Non-Adiabatic Mechanism for Photosynthetic Energy Transfer and All-Optical Determination of Concentration using Femtosecond Lasers

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NON-ADIABATIC MECHANISM FOR PHOTOSYNTHETIC ENERGY TRANSFER AND ALL-OPTICAL DETERMINATION OF CONCENTRATION USING FEMTOSECOND LASERS

by

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B.S., INDIAN INSTITUTE OF TECHNOLOGY KANPUR 2009
M.S., INDIAN INSTITUTE OF TECHNOLOGY KANPUR, 2009

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Department of Chemistry and Biochemistry

2014
This thesis entitled:
Non-Adiabatic Mechanism for Photosynthetic Energy Transfer and All–Optical Determination of Concentration using Femtosecond Lasers
has been approved for the Department of Chemistry and Biochemistry

______________________________
David M. Jonas

______________________________
Joel D. Eaves

Date ___________

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
Development of efficient light-harvesting technologies hinges on our understanding of the fundamental physics of light-harvesting in both natural and artificial systems. This work addresses the following topics, i.) the mechanism underlying the remarkably efficient electronic energy transfer in natural light harvesting antennas, ii.) a femtosecond time-resolved photonumeric technique to quantitatively characterize transient chemical species.

A non-adiabatic model for photosynthetic energy transfer in light harvesting antennas is proposed. Light harvesting antennas use a set of closely spaced pigment molecules held in a controlled relative geometry by a protein. It is shown that in the Fenna-Matthews-Olson (FMO) antenna protein, the antenna found in green sulfur bacteria, the excited state electronic energy gaps are resonant with a quantum of vibrational energy on its pigment, bacteriochlorophyll a. Through a dimer model loosely based on FMO, it is shown that such a resonance leads to an unavoidable nested non-adiabatic energy funnel on the excited states of photosynthetic antennas. The non-adiabatic model presented here leads to enhanced vibrational oscillations on the ground electronic state of these antennas, the 2D spectroscopic signatures and oscillation frequencies of which are consistent with all the reported 2D signatures of long-lived oscillations, including the ones that are not explained by prior models of excited state electronic energy transfer. Extensions that account for both resonant and near-resonant pigment vibrations suggest that photosynthetic energy transfer presents a novel design in which electronic energy transfer proceeds non-
adiabatically through clusters of vibrations with frequencies distributed around electronic energy
gaps.

The latter part of the thesis presents absolute measurements of femtosecond pump-probe
signal strength. The experiments demonstrate quantitative time-resolved measurement of
absolute number of excited state molecules. Based on these measurements, an all-optical
technique that simultaneously determines concentration and extinction coefficient of an unknown
sample is presented. Unlike prior such analytical techniques, the present photonumeric method
does not require any sample isolation, physical handling or in situ calibrant. In principle, the
experimental and theoretical framework developed allows extensions towards characterization of
transient chemical species.
ACKNOWLEDGEMENTS

My academic journey so far has only been possible because of the encouragement and motivation that I have enjoyed from a number of people. It was the motivation from my father who has a Ph.D. in Organic Chemistry himself, my undergraduate advisor Prof. Debabrata Goswami and my graduate student mentor S.K. Karthick Kumar, which ultimately led me to graduate school. Getting a chance to work with Prof. David Jonas during graduate school has made it a very privileged experience.

High quality research standards set by Prof. David Jonas have been a source of continuous motivation. The art of expert critical thinking is quite rare in the scientific community; David strives hard to perfect that art and to pass it on to his students. I believe that it has played (and will play in the future) a key role in my metamorphosis into a careful scientist and a critical person in general. His patient disposition has allowed me to learn from my mistakes and made my Ph.D. experience a cherished one. Motivation from other professors in the program, especially Prof. Joel Eaves, has also played a major role in shaping my research. I am also very grateful to have worked with a number of great people in the Jonas group. I have especially benefited from the guidance of Jonas group seniors Dr. Byungmoon Cho, Dr. William Peters, Dr. Trevor Courtney, Dr. Robert Hill and Dr. Danielle Buckley. Discussions with Jonas group members Austin Spencer, Dmitry Baranov, Samuel Park, Jisu Ryu and Anna Curtis have been very fruitful towards my own learning.

Coffee time discussions with Dmitry Baranov and, post soccer discussions over beer with Paul Teichen and Dmitry Baranov about research and life in general, have been the mark of these five years and will be greatly cherished. I must also thank my friends since undergraduate days, Dr. Abhik Kumar Das and Dr. Man Prakash Gupta, whom I have always looked up for support
and guidance. I could not have asked for better roommates, Aditya Zutshi and Rohan Singh, who have also played a big role in making my graduate school experience a truly enjoyable one. Finally, I am thankful to my parents, Suresh and Leela Tiwari, and my brother, Abhishek Tiwari, for standing behind all the decisions that I have taken so far in my journey, and providing encouragement during the high and low times of my academic journey.
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CHAPTER 1
INTRODUCTION

The efficiency of photosynthetic light harvesting\textsuperscript{1,2} has long inspired research on natural light harvesting systems\textsuperscript{3} from photosynthetic organisms such as plants, bacteria, and algae. Natural light-harvesting antennas\textsuperscript{5}, typically contain chlorophylls in close proximities of \textasciitilde10-20 Å, held together at specific relative orientations by a protein. Under low light conditions, the quantum efficiency of electronic energy transfer from the site of light absorption to the reaction center for charge separation and subsequent chemical reactions, can be 100 % within experimental error. An understanding of the fundamental physics of this remarkably efficient energy transfer is required in order to replicate the natural design principles in artificial light-harvesting systems.

Photosynthetic pigments have a large number of intramolecular vibrational modes. Förster\textsuperscript{6} established an adiabatic framework for the theory of energy transfer, assuming that light electrons instantaneously follow the vibrational motions of the heavy nuclei everywhere except in small ranges of vibrational coordinates where the adiabatic approximation\textsuperscript{7} fails. Recently we have shown\textsuperscript{4} that the adiabatic framework for energy transfer completely breaks down in some antennas, and proposed a non-adiabatic mechanism for photosynthetic energy transfer in which electronic and vibrational motions are not separable. The experimental signatures of this mechanism are consistent with all the previously reported signatures from 2D spectroscopic experiments\textsuperscript{8-11} on photosynthetic antennas and artificial polymers\textsuperscript{12}, including the ones that are not explained by recent theories of electronically coherent energy transfer.

Characterizing energetic relaxation and dissipation channels on excited electronic states of molecules is important for a mechanistic or analytical understanding of the chemistry
involved. An absolute quantification of transient chemical species and excited electronic population can be useful in a number of problems such as characterizing a possible dark state in the rapid electronic relaxation from $S_2$ to $S_1$ singlet state in carotenoids$^{13,14}$, or characterizing radiationless cis-trans photoisomerization$^{15}$. Traditional time-resolved methods such as flash photolysis$^{16}$ or pump-probe techniques$^{17}$ using pulsed lasers have only achieved a certain degree of control and accuracy, and often rely on relative determination$^{18,19}$ of extinction coefficient and electronic populations. Relative estimates of pump-probe signal strength lie at the heart of controversial reports$^{20,21}$ of high yields of generation of multiple low-energy electron-hole pairs from one high energy electron-hole pair in semiconductor nanocrystals.

Recently we have developed a general theoretical framework for determining the absolute pump probe signal, that is, the absolute change in the number of transmitted probe photons caused by molecular absorption of photons from the pump, and demonstrated its validity on a dye, fluorescein, with better than 10% error between theory and experiment. We have also extended this approach to demonstrate a novel all-optical technique that combines a linear and a non-linear spectroscopic measurement to simultaneously determine an unknown concentration and extinction coefficient without any sample isolation or in situ calibrant. By comparison to the gravimetric$^{22}$ concentration determined by measuring mass and volume, the technique can also be used to optically determine sample mass purity.

1.1 Photosynthetic Energy Transfer

1.1.1 Natural Light-Harvesting Pigments and Antennas

Photosynthetic pigments that play the major role in gathering and transferring the photon energy to the reaction center come in wide varieties. Remarkably, in photosynthetic organisms
such as plants, bacteria and algae, the pigments are synthesized through similar biochemical pathways resulting in pigments based on chlorin or bacteriochlorin ring structures, or open forms of it (bilins). Fig. 1.1 shows a few of the wide variety of antenna pigments found in photosynthetic organisms from vastly different natural environments. The chlorophyll or bacteriochlorophyll pigments alone are present in a number of forms such as $\text{Chl}\ a$-$d$ and $\text{BChl}\ a$-$g$, depending on the particular species and the antenna complex in question$^5$. Both the light harvesting antenna complexes of purple bacteria$^5$ and the Fenna-Matthews-Olson (FMO) antenna complex of green sulfur bacteria$^5$ contain of $\text{BChl}\ a$, whereas light-harvesting complex (LHC II) in plants$^5$ contains $\text{Chl}\ a$ and $\text{Chl}\ b$. The antenna complexes in cyanobacteria and marine algae contain bilins, the linear, open-chain form of chlorin-like rings, as the major photosynthetic pigments.

Fig. 1.2 compares the porphyrin macrocycle to the chlorin and bacteriochlorin macrocycles. Both chlorophylls and bacteriochlorophylls are based on a porphyrin macrocycle with a central Mg atom coordinated to four nitrogen atoms. In the chlorin macrocycle found in chlorophylls, one of the double bonds in the symmetric porphyrin ring is reduced. In bacteriochlorins, the conjugation in the chlorin ring is further reduced by one double bond. In the simplest molecular orbital picture, the lowest energy electronic transition is a $\pi-\pi^*$ transition. The absorption spectrum of these pigments can be better understood in terms of Gouterman “four orbital” model, that approximates the configuration interaction to only the two highest lying occupied molecular orbitals (HOMOs) and the two lowest unoccupied molecular orbitals (LUMOs). The resulting lowest energy band is the $Q_y$ band, in which the largest electronic density changes occur when the exciting electric field is polarized along a y molecular axis. The absorption band of bacteriochlorophyll is situated near ~ 800 nm and the corresponding
Figure 1.1. Structures of the wide variety of photosynthetic pigments found in plants (chlorophylls a and b, Chl a,b), bacteria (bacteriochlorophyll a, BChl a) and marine algae (bilins). All the pigments are based on a reduced porphyrin macrocycle or an open form of it in case of bilins, and have delocalized $\pi$ electrons due to an extended conjugation.
Figure 1.2. Comparison of the porphyrin macrocycle with the reduced, less symmetrical forms of it (chlorin and bacteriochlorin), which are present in the photosynthetic pigments shown in Fig. 1.1. (Adapted from the free online source www.commons.wikimedia.org)
absorption band in FMO or LH2 has been extensively investigated spectroscopically as a probe of the mechanism of energy transfer between the pigments.

