagram $R_2$ in eq 8.5. An additional complication for vibrational transitions with arbitrary orientation in the microscopic frame is that orientational relaxation of the nonlinear polarization accompanies coherence or population exchange processes. Each particular interaction sequence must be scaled by the projection of the light fields onto the transition moments in the molecular frame. To fully include all the vibrational and orientational effects, we choose to propagate the density matrix under the Redfield tensor for each time period whilst acting repetitively with the dipole operator for a field interaction and the complete relaxation operator for the following time period.

In practice this is implemented as follows. We begin with an $n \times n$ reduced density matrix $\rho$ for the $n$ level vibrational system, and an $n \times n$ dipole operator $M$ incorporating all the allowed transitions among the different elements of the density matrix. The dipole operator is expressed in terms of the components of the vibrational transition moments in the molecular body-fixed frame. The third–order nonlinear signal is simulated starting with an equilibrium density matrix that assumes $\rho_{00}$ is the only non-zero element, as only the ground state is initially occupied for the case of high-frequency vibrations. For each interaction, the density matrix is multiplied by $M$ from the right or left side depending on whether the phase of interaction dictated by the wave-vector matching condition dictates a bra or ket side interaction. For the purposes of propagating the density matrix under the influence of the tetradic Redfield tensor according to eq. 8.3, we express $\rho$ as a column vector whose length is dictated by the total number of elements ($n^2$, for an $n$ level system). The Redfield tensor $\Gamma$ is written as an $n^2 \times n^2$ matrix whose elements contain all the information about the relaxation pathways coupling density matrix elements and the oscillation frequencies of the different elements of $\rho$. The right hand side of eq. 8.3 is obtained by multiplying $\rho$ by $\Gamma$, yielding a system of coupled equations that are numerically solved for the propagation to a particular experimental time delay. The result is a series of vectors representing $\rho$ at a particular time point. Each of these vectors is re-written in a matrix form and multiplied by $M$ and the procedure is repeated till we have performed three field-matter interactions. The solutions are calculated for a range of $\tau_1$ and $\tau_3$ from 0 to 8 ps, and a fixed value of $\tau_2$. After the third field matter-interaction, each point in the data matrix $(\tau_1, \tau_3)$ for a particular value of $\tau_2$ is obtained by performing a trace over the density matrix obtained for that value of $\tau_1$, $\tau_2$, and $\tau_3$. The trace at each point contains a number of contributions indexed by the microscopic frame directions of the four transition dipole moments sampled during the interaction with the fields. The
laboratory frame orientational response is obtained by multiplying the vibrational response functions with the appropriate orientational averages, given by \( \tilde{\gamma}_{ijkl}(\tau_j,\tau_k,\tau_l) \). The data matrix in \((\tau_1,\tau_3)\) is 2D Fourier transformed to obtain the 2D IR spectrum.

The above recipe was implemented to simulate the 2D IR rephasing spectra of RDC dissolved in hexane. The elements of the Redfield operator responsible for the population exchange between the symmetric and asymmetric states were given by the rates \( \gamma_{aa,ss} \) and \( \gamma_{ss,aa} \), whereas relaxation to the ground state is expressed as \( \gamma_{aa,00} \) and \( \gamma_{ss,00} \). The dephasing of the fundamental transitions and four transitions to the doubly excited states dictating the linewidths in the 2D spectrum have rate constants \( \gamma_{ij,ij} \). The dephasing of the AS coherence that evolves during \( \tau_2 \) damps with a rate \( \gamma_{as,as} \). Finally, since coherence transfer can occur during any of the time-periods, we must consider coherence transfer between the \( 0,s \) and \( 0,a \) coherences (\( \gamma_{0a,0a} \) and \( \gamma_{0s,0s} \)), and between the combination band and overtones (\( \gamma_{2aa,11a} \); \( \gamma_{11a,2aa} \); \( \gamma_{2ss,11s} \); \( \gamma_{11s,2ss} \)). All these processes have been duly noted in Figure 8-6 and are related to one another using detailed balance relationships. We only include coherence transfer processes that result in the change of one vibrational quantum, and neglect all higher order processes. We do not include any relaxation pathways between the diagonal and off-diagonal elements of the density matrix. This results in a 15 x 15 Redfield matrix.

**Figure 8-6**: Six vibrational system eigenstates and the dominant bath-induced relaxation processes considered for the two strongly coupled C=O stretching vibrations of RDC. Double headed arrows represent the dephasing of coherences, with a damping rate \( \gamma_{ij,ij} \). Curved arrows represent population relaxation to the ground state or cross relation between the excited states (\( \gamma_{ii,jj} \)). Squiggly arrows represent coherence transfer between coherences prepared during \( \tau_1 \) or \( \tau_3 \). The transition frequencies used to simulate the spectra are:
\[
\begin{align*}
\omega_{a,0} &= 2012.5 \text{ cm}^{-1}, & \omega_{s,0} &= 2087.5 \text{ cm}^{-1}, & \omega_{2a,a} &= 1987 \text{ cm}^{-1}, & \omega_{2s,s} &= 2092 \text{ cm}^{-1}, & \omega_{as,a} &= 2074.5 \text{ cm}^{-1}, \\
\omega_{as,s} &= 1970 \text{ cm}^{-1}, & \omega_{2a,s} &= 1933 \text{ cm}^{-1} & \omega_{2s,a} &= 2142 \text{ cm}^{-1}.
\end{align*}
\]
From 2D IR data presented in Figs. 8-2-5, we can estimate the value for many of the relaxation rate constants $\gamma$. The long time decay of all the peaks in Figure 8-4 is representative of $\gamma_{aa,00}$ and $\gamma_{ss,00}$, whereas the population exchange rates $\gamma_{aa,ss}$ and $\gamma_{ss,aa}$ are indicated by the rise of the cross peak amplitude and the fast decay of the diagonal peaks during $\tau_2$. The dephasing rates of the fundamental transitions $\gamma_{a0,a0}$ and $\gamma_{a1,a1}$ are dictated by the 2D IR linewidths and the dephasing of the $a,s$ coherence ($\gamma_{as,as}$) is given by the decay of the quantum beats in the pump-probe experiment. The magnitude of the coherence transfer rates amongst the various transition frequencies determines the amplitudes of the relaxation-induced peaks in the 2D IR spectrum at $\tau_2=0$. The coherence transfer rates also affect the peak positions in the 2D IR spectrum. It has been shown, that the rates of coherence transfer must exceed the energy splitting between the coupled transitions, and occur at a faster rate than the dephasing times of the individual transitions. With these observations, we find that we can model the 2D IR data with a set of seven relaxation parameters: $1/\gamma_{ss,00} = 1/\gamma_{aa,00} = 50$ ps; $1/\gamma_{ss,aa} = 3$ ps; $1/\gamma_{00,00} = 10$ ps; $1/\gamma_{as,as} = 2.8$ ps; $1/\gamma_{0s,0a} = 0.35$ ps; $1/\gamma_{2aa,11a} = 0.15$ ps; and $1/\gamma_{2ss,11s} = 0.1$ ps. The values of the transition frequencies used in the simulation are noted in the caption of Figure 8-6. The other non-zero matrix elements are related to the above parameters using the detailed balance relations. The dephasing of transitions involving the doubly excited vibrational states are related to the fundamentals by approximate harmonic scaling relations.

The dipole operator for RDC in hexane is written in the harmonic approximation, accounting for all the transitions between the zero and one-quantum states ($\mu^{a0}, \mu^{s0}$) and those between the one and two quantum states ($\mu^{11a}, \mu^{11s}, \mu^{2ss}, \mu^{2aa}$). From our earlier studies we know that $\mu^{a0}$ and $\mu^{s0}$ are mutually orthogonal. Additionally, we assume that the transition dipoles $\mu^{11a}$ and $\mu^{2ss}$ are aligned along $\mu^{a0}$, while $\mu^{11s}$ and $\mu^{2aa}$ are aligned along $\mu^{s0}$. The amplitudes of the various elements in the transition dipole operators are given by the harmonic relations where: $|\mu^{11a}| = |\mu^{11s}| = |\mu^{2ss}| = |\mu^{2aa}| = \sqrt{2} |\mu^{a0}|$ and $|\mu^{s0}| = |\mu^{a0}|$. A detailed description of the modeling of the 2D IR spectrum independent of vibrational cross relaxation processes is given in chapters 4 and 7.

The results for the simulations using the parameters mentioned above are plotted as the amplitudes of the six peaks in the 2D IR rephasing spectrum as a function of $\tau_2$ along $\omega_1 = \omega_{ss,0}$, in the second column of Figure 8-4. The modeling reproduce most of the observed experimental results of the absolute value 2D IR spectra including the bimodal decay of the diagonal peaks, the growth and oscillation of the cross-peaks and the finite amplitude of the relaxation-induced peaks at zero waiting time. Along with reproducing the experimental amplitudes, the choice of relaxation parameters also gives excellent agreement for the real part of the rephasing spectrum.
In modeling, we notice that the phase twist and sign of the relaxation-induced peaks is extremely sensitive to the relative magnitudes of the coherence transfer rates. 