Because of an extended conjugation present in these pigments, a $\pi$ to $\pi^*$ electronic excitation is only accompanied by small changes in bond character or geometry. Hence the Franck-Condon vibrational wavefunction overlap factors, which describe the probability of vibrational excitation accompanying an electronic excitation, are small. All the photosynthetic pigments mentioned above also have a large number of Franck-Condon (FC) active vibrations\textsuperscript{23-29}, although for the above reason many such vibrations are very weakly coupled to an electronic excitation. In case of $BChl$ $a$ for example, there is an abundance of low-frequency vibrations in the $< 500$ cm\textsuperscript{-1} range\textsuperscript{26}, with very weak stabilization energies of approximately 5 cm\textsuperscript{-1} or less\textsuperscript{23,24,28}. Several fluorescence line-narrowing measurements from independent groups\textsuperscript{23,24,28,29} show that even within the low-frequency vibrational range, there are clusters of closely spaced vibrations, for example, the 160-200 cm\textsuperscript{-1} vibrational range in $BChl$ $a$. Fig. 1.3 has been adapted from refs.\textsuperscript{24,27} and shows the resonance Raman and fluorescence line-narrowing spectra of the large number of low-frequency FC active vibrations in the ground electronic state of $BChl$ $a$. The vibrational cluster in the 160-200 cm\textsuperscript{-1} range appears in the fluorescence line-narrowing spectrum shown in the lower panel. The spectrum is obtained by red edge excitation of the lowest energy absorption band corresponding to the lowest energy exciton in FMO. Within the antenna complex, these vibrational frequencies and their damping can be modified by the electrostatic interactions with the surrounding protein environment\textsuperscript{30,31}. In the FMO antenna, a strong excitation wavelength dependence of the coupling of the protein vibrations to the lowest energy electronic transition has been observed\textsuperscript{23,24}. Energy transfer mediated by coupling between $BChl$ $a$ pigments is proposed\textsuperscript{32} to be likely responsible for this wavelength dependence.
Figure 1.3. (Top) $Q_y$ excitation Resonance Raman spectrum (adapted with permission from ref.27) of BChl a films. The figure shows all the Raman active ground electronic state vibrations in the BChl a pigment. (Bottom) Fluorescence line-narrowing spectrum (adapted with permission from ref.24) of BChl a in the FMO antenna complex at 4 K from 827.1 nm excitation at the red edge of the lowest energy exciton in FMO. The figure shows all the ground state vibrations of coupled BChl a in the FMO protein with a broad pedestal of several vibration frequencies in the range 160-200 cm$^{-1}$. 
Light-harvesting antenna proteins are found in most chlorophyll-based photosynthetic organisms. The pigments are arranged in highly specific three dimensional spatial arrangements with respect to each other. The antenna proteins are spatially arranged so that they transfer energy to a reaction center. Some aspects of antenna protein organization depend on the light conditions the organism grows in. Antenna proteins are primarily responsible for increasing the effective cross-section for photon absorption from sunlight by increasing the number of pigments associated with each reaction center. The captured electronic energy is funneled to the reaction center within a few picoseconds with a near unity quantum efficiency in many cases. The reaction center uses these excited electronic states to drive photochemical reactions that ultimately produce and store energy for the organism.

Photosynthetic organisms show a remarkable variety of antenna proteins, suggesting several independent evolutionary origins of antennas towards achieving a common adaptation for a light-gathering system. Most antenna proteins are pigment-protein complexes, where the chlorophylls are specifically associated with proteins in a unique arrangement. Antennas are broadly divided into two categories, ones in which the proteins and their associated pigments are deeply buried in a lipid bilayer membrane (integral membrane proteins), and those in which the pigments do not span the membrane and are only attached to one side of the lipid bilayer (peripheral membrane proteins). The integral membrane proteins can be further divided based on their relative position in the energy transfer chain and the structure.

An example of an integral membrane protein is the LH2 light harvesting antenna found only in certain purple bacteria grown under low-light conditions such that their ratio with respect to the reaction center is not specific. LH2 is comprised of bacteriochlorophyll a (BChl a) molecules and carotenoids along with α and β protein subunits. The BChl a molecules are
Figure 1.4. X-ray crystal structure of the B850 and B800 rings in the LH2 antenna complex from purple bacteria. a.) B850 ring of 9 BChl a dimeric subunits b.) B800 ring of 9 monomeric BChl a subunits. The surrounding α helical protein chains are shown in yellow and green. Accompanying carotenoid pigments and β sheet protein chains have been removed for clarity. (Adapted with permission from ref. 34)
arranged in two rings\textsuperscript{34}, B800 and B850 of approximately 65 Å diameters. The rings are named according to the approximate wavelengths (800 nm vs. 850 nm) of their $BChl$ $a$ $Q_y$ electronic absorption band. The B800 ring comprises of 8 or 9 largely monomeric $BChl$ $a$ molecules arranged in a symmetric fashion with relative distances of approximately 20 Å. The B850 ring comprises of 8 or 9 $BChl$ $a$ dimeric sub-units with similar distances of approximately 10 Å both within a dimeric unit and between adjacent units. Fig. 1.4 has been adapted from ref.\textsuperscript{34} and shows the dimeric B850 ring in the upper panel, and the monomeric B800 ring in the lower panel.

In contrast to the LH2 antenna protein, the Fenna-Matthews-Olson (FMO) antenna protein is an example of a peripheral antenna from green sulfur bacteria which are found in extremely low-light conditions. The FMO antenna protein was the first chlorophyll-based antenna to have its structure determined by X-ray crystallography\textsuperscript{35}, and has been extensively studied using both spectroscopy\textsuperscript{8,9,24,28,36,37} and theory\textsuperscript{30,31,38-41}. FMO transfers the energy from chlorosomes that first absorb sunlight to the reaction center for photochemistry. Each monomeric sub-unit in the FMO trimer contains of 7 $BChl$ $a$ molecules separated by a distance of approximately 20 Å. A possible eighth $BChl$ $a$ molecule lies outside the monomeric sub-unit and has only been recovered at a ratio of $\sim 1$ $BChl$ $a$ molecule per trimer\textsuperscript{42}. It is possible that another two have been washed off during protein isolation and purification. Fig. 1.5 has been adapted from ref.\textsuperscript{5} and shows the three monomers in the FMO complex, with the $BChl$ $a$’s embedded within the protein.

The $Q_y$ band in the low-temperature linear absorption spectrum of photosynthetic antennas has a number of delocalized excitons that is equal to the number of pigments in the antenna. For example, the FMO antenna complex has 7 excitons corresponding to the 7 pigment sub-unit in the FMO trimer. However, the low temperature absorption spectrum of FMO\textsuperscript{30}
Figure 1.5. (Top) Ribbon diagram of the three-fold symmetric Fenna-Matthews-Olson antenna complex from green sulfur bacteria. The C$_3$-symmetry axis shown by the arrow head is perpendicular to the plane of paper. Each monomeric sub-unit of the trimer has 7 BChl $a$ molecules embedded in the $\alpha$-helical (green) and $\beta$ sheet (blue) protein chains. (Bottom) Position of the BChl $a$ pigments within the monomeric subunit with the dashed line representing the C$_3$-symmetry axis. Combined quantum chemical and classical electrostatic calculations suggest that the $\alpha$-helices (green) interact electrostatically with the pigment to create an energy sink at pigment 3. (Adapted with permission from ref. 31, copyright (2007) National Academy of Sciences, U.S.A.).
shows only three distinct splittings, with other excitonic splittings hidden under the lineshapes dominantly broadened by static inhomogeneities in the excited state pigment energy gaps\textsuperscript{30} caused by the electrostatic protein environment. These excitonic splittings span the same energetic frequency range as the low-frequency intramolecular pigment vibrations, with the first excitonic splitting in FMO almost resonant with the 160-200 cm\textsuperscript{-1} vibrational cluster in \textit{BChl a}. Similarly, the \textasciitilde 700 cm\textsuperscript{-1} energy gap between B800 and B850 rings in the LH2 antenna complex\textsuperscript{43} sits pretty close to a \textit{BChl a} vibrational frequency of \textasciitilde 730 cm\textsuperscript{-1}\textsuperscript{27}.

\textbf{1.1.2 Energy Transfer Framework}

Photosynthetic antennas function to increase the effective cross-section for capturing sunlight and transfer the energy to a reaction center for photochemistry. The initial models of energy transfer from the antenna pigments towards the reaction center treated the problem as an inefficient diffusion problem\textsuperscript{1}. In such a model the energy transfer can take infinite number of steps before reaching the reaction center. Vibrational energy dissipation in antenna pigments occurs through collisions of the vibrating pigment nuclei with the condensed protein environment in a timescale on the order of picoseconds. A diffusional transfer of energy through an ensemble of pigments would be too slow such that the electronic energy will be completely dissipated in the environment first, without even reaching the reaction center. The overall antenna is usually arranged in the form of an energy funnel with most (but not always all) steps being downhill energy transfer towards the reaction center\textsuperscript{44}. Combined quantum chemical and classical electrostatic calculations\textsuperscript{31} based on the high resolution X-ray structure\textsuperscript{35} of the FMO antenna protein suggest that the pigments are roughly arranged in the form of an energy transfer funnel with the pigment closest to the reaction center absorbing the lowest photon energy. Fig.
1.5 has been adapted from ref. 31 and shows the three-dimensional arrangement of pigments in a monomer sub-unit of the FMO trimer with the pigment closest to the reaction center having the least absorption energy due to electrostatic interactions with specific amino acid residues on the α-helices. Since the pigments are in close proximities of ~10-20 Å, their electronic densities are electrostatically coupled with a dipole-dipole interaction that roughly scales as the inverse cube of the distance between the pigments. A diffusional model of energy transfer neglects the above mentioned energetic funnel arrangement between the pigments.

1.1.2.1 Adiabatic versus Non-adiabatic vibrational-electronic motions

In many problems in chemistry, fast electronic motion is separable from the slow motions of the nuclei (the Born-Oppenheimer approximation). With this approximation, the Schrödinger equation for the electronic motion is solved for a series of fixed nuclear frameworks, that is, zero nuclear kinetic energy. The resulting electronic wavefunction \( \psi_{\text{elec}}(\vec{r}_{\text{elec}}, \vec{R}_{\text{nuc}}) \) and energy \( E(\vec{R}_{\text{nuc}}) \) depend on the nuclear coordinates parametrically. For Schrödinger’s equation for nuclear motion, the above electronic energy provides a potential energy surface for the nuclei to vibrate and, together with the nuclear kinetic energy, form the vibrational-rotational Hamiltonian. The resulting overall wavefunction of the system is a separable product of electronic and nuclear wavefunctions, \( \psi_{\text{elec}}(\vec{r}_{\text{elec}}, \vec{R}_{\text{nuc}}) \chi_{\text{nuc}}(\vec{R}_{\text{nuc}}) \), with each electronic state supporting a complete set of vibrational eigenfunctions. This separable product assumes that the fast electrons follow the nuclei adiabatically so that slow changes in nuclear framework cannot cause changes in the electronic quantum state. The adiabatic electronic wavefunction can change with molecular geometry, but all vibrational states have a common adiabatic electronic wavefunction at each geometry. If the separable product wavefunction is
substituted into the original Schrödinger equation, it is found that it is a solution if the effect of
the nuclear kinetic energy operator on the electronic wavefunction is negligible. In particular,
two terms \( \chi_{nuc} \frac{\partial^2 \psi_{elec}}{\partial R^2} \) and \( \frac{\partial \chi_{nuc}}{\partial R} \frac{\partial \psi_{elec}}{\partial R} \) are neglected in the adiabatic approximation\(^7\). In certain
situations, such as when the electronic potential energy surfaces corresponding to different
electronic states intersect (conical intersection\(^{46}\)), the adiabatic electronic wavefunction can
become a strong function of nuclear coordinates so that the Born-Oppenheimer approximation
breaks down around the region of intersection. In such cases, vibrational motions can promote
rapid electronic equilibration between the two electronic states\(^{47,48}\). The true non-adiabatic
vibrational-electronic wavefunctions can no longer be separated into a product of an electronic
wavefunction that supports a complete set of vibrational wavefunctions. In this case, two non-
adiabatic eigenfunctions may have different electronic character at the same geometry. Such
conical funnels are responsible for the excited state photochemistry of a large number of organic
molecules\(^{48}\).