The origin of the coherence transfer between the various transitions results from the coupling of the high-frequency modes sampled in the experiments to dark states of the bath. The most likely origin of this coupling is a mutual interaction of the $a$ and $s$ states with vibrations of the acetylacetonate ligand ($l$) of RDC, in an interaction of the form $H_{5a} \sim (Q_a + Q_s)q_l$. To test this, DFT calculations were performed for us on the isolated RDC molecule using Amsterdam Density Functional package to optimize the structure and obtain accurate harmonic frequencies. The calculated splitting between the $A$ and $S$ states was 64 cm$^{-1}$, in close agreement with the gas phase value. Modeling of the occupied $d$ orbitals shows that the orbitals of $d_{z^2}$ and $d_{xz}$ character are strongly mixed both into the antibonding $\pi$ orbitals of the terminal COs and the carbons and oxygens of the aromatic acac ring. These observations indicate that $d-\pi^*$ back-bonding in this system involves not only the terminal CO groups, but also the $\pi^*$ orbitals of the ligand COs and acac ring. Previous DFT calculations on RDC have shown little coupling of the carbonyl stretches to the Rh-C bond, but have not specifically addressed the influence of the ligand and obtain only a 28 cm$^{-1}$ $a,s$ splitting.

8.5 Discussion

The experimental results presented in this chapter shed light on the signatures of vibrational relaxation including coherence and population transfer in 2D IR spectra of two strongly coupled
vibrations. The presence of an extra peak (peak 5 in Fig. 8-2 and 8-8) due to coherence transfer during \( \tau_1 \) and \( \tau_3 \) is of particular significance in demonstrating the ability of 2D IR spectroscopy to associate peak positions and amplitudes with specific sequences of field-matter interactions. Our ability to cleanly detect vibrational coherence transfer processes is a result of our choice of experimental technique and model system. Performing the experiments with broadband pulses in the time-domain as opposed to the narrow-band double resonance experiments allows us to be sensitive to Liouville pathways which involve interactions with four different molecular dipoles, and enables us to measure the rephasing and non-rephasing signals separately.\(^{54,64}\) Additionally, our choice of model system of RDC in hexane where the off-diagonal anharmonicity is twice the diagonal anharmonicity \( (\Delta_{AS} \sim 2\Delta_S \sim 2\Delta_A) \) ensures the unique placement of peak 5 free of interfering effects from the other stronger resonances. It is important to stress that the results from the modeling of the data as presented in Section 8.4 have several implications for the analysis of 2D IR spectra and the manipulation of experimental parameters to simplify congested 2D IR spectra.

From our modeling of this system, we have found that the coherence transfer rate scales roughly linearly with the amplitude of the relaxation-induced peak 5. This relationship can be understood through Figure 8-8, which illustrates a modified Liouville pathway at \( \tau_2 = 0 \). This is one pathway that contributes to the amplitude of peak 5 at \((\omega_1, \omega_3) = (\omega_{S,0}, \omega_{AS,A})\) marked in Figure 7, as a result of vibrational coherence transfer occurring during \( \tau_1 \) and \( \tau_3 \). We notice that coherence transfer occurs between \( \omega_{0s} \) and \( \omega_{0a} \) and between \( \omega_{2a,a} \) and \( \omega_{11,a} \) during \( \tau_1 \) and \( \tau_3 \) respectively. The figure clearly shows that the light-matter interactions occur with the system eigenstates resulting in the absence of cyclic symmetry for the dipole product: \( \mu_0^s \mu_a^0 \mu_2^{2aa} \mu_{a11}^{a11} \). It is clear that the contribution of this Liouville-pathway depends on the coherence transfer rates, \( \gamma_{0s,0a} \) and \( \gamma_{2aa,11a} \) and if these were zero, peak 5 would cease to exist.

![Figure 8-8](image_url)

**Figure 8-8.** An example of a Liouville-pathway giving rise to peak 5 indicated by arrows in Figure 8-8. Starting from the equilibrium density matrix \( \rho_{00} \), solid horizontal arrows indicate the interactions with the external light fields and the elements of the dipole operator are shown in red. The dashed vertical arrows indicate the coherence transfer processes occurring during \( \tau_1 \) and \( \tau_3 \). Noting that \( \mu^s \) and \( \mu^0 \) are orthogonal, the nonlinear orientational average for this pathway is given by \( \mathcal{Y}_{ZZZ}^{a11} (\tau, \tau, \tau) \).
The process of coherence transfer between the eigenstates of the system reflects the fact that the system coordinates interact significantly with coordinates that are “dark” to the experiment. These dark states, nominally in the bath Hamiltonian in eq. 8.2, may have intra- or inter-molecular origins and the strong, indirect couplings lead to an oscillation of amplitude between coherences involving the $a$ and $s$ state, at a rate given by the system-bath coupling. Thus, the bath-mediated coherence transfer amongst the states in the system Hamiltonian of eq 8.2 leads to a new set of bath-renormalized, experimental eigenstates, which dictate the positions of the various resonances in the 2D IR spectrum. This set of observed eigenstates are equivalent to the description of “dressed states” that would be obtained from a Van Vleck transformation of the system eigenstates.\textsuperscript{65}

Coherence transfer among the fundamental transitions during the experiment results in the motional narrowing of the two asymmetric and symmetric stretches. In modeling the 2D IR spectra of Fig. 8-2, the transition energies of the first two excited states in the system Hamiltonian were taken to be 2012.5 cm\textsuperscript{-1} and 2087.5 cm\textsuperscript{-1}. The coupling to the bath mode leading to coherence transfer between these two system states with a rate of $1/\gamma_{0a,0a} = 0.35$ ps\textsuperscript{-1} results in the experimentally observed frequencies of $\omega_{A,0}$ and $\omega_{S,0}$ equal to 2015 cm\textsuperscript{-1} and 2085 cm\textsuperscript{-1} respectively. The influence of coherence transfer on the observed resonance positions therefore has a noticeable impact on the determination of structural variables in 2D IR experiments. Its influence on the energies of the system eigenstates affects the perceived couplings between local-mode coordinates in a system Hamiltonian used to extract transient structural information from the spectrum.\textsuperscript{43,50}

The other striking consequence of coherence transfer processes is their influence on the determination of orientational variables. Coherence transfer between fundamental vibrational transitions during $\tau_1$ leads to a change in the anisotropic orientational distribution of transition moments prepared by the initial pulse. This means that the orientation of observed transition dipole moments associated with the system eigenstates are dependent on the coherence transfer rate. Much as it acts to shift the system eigenenergies through their interaction with the bath, coherence transfer likewise leads to new effective transition dipole moment orientations that reflect bath-renormalized system eigenvectors.

Independent of relaxation processes, the ratio of the amplitudes of the cross peaks in polarization-selective 2D IR experiments can be used to obtain the projection angle between two coupled transition dipole moments as described previously.\textsuperscript{50,59} Figure 8-9 shows how the ratio of the cross peak amplitudes for the case of RDC in the ZZYY (perpendicular) and ZZZZ (parallel) polarization geometries depends on the projection angle between the transition dipole with and without including the effects of coherence transfer. We see that coherence transfer leads to the motional narrowing of the transition energies and the orientational narrowing of the transition dipole
directions. The net result is the observation of smaller projection angles than those observed in the absence of relaxation processes.

Figure 8-9: The dependence of the peak amplitude ratios on the projection angle between two transition dipole moments for cross peak 2 and diagonal peak 1 in a 2D IR anisotropy measurement. The solid line represents the amplitude ratio with no relaxation processes, and the dashed line includes coherence transfer at $1/\gamma_{\text{obs}} = 0.35 \text{ ps}^{-1}$. The displayed amplitude ratios are obtained from simulating 2D IR correlation spectra for the ZZYY (⊥) and ZZZZ (∥) polarization geometries at $\tau_2 > 0$. These results are specific for the chosen coherence transfer rate in a system of two strongly coupled vibrations.

The presence of coherence transfer also holds implications for designing experimental strategies in simplifying congested 2D IR spectra. Recently, it has been shown that polarization conditions of the input fields can be manipulated to suppress diagonal features in congested 2D IR rephasing spectra. This relies on the fact that the diagonal peaks do not involve interactions with different vibrational manifolds during the experiment. The presence of coherence transfer will prevent this from being true because the diagonal contributions in the rephasing spectrum can result from interactions between two different vibrational manifolds giving them an angular dependence. The redistribution of the amplitude among the resonances in a 2D IR spectrum as a result of coherence transfer suggests that care must be taken in designing experiments to simplify congested spectra. On the other hand, polarization selectivity may be used to enhance and suppress the relaxation-induced peaks.