### 1.1.2.2 Förster’s Adiabatic Energy Transfer Framework

Energy transfer between electronically coupled molecules has been understood under
Förster’s adiabatic framework for energy transfer\(^6\). Based on the strength of electronic coupling
\( J \) between the molecules versus the electronic stabilization energy \( \lambda \) from vibrations on a
molecule, the adiabatic energy transfer problem can be classified under Förster’s weak, strong or
intermediate\(^6,49\) coupling regimes. In the strong electronic coupling limit \( J > \lambda \), the weak effect
of vibrations is treated perturbatively, and the electronic energy can coherently hop back and
forth between the pigments, before the transport can be made permanent by vibrational
relaxation in the bath. The excitation is essentially delocalized between the molecules. In
contrast, in the very weak coupling limit where $J < \lambda$, the strongly coupled vibrations dissipate excess energy quickly enough that the electronic energy transfer between the pigments can be treated in the incoherent rate limit, with no coherent transport back and forth between the pigments. In this limit, the excitation is localized on the individual molecules and the transport resembles electronic energy incoherently hopping between the pigments. Landau-Zener electronic curve crossing problems\(^5\)\(^0\) are treated under this limit, such that vibrational motion to a localized region where electronic energy can non-adiabatically hop between the potential energy surfaces is still understood under the adiabatic framework. Experiments on bacterial reaction centers by Vos et al.\(^5\)\(^1\),\(^5\)\(^2\) showed that vibrational coherence persists, both during and after the charge separation process in reaction centers, suggesting the possibility of coherent vibrational motion in energy and charge transfer in similar photosynthetic systems. The intermediate coupling regime where $J \sim \lambda$ is not well understood under Förster’s adiabatic framework because there is a strong change in the electronic character as a function of vibrational coordinate combined with large amplitude ‘vibrational functions’ in that region\(^6\).

Protein structure determination along with quantum chemistry and classical dynamics simulations\(^3\)\(^0\),\(^3\)\(^1\),\(^3\)\(^8\),\(^5\)\(^3\)-\(^5\)\(^5\) of antenna proteins show that the strength of electrostatic Coulomb coupling between the pigments varies between antennas. For LH2 itself\(^4\)\(^3\)\(^4\)-\(^5\)\(^6\), the pigments in the B800 ring are largely monomeric (weak electronic coupling limit), whereas for the B850 ring, each monomer in the dimer subunit is strongly electronically coupled to the other monomer, and also to the adjacent dimer units. For FMO, the coupling between the $BChl$ $a$ pigments places the system in between the two better understood coupling regimes, that is, in the intermediate coupling regime. As is shown\(^4\) later in Chapter 2 of this thesis, in the intermediate coupling regime in FMO, the pigment vibrations and electrons cannot be treated independently of each
other. Certain delocalized pigment vibrations can become strongly coupled with the electronic motion and assist in energy transfer between the pigments.

1.1.3 Two-dimensional Spectroscopy of Antennas and Interpretations

Photosynthetic antennas have been extensively studied by linear and non-linear spectroscopic techniques. Two-dimensional electronic spectroscopy\(^{57,58}\) is a non-linear four-wave mixing optical technique that can, in principle, resolve couplings between electronic states by mapping out the absorption and emission frequencies of a vibrational-electronic manifold of states onto correlated excitation and detection axes. Excited electronic states that are coupled and arise from a common ground state can produce cross-peaks or diagonal peaks in the 2D spectrum, which resolves the couplings.

In a 2D experiment, three non-collinear femtosecond pulses (wave vectors \(k_a, k_b\) and \(k_c\)) are crossed inside the sample, and the radiated four-wave mixing field is measured along the phase-matched wavevector direction \(\mathbf{k}_s = \mathbf{k}_c + \mathbf{k}_b - \mathbf{k}_a\). The radiated signal is measured as a function of \(\tau\) (the delay between pulses \(a\) and \(b\)) and \(t\) (the time after pulse \(c\)) for a fixed waiting time \(T\) (the interval between the second and third pulses). Fourier transforming with respect to \(\tau\) and \(t\) axes yields the excitation and detection axes, respectively. In general, 2D spectra contain two positive contributions: reduced absorption from the ground state and stimulated emission from the excited state; in addition, there are negative contributions from excited state absorption. When there are multiple singly excited electronic states present in the system the first two pulses can create superpositions of excited states that evolve with the waiting time \(T\). The oscillatory superpositions show up as oscillations in the 2D peaks as a function of \(T\). Such oscillations can indicate vibrational, electronic or vibrational-electronic quantum coherences on the excited
electronic states of the system, or vibrational quantum coherence on the ground electronic state. On femtosecond excitation timescales, the slow vibrational motions of the protein environment are frozen out. The frozen phonons in the low-frequency phonon sideband of the protein and the static distribution of the excited state pigment electronic energy gaps contribute towards the inhomogeneous broadening of the 2D lineshape along the 2D diagonal. Fig. 1.6 shows a simulated ground state 2D spectrum corresponding to the non-adiabatic model presented in chapter 2 of this thesis for a fixed waiting time $T$. The convention followed in this work for the 2D peak labels is also shown. The diagonal peaks (DPs) are marked with an ‘o’, while the cross peaks (CPs) are marked with an ‘x’.

The FMO antenna complex was the first photosynthetic antenna protein to be studied by 2D electronic spectroscopy by Fleming and co-workers in 2005 \cite{fleming2005}, and several energy transfer pathways in FMO were reported. In 2007 \cite{fleming2007}, the same group reported oscillatory 2D peaks. As mentioned in section 1.1.1, the $BChl$ a vibrations of isolated pigments have very weak FC factors such that the probability of vibrational excitation upon electronic excitation is very small. Thus, FC excited vibrations alone are not expected to produce the reported oscillation amplitudes in the 2D spectra. Curiously, the reported picosecond decay times for the 2D peak oscillations are typical of purely vibrational coherences \cite{fleming2005}. Coherent oscillations in electronic density in a condensed phase electrostatic environment usually dephase within $\sim 100$ femtoseconds. To me this suggests two possible explanations: either electronic coherence is somehow protected \cite{fleming2005} by the protein environment so that it becomes ‘long-lived’ or vibrational coherence is somehow more strongly excited in the antenna than in the isolated pigment so that it can produce the reported amplitude of the 2D oscillations. Fleming and co-workers suggested only the first alternative and interpreted the oscillatory signatures as purely electronic coherence.
Figure 1.6. A simulated ground state 2D spectrum corresponding to the non-adiabatic model presented in chapter 2 of this thesis. The peak labels DP and CP stand for diagonal peak and cross peak respectively. In the convention that is followed in this work, the cross-peak closer to the excitation axis is labelled as CP12, while the cross-peak closer to the detection axis is labelled as CP21. The 2D spectrum is calculated for a fixed waiting time $T$, and the peak amplitudes and widths evolve as a function of $T$. Note that some papers show detection axis as vertical and excitation axis as horizontal. When multiple cross peaks are present in multi pigment antennas, the cross peaks between the diagonal and the excitation axis are labelled here as of CP12 type. This cross peak type label is independent of how the 2D spectra are plotted.
Before my 2013 paper, all subsequent work had been based on variations of this hypothesis involving excited state coherence. It was suggested that such a “wavelike” energy transfer allows the excitation to be delocalized simultaneously on multiple pigments (like a Schrödinger’s cat simultaneously delocalized between dead and alive states), and thus perform an efficient quantum search of the energetic landscape for the lowest energy pigment that is closest to the reaction center. Subsequent to the initial measurements on FMO, Fleming and co-workers also reported polarization signatures predicted for electronic coherence in LHC II, the light harvesting antenna complex in plants. Scholes and co-workers reported oscillations that lived for at least 300 fs in a conjugated polymer, MEH-PPV, used in organic solar cells; they attributed these oscillations to ‘long-lived’ electronic coherence. Later, they also reported ‘long-lived’ oscillations that lasted for at least ~300fs in photosynthetic antennas of marine algae at room temperature.

Subsequent measurements on FMO by Engel and co-workers reported that the ‘long-lived’ oscillations persist for more than 400 fs at room temperature, and that oscillations on the 2D cross-peak closer to the excitation axis oscillate with a ~90° phase relationship with respect to the 2D diagonal peak. Both these signatures were incompatible with a purely electronic coherence model. To explain the unexpected diagonal peak oscillations, a quantum energy transport model was proposed where energy exchange with the bath can be reversible rather than just dissipative. The phase relationship explanation was based on the 90° phase relation between a diagonal and an off-diagonal density matrix element, which do not necessarily map onto the 2D diagonal and off-diagonal peaks. Several long-lived oscillation frequencies were extracted from the FMO 2D spectra, but the specific oscillation frequencies present were not explained by any model except an ad hoc purely excitonic model where the pigments are
electronically coupled by an electrostatic Coulomb interaction. Control experiments on a single chromophore sub-unit of the marine algae light harvesting protein PE545 antennas\textsuperscript{65}, and isolated BChl a in solution\textsuperscript{66} were also reported to argue that the coherence observed in antennas spans more than one pigment and that the oscillation frequencies reported are not readily detectable in isolated pigments. In summary, the above 2D experiments suggested two key facts –

1. Coherence spans more than one pigment and is readily detectable with multiple pigments.
2. Oscillations cannot be vibrations arising from pigment geometry changes alone because the isolated pigment vibrations are too weakly coupled to electronic excitation to dominate the 2D spectrum.

Correlated fluctuations in the protein environment were proposed\textsuperscript{67} as a possible mechanism for protecting “long-lived” coherence protection. However, calculations based on classical vibrational motion\textsuperscript{39} have not reported the necessary correlated fluctuations in the protein environment. Experiments such as pump-probe or 2D spectroscopy measure an ensemble average. Third-order 2D spectroscopy experiments can remove inhomogeneity on the anti-diagonal by correlating the excitation and detection frequencies within the ensemble. This can also remove inhomogeneities that are correlated to the excitation or detection frequency. However, this does not necessarily remove inhomogeneity in the energy gap between two excited states. An inhomogeneous excited state electronic energy gap distribution can cause any purely electronic oscillations to dephase (there is no rephasing of the waiting time $T$ in a third order 2D spectroscopy experiment). Pump-probe experiments by Struve and co-workers\textsuperscript{68,69} prior to 2D spectroscopy suggest that any purely electronic oscillations on the excited state of FMO will dephase within ~200fs, much faster than the decay of reported “long-lived” oscillations that
continue for at least 2 ps. Recently, vibrational-electronic mixing\textsuperscript{70,71} has been suggested as possible explanation for the long-lived nature of these oscillations. It was suggested\textsuperscript{70} that vibrational-electronic mixing between pigments allows the excited state coherence to have a largely intra-pigment or vibrational character, and thus oscillations that persist for longer than expected. Both electronic and vibrational-electronic coherence mechanisms assume that the reported signatures come from the excited states of the antennas.

### 1.1.4 Vibrational-Electronic Resonance and Non-adiabatic Energy Transfer

In chapters 2 and 3 of this thesis a new mechanism for energy transfer in photosynthesis has been proposed. A dimer model, with pigments $A$ and $B$, based on a pair of excitons in the FMO complex with all crucial parameters dictated by readily interpretable experiments on FMO is presented. By comparing excitonic splittings in the low temperature absorption spectrum of FMO\textsuperscript{30}, and the FC active vibrations in $BChl$ $a$ as seen in the resonance Raman\textsuperscript{27} and fluorescence line-narrowing studies\textsuperscript{23,24} (see Fig. 1.3) a vibrational-electronic resonance in FMO is established. The dimer model presented incorporates this resonance between the excitonic splitting in the antenna and a quantum of vibrational energy on the pigments. The ground state of the dimer with no pigment excited is represented as $|0_A\rangle|0_B\rangle$ while the singly excited electronic states are $|A\rangle|0_B\rangle$ and $|0_A\rangle|B\rangle$, representing electronic excitation on pigments $A$ and $B$ respectively. The doubly excited state with both pigments excited is represented by $|A\rangle|B\rangle$.

Through a transformation from localized intra-pigment vibrational coordinates $\hat{q}_A$ and $\hat{q}_B$ on pigments $A$ and $B$, respectively, to correlated and anti-correlated vibrational coordinates ($\hat{q}_+ = (\hat{q}_A + \hat{q}_B) / \sqrt{2}$ and $\hat{q}_- = (\hat{q}_A - \hat{q}_B) / \sqrt{2}$, respectively), that are delocalized over both pigments, it is shown that anti-correlated nuclear motions between the two pigments, for
example, bacteriochlorin ring expansion on one pigment versus ring contraction on the other, can change the excited state electronic energy gap between the pigments. Such a tuning of energy gap mediates strong vibrational-electronic mixing between the two excited electronic states and drives non-adiabatic electronic energy transfer between them. Correlated nuclear motions on the two pigments do not affect the excited state electronic energy gap between them. The contribution of correlated motions is adiabatically separable from the total Hamiltonian, and has no effect on the energy transfer between the pigments.

The anti-correlated tuning coordinate is akin to the energy tuning coordinate for a conical intersection\textsuperscript{46}, although due to an approximately constant off-diagonal coupling in the energy transfer Hamiltonian, the off-diagonal coupling may not go to zero. It is unlikely that the pigments can rotate within the rigid protein environment in order to make the coupling go to zero without disrupting the antenna all together. Thus, even with certain similarities, photosynthetic energy transfer is different from a conical intersection problem\textsuperscript{72}. It is shown that a combination of vibrational-electronic resonance and nesting of excited state excitonic curves due to small vibrational displacements in these pigments strongly mixes the electronic and nuclear motions over a wide range of vibrational coordinates. Fig. 1.7 shows the ground and first excited state electronic potential energies for the dimer model presented in chapters 2 and 3 of this thesis. The electronic potential energies are shown in the electronically localized (site diabatic) basis and the electronically delocalized (adiabatic excitonic) basis. Due to vibrational-electronic resonance in the FMO antenna complex, the energy gap between the adiabatic electronic potential energy curves in the middle panel is resonant with a quantum of vibrational energy on the lower energy exciton $\alpha$. The two resonant vibrational levels on the adjacent electronic potential energy surfaces are strongly mixed due to the non-adiabatic coupling between them, and the exact non-
adiabatic vibrational-electronic energies corresponding to the resonant states are split by $\sim 30$ cm$^{-1}$.

**Figure 1.7.** Electronically localized, electronically delocalized, and non-adiabatic levels and the absorption spectrum for the model dimer presented in chapters 2 and 3 of this thesis. (Electronically Localized) Electronic potential energy surfaces and their vibrational energy levels in the electronically localized (site diabatic) basis and the electronically delocalized (adiabatic excitonic) basis. When none of the pigments is electronically excited the electronic state $|0_{s}\rangle|0_{g}\rangle$ is represented as $|0\rangle$ in the figure. On the first excited electronic state, only the excited pigment is shown in the electronic state labels, such that electronic states $|A\rangle|0_{s}\rangle$ and $|0_{s}\rangle|B\rangle$ are represented as $|A\rangle$ and $|B\rangle$, respectively. The lowest vibrational level on the ground electronic state is taken as the zero of energy. The relative energies in the ground or the excited electronic energy states are drawn to scale, whereas the energy gap between the ground and excited electronic states is not drawn to scale. This is shown as a discontinuity on the energy
axis. The excited state electronic potential energy curves are displaced in opposite directions along a delocalized vibrational coordinate on which the two pigments vibrate in an anti-correlated fashion. Based on the parameters coming from resonance Raman and fluorescence line-narrowing experiments on the bacteriochlorophyll $a$ pigment in the FMO antenna complex, the vibrational quantum of energy for all the electronically localized curves is 200 cm$^{-1}$. In the localized electronic basis $|A\rangle,|B\rangle$, the excited state curves are separated by a static 150 cm$^{-1}$ splitting. $v_\pi = 0$ ($v_\pi = 1$) level energies are shown as solid (dashed) horizontal line segments attached to each curve. All three localized curves have the same curvature (hence harmonic frequency). The excited electronic state curves $|A\rangle$ and $|B\rangle$ are displaced in equal and opposite directions along the anti-correlated vibrational coordinate such that the curves cross at $q_\pi \sim 2.4$ (The classical turning points for the zero point level on the ground state are located at $q_\pi = \pm 1$). These displacements correspond to the 5 cm$^{-1}$ vibrational stabilization energy for the individual pigments that is based on the fluorescence line-narrowing experiments. (Electronically Delocalized) When the Coulombic coupling of 66 cm$^{-1}$ between the pigments is included, the adiabatic approximation leads to delocalized electronic states. In the delocalized adiabatic electronic basis $|\alpha\rangle,|\beta\rangle$, the slow nuclear motions are separable from fast electronic motions and the Coulombic coupling is diagonalized, such that the adiabatic curves $|\alpha\rangle$ and $|\beta\rangle$ have an avoided crossing instead of a crossing at $q_\pi \sim 2.4$. Compared to the equal curvatures on the diabatic curves $|A\rangle$ and $|B\rangle$, the adiabatic electronic potential energy curves $|\alpha\rangle$ and $|\beta\rangle$ have a smaller and a larger curvature, respectively. Curvature of the ground electronic state stays constant. The energy gap between the excited curves $|\alpha\rangle$ and $|\beta\rangle$ is resonant with the vibrational frequency, that is, one quantum of vibration (shown as a dashed black line) on the lower electronic energy curve $|\alpha\rangle$ is resonant with the energy level that has zero quanta of vibration on the higher electronic energy curve $|\beta\rangle$. (Non-Adiabatic Levels) These resonant levels on different electronic states are non-adiabatically coupled by the anti-correlated vibration of the dimer, such that the two resonant levels are split (as shown by dashed red lines). Because of strong non-adiabatic mixing of nuclear and electronic motions, the notion of an electronic potential energy surface for the nuclei to vibrate upon breaks down, and the exact energies do not have an associated potential energy curve. This vibrational-electronic mixing causes a rapid change in the electronic character of the dimers (which is shown in color in Fig.1.8 and chapter 2 of this thesis) as a function of the anti-correlated vibrational coordinate. The rightmost panel shows the absorption cross-section $\sigma(\omega)$ for such a dimer, where the transition intensity for the transition from the lowest vibrational level on the ground electronic state to the higher energy vibrational-electronic level is split into two nearly equal intensity peaks. This splitting is completely obscured due to lineshape broadening (shown in red) caused by both intramolecular vibration and the protein environment.
This splitting manifests itself in the absorption spectrum (shown in the right most panel), but is obscured due to line broadening (shown in red) caused by the protein environment. Due to strong vibrational-electronic mixing between the resonant levels, changes in nuclear geometry along the anti-correlated vibration also cause a rapid change in the electronic character of the dimer. Fig. 1.8 (middle panel) shows, for an adiabatic calculation that neglects the nuclear momentum by neglecting the effect of the nuclear kinetic energy operator on the adiabatic electronic wavefunction, how the electronic character of the dimer system varies along \( \hat{q} \) as shown by the slow color variation. For an exact calculation that does not neglect the nuclear momentum, the Born-Oppenheimer approximation completely breaks down over a range of vibrational coordinates. This is seen by rapid change in color in the thick lines (levels not attached to any curve) in the right most panel of Fig. 1.8. Rapid color variation indicates strong variation in electronic wavefunction as a function of anti-correlated vibrational coordinate. Because the electronic and nuclear motions cannot be solved for separately, there are no nuclear potentials associated with the exact vibrational-electronic eigenstates. The color wheel in Fig. 1.8 represents the excited state electronic character of the dimer system which varies smoothly between pigment \( A \) excited (represented by electronic state \( |A\rangle \)), pigment \( B \) excited (represented by electronic state \( |B\rangle \)), through all linear combinations (quantum coherent superpositions) of these states. Fig. 1.8 shows the excited state adiabatic potential energy curves and energy eigenvalues along with the exact non-adiabatic energy eigenvalues, where the small \( \sim 30 \text{ cm}^{-1} \) splitting between the resonant levels is due to the non-adiabatic coupling. The electronic character is represented more physically in Fig. 1.9, where the colored pigment is the electronically excited pigment and gray represents a pigment in its electronic ground state. When both pigments are colored, the excitation is delocalized on both pigments.
Figure 1.8. (left panel) Isolated pigment (dashed potential curves and levels), (middle panel) coupled adiabatic (solid potential curves and levels) and (right panel) exact non-adiabatic (levels not attached to any curve) excited state vibrational-electronic levels for the dimer model presented in Chapter 2. \( \nu \) is the quantum number of the anti-correlated vibration. Color shows the electronic state character, which is a linear combination of the two pigment states, at each coordinate. The electronic character does not change for isolated pigments and varies slowly for adiabatic states. Rapid changes in electronic character for the non-adiabatic levels over the entire coordinate range indicate a complete breakdown of the adiabatic framework.
Figure 1.9. Coupled adiabatic excited state potential energy curves and vibrational-electronic energy levels, and exact non-adiabatic vibrational-electronic energy levels (levels not attached to any curve) for the dimer model presented in Chapter 2 of this thesis. The color shows the pigments excited (orange for one and blue for the other, with quantum superpositions of intermediate color). At top and bottom of the wheel, gray represents a pigment in its electronic ground state, while the green colored pigment with a ‘hollow’ ring represents its negative phase contribution in the quantum superposition. The pigment sizes represent an anti-correlated ring breathing vibration, with the bacteriochlorin ring (contracted) expanded in the electronically (un)excited pigment. For nearly equal quantum superpositions of the two pigments (pink and green), the rings are approximately of equal sizes. The electronic character of the exact non-adiabatic vibrational-electronic levels (wide bars) changes rapidly with anti-correlated vibration of the pigments, driving photosynthetic energy transfer outside the adiabatic potential surface framework (curves).
A pigment with hollow coloring represents its negative phase contribution to the linear superposition. Because of non-adiabatic vibrational-electronic mixing, the electronic character of the excited state vibrational-electronic eigenstates between the \( \nu = 0 \) classical turning points (-1 to 1 in terms of the dimensionless displacement \( d \) along the anti-correlated nuclear coordinate in Fig. 1.8), changes rapidly from nearly a dimer with only pigment \( A \) excited (red-orange) to a dimer with excitation delocalized nearly equally on both pigments (blue-green).