In addition to the effects of coherence transfer, the experiments in this chapter have been sensitive the coherent dynamics of the superposition between the fundamental vibrations during $\tau_2$. This implies that for a system of two coupled vibrations, the choice of a waiting time to measure a 2D IR spectrum may dictate the presence or absence of a cross peaks. As shown in the results, at early
waiting time ($\tau_2 \leq 2\text{ps}$), the amplitude of the cross-peaks in the rephasing spectrum and that of the diagonal peaks in the non-rephasing spectrum is maximum at $\tau_2 = n\pi/2\omega_{AS}$, where $n$ is an even integer. This provides us with a handle to selectively measure rephasing and nonrephasing Liouville pathways in 2D IR experiments.\(^{64,67-69}\) The modulation of the peaks and the presence of coherence transfer will not be seen in double-resonance experiments employing a narrow-band pump pulse. These processes require the presence of broadband pulses, which can excite a coherence superposition of the fundamental vibrations. The fast population transfer among the excited states during $\tau_2$, also affects the relative amplitudes of the various peaks.\(^{57}\) For pump-probe experiments performed in the magic angle geometry, the fast population transfer between the different vibrational manifolds leads to the non-single exponential measurement of the orientational diffusion constant.

The manifestation of all possible relaxation processes in the 2D IR spectra of two strongly coupled vibrations interacting with a bath suggests that these effects have to be incorporated into the existing theories of four-wave mixing spectroscopy. The response function formalism for third order nonlinear spectroscopy typically dictates an energy conservation statement relating the frequency of the radiated field to the three interaction energies, as in $\omega_s = -\omega_1 + \omega_2 + \omega_3$. Due to the influence of the bath, the presence of coherence transfer among the system eigenstates violates this condition. Similarly, the rules for simplifying the orientational response functions by invoking symmetry relations between the interchange of the laboratory and molecular fixed indices\(^{59}\) no longer exist. The inclusion of coherence transfer and population transfer makes it impossible to write analytic solutions to the nonlinear vibrational and orientational response functions even in the Markovian limit using the current theoretical tools.

The increased complexity in dealing with multiple coordinates, orientations, and relaxation processes, emphasizes the need to move away from the approach of calculating 2D responses from an explicit sum of pathways over the system eigenstates. Rather, computational methods are required that propagate the density matrix directly with all interaction and relaxation processes and orientational averages included. In general, one would like to incorporate the effects of the system-bath interactions in the local mode picture, allowing a direct modeling of time-dependent conformational fluctuations in solution. The nonlinear exciton equations presented by Mukamel and co-workers have been developed with this in mind.\(^{70}\)
8.5 Concluding Remarks

The 2D IR spectra presented here allow four types of vibrational relaxation processes to be characterized. Vibrational population relaxation, dephasing, population transfer and coherence transfer were characterized from the time-varying amplitude of the various peaks in a 2D IR relaxation experiment. Extra relaxation-induced resonances in the 2D spectrum for a zero waiting time are clear signatures of vibrational coherence transfer, whose amplitude scales with the transfer rate. As a motional narrowing mechanism, when coherence transfer is a significant, it must be included in an accurate analysis of molecular structure from 2D IR data. The data for RDC in hexane are reasonably modeled using Redfield theory. Our analysis has involved the numerical propagation of the density matrix during each experimental time period accounting for all pertinent vibrational and orientational relaxation processes. While the model system of RDC in hexane offers narrow homogeneous lineshapes characteristic of a rapidly fluctuating bath, we realize that it is a special case where we have been able to treat the 2D IR lineshape simply and model the data using a set of rate equations. More generally, such a separation of timescale does not exist and one has to account for relaxation processes resulting in complex lineshapes. We hope that this study stimulates further activity into incorporating the effects of coherence and population transfer into existing theories of nonlinear spectroscopy.
References

Chapter 9

Thermal Denaturation of RNase A

9.1 Introduction

The protein-folding problem can be described as trying to solve for an ensemble of structures over many timescales ranging from picoseconds (10^{-12} s) to seconds. This chemical reaction occurs over a vast range of length and timescales making it difficult to define structural coordinates that can describe the reaction as it proceeds. 1-7 There exists no single experimental technique that can capture structural changes at high spatial and time resolution occurring over 12 decades in time in solution. Recent advances show that picosecond time-resolved x-ray diffraction promises to be an exciting technique for studying time-dependent structural processes in biological crystals with high spatial resolution.8 Multi-dimensional nuclear magnetic resonance (NMR) spectroscopy is a well-established experimental technique to characterize structures on a millisecond timescale.9,10 The inherently fast timescales of optical and IR spectroscopic techniques makes them an appealing choice to study the structural dynamics of protein folding/unfolding. However, traditional optical and infrared spectroscopic methods lack the structural resolution of X-ray and NMR.

The development of 2D IR spectroscopy based on the concept of 2D NMR, has opened up the possibility of quantitatively describing molecular structure and dynamics in solution on a picosecond timescale.11-13 The previous chapters of this thesis provide the experimental and theoretical background for collecting and interpreting intuitive 2D IR correlation spectra. In particular, Chapter 7 demonstrates how structural information in the form of vibrational couplings and angles between coupled vibrations can be obtained on a 20 ps timescale. In this chapter, I describe the application of 2D IR spectroscopy and other nonlinear IR tools in probing the thermal denaturation of RNase A.

We use the Amide I vibration to probe the conformational changes accompanying the thermal denaturation of proteins. The Amide I vibrations result from the couplings between the individual
amide vibrations of each peptide unit along the protein backbone. For a single peptide unit, the amide I vibration is primarily the C=O stretching motion with some contribution from CN stretching and the CCN deformation motions. These vibrational states are delocalized along many peptide units and are sensitive to the different secondary structures. Qualitative experimental methods have established a correlation between secondary structural elements and their characteristic Amide I absorption frequencies such that $\alpha$-helices ($\sim 1650 \text{ cm}^{-1}$), $\beta$-sheets ($\leq 1640 \text{ cm}^{-1}$ and $\geq 1680 \text{ cm}^{-1}$) and random coils ($\sim 1640-1650 \text{ cm}^{-1}$). Among these, the absorption frequencies of the AP $\beta$-sheet are the most distinct showing two transitions, a strong one between 1610-1640 cm$^{-1}$ and a weak one between 1680-1700 cm$^{-1}$. We have made recent theoretical and experimental efforts to understand the signatures of AP $\beta$-sheet structure in a 2D IR correlation spectrum. Our efforts reveal that the 2D IR spectra of AP $\beta$-sheet containing proteins show a cross peak between the low and high frequency amide I transitions. Additionally, the position, amplitude and shape of the various spectral features are extremely sensitive measures of the couplings, disorder and angles between these two transitions. In this chapter, we build on these results to use 2D IR spectroscopy as a tool for probing the thermal denaturation of RNase A.

Ribonuclease A is a 124-residue enzyme, which catalyzes the degradation of RNA. It is a relatively small, globular and widely studied single-domain protein whose x-ray structure is pictured in Figure 9-1. Its main secondary structural elements include a V-shaped anti-parallel $\beta$-sheet with three strands on one side and four on the other, and three short helices. The secondary structural elements are held together by four disulfide bonds, accounting for the exceptional stability of the protein. The amino acid sequence includes eight fluorescent tyrosine (Tyr) residues and four proline (Pro) residues. The cleft between the arms of the V-shaped AP $\beta$-sheet forms the active site of this enzyme. The NMR solution structure of RNase A has been determined and is found to be very similar to the X-ray structure. Molecular dynamics simulations reveal that the protein undergoes large-scale motions involving the breathing of the active site region. The radius of gyration for RNase A in solution is estimated to be 15 Å.

The abundance of detailed structural information available on RNase A makes it an excellent model system to test new experimental methods for probing the structural dynamics of protein unfolding/folding. Infrared, Raman and optical CD studies have shown that the thermal denaturation of RNase A is a reversible process with a melting temperature of $\sim 65 ^\circ \text{C}$. Recent FTIR and CD measurements have revealed that the folding/unfolding of RNase A is not a simple two-state process because it includes a pre-transition around 47 °C. It has been suggested that the pre-transition involves the unwinding of the helix at the C terminus along with the destabilization of the $\beta$-strand involving residues 43-49. Previous studies have shown that the thermally denatured states of RNase
A retain a sizeable amount of the native secondary structure.\textsuperscript{28,29} This is not altogether surprising, as the disulfide bonds, which hold the protein together, remain intact at high temperatures.\textsuperscript{30} This view is supported by the measurement of the radius of gyration of the thermally denatured states, which increases from 20 Å to 30 Å under reducing conditions.\textsuperscript{22}

![X-ray structure of Ribonuclease A](image)

**Figure 9-1.** (Color) The X-ray structure of Ribonuclease A (PDB ID: 1FS3, Ref. 19) showing a seven-stranded V-shaped AP β-sheet containing the active site and three short helices.

Temperature jump and stopped-flow studies using fluorescence and UV absorption detection and pulsed quench-flow H/D exchange along with 2D NMR have shown that the unfolded disulfide-intact RNase A exists as a mixture of species, which refold over multiple timescales. It has been proposed that the refolding involves intermediate species and the rate-limiting step is dictated by proline isomerization.\textsuperscript{23,31-34} The thermal unfolding pathway studied using stopped flow methods, also reveals multiple timescales.\textsuperscript{35,36} The fast timescale is on the order of 16 ms, while the slower timescale involving species with proline residues in non-native conformations is around 50-200 s. Faster studies using a laser initiated temperature jump and IR spectroscopy as a probe have shown changes on sub-nanosecond timescales and those using Raman spectroscopy as a probe show a disruption of structure in the active site region by 200 µs and the modifications around the surface in 200 ns.\textsuperscript{37,38}

It is difficult to systematically compare all the existing data on the thermal denaturation of RNase A because of differing experimental conditions and sensitivities of the experimental probes. Some of the unanswered questions include: What is the structural make-up of the denatured and natured ensembles? In light of the recent FTIR and CD thermal denaturation data, is RNase A a two-state folder? What are the first steps in the thermal denaturation of RNase A?
It is my hope that the experimental methodologies based on nonlinear IR spectroscopy, presented in this chapter and a careful quantitative analysis of the data will provide clues to answering some of these questions. The chapter is organized into two main halves. The first half deals with equilibrium nonlinear IR studies on the thermal denaturation of RNase A. In this section I highlight the inherent advantage of using 2D IR spectroscopy and its projections over conventional FTIR for monitoring structural changes accompanying the thermal denaturation of proteins. The second half uses a nanosecond near IR laser to create a rapid temperature jump in the protein solution and probes the following conformational changes with a nonlinear IR spectroscopic probe. The experiment is extremely sensitive to minute spectral changes and reveals a sub-µs timescale in the thermal denaturation of RNase A.

9.2 Equilibrium nonlinear IR studies of the thermal denaturation of RNase A

9.2.1 Experimental

The basic experimental set-up for the 2D IR experiments presented here is identical to that described in Chapter 5 and illustrated in Figure 5-2. The experiments on the Amide I band of RNase A are performed with 90 fs, mid-IR pulses centered at 6 µm. These pulses have a bandwidth of ~160 cm\(^{-1}\) which is sufficient to excite the fundamental (0→1) and the anharmonically shifted overtone (1→2) transitions of interest. Three time-coincident ~150 nJ pulses, \(E_\alpha\), \(E_\beta\) and \(E_\chi\), are arranged in a “boxcar” geometry and focused down to a 100 µm diameter at the sample. The polarization and intensity of each of the beams were controlled using ZnSe wire grid polarizers and MgF\(_2\) half-wave plates. The relative timings between the three pulses are controlled using retroreflectors mounted on motorized linear stages (Aerotech, ANT-50L) with a resolution of 10 nm (0.067 fs), an accuracy of 300 nm (2 fs) and repeatability of 50 nm (0.33 fs). The nonlinear signal field is generated in the phase-matched direction \(k_s=-k_\alpha+k_\beta+k_\chi\). The signal is isolated after the sample and overlapped spatially and temporally with a local oscillator field. Heterodyne detection is performed by dispersing the two beams in a 190 mm monochrometer with a 40-lines/mm grating. The dispersed signal is collected at the focal plane with a liquid nitrogen cooled MCT array detector consisting of 64 pixels. Rephasing (\(k_s=-k_1+k_2+k_3\)) and non-rephasing (\(k_s=k_1-k_2+k_3\)) experiments are performed for \(\tau_2=0\) by altering the time sequence of the first two pulses in the ZZYY or crossed polarization geometry.
Arrays of dispersed heterodyned signals with a spectral resolution of ~4 cm\(^{-1}\) in the \(\omega_3\) dimension are collected as a function of \(\tau_1\) in 4 fs steps for both rephasing and non-rephasing configurations. The data is collected for \(\tau_1\) delays ranging from –0.6 to 2ps and from –0.6 to 1ps for the rephasing and non-rephasing experiments respectively. A Fourier transform along the \(\tau_1\) axis yields the individual 2D rephasing and non-rephasing spectra, and the sum of these gives the 2D IR correlation spectrum. The resolution in the \(\omega_1\) dimension after Fourier transformation is 1 cm\(^{-1}\).

Dispersed pump-probe spectra were obtained using the tracer beam as the probe and \(E_x\) as the pump at \(\tau_2\)=0 to help us phase the 2D IR spectra.

**Figure 9-2.** (Color) Phasing procedure for the 2D IR spectra of RNase A at 50°C. The left panel shows the slices of the rephasing (green) and non-rephasing (red) data matrices collected as a function of \(\tau_1\) and \(\omega_3\), which matches the dispersed pump-probe (black) at \(\tau_2\)=0. The values of \(\Delta\tau_1\) for rephasing and non-rephasing signals are 6fs and –2 fs respectively. The right panel shows the projection of \(\tilde{S}_\omega\) (black, dashed) along \(\omega_1\) to the dispersed pump probe (black, solid) data. The values of \(\Delta\tau_3\) and \(\phi\) are (6fs, 0.2) and (7fs and 0.25) for the rephasing and non-rephasing spectra respectively.

The treatment of the raw data to yield properly “phased” 2D IR correlation spectra has been described previously in Chapter 5. We use a slightly different method to phase the data presented in this chapter, as the \(\tau_1\) timing is no longer externally calibrated due to the superior performance of the new Aerotech stages. The first step of the phasing procedure involves using the dispersed pump-probe trace at \(\tau_2\)=0 to find the error in the \(\tau_1\) timing (\(\Delta\tau_1\)) for the rephasing and non-rephasing signals.
collected as a function of $\omega_3$ and $\tau_1$. We make use of the fact that the slices corresponding to $\tau_1 = 0$ of the rephasing and non-rephasing data matrices are each equal to the dispersed pump-probe array at $\tau_2 = 0$ and identical to each other. This allows us to correct for the finite values of $\Delta \tau_1$ for the individual rephasing and non-rephasing signals. To make sure that we have phased the data correctly, we transform the individual signals along $\tau_1$, and sum their real parts to obtain an absorptive 2D IR correlation spectrum, $\tilde{S}_C$. We then compare the projection of $\tilde{S}_C$ along $\omega_1$ to the dispersed pump probe data and make sure that they are identical. If this is not the case, then we multiply the complex value rephasing and non-rephasing 2D spectra individually with the following phase factor:

$$\exp(i \omega_3 \Delta \tau_3 + \phi).$$

Note that the value of $\Delta \tau_3$, which accounts for our inability to time the signal and LO fields exactly, can be different for the rephasing and non-rephasing signals. The variable $\phi$ accounts for phase errors arising from the imbalances in the interferometer. After multiplying the complex rephasing and non-rephasing 2D spectra as described above we again sum their real parts and compare the projection of $\tilde{S}_C$ along $\omega_1$ to the dispersed pump probe data. This is done iteratively till we are satisfied with the comparison between the projection of $\tilde{S}_C$ along $\omega_1$ and the dispersed pump probe data. Figure 9-2 shows an example of the results from the phasing procedure described above.

Dispersed pump-probe spectra were obtained using the tracer beam as the probe and $E_z$ as the pump at $\tau_2 = 0$. The dispersed vibrational echo measurements were obtained in the all-parallel (ZZZZ) polarization geometry by dispersing the homodyne signal field at $\tau_1 = \tau_2 = 0$ in the monochrometer as described above. The FTIR spectra were collected on a Mattson Infinity Gold FTIR spectrometer with a frequency resolution of 2 cm$^{-1}$. For all these measurements, the sample was placed in a home-built, temperature-controlled cell consisting of two 1mm thick CaF$_2$ windows with a 50 $\mu$m thick Teflon spacer in between them. The temperature of the cell could be controlled to within 0.1 °C.