The consequences of such vibrational-electronic eigenvectors for the 2D spectrum are dramatic. It is shown that the excitation of ground state Raman coherence that was previously very weak because of small FC factors associated with intra-pigment vibrations, is now enhanced because of vibrational-electronic mixing on the excited state. This delocalized coherent vibrational motion can only be excited when two or more pigments are electronically coupled to each other. In chapter 2 the signatures of non-adiabatically excited anti-correlated ground state vibrational wavepackets are systematically compared with all signatures associated with “long-lived” quantum coherence. Fig. 1.10 shows the experimental signatures that are reproduced (in green) against the number of signatures not reproduced (in red) by prior models of energy transfer, that is, purely electronic coherence\(^{49}\) and quantum transport\(^{63}\) models. The previous models are compared with the signatures from the non-adiabatic excitation of ground state vibrational wavepackets. Non-adiabatic vibrational-electronic resonance model for photosynthetic energy transfer is the model most consistent with reported experimental 2D data so far (also see Table S2 of ref. \(^4\)). Ground state signatures are consistent with certain reported signatures that are not explained by any of the previous models.
Figure 1.10. Status of photosynthetic energy transfer signatures reported from 2D experiments on natural as well as artificial systems. Green indicates the signatures that are reproduced by theoretical models, while red indicates experimental signatures that are not consistent with theoretical predictions. Electronic coherence and quantum transport models consider the reported experimental signatures as coming from the excited electronic states of the antennas while the non-adiabatic model explains the reported signatures as coming from the ground electronic state of the antenna. The figure has been adapted from Table S2 of ref. 4.
One of the predictions of the model about larger CP12 (2D cross-peak closer to the excitation axis in Fig. 1.6) oscillation amplitude compared to CP21 (2D cross-peak closer to the detection axis in Fig. 1.6) is also consistent with the reports\(^{11}\) of 2D spectroscopy on a photosynthetic antenna from marine algae. In all studies of the FMO antenna complex, only CP12 oscillations have been reported. Interestingly, all the reported frequencies sampled on cross-peaks of type CP12, the cross-peak type that is predicted to beat the strongest, match (see Table S1 of ref. \(^{4}\)) with \(BChl\ a\) ground state FC active vibrational frequencies. These frequencies were not reported in the 2D spectra of isolated pigments, which is consistent with resonant non-adiabatic enhancement of ground state vibrational wavepackets. Such an enhancement has been recently reported in the bacterial reaction centers\(^{73}\).

In chapter 3, a generalized theoretical framework for several intra-pigment vibrational modes and protein vibrational modes delocalized over both pigments is developed. An expression for a generalized tuning coordinate is obtained. The framework is then simplified to show that ground state 2D signatures are robust to an additional protein environmental mode. For the simplest case of a dimer model with identical vibrations on each pigment, a Bloch dephasing model for 2D lineshapes shows that diagonal and cross-peaks are split into several closely spaced mixed vibrational-electronic peaks. A dimer with two intra-pigment vibrational modes on each pigment is also considered. Apart from the resonant 200 cm\(^{-1}\) mode, the model has an additional 180 cm\(^{-1}\) near-resonant mode. The resulting non-adiabatic effects are cumulative and their consequences in the 2D spectrum are shown to be nearly additive. The oscillation amplitude of the ground state cross-peak predicted to beat stronger, increases by more than 2 times in the presence of an additional near-resonant mode. Thus, in the actual FMO complex with several near-resonant vibrations, the stronger ground state cross-peak oscillations are expected to
dominate the long-lived signatures. These ground state signatures will persist even if all the excited states of the antenna were somehow quenched after excitation of anti-correlated ground state vibrational wavepackets.

1.1.5 Connections with previous models and experiments, and competing theories

Experiments and modeling of systems with near-degenerate excited states provides crucial insights into the dynamics of excited state and ground state vibrational wavepackets. Previous pump-probe polarization anisotropy experiments\textsuperscript{74,75}, which measure the realignment of the electronic transition dipole subsequent to a polarization-selective pump excitation, on a four-fold symmetric silicon naphthalocyanine (SiNc) molecule revealed Jahn-Teller conical intersection dynamics on the excited state. Electronic reorientation on the excited state causes a rapid anisotropy decay on a timescale of $<100$ fs in the measured electronic anisotropy. Analysis of the pump-probe transients shows that vibrational wavepackets along asymmetric vibrational coordinates on the excited electronic state rapidly dephase through non-adiabatic dynamics, whereas those on the ground electronic state continue to oscillate on picosecond timescales. In contrast, non-adiabatic dynamics does not affect the excited state dephasing of symmetric vibrational wavepackets. In a conical intersection, one asymmetric vibrational coordinate tunes the excited state energy gap (tuning coordinate) and another asymmetric vibrational coordinate mixes the electronic states together (coupling coordinate). The symmetric vibrations form the “seam space” of the intersection and do not drive non-adiabatic dynamics. In a dimer, the anti-correlated vibrational coordinate is the tuning coordinate for energy transfer. As mentioned in Section 1.1.4, the off-diagonal coupling in energy transfer is approximately constant and it is unlikely that a rigid protein environment can rotate the pigment so as to make the coupling go to
zero. The correlated vibration in energy transfer forms a part of the conical intersection seam and is not responsible for any non-adiabatic dynamics. Modeling the near-degenerate electronic states in naphthalocyanines\textsuperscript{76} shows that static inhomogeneities in the energy gap, such as those present in the photosynthetic antennas, can wipe out excited state asymmetric vibrations but do not affect the ground state vibrations. Thus, in the energy transfer problem where both excited state non-adiabatic dynamics and static inhomogeneities in the energy gap are present, the expectation that excited-state vibrational-electronic beats can dephase rapidly is natural. The excited state relaxation processes and timescales in the presence of non-adiabatic dynamics are not well understood, although consistent signatures of the ground state anti-correlated vibrational wavepackets with the reported “long-lived” signatures in antennas suggests rapid excited state relaxation.

Many parallel theories of photosynthetic energy transfer have also incorporated the role of intra-pigment vibrations\textsuperscript{70,71,77}. By using a vibronic-exciton model\textsuperscript{77}, Moran and co-workers first showed that vibrational-excitonic resonance can increase the energy transfer rate in allophycocyanin, one of the cyanobacterial antennas, by an order of magnitude, versus C-phycocyanin, another such antenna that does not have this resonance. Mancal and co-workers have used a similar model\textsuperscript{70} but neglecting the vibrations of the unexcited pigment, for example, unexcited pigment $A$ in the excited electronic state $|0_A⟩|B⟩$, Mancal and co-workers proposed that the reported oscillations in FMO are vibrational-electronic coherences that arise from the excited electronic state with a largely intra-pigment vibrational nature which allows them to oscillate on picosecond timescales. Crucially, by neglecting the vibrations of the unexcited pigment, the ground electronic state contributions were neglected. A similar model by Plenio and co-workers\textsuperscript{71} proposed that certain vibrational modes in the spectral density can assist in
spontaneous regeneration of excited state electronic coherences for longer times. All the above prior studies based on a vibronic-exciton model neglect the role of vibrational relaxation on the ground electronic state of the donor after energy transfer to the acceptor (note that this is an electronically excited state of the antenna). These models assume that the electronically excited donor pigment $B$ in the dimer electronic state $|0_A\rangle|B\rangle$ gets both electronically and vibrationally de-excited by transferring its electronic energy to pigment $A$. As a result, the ground electronic state of pigment $B$ in the dimer’s excited electronic state $|A\rangle|0_B\rangle$ has no vibrational excitation to relax. In chapter 3, it is shown that the basis set used for treating anti-correlated vibrations allows for vibration to be left behind on the donor and for vibrational relaxation processes on the ground state of the donor to remove the excess energy needed for energy transfer back to the donor. The role of this vibrational relaxation is one way to make the energy transfer to the acceptor permanent. Although the ground state 2D signatures, such as stronger CP12 oscillations compared to CP21 (Fig. 1.6 and 1.10), do not depend on this excited state vibrational relaxation process, allowing for vibrational excitation on the ground state of the unexcited pigment is necessary to correctly reproduce the ground state 2D signatures.

1.1.6 Implications for a Design Principle

Besides FMO, there is a possibility of a non-adiabatic vibrational-electronic resonance mechanism coming into play for energy transfer between the B800 and B850 rings in the LH2 antenna complex of purple bacteria, with $\sim 730$ cm$^{-1}$ vibrational frequency of $BChl$ $a$ being close to the $\sim 700$ cm$^{-1}$ energy gap between the rings. The energy transfer between the rings happens on a $\sim 0.7$ ps timescale$^{5,43}$, and the possibility of a non-adiabatic vibrational-electronic resonance mechanism for energy transfer has not yet been considered. Recently, enhanced ground state
vibrational coherences due to non-adiabatic vibrational-electronic resonance have been reported in the electronic energy transfer from H (bacteriopheophytin) to B (accessory bacteriochlorophyll) pigments in the reaction center from purple bacteria. A similar vibrational-electronic resonance is also known in allophycocyanin, the cyanobacterial antenna complex. Thus, electronic energy gaps lying close to FC active vibrational frequencies might be one of the designs adopted by nature in a variety of photosynthetic organisms. Figure 1.10 shows that 2D signatures of vibrational-electronic resonance have been reported (as putative signatures of electronic coherence) in antenna complexes responsible for over 50% of the solar light harvesting on the planet.

The nested non-adiabatic funnel on the excited states of photosynthetic antennas that results due to vibrational-electronic resonance presents a new design parameter that was not previously considered. The additivity of 2D signatures of non-adiabatic effects due to several near-resonant modes points towards designs where excited state electronic energy gaps in an ensemble of molecules should be distributed around clusters of closely spaced FC active vibrational frequencies. The nested funnel provides directed energy transfer towards the lowest vibrational state on the acceptor, which is almost adiabatic because it does not have an adjacent resonant vibrational level from the donor electronic state. Thus, an assembly of such molecules should in principle allow for a long range non-adiabatic transport towards the lowest energy acceptor molecule. Such designs might prove useful in the field of organic solar cells, where the role of morphological disorder in excitonic transport along the polymer chain and interfacial charge separation are key.
1.2 Optical Characterization of Transient Chemical Species

1.2.1 Absolute Measurements of Femtosecond Pump-probe Signal Strength

Absolute concentration determination of transient chemical species is important for a mechanistic and analytical understanding of chemical reactions. Flash photolysis\textsuperscript{16} has allowed investigations of transient chemical species such as radicals, with further extensions using actinometry\textsuperscript{82,83} allowing yield determination of non-radiative triplet states in chlorophylls. Later, pump probe spectroscopy\textsuperscript{17} using pulsed lasers have provided a better time-resolution of ultrafast chemical reactions. However, analysis of transient changes in the transmitted probe spectrum due to the pump only allows for relative determinations of absorption cross-section and yields.