The RNase A sample was purchased from Sigma Aldrich (R5500). It was dissolved in a phosphate buffer solution of pH$^*$ = 7 (uncorrected pH) for a final concentration of 16.5 mg/mL. The sample had a peak OD of 0.2 for the 2D IR and dispersed pump-probe experiments and $\sim$0.5 for the dispersed echo experiments. All the samples were incubated at 60°C for 1 hr prior to performing the experiments to allow for complete H/D exchange.
9.2.2 Results and Discussion

9.2.2.1 IR vibrational spectroscopy of proteins containing AP β sheets

Anti-parallel (AP) β sheets have distinct spectral signatures in the Amide I region of proteins. FTIR spectra of model proteins and peptides show two stretching modes, a strong transition $\nu_{a-}$, which lies between 1610 and 1640 cm$^{-1}$ and a weak transition $\nu_{a+}$ between 1680 and 1700 cm$^{-1}$. While these modes are easily distinguishable in homo-polypeptide chains, they are more difficult to see in proteins where different secondary structural elements are present and there is considerable conformational disorder within the β sheet regions of the proteins. Using a vibrational exciton model of an idealized AP β sheet, we have shown that the 2D IR spectrum is a sensitive probe of the size, angle and disorder in the protein structure.$^{17,18}$

Figure 9-3 (Color) (Left) 2D IR spectrum of poly-L-lysine at high pD obtained in the crossed-polarization geometry. The FTIR spectrum is shown in the top panel and the right panel displays the slice along $\omega_3$ from the 2D IR spectrum corresponding to $\omega_1=1611$ cm$^{-1}$. Nineteen equally spaced contours are plotted between −20% and 20% of the peak maximum. (Right) 2D IR correlation spectrum of RNase A at 25 °C obtained in the crossed polarization geometry. The FTIR spectrum is shown in the top panel and the left panel displays the slice along $\omega_3$ from the 2D IR spectrum corresponding to $\omega_1=1636$ cm$^{-1}$. Twenty-one equally spaced contours are drawn from −60% to 60% of the maximum amplitude in the 2D spectrum.

We have used Poly-L-Lysine (PLL) at high pH, as a model system for studying the signatures of AP β-sheet structure in 2D IR spectra.$^{18}$ A 2D IR spectrum of PLL at high pH is plotted in the left panel of Figure 9-3. The FTIR spectrum displayed on the top panel, shows the two Amide I resonances at 1611 cm$^{-1}$ ($\nu_a$) and 1680 cm$^{-1}$ ($\nu_{a+}$). Ignoring the diagonal features in the middle of the
2D IR spectrum, we see the eight-peak structure expected for a spectrum of two coupled vibrations consisting of pairs of oppositely signed diagonal and cross peaks. One of the interesting observations is the elongation of the cross-peaks in the $\omega_3 = \nu_a$ dimension. This observation supported by preliminary calculations, suggests that the $\nu_a$ vibrational mode, which lies parallel to the inter-strand axis of an extended $\beta$-sheet, is extremely sensitive to the conformational disorder present in the system.\textsuperscript{18,39}

2D IR correlation spectra of proteins containing varying amounts of $\beta$ sheet structure show a cross peak or a ridge along $\omega_3 \sim 1680$ cm\textsuperscript{-1} and have a characteristic Z-shaped contour profile.\textsuperscript{18,39} Note that it is no longer correct to label the distinguishable vibrational modes as $\nu_a$ and $\nu_{a^+}$ because the proteins have overlapping contributions from many different secondary structural elements. The right panel of Figure 9-3 displays a 2D IR correlation spectrum of RNase A taken at 25°C in the crossed polarization geometry. The FTIR spectrum in the top panel shows two distinguishable transitions of the AP $\beta$-sheet at 1636 cm\textsuperscript{-1} and 1683 cm\textsuperscript{-1} which we will refer to as $\nu_1$ and $\nu_2$ for the rest of this chapter. The shoulder at 1683 cm\textsuperscript{-1} corresponds to the trough in the temperature-dependent FTIR difference spectra shown in Figure 9-4. The 2D IR spectrum of RNase A is very different from that of PLL. Notice that we do not see the eight-peak structure, which is characteristic of a 2D IR spectrum of two spectrally resolved, coupled vibrational modes. One of the cross-peaks between the two modes of the AP $\beta$ sheets is seen as a ridge along $\omega_3 = 1680$ cm\textsuperscript{-1} in the top left corner of the 2D IR correlation spectrum. The presence of the other cross peak is indicated by the decrease in intensity of the strong overtone band along the diagonal axis leading to its peculiar curvature. The spectral resonances along the diagonal are elongated indicative of strong inhomogenous broadening. The interference effects between the diagonal and cross-peaks lead to the Z-shaped 2D IR spectral profile, characteristic of an AP $\beta$ sheet containing protein. The cross peak at $\omega_1 < \omega_3$ is seen clearly in the slice along $\omega_1 = 1636$ cm\textsuperscript{-1} as plotted on the right panel of Figure 9-3.

Although we have focused our discussion on the markers for the AP $\beta$ sheet structure, the FTIR and 2D IR spectra of the Amide I region contain contribution from the other secondary structural elements present in RNase A. Additionally, the conformational disorder present in the various parts of the protein and its interactions with the solvent also dictates the observed 2D IR lineshapes.

Despite suffering from spectral congestion, it is clear to see that the 2D IR correlation spectrum provides a wealth of additional information compared to the FTIR spectrum, by virtue of accessing a second frequency dimension in a coherent manner. The Amide I band in FTIR spectroscopy has been used to follow the denaturation of proteins due to temperature, pressure and chemical denaturants. Often times, the changes in the FTIR spectrum are too minute and difficult to
correlate to structural changes. This has led to a battery of spectral decomposition techniques to try and glean structural information, in particular the amount of secondary structure in a denatured state vs. a natured state.⁴⁰ We compare temperature-dependent FTIR spectra along with 2D IR spectra and other nonlinear IR probes to test their abilities as probes of thermal unfolding of proteins.

### 9.2.2.2 Temperature-dependent FTIR spectra of RNase A

![Figure 9-4](Color) (Left, top) Temperature dependent FTIR spectra of RNase A. (Left, bottom) Difference FTIR spectra of RNase A where each spectrum from the top panel has been subtracted by the one taken at 48.7°C. (Right) A melting curve following the temperature-dependent amplitude of the spectral amplitude at 1632 cm⁻¹ indicated by the dotted line in the FTIR spectra.

Figure 9-4 shows the temperature dependent FTIR spectra of RNase A at pH 7, in the range of 50°C-75°C and the difference spectra with respect to the one at 50°C. The spectrum at 50°C is asymmetric with a broad maximum in the region of 1636 cm⁻¹ and a shoulder at 1680 cm⁻¹. Both these regions correspond to the main Amide I transitions of the AP β-sheet structure (ν₁ and ν₂). As the temperature increases, the overall shape of the spectrum becomes more symmetric and the spectrum shifts to higher frequencies with the main peak being centered at 1649 cm⁻¹ at 75 °C. The
changes at different frequencies can be seen clearly in the lower panel depicting the difference between the high temperature FTIR spectra and the spectrum obtained at 50 °C. We notice that as the temperature increases, there is a decrease in the frequency centered around 1632 cm\(^{-1}\) and increase in the regions centered at 1666 cm\(^{-1}\) and 1690 cm\(^{-1}\).

If we attempt to correlate these observations to changes in the secondary structural elements of RNase A using empirical relations we notice a depletion of the \(\beta\)-sheet structure and an increase in the \(\alpha\)-helical, turns and random coil structures. The maximum change that we see in the FTIR difference spectra of \(\Delta T = 25 \, ^\circ\text{C}\), corresponds to \(\sim 10\%\) of the maximum absorbance in the Amide I resonance at 50 °C. The melting curve obtained by plotting the temperature-dependent FTIR amplitude at 1632 cm\(^{-1}\) reveals a melting temperature of \(\sim 63 \, ^\circ\text{C}\). Previous FTIR studies, where the spectra have been analyzed using factor analysis have shown that there is a pre-transition at \(\sim 47 \, ^\circ\text{C}\), which involves the \(\beta\)-sheet as well as the \(\alpha\)-helical regions.\(^{27}\) Our very preliminary analysis illustrated in Fig 9-4 does not reveal such a pre-transition, but a more thorough analysis may prove otherwise.

9.2.2.3 Temperature-dependent 2D IR spectra of RNase A

The positions, amplitudes and lineshapes of the various resonances in the 2D IR correlation spectra of RNase A contain all the information about, vibrational anharmonicities, couplings and disorder present in the Amide I region of RNase A. As the temperature increases, the protein loses its native contacts and its structure is disrupted. In the earlier section we observed that the FTIR spectra showed minor changes in the position, amplitude and lineshape as it tracked the thermal denaturation of RNase A. In this section, we will show how the conformational changes accompanying the thermal denaturation of RNase A are manifested in 2D IR spectra, paying close attention to the fate of the cross-peak. Figure 9-5 shows the temperature dependent 2D IR correlation and nonrephasing spectra of RNase A from 25°C to 75°C.

We shall first focus our attention on the temperature-dependent 2D IR correlation spectra of RNase A obtained from the sum of 2D rephasing and nonrephasing IR spectra and displayed on the left panel of Figure 9-5. Since RNase A has a significant amount of AP \(\beta\) sheet structure, the 2D IR correlation spectrum of the native state at 25 °C exhibits a Z-shaped contour profile as discussed earlier. Using that spectrum as our starting point, we see that as the temperature increases, the positive lobe along the diagonal moves to higher frequency, its anti diagonal width increases and the Z-shaped contour profile disappears and the overall spectral shape becomes more symmetric. The disappearance of the Z-shaped contour profile indicates that the cross peak between the \(\nu_1\) and \(\nu_2\)
vibrational modes of the AP β sheet structures disappears as the protein denatures. The disappearance of the cross peak is highlighted in the slices of the 2D IR spectra taken along $\omega_1 = 1636 \text{ cm}^{-1}$ and plotted adjacent to each spectrum. At 75 °C, there is no distinct cross-peak seen along $\omega_1$ for $\omega_1 = 1636 \text{ cm}^{-1}$.