Excited electronic states of molecules can have a number of non-radiative electronic population relaxation channels, the timescales and absolute cross-sections of which are not deducible without knowing the expected absolute signal strength from a known radiative cross-section. Absolute expected signal strength can be used an absolute reference point to back out the ultrafast non-radiative cross-sections in problems such as the possibility of a dark state in the rapid electronic relaxation from S\textsubscript{2} to S\textsubscript{1} singlet state in carotenoids\textsuperscript{13,14}, or characterizing radiationless cis-trans photoisomerization\textsuperscript{15} through a conical intersection. Approximations regarding the pulse propagation in the sample, pulse spectrum or the transverse beam profile only provide a certain degree of accuracy in estimating the absolute signal strength. However, relative estimates of pump-probe signal strength lie at the heart of certain phenomenon such as the controversial reported\textsuperscript{20} determination of carrier multiplication yields\textsuperscript{21,84} in semiconductor nanocrystals. Recent reports of several carrier loss channels\textsuperscript{85-87} in semiconductor nanocrystals highlight the need for an absolute quantification of the signal strength in order to time track the
hot carriers\textsuperscript{88}. Similarly, in phenomena such as singlet fission\textsuperscript{89}, absolute quantification of triplet yields and cross-sections can be a useful reference point.

Beer’s law is a convenient technique for measuring\textsuperscript{90-92} an unknown concentration but relies on a known extinction coefficient. Similarly, techniques for measurement of an unknown extinction coefficient ultimately rely upon a concentration of the sample known from an absolute method. For example, absolute gravimetric methods\textsuperscript{22,93} involve measurements of mass and volume, and absolute coulometric methods\textsuperscript{93} involve measurements of number of electrons. Insufficient time resolution, sample isolation or physical handling is also a major limitation of such analytical techniques. Similarly, determination of extinction coefficients of chemical intermediates either relies upon a known concentration\textsuperscript{94} or extinction coefficient\textsuperscript{82,95} of the reactants, or comparisons with coproducts which have a known stoichiometric ratio and extinction coefficient\textsuperscript{96}. In this thesis, a new absolute “photonumeric” analytical technique for determining concentration solely based on measurements of photon numbers is introduced.

The difficulty in simultaneously determining a molecule’s concentration $C$ in the starting energy level, and its extinction coefficient $\varepsilon$ (which is ultimately related to the square of its transition dipole moment $\mu$ that connects the initial and final levels), is that absorbance depends only on the product $\varepsilon C$. If $\varepsilon$ and $C$ are both unknown, one cannot tell if a sample has many weak absorbers or a few strong absorbers. Similarly, in an ideal pump-probe measurement (which is a non-linear optical measurement involving two photons per molecule), the pump-probe signal $S_{pp}$ is proportional to $\varepsilon^2 C$ and one encounters the same ambiguity. If absolute measurements of absorbance and pump-probe signal strength are combined for the same sample, the ambiguity can be resolved so that the extinction coefficient and the concentration of the absorbers in a
sample can be determined simultaneously. The need to account for or prevent any dynamical changes in the pump-probe signal requires a time-resolved measurement.

Multiplex spectroscopy demonstrated by Germann and Rakestraw\textsuperscript{18} combined linear and non-linear spectroscopic measurements to simultaneously determine transition dipole moments and absolute concentrations of transient gaseous HCl in a combustion reaction. Later extensions\textsuperscript{19} using two-dimensional infrared spectroscopy as the non-linear measurement, have also demonstrated simultaneous determinations of transition dipole strengths and concentrations. The above techniques do not measure the absolute non-linear optical signal or the factors in the proportionality constant. This required introduction of an \textit{in situ} internal calibrant with a known cross-section and concentration into the sample itself. The calibrant must be suitable for the excitation wavelengths used, which is a major limitation of these techniques. Furthermore, any differences in the dynamics between the sample and the calibrant must be eliminated or addressed. No theory has been developed to account for dynamical differences between the sample and the calibrant. Thus, these techniques have been demonstrated only relative to the \textit{in situ} calibrant.

Chapter 4 of this thesis presents an optical technique\textsuperscript{97} that measures the absolute number of excited state molecules. Compared to the prior works\textsuperscript{18,19}, the theoretical and experimental framework presented here differs in following aspects: 1. instead of an internal calibrant, the experiment relies on an absolutely calibrated pump-probe signal with careful determinations of all the experimental parameters needed for calculating the theoretically expected signal, 2. the exponential attenuation of the pump and probe pulses as the travel through the sample is accounted for, 3. weak pump and probe pulses each act linearly in the sample to generate the non-linear pump-probe signal, 4. measurement of the electronic polarization anisotropy allows
for explicit incorporation of polarization effects. A general theoretical framework for a multi-
level system is developed which is then simplified for a vibrationally relaxed two-level system
that obeys the Condon approximation\textsuperscript{98}. Such a simplification allows for a quantitative
theoretical prediction with the least number of measurable experimental parameters which are
the sample concentration, absorption and emission cross-sections, transverse beam profile at
pump-probe overlap, laser spectrum, population relaxation lifetime\textsuperscript{99}, and the pump-probe
polarization anisotropy of the system. Experiments on a fluorescein dianion are discussed for
which the calculated pump-probe signal matches the measured pump-probe signal to better than
10 % error. Concentration dependence of the pump-probe signal is also consistent with the
theoretical prediction. The agreement demonstrates that the theoretical characterization of the
pump-probe signal over the transverse spatial profile of the beam, over the entire laser spectrum,
and for each slice in the sample is valid.

1.2.2 All-Optical Determination of an Unknown Concentration

Extending this approach in chapter 5, an optical technique\textsuperscript{100} that simultaneously
measures an unknown concentration and extinction coefficient is presented. The technique
combines a linear absorption measurement with a non-linear pump-probe signal measurement to
determine the two unknown quantities. Unlike previous such techniques for measurement of
concentration and extinction coefficient\textsuperscript{18,19,82,93,101}, the present technique does not require any
sample isolation or an \textit{in situ} calibrant in the sample, and can also be extended for determination
of unknown concentrations of transient species in solution. By comparing the optically
determined concentration with the mass and volume prepared concentration, sample purity can
also be determined. As shown in Fig. 1.11, the expected signal concentration can be plotted as a
function of the sample OD and the measured absolute pump-probe signal.
Figure 1.11. Surface plot of the calculated optically determined concentration $C$ as a function of the linear absorbance $A$ and the non-linear pump-probe signal $S_{pp}$ from a sample of fluorescein in basic methanol. The surface was calculated using Eqn. 5 of ref. $^{100}$, and normalizing the pump-probe signal by the pump and probe powers. The experimental error in concentration determination can be optimized by changing $A$ and $S_{pp}$ such that they correspond to a flatter slope on the concentration surface. (Figure adapted with permission from ref. $^{100}$ )
Based on the position of the theoretically calculated concentration for a given combination of sample OD and the pump-probe signal, the surface slope at that point can be used to determine the optimal experimental parameters for which the measured concentration will have the least amount of error.

The agreement between theoretical and experimental frameworks for an example two-level system, fluorescein dianion, sets it as a reference point for future measurements on more complicated molecular or quantum dot systems. For example, excited state cis-trans isomerization through a conical intersection in cyanine dyes hints towards a possibility of ultrafast frequency dependent non-radiative cross-section. Taking the known excited state cross-section into account, the non-radiative cross-section can be backed out using the theoretical and experimental framework presented. Similarly, the effect of passivating surface traps on the carrier loss channels in semiconductor nanocrystals might be systematically characterized using absolute measurements of pump-probe signal strength.

1.3 Organization of the Thesis

Chapter 2 presents a non-adiabatic model for photosynthetic energy transfer. Using a dimer model that is loosely based on a pair of excitons in the FMO antenna complex, it is shown that anti-correlated motions along a vibrational coordinate delocalized over both pigments lead to a strong non-adiabatic vibrational-electronic mixing on the excited state. On the ground state of the antenna, this leads to an enhanced excitation of Raman coherence, the frequencies and 2D signatures of which are consistent with all reported signatures of “long-lived electronic coherence” in photosynthetic antennas.
Chapter 3 extends the dimer model presented in Chapter 2 to build a generalized theoretical framework for a dimer with several intra-pigment vibrational modes as well as protein modes delocalized over both pigments. A generalized energy gap tuning coordinate, the one that drives non-adiabatic energy transfer, is established. The 2D signatures of the generalized model are calculated for some simplified cases such as a dimer with an environmental mode, and a dimer with a resonant and a near-resonant FC active vibration on each pigment. It is shown that the ground state signatures discussed in Chapter 2 are in fact enhanced by ~2 times in the presence of additional near-resonant vibrational modes, suggesting additivity of excited state non-adiabatic effects. The role of vibrational relaxation on the ground state of the donor after it has transferred its electronic energy to the acceptor, (missing in other theoretical models in the literature) is also discussed.

Chapter 4 presents absolute measurements of pump-probe signal strength. A generalized theoretical framework for calculating the pump-probe signal for a multi-level system is developed. The framework is simplified for a vibrationally relaxed two electronic level system that is assumed to have no non-radiative cross-sections and obey the Condon approximation. The resulting expression for the pump-probe signal can be written in terms of the sample concentration, absorption and emission cross-sections, transverse pump and probe profiles at overlap, laser spectrum, population relaxation lifetimes and the electronic anisotropy. Experiments on fluorescein in basic methanol demonstrate the agreement between the theory and experiment to better than 10% error. The experiment demonstrates quantitative measurement of excited state populations.

Chapter 5 modifies the theoretical framework developed in Chapter 4 to determine an unknown concentration in terms of a linear absorption spectrum measurement and a non-linear
pump-probe signal measurement. This time-resolved all-optical technique can simultaneously measure an unknown concentration and extinction coefficient without the need for sample isolation or an in situ calibrant. Experiments on fluorescein are used to demonstrate the technique. By comparing the optically determined concentration to the mass and volume prepared concentration, sample purity is also determined. In principle, this technique can be extended to measure the concentration of unknown transient species.

References


CHAPTER 2

ELECTRONIC RESONANCE WITH ANTI-CORRELATED PIGMENT VIBRATIONS DRIVES PHOTOSYNTHETIC ENERGY TRANSFER OUTSIDE THE ADIABATIC FRAMEWORK

The delocalized, anti-correlated component of pigment vibrations can drive non-adiabatic electronic energy transfer in photosynthetic light harvesting antennas. In femtosecond experiments, this energy transfer mechanism leads to excitation of delocalized, anti-correlated vibrational wavepackets on the ground electronic state that exhibit not only two-dimensional spectroscopic signatures attributed to electronic coherence and oscillatory quantum energy transport but also a cross-peak asymmetry not previously explained by theory. A number of antennas have electronic energy gaps matching a pigment vibrational frequency with a small vibrational coordinate change upon electronic excitation. Such photosynthetic energy transfer steps resemble molecular internal conversion through a nested intermolecular funnel.

The contents of this chapter have been adapted from the paper titled “Electronic Resonance with Anti-correlated Pigment Vibrations Drives Photosynthetic Energy Transfer Outside the Adiabatic Framework”, which was published in January 2013 in the Proc. Natl. Acad. Sci. USA of the United States of America. The supplementary information accompanying this paper has been adapted in the appendix to this chapter.

2.1 Introduction

Photosynthesis, which powers life on our planet, is initiated when sunlight is captured by antenna proteins containing light absorbing pigments. The antenna protein positions the pigments to couple them and alters their electronic energies to direct electronic energy transfer toward a reaction center, which stores this energy chemically. Photosynthetic energy transfer is
remarkably fast and efficient, often with quantum yields equal to one within experimental error\textsuperscript{1,2}. Even with atomic resolution structures of several antennas and advances in quantum chemistry that provide the electronic structure and couplings between pigments\textsuperscript{3}, full understanding of the energy transfer mechanism and design principles has remained elusive.