Figure 9-5. (Color) Temperature-dependent 2D IR correlation spectra (left) and absolute value non-rephasing spectra (right) of RNase A obtained in the crossed-polarization geometry. The left panel of each 2D IR spectrum displays the slice along $\omega_3$ corresponding to $\omega_1=1636 \text{ cm}^{-1}$. All the 2D IR spectra have been normalized to the maximum positive amplitude of the corresponding spectrum at 25 °C. Twenty-one equally spaced contours are drawn between –60% to 60% of the maximum amplitude for all the 2D IR correlation spectra. Eleven equally spaced contours are drawn from 0-60% of the maximum amplitude for all the absolute value nonrephasing 2D IR spectra. These spectra indicate that the maximum change in the protein solution occurs between 65 and 75°C.
The absolute value non-rephasing 2D IR spectra plotted in the right panel of Figure 9-5 show the same effects as the correlation spectra. However, the disappearance of the cross peaks by 75 °C temperatures is extremely clear in the nonrephasing 2D IR spectra especially for the cross peak located at \( \omega_1<\omega_3 \). The absolute value nonrephasing experiment is insensitive to line broadening effects and this leads to considerable less amplitude along the diagonal in the 2D IR spectrum. Additionally, all the peaks in the 2D IR nonrephasing spectrum are tilted along the off diagonal. This means that there is less destructive interference between the diagonal and cross-peaks resulting in the latter being clearly resolved as seen in the 2D IR spectrum at 25 °C. The ratio of the amplitudes of the cross-peaks to diagonal peaks is enhanced in the crossed polarization geometry allowing us to see the cross-peaks clearly in the absolute value non-rephasing spectra.

![Figure 9-6.](image)

**Figure 9-6.** (Color) 2D IR difference spectrum obtained by taking the difference of the 2D IR correlation and spectrum at 72.2 °C and that obtained at 25 °C. The spectrum has been normalized to its maximum intensity and 21 equally spaced contours are plotted from –40% to 40%.

The disappearance of the cross-peaks, and the shift of the \( \nu_1 \) frequency to the blue is shown clearly in the difference 2D IR spectra plotted in Figure 9-6. These spectra are obtained from the subtraction of the 2D IR spectrum obtained at 75 °C from the one measured at 25 °C. The 2D difference plots reveal the disappearance of the eight-peak structure due to the thermal denaturation of RNase A. More importantly, they suggest that 2D IR spectroscopy is sensitive to AP \( \beta \)-sheet structures in a globular \( \alpha/\beta \) protein such as RNase A. From the qualitative comparison of the FTIR and 2D IR temperature-dependent spectra of RNase A, it is clear that the latter technique holds much promise for studying thermal denaturation of proteins. A quantitative analysis of the spectroscopic observables in the 2D IR spectra has the potential to reveal the structural heterogeneity in the protein sample along the route to denaturation. The sensitivity of the position, amplitude and lineshape of the cross-peaks between the \( \nu_1 \) and \( \nu_2 \) modes of the AP beta sheets to the thermal denaturation of RNase A is an extremely promising and exciting result. Additionally a careful analysis will reveal if there is
a pre-transition before 50 °C for the thermal denaturation of RNase A. However, we need to do a systematic analysis of the cross-peak characteristics as a function of size and conformational variation of AP β sheets before we can use it as a true structural marker.

9.2.2.4 Temperature-dependent dispersed vibrational echo (DVE) and dispersed pump-probe (DPP) spectra

In this section I will show that the homodyne dispersed vibrational echo (DVE) and the dispersed pump-probe (DPP) experiments are both extremely sensitive to the changes in the Amide I band accompanying the thermal denaturation of RNase A. This relies on the fact that both these 1D measurements can be related to the 2D IR spectrum. All of these experiments are a measure of the third-order nonlinear signal field ($E_{\text{sig}}^{(3)}$) generated by the third-order nonlinear polarization ($P^{(3)}$) in the phase-matched direction $k_s$. In the limit of delta – function input pulses (impulse response) and perfect phase-matching conditions, the signal field can be expressed as a function of the three experimental time periods ($\tau_1$, $\tau_2$, and $\tau_3$):

$$E_{\text{sig}}^{(3)}(k_s, \tau_1, \tau_2, \tau_3) = \frac{\ell}{n(\omega_3)c} i\omega_3 P^{(3)}(k_s, \tau_1, \tau_2, \tau_3)$$

(9.1)

In the above expression, $\omega_3$ is the signal frequency, $n$ is the index of refraction, $c$ is the speed of light, and $\ell$ is the interaction length. Note that the signal field contains both birefringent (in-quadrature) and dichroic (in-phase) contributions. For a resonant experiment, we would expect the dichroic experiment to be dominant.

The experimental characterization of the femtosecond nonlinear IR signal field is achieved by using a heterodyne detection scheme, which requires a well-characterized local oscillator ($E_{\text{LO}}$) field. In the experiments presented in this thesis, we spectrally disperse the signal and LO fields in a monochromator, which effectively Fourier transforms the signal along the detection time period ($\tau_3$) to its Fourier transform pair, $\omega_3$. Ignoring any timing errors, the experimentally obtained 2D IR correlation spectrum can be written as:

$$\tilde{S}_{\text{C}}(\omega_1, \omega_3, \tau_2) \propto \text{Re} \left[ \int_{-\infty}^{\infty} E_{\text{LO}}^{(3)}(\omega_3) E_{\text{sig}}^{(3)}(k_R, \tau_1, \tau_2, \omega_3) e^{i\omega_3 \tau_2} d\tau_2 + \int_{-\infty}^{\infty} E_{\text{LO}}^{(3)}(\omega_3) E_{\text{sig}}^{(3)}(k_N, \tau_1, \tau_2, \omega_3) e^{i\omega_3 \tau_1} d\tau_1 \right]$$

(9.2)
In the above expression, the wave-vectors $k_R$ and $k_{NR}$ represent the phase-matching conditions for the rephasing and non-rephasing signals. The two-pulse dispersed pump-probe signal, where the transmitted probe field ($E_{pr}$) acts as the intrinsic local oscillator field, is written as:

$$\tilde{S}_{DPP} (\omega_3, \tau_2, \tau_1 = 0) \propto \text{Re} \left[ E_{pr} (\omega_3) E_{\text{sig}}^{(3)} (k_{pr}, \tau_2, \tau_1 = 0, \omega_3) \right]$$

(9.3)

For a two-pulse dispersed pump-probe experiment, the first two interactions are with the same pump pulse and $\tau_1 = 0$. Finally, the dispersed echo measurement, which is a homodyne signal, is given as the square of the third-order nonlinear signal field at $\tau_1 = 0$,

$$\tilde{S}_{DVE} (\omega_3, \tau_2, \tau_1 = 0) \propto \left| E_{\text{sig}}^{(3)} (k_R, \tau_1 = 0, \tau_2, \omega_3) \right|^2$$

(9.4)

Since the dispersed vibrational echo, is a homodyne measurement, it includes contributions from the dichroic as well as the birefringent response of the field-matter interactions.

Using the projection-slice theorem, we can relate the DPP and DVE signals to projections of the 2D IR correlation spectrum along $\omega_1$. It has been established that in the limit of weak sample absorbance, the dispersed pump-probe measurements can be obtained from the 2D IR correlation spectrum by using the following expression:

$$\tilde{S}_{DPP} (\omega_3, \tau_2) = \frac{\int_{-\infty}^{\infty} \tilde{S}_C' (\omega_1, \omega_3, \tau_2) d\omega_1}{E_{LO} (\omega_3)}$$

(9.5)

In most cases, it is valid to approximate $E_{pr(LO)} (\omega_3) = \sqrt{I_{pr(LO)} (\omega_3)}$, where $I_i$ is the measured intensity of the field, $E_i$. The fields $E_{pr}$ and $E_{LO}$ do not cancel each other in the above expression as they travel different paths before combining with the signal. Namely, the probe field is transmitted through the sample, while the LO is overlapped with the signal field after the sample. The eq 9.5 forms the basis for all the “phasing” methods used to correct for timing uncertainties in our heterodyne-detected experiments. Similarly, the DVE spectrum can be related to the absolute value square of the projection of the complex 2D IR spectrum along $\omega_1$ following equation as shown below,

$$\tilde{S}_{DVE} (\omega_3, \tau_2, \tau_1 = 0) \propto \left| \frac{\int_{-\infty}^{\infty} \left( \tilde{S}_C' (\omega_1, \omega_3, \tau_2) + iS_C^* (\omega_1, \omega_3, \tau_2) \right) d\omega_1}{E_{LO} (\omega_3)} \right|^2$$

(9.6)

where,
\[ \tilde{S}_C^* (\omega_1, \omega_3, \tau_2) \propto \text{Im} \left[ \int_{-\infty}^{\infty} E^{(3)}_{\text{LO}} (\omega_3) E^{(3)}_{\text{SR}} (\mathbf{k}_R, \tau_1, \tau_2, \omega_3) e^{i\omega_1 \tau_1} d\tau_1 + \int_{-\infty}^{\infty} E^{(3)}_{\text{LO}} (\omega_3) E^{(3)}_{\text{SR}} (\mathbf{k}_{\text{NR}} \tau_1, \tau_2, \omega_3) e^{i\omega_1 \tau_1} d\tau_1 \right] . \] (9.7)

Equation 9.6 is not theoretically rigorous because we do not experimentally measure the birefringent part of the response function contained in \( \tilde{S}_{\text{DVE}} \).