Femtosecond two-dimensional (2D) electronic spectroscopy\textsuperscript{4} has revealed not only energy transfer pathways\textsuperscript{2,5} but also oscillations indicative of quantum mechanical coherence\textsuperscript{2,6}(with a single system having two or more distinct properties at the same time, like Schrödinger’s cat) that can persist throughout the energy transfer process (2D signatures of photosynthetic energy transfer). This coherence spans more than one pigment in the antenna\textsuperscript{6,7}. Curiously, it has many signatures in common with coherence between electronic states, yet the longest lifetimes at cryogenic temperatures are typical of coherence between vibrational states, inspiring studies to elucidate its origin and role in photosynthetic light harvesting\textsuperscript{8-12}.

Following Förster\textsuperscript{13}, electronic energy transfer is conventionally considered in an adiabatic framework (the Born-Oppenheimer approximation\textsuperscript{14}), where the motions of fast electrons are separable from slow vibrations. In this framework, each electronic state has a potential energy surface on which molecules vibrate and non-adiabatic changes in electronic state occur only when the molecules vibrate to a place on the potential surface where the adiabatic approximation breaks down. Within the adiabatic framework, an energy transfer step can be either adiabatic (Förster’s strong coupling) or non-adiabatic (Förster’s very weak coupling). Although Förster recognized that the adiabatic framework would be appropriate for electronic energy transfer in the very weak and strong coupling limits, but not necessarily in between\textsuperscript{13}, he also argued that the very weak and strong coupling limits overlapped for systems with continuous spectra (such as antennas).
“Conical funnels”\textsuperscript{15}, where electronic potential energy surfaces approach so closely that the adiabatic approximation breaks down, play an important role in photochemistry\textsuperscript{15,16}, and often funnel molecules to lower energy electronic states (internal conversion). A conical funnel may be either a conical intersection between adiabatic potential surfaces or a “near miss”, but must allow a change in electronic state before vibrational equilibration.\textsuperscript{15} Recent experiments on molecules\textsuperscript{17} have found that femtosecond pulse driven passage through a conical funnel generates vibrational wavepackets on the ground electronic state that exhibit signatures of coherence between the excited electronic states connected by the funnel. In these experiments, quantum vibrational wavepacket width promotes non-adiabatic motion through the funnel and signatures of electronic and vibrational coherence on the excited state can be rapidly suppressed by weak non-adiabatic coupling\textsuperscript{17}. This observation naturally raises the question of whether similar non-adiabatic dynamics drive photosynthetic energy transfer.

In antennas, several reported 2D signatures of photosynthetic energy transfer match calculations for coherence between excited electronic states and oscillating electronic state populations (quantum transport - QT)\textsuperscript{10,18}. Antenna 2D spectra have overlapping excitonic resonances with oscillatory phase-twisted 2D peakshapes extending beyond resonance; methods for locating peaks and determining phase relationships are contested\textsuperscript{19,20}. Some 2D signatures are incompatible with the usual adiabatic (Franck-Condon – FC)\textsuperscript{14} excitation of vibrational wavepackets. While QT was proposed to explain oscillatory diagonal peaks\textsuperscript{18}, others argued that picosecond beat decay indicates a vibrational origin with amplitudes enhanced by vibrational-electronic coupling\textsuperscript{21}. Here we show that one should expect small amplitude vibrational wavepackets in which two pigments vibrate out of phase, that they give rise to non-adiabatic vibrational electronic mixing between the excited states, and that this mixing leads to delocalized
anti-correlated vibrational wavepackets on the ground electronic state that exhibit the reported signatures. The anti-correlated vibrational wavepackets also generate signatures that have not been explained by previous models incorporating electronic coherence, QT, or vibrations. The model reproduces these signatures when the frequency of a FC active vibration is nearly resonant with the donor-acceptor electronic energy gap; this vibrational-electronic resonance drives energy transfer through non-adiabatic dynamics entirely outside the adiabatic framework.

2.2. Model

The dimer model used for this study starts with two pigments (A and B), each with an identical intramolecular harmonic vibration. Using energy in frequency units, the Hamiltonian for pigment A is

$$\hat{H}_A = \frac{1}{2} \omega_A (\hat{q}_A^2 + \hat{p}_A^2) \hat{l}_A + (E_A - \omega_A d_A \hat{q}_A) |A\rangle\langle A|$$

(2.1)

where $\hat{l}_A$ is the identity operator, $\hat{q}_A$ and $\hat{p}_A$ are the dimensionless position and momentum operators of the vibration with frequency $\omega_A$, $|A\rangle$ is the excited electronic state with vertical excitation energy $E_A$, and $d_A$ is the FC displacement of the vibration. The Hamiltonian for pigment B has the same form, but with a vertical excitation energy difference, $\Delta = (E_B - E_A)$. If either pigment is excited, the two pigments interact through a Coulombic coupling, which can be calculated by the transition dipole approximation at long range; therefore

$$\hat{H}_{\text{dimer}} = \hat{H}_A + \hat{H}_B + J(|A\rangle\langle B| + |B\rangle\langle A|)$$

where the coupling $J$ is assumed to be independent of the intramolecular vibrational coordinates. The two pigment system has a single electronic ground state (no pigments excited), two singly excited electronic states (one for each pigment), and a doubly excited electronic state (both pigments excited). The electronic states of coupled
pigment systems are referred to as (Frenkel) excitons. The singly excited (single exciton) electronic states are delocalized to some extent by the coupling, and the doubly excited state energy differs from $E_B + E_A$ by the bi-exciton binding energy.

The above Hamiltonian is standard in energy transfer. An exact non-adiabatic treatment of the resulting states, their consequences in the 2D spectrum (including signals from the ground electronic state), and role in photosynthetic energy transfer are presented here. For a non-adiabatic treatment, it is useful to project the localized intramolecular vibrational coordinates into correlated and anti-correlated vibrational coordinates, $q_+ = (q_A + q_B)/\sqrt{2}$ and $q_- = (q_A - q_B)/\sqrt{2}$, that are delocalized over both pigments. Förster recognized that the delocalized, anti-correlated vibration, since it tunes the electronic energy gap between pigments, is the relevant vibrational coordinate for driving energy transfer at all coupling strengths, regardless of the extent of electronic delocalization. In the language of conical intersections, $q_-$ is the “tuning coordinate” $g$. In a delocalized, correlated intramolecular vibration, every bond length and angle vibrates with exactly the same phase on both molecules; in the delocalized, anti-correlated intramolecular vibration, every bond in one molecule contracts while it expands in the other (and every bond angle bends in the opposite sense). Proteins can alter pigment vibrations, in this case, correlated and anti-correlated vibrations need not be normal modes or vibrate with equal magnitude on two coupled pigments. This change of vibrational coordinates does not assume that vibrations are delocalized, but reveals that only the projection of a vibration onto the delocalized, anti-correlated coordinate drives and can be driven by non-adiabatic mixing. The non-adiabatic interactions are thus more clearly seen by writing the single exciton Hamiltonian as $\hat{H}_1 = \hat{H}_{\text{cor}} + \hat{H}_{\text{int}}$, with
\[
\hat{H}_{\text{corr}} = \frac{1}{2}(E_A + E_B) + \omega (\hat{q}_+^2 + \hat{p}_+^2) - \sqrt{2} \omega d \hat{q}_+ \hat{I}_1
\]  \quad (2.2)

and
\[
\hat{H}_{\text{int}} = \frac{1}{2} \omega (\hat{q}_+^2 + \hat{p}_+^2) \hat{I}_1 + \left[ \left( \frac{\Delta}{2} - \frac{\omega d \hat{q}_-}{\sqrt{2}} \right) \right] \hat{A} \langle A \hat{A} \rangle + J \hat{A} \langle B \hat{B} \rangle
\]  \quad (2.3)

\hat{H}_1 \text{ uses a basis where the vibrational coordinate is delocalized and the electronic states are localized. Since } \hat{H}_{\text{corr}} \text{ depends only on } q_+ \text{ and } \hat{H}_{\text{int}} \text{ depends only on } q_-, \text{ the adiabatic correlated dynamics are exactly separable from the non-adiabatic anti-correlated dynamics. Thus, a correlated vibration, while it disrupts the quantum phase relationship between the ground electronic state and both single exciton states, does not alter the potential energy difference between single exciton states, does not disrupt their quantum phase relationship, and plays no role in non-adiabatic dynamics. (That is why correlated vibrations oscillate coherently on the excited state throughout the energy transfer process\textsuperscript{23,24}.)} \text{ In contrast, an anti-correlated vibration affects the excited states of pigments } A \text{ and } B \text{ oppositely in } \hat{H}_{\text{int}}, \text{ tunes their potential energy difference, disrupts their quantum phase relationship, and drives non-adiabatic dynamics. Both coordinates affect the absorption lineshape and 2D peakshapes. Because the anti-correlated vibration is delocalized over both coupled pigments, it cannot drive or be driven by non-adiabatic dynamics for a single pigment system, explaining the results of refs.\textsuperscript{6,7}. In contrast to the adiabatic approximation, which first solves for electronic states of the dimer at fixed vibrational coordinates, the non-adiabatic treatment simultaneously solves for mixed vibrational-electronic states (see Appendix).}
Model parameters are very roughly based on one pair of excitons in the Fenna-Matthews-Olson (FMO) complex from green sulfur bacteria\textsuperscript{1}. Its pigment, BChl \textit{a}\textsuperscript{22,25}, has FC active vibrations with frequency $\omega \approx 200$ cm\textsuperscript{-1} and a stabilization energy of $(1/2)\omega d^2 \approx 5$ cm\textsuperscript{-1}. In the dimer model, the transition dipoles of the two pigments are perpendicular. The coupling is $J = 66$ cm\textsuperscript{-1} (typical in FMO\textsuperscript{2}). The difference in electronic energy between the two pigments, $\Delta$, has a static distribution with average $\langle \Delta \rangle = 150$ cm\textsuperscript{-1} (also typical), and variance $\sigma_{\Delta}^2$. $\langle \Delta \rangle$ and $J$ are chosen so the average energy gap between excitons ($\langle \Delta_{EX} \rangle = \langle 2[(\Delta/2)^2 + J^2]^{1/2} \rangle$) matches the vibrational energy $\langle \Delta_{EX} \rangle \approx \omega$. Based on the excitonic peaks in FMO absorption\textsuperscript{26}, such a resonance occurs between the first exciton and an exciton $\sim 200$ cm\textsuperscript{-1} higher; additional resonances seem likely (Tab. 2A.1). The exciton splitting inhomogeneity, $\sigma_{\Delta EX} = 26$ cm\textsuperscript{-1}, is slightly smaller than that determined at 19K from the anisotropy for a pair of excitons separated by $\sim 150$ cm\textsuperscript{-1} in FMO\textsuperscript{27}. Because correlated vibrations do not affect the non-adiabatic dynamics, the damping of correlated vibrations can be incorporated via the non-linear response function formalism\textsuperscript{17}. A critically damped Brownian oscillator with a frequency of 70 cm\textsuperscript{-1} and a stabilization energy of 30 cm\textsuperscript{-1} was used to represent the correlated component of the low energy phonon sideband (this stabilization energy reproduces the Stokes’ shift at 80K\textsuperscript{2}).