Figure 9-7 shows the temperature dependent DPP (left) and DVE (right) spectra obtained at \( \tau_2=0 \) and \( \tau_1=\tau_2=0 \) respectively. For the DPP spectra we see that as the temperature increases (bottom to top), the three-peak structure in the positive lobe starts to disappear, the overall amplitude decreases and the positive and negative lineshapes look symmetric. This directly corresponds to the spectral changes in the 2D IR correlation spectra where the Z-shaped contour profile disappeared at high temperatures leaving two almost symmetric and oppositely signed peaks along the diagonal.

The DVE spectra show similar dramatic changes. At the lowest temperature, we see a two-peak structure, which looks remarkably similar to the FTIR spectrum of poly-L-lysine at high pH where the two features directly correspond to the \( \nu_1 \) and \( \nu_2 \) amide I modes of the AP \( \beta \) sheets. In the DVE spectrum at 50 °C we see two peaks at 1620 cm\(^{-1}\) and 1680 cm\(^{-1}\). As discussed above, it is not straightforward to relate a projection of the 2D IR spectrum to the DVE signal. Nonetheless, it is
easy to see that the peak at 1680 cm$^{-1}$ corresponds to the high frequency Amide I mode of the AP $\beta$ sheet, while the low frequency peak results from the destructive interference of the positive and negative features in the 2D IR spectra. The nonlinear scaling of the transition dipole moment in the DVE spectra results in far greater temperature–dependent changes as opposed to the linear FTIR spectra. As the temperature increases, we see that the relative ratio of the low and high frequency features in the DVE spectra drops, the low frequency peak moves to $\sim$ 1630 cm$^{-1}$ and the spectral amplitude in the central region around 1650 cm$^{-1}$ increases.

Although, the spectra displayed in Figure 9-7 are one dimensional (1D) in nature, they are more sensitive to the changes in the Amide I region accompanying the thermal denaturation of RNase A than the traditional linear FTIR spectra shown in Figure 9-4. Despite being 1D in nature the DPP and the DVE spectra can be easily related to projections of the 2D IR spectra making them easy to interpret in terms of the 2D spectral signatures.

9.3 Using nonlinear IR spectroscopy to study early events in the thermal denaturation of RNase A

While the temperature-dependent equilibrium measurements can characterize the native and denatured vibrational states of RNase A, they do not tell us anything about the timescales involved in the unfolding reaction and the role if any of intermediate species. To learn about the structural dynamics of protein unfolding reactions, we perform nanosecond T-jump experiments and use the DVE experiments in the Amide I region to probe the conformational changes occurring in the protein solution. The choice of DVE as an experimental probe satisfies the following conditions: (1) it is a background free signal so we do not have to worry about the effects of the temperature dependent changes of the D$_2$O solution masking the dynamics of the Amide I signal and (2) it is a 1D measurement, making it feasible to average for a long time to obtain a ratio of S/N $>$ 10. Because of the lower repetition rate of the T-jump laser (20 Hz), our experimental time increases by a factor of 50 to obtain the data quality seen in figures 9-4 to 9-7. This fact does not currently make the 2D IR experiment a feasible probe for the transient experiments. Among the 1D experiments, the DVE is the only background free experiment. Using the DPP experiment to follow the T-jump induced conformational changes would entail a subtraction of a reference DPP spectrum taken with only the D$_2$O buffer solution at each time point. The following sections illustrate the experimental set-up and qualitatively describe the preliminary results.
9.3.1 Experimental Set-up

The transient measurements use a 20 Hz nanosecond Nd:YAG laser to initiate a fast temperature jump and three femtosecond mid IR fields to probe the resultant changes in the solution. The delay between the T-jump laser and the fs pulse train can be controlled from 1 ns - 1 ms in fine steps and then in steps of 1 ms. The temperature jump remains constant for ~300 μs providing a short time window to initiate and probe denaturing processes.

Modifications were made to the existing set-up to study early events in the thermal denaturation of RNase A. This experimental study required a nanosecond laser to create an abrupt temperature jump (T-jump) and a spectroscopic probe to follow the resultant conformational changes in the protein solution. The temperature jump is created by exciting the OD overtone stretch at 2 μm (ε = 10 cm⁻¹) of the D₂O buffer solution. The relaxation of the vibrational energy results in the heating of the surroundings on a picosecond timescale. This is experimentally realized using a Q-switched, 20 Hz, Nd:YAG laser (Opotek). The 1064 nm output is frequency doubled and the 532 nm light pumps a BBO based OPO. The idler beam (1.98 μm) is used to create the T-jump. The near IR beam emerging from the OPO is highly divergent and is expanded and collimated to a 1.5” diameter using a 1.5: 1 telescope. The telescope consists of a pair of plano-convex BK7 lenses with a custom ¼-λ MgF₂ AR coating and focal lengths of 10 and 15 cm. The beam after the telescope is still slightly divergent and we need a short focal length lens to focus it tightly at the sample. The experimental set-up near the sample area is shown in the bottom panel of Figure 9-8. The 5 mJ, 8ns, 20Hz idler beam at 1.98 μm is focused down to a 0.5 mm diameter at the sample with the help of a 10 cm lens (L). This geometry enables us to create temperature jumps of ~8-10°C in the protein solution. We use the dispersed vibrational echo (DVE) – a nonlinear IR tool to probe the conformational changes following the temperature jump. As described in the previous section, three time-coincident pulses cross in the sample to generate a nonlinear signal, which is dispersed onto an MCT array detector. To perform the transient measurements, we need to spatially and temporally overlap the 1.98 μm, 20 Hz T-jump pulse with the three time-coincident 6 μm, 1 kHz pulses.

The spatial overlap is achieved by using an iris centered on the focus of the three 6 μm pulses and the adjusting the position of the lens and the pointing of the T-jump pulse to allow for maximum throughput. The timing diagram of the 6 μm and 2 μm light relative to each other is shown in the top panel of Figure 9-8. We see that the 6 μm light arrives at the sample ~300 μs before the 2 μm light. In order to time the pulses without introducing additional timing jitter into our set-up we need a stable 1 kHz source. Our source is the RF driver in the femtosecond oscillator running at ~40.8 MHz. The
RF frequency is divided 41000 times to yield a stable kHz source (996.67 Hz), which is used to trigger the regenerative amplifier. The 1 kHz reference frequency is divided by 50 using a frequency divider (Pulse Research Labs) to yield the 20 Hz trigger. To be able to time the 6 µm and 2 µm pulses relative to one another, we provide variable 20 Hz triggers to the flash lamp and Q-switch drivers of the Nd:Yag laser with the help of a SRS electronic delay generator. The timing between the pulses is found by placing a fast Si photodiode (EOT) at the crossing of all the beams and observing the 6 µm and 2 µm pulses on a 1GHz oscilloscope. The signal from the 6 µm light is observed because of multi-photon transitions across the Si band gap, while the near IR light is scattered onto the photodiode. The SRS delay generator is used to control the fine timing between the T-jump laser and the mid IR probe pulses.

Figure 9-8  (Color) (Top) The timing sequence for the 1 kHz and the 20 Hz laser systems. We supply the 1 kHz triggers to the evolution, the pockel cell driver and the frequency divider. The 20 Hz signal produced by the frequency divider serves as an input to the SRS delay generator, which controls the timing between the 20 Hz and 1 kHz pulse trains. (Bottom) Experimental set-up for the transient experiments. M: mirror; L: 10 cm lens; PM: parabolic mirrors; BS': ZnSe window, S: sample and A: analyzer.
The uniform temperature jump created in our interaction volume of ~ 9 nL is calibrated by measuring the transmission change of the tracer beam. There is a broad D$_2$O absorbance background covering the bandwidth of the femtosecond 6 µm pulses. Taking spectra of the tracer pulses by systematically increasing the temperature of the D$_2$O buffer solution we find that the change in absorbance per degree per pathlength is given by $\Delta$OD/$\Delta$T/cm = 0.2 between 1500-1650 cm$^{-1}$. For the experiments described in this chapter the starting temperature, $T_0$=63°C.

The data collection scheme is described in Figure 9-9. To detect the slightest changes induced in the sample by the T-jump, we measure the difference signal, $\Delta S=S(T,\tau)-S(T_i,\infty)$ for a particular value of $\tau$. The final temperature attained as a result of the interaction of the sample with the near IR laser is designated as $T$. Since we use the DVE spectrum as a probe to follow the T-jump induced conformational changes in the protein solution, the difference signal is defined as:

$\Delta \tilde{S}_{\text{DVE}}=\tilde{S}_{\text{DVE}}(T,\tau)-\tilde{S}_{\text{DVE}}(T_i,\infty)$. The red pulse labeled 1 in the kHz pulse train is the pulse immediately following the T-jump laser and the DVE spectra taken using three replicas of that pulse can track changes from 10 ns – 1 ms. The pulses labeled 2-10 which also constitute the 20 Hz signal can only track changes in 1 ms time intervals. By 10 ms, the temperature of the solution has equilibrated to its $T_0$ value of 65°C. The spectra obtained from the 6µm pulses (11-50) colored green.
will be referred to as the 1kHz signal representing the reference signal, $\tilde{S}_{\text{DVE}}(T, \infty)$. The $\tilde{S}_{\text{DVE}}(T, \tau)$ and $\tilde{S}_{\text{DVE}}(T, \infty)$ signals are collected separately and then subtracted from each other for each value of $\tau$ to highlight the early events in the thermal denaturation of RNase A. At each value of $\tau$, we also block the probe light at 6 $\mu$m and collect the 2 $\mu$m background signal. This is essential because there is (1) a time-dependent electronic RF noise at 20 Hz and (2) the third diffraction order of the 2 $\mu$m light, which makes it through to the MCT array detector and needs to be subtracted as it masks the DVE signal.

### 9.3.2 Results

![Figure 9-10](image)

**Figure 9-10.** (Color) (Right) Transient difference DVE spectra following a T-jump of ~10 °C with the starting temperature at 65 °C. (Left) The equilibrium difference DVE spectra obtained from the difference of the spectra at 75 °C from the one at 65 °C. The Y-axis on both channels represents the counts on the 64 channel MCT array detector.

The results from the T-jump experiments are plotted in the right panels of Figure 9-10 for various values of the delay $\tau$ between the 2 and 6 $\mu$m pulses. The left panel shows an equilibrium difference DVE spectrum corresponding to the temperatures accessed in the transient experiments. We see maximum changes at 1625 cm$^{-1}$, 1640 cm$^{-1}$ and 1670 cm$^{-1}$. A first glance at the transient difference DVE spectra reveals a completely different shape and magnitude from the equilibrium difference spectrum. The very presence of a non-zero difference DVE spectrum at $\tau<10$ ns tells us that there are sub-nanosecond changes taking place in our solution following an abrupt temperature jump. The changes in the transient difference DVE spectra reach their maximum by ~ 1 $\mu$s after
which there is a decay of the peak at 1630 cm\(^{-1}\). One of the interesting observations is the growth of the negative feature at 1625 cm\(^{-1}\), which coincides with the decay of the peak at 1630 cm\(^{-1}\). The difference DVE spectra at \(\tau\>1\) ms show the protein solution slowly returning to its original conformation at 65 °C. By 10 ms, the solution has re-equilibrated and the signal level is close to zero.

These minute changes are seen more clearly by plotting the amplitudes at four spectral regions indicated by arrows on a trace of the DVE at 63 °C as a function of the delay \(\tau\) in the lower plot of Figure 9-11. To allow for an accurate comparison with the instrument response function of the T-jump laser, we plot the change in absorption of the tracer beam at 1630 cm\(^{-1}\) and the corresponding temperature of the solution as a function of \(\tau\) on the top panel of Figure 9-11. The temperature jump of \(~10\) °C is achieved by 10 ns. It drops by \(~10\)% of its maximum value by 300 \(\mu\)s and returns to the starting temperature by 10 ms. The observed temperature profile is in keeping with the calculations performed by Dyer et al.\(^{41}\) We do not see any photo-acoustic effects in the measurement of the temperature profile as mentioned in previous T-jump studies.\(^{42}\)

\[\text{Figure 9-11. (Color) (Top) The time profile of the temperature jump created in the D2O buffer solution. The Y axis on the left depicts the change in absorption of the tracer beam at 1630 cm}\(^{-1}\) and the actual temperature of the solution is plotted on the right axis. (Bottom) Time - dependent amplitudes for the normalized difference DVE spectra at 1598 cm\(^{-1}\) (blue), 1625 cm\(^{-1}\) (green), 1650 cm\(^{-1}\) (red) and 1679 cm\(^{-1}\) (purple). The estimated error bars for each point in the lower plot are 0.5%, which is equal to the interval between successive tick marks.}\]
The traces in the lower plot of Figure 9-9 indicate that the rise time of the nonlinear IR response to the T-jump is limited by our experimental instrument response time of 8 ns. We also see that the mid IR response is complicated, non-single exponential in nature and does not follow the temperature jump profile for 10 ns<\(\tau<300\) µs. In particular, we see that there is decay of all the spectral components where \(\omega_3 > 1600\) cm\(^{-1}\) between \(\tau =1-3\) µs. During that corresponding time period, the spectral change at 1598 cm\(^{-1}\) goes below zero indicting that the peak position of the DVE spectrum is moving to higher frequencies in response to the transient T-jump. For \(\tau>300\) µs, all the traces approach the zero baseline value following the T-jump profile.

9.3.3 Discussion

At this point, we are far away from extracting transient structural information from the results presented in this chapter. However, this initial study does indicate the following: (1) the DVE spectrum is a sensitive and viable tool to probe conformational changes in protein following an abrupt temperature jump, (2) there are sub-nanosecond changes in the Amide I region indicated by the non-zero value of the difference DVE spectrum at \(\tau\sim0\), and (3) there is a change on the timescale of 1 µs in all the regions of the Amide I band especially the β-sheet region as noted by the change in the low and high frequency ends of the DVE spectrum at 1600 and 1679 cm\(^{-1}\) respectively.

The presence of the sub-nanosecond response is in keeping with a previous study on RNase A by the Hochstrasser group.\(^{37}\) The sub µs timescales observed in our data agrees with the fast timescales observed in studies of cytochrome c and a small protein BBA where the response to a ns T-jump was probed using Trp fluorescence.\(^{33,44}\) The results from these studies suggested that this fast timescale can be associated with the hydrophobic collapse of the polypeptide chain.\(^{45,46}\) More insight will be gained from performing experiments with different starting and ending temperatures and with differing magnitudes of the temperature jump. Also a quantitative analysis of the equilibrium 2D IR data will be crucial in extracting microscopic information from the transient experiments presented in this section. Our initial studies suggest that the fast response in this 124-residue protein with 4 disulfide bonds reflects the rearrangement of the solvent shell caused by disruption of the tertiary contacts. It is clear from the differing magnitudes of the equilibrium and transient difference spectra that the major structural changes occur on a timescale longer than 1 ms. The thermal unfolding pathway studied using stopped flow methods, has revealed multiple slow timescales on the order of 10’s of milliseconds. There is an even slower timescale involving species with proline residues in non-native conformations around 50-200 s.
As shown in Figure 9-11, the maximum change in the transient difference spectra is just 8% of the maximum amplitude of that spectral component in the reference DVE spectrum. This suggests that we are developing an extremely sensitive spectroscopic probe to study fast structural changes in the thermal denaturation of proteins. On the flip side, our detected response will be extremely sensitive to experimental conditions such as intensity fluctuations in the near IR and mid IR laser systems and the choice of excitation geometry to create a temperature jump. Additionally, the difference measurement of \( \Delta \tilde{S}_{DVE} = \tilde{S}_{DVE}(T, \tau) - \tilde{S}_{DVE}(T, \infty) \) will be affected by the increase in transmission of the signal field as well as the three input fields at higher temperatures. We can try and account for that using the measured temperature profile over the entire spectral range of our femtosecond pulses. The effects of absorption of the signal field and input fields as they travel through the sample may also have to be taken into account.

In conclusion, we have presented a new and sensitive approach to study fast structural changes accompanying the thermal denaturation of proteins. We create a fast temperature jump in our protein solution and then probe it using dispersed vibrational echo spectroscopy. This nonlinear IR probe proves to be an extremely sensitive measure of minute transient spectral changes in the Amide I region on a sub-\(\mu\)s timescale. We anticipate that a careful and self-consistent analysis of the equilibrium and transient measurements will provide clues into the structural changes accompanying the thermal denaturation of RNase A.
References

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