Fig. 2.1 shows that the vibrational and electronic states of $\hat{H}_{\text{int}}$ in the adiabatic approximation gradually change electronic character with the anti-correlated vibrational coordinate. The derivatives of these adiabatic wavefunctions lead to non-adiabatic couplings that must be included to calculate the exact states\textsuperscript{16,17}. 
Figure 2.1. Isolated pigment (dashed potential curves and levels), coupled adiabatic (solid potential curves and levels) and exact non-adiabatic (levels not attached to any curve) excited state vibrational-electronic levels for $\hat{H}_{\text{int}}$ of the dimer model with $\Delta_{EX} = \omega$. $v.$ is the quantum number of the anti-correlated vibration. Color shows the electronic state character, which is a linear combination of the two pigment states, at each coordinate (see Appendix). The electronic character does not change for isolated pigments and varies slowly for adiabatic states. Rapid changes in electronic character for the non-adiabatic levels over the entire coordinate range indicate a complete breakdown of the adiabatic framework.
The exact non-adiabatic states (which are both vibrationally and electronically mixed) exhibit a rapid variation in their electronic character over the entire range of coordinates, even though the adiabatic energy levels are reasonably accurate. This breakdown of the adiabatic framework affects both the 2D spectra and the photosynthetic energy transfer process.

With a constant Coulombic coupling between pigments, the adiabatic potentials for excitons in Fig. 2.1 do not intersect and there is no conical intersection (pigments must translate or rotate relative to each other by more than the protein allows in order to send the coupling to zero). However, the non-adiabatic changes in electronic character with anti-correlated vibrational coordinate indicate electronic state changes within half a vibrational period, so there is a funnel. Since Eq. (2.2) indicates the potentials are identical in correlated vibrational coordinates, extending the curves in Fig. 2.1 into 2D surfaces reveals a non-conical funnel in which adiabatic potential surfaces arising from two different molecules are nested inside each other in all intramolecular coordinates; the adiabatic framework breaks down everywhere inside this nested intermolecular funnel. Nesting requires that the vibrational displacement be small compared to the zero-point amplitude, while non-adiabatic coupling requires vibrational displacement or vibrational dependent coupling.

2D spectra are generated by crossing three non-collinear femtosecond pulses (wave vectors \( k_a, k_b \) and \( k_c \)) inside the sample, measuring the four-wave mixing field radiated with wavevector \( k_s = k_c + k_b - k_a \) as a function of \( \tau \) (the delay between pulses \( a \) and \( b \)) and \( t \) (the time after pulse \( c \)) at a fixed \( T \) (the interval between the second and third pulses), and Fourier transforming with respect to \( \tau \) and \( t \). For scans over all \( \tau \), the real part of the 2D spectrum (absorptive 2D spectrum) reveals net changes in sample absorption as a function of both the excitation (\( \omega_\tau \)) and detection (\( \omega_t \)) frequencies. Some of the coherence signatures in 2D
spectroscopy depend on whether \( \tau \) is positive (pulse \( a \) is before pulse \( b \) for “rephasing” 2D spectra) or negative (pulse \( b \) is before pulse \( a \) for “non-rephasing” 2D spectra), and on the physically meaningful relative signs of the frequencies. Figure 2.2 shows an all parallel pulse polarization 2D spectrum for the dimer model at \( T=0 \) (it is simpler than the 2D spectrum of FMO, which involves 7 single exciton states and 21 bi-exciton states). Oscillations in the marked 2D peaks as a function of \( T \) indicate quantum coherence. 2D spectra generally contain contributions from reduced ground state absorption, excited state stimulated emission, and excited state absorption (total 2D spectrum).

In a 2D experiment, the first pulse pair can create vibrational coherence on the ground state (via stimulated Raman scattering) along with both vibrational and electronic coherence on the excited state. An adiabatic picture predicts that vibrational coherence will only cause weak modulations of the 2D spectra because of small FC vibrational displacements in antenna pigments. Furthermore, the adiabatic approximation predicts that vibrational coherence will have the same polarization signatures as electronic state populations. The non-adiabatic wavefunctions illustrated in Fig. 2.1 generate dramatically different behaviour. Stimulated Raman excitation of ground state vibrational wavepackets can involve sets of transition dipoles identical to those involved in excitation of electronic coherence. This electronically enhanced contribution to 2D spectra can be appreciated from Fig. 2.3, which uses wave-mixing diagrams (see Appendix) to show the states and fields for four terms in the nonlinear optical response from the non-adiabatically coupled states of \( \hat{H}_{\text{int}} \). The first (leftmost) frequency in the diagrams is \( \omega_r \) (excitation) and the last (rightmost) frequency is \( \omega_f \) (detection). Pathways corresponding to the diagonal peaks (DP1 and DP2) and the cross-peaks (CP12 and CP21) are arranged according to their position in the 2D spectrum shown in Fig. 2.2. For the pathway contributing to CP12,
Figure 2.2. Real part of the ground electronic state contribution to the “rephasing” 2D electronic spectrum for the dimer model with $(E_A + E_B)/2 = 11,574$ cm$^{-1}$ at a temperature of 80K. Here, the vertical axis is the excitation frequency $-\omega_\tau$, and the horizontal axis is the detection frequency $\omega_t$. The amplitude of the 2D spectrum for each frequency pair is indicated by color and via contours at the 0, 2, 4, 6, 8, 10-90 % levels. Positive and negative contours are solid and dashed, respectively. The waiting time is $T = 0$ fs. The 2D spectra are dominated by 4 resolved peaks, which oscillate in amplitude and shape with $T$. Diagonal peak maxima are marked with an o (lower left - DP1, upper right - DP2); cross-peak maxima are marked with an x (upper left - CP12, lower right - CP21).
Figure 2.3. Wave-mixing pathways (see Appendix) for the oscillatory ground state 2D signal ($D_3$ in ref. (4)) showing resonant enhancement by non-adiabatic coupling of vibrational and electronic levels. For each diagram, the vertical axis is energy and time runs from left to right (neither drawn to scale). The pathways are arranged to correspond with peaks in the “rephasing” 2D spectrum (Fig. 2.2). Delocalized, anti-correlated vibrational levels on each electronic state are indicated by solid lines for $v_\neq 0$, dashed for $v_\neq 1$, and dotted for $v_\neq 2$; their orange and blue colors indicate localized electronic basis states on pigments $A$ and $B$. As in Fig. 2.1, resonant pairs of levels couple to form the non-adiabatic states (the first pair is roughly $|A\rangle|v_\neq 1\rangle \pm |B\rangle|v_\neq 0\rangle/\sqrt{2}$). The orange (blue) vertical lines in the figure represent field-matter interactions utilizing the $A$ ($B$) electronic character of a mixed level, with no change in $v_\neq$, yielding a vibrational overlap integral approaching one. Thus, CP12 is fully electronically enhanced at every step, with all frequencies and transition dipole directions matching those for purely electronic coherence (Fig. 2A.1). Vertical lines in black represent weaker field-matter interactions – these have small vibrational overlap or lack vibrational-electronic resonance. As a result, oscillations of DP1, DP2, and CP21 are not fully electronically enhanced.
extensive mixing between $v_0 = 0$ on $B$ and $v_1 = 1$ on $A$ (illustrated in Fig. 2.1) along the anti-correlated coordinate allows vibrational coherence on the ground state to be excited exclusively through strong electronic transitions with $\Delta v_\perp = 0$ basis state character; the frequencies (arrow lengths) and transition dipole directions (orange vs. blue arrows, using the color scheme of Fig. 2.1) are all the same as those for excited state electronic coherence (Fig. 2A.1). The other paths are similar, but not fully electronically enhanced at each step (black arrows); for example, in DP1 and CP21, interaction with pulse $b$ depends on non-resonant mixing of $|v_\perp = 0\rangle$ on $A$ and its vibrational overlap with $|v_\perp = 1\rangle$ on $G$. As a result, ground state vibrational wavepackets closely mimic signatures of excited state electronic wavepackets. In particular, the transition dipole sequences in Fig. 2.3 show that non-adiabatic excitation of anti-correlated ground state wavepackets will generate oscillations which survive the polarized pulse sequence used on Light Harvesting Complex II (LHCII) from green plants $^{28}$.

### 2.3. Results

Three predicted signatures of electronic coherence between excited states in 2D spectra have been reported for antennas. Signature 1, diagonal peak amplitude beating, was first reported in the absorptive 2D spectra of FMO $^{29}$; Fig. 2.4 shows signature 1 also arises from ground state vibrations for both diagonal peaks in the 2D spectra. Signature 2, a negative cross-peak CP12 beating frequency (for positive $\omega_t$) in the rephas ing 2D spectrum, has been reported for PC645 (the phycocyanin PC645 antenna from the marine cryptophyte Chroomonas CCMP270) $^{30}$, Fig. 2.3 shows signature 2 is also reproduced for non-adiabatic excitation of ground state vibrations (see Appendix and Fig. 2A.1). Signature 3, cross-peak amplitude modulations that occur only in “rephasing” and not in “non-rephasing” 2D spectra, can arise from electronic coherence $^{30}$.
Figure 2.4. Absolute amplitudes and relative phase relationships between the diagonal (DP1 and DP2) and cross-peaks (CP12 and CP21) as a function of waiting time $T$ in the “rephasing” (top) and “non-rephasing” (bottom) ground electronic state contributions to the 2D spectra for the dimer model. The transients are offset (additive constants in boxes) to show phase relationships, but not multiplicatively scaled. The peaks oscillate at a vibrational frequency of 200 cm$^{-1}$ which is in resonance with the excitonic energy gap. In the rephasing 2D spectra, the cross peak beats are 160° out of phase with each other, CP12 beats are 120° ahead of the diagonal peaks and CP12 beats are ~ 14 times stronger than CP21.
without QT. Ref. 30 reports CP12 beats with a signal to noise ratio of 2.4:1 in rephasing, but no CP12 beats (signal to noise 1.2:1) in the “non-rephasing” 2D spectra of PC645. Fig. 2.4 (top vs. bottom) compares the beating amplitudes in the “rephasing” and “non-rephasing” 2D spectra - CP12 beats are ~5x times weaker in the non-rephasing 2D spectrum, which is also consistent with experiment. For non-adiabatic excitation of ground state vibrations, CP12 beats should be detectable with higher signal to noise non-rephasing 2D spectra. The ground state vibrational origin for these 3 signatures naturally explains their robust behaviour at physiological temperature, as recently reported for FMO 31 and PC645 30.

A ~180° phase difference between opposite cross-peak beats has been reported in “rephasing” 2D spectra of PE545 (the phycoerythrin PE545 antenna from the marine cryptophyte Rhodomonas CS24) and attributed to electronic coherence 32. Contradicting this interpretation, for isolated peaks probed on center, Butkus et al. show opposite 2D cross-peaks oscillate in phase for both electronic coherence and adiabatic excitation of vibrations 20. A 160° phase difference arises from non-adiabatic excitation of anti-correlated ground state vibrations (Fig. 2.4 top panel).

The calculations predict another significant experimental difference. According to models of electronic coherence 2 and QT 10, opposite cross-peaks (related by reflection across the 2D diagonal, such as CP12 and CP21) are expected to oscillate with equal amplitudes in a “rephasing” 2D spectrum (see Fig. 5 (top panel) in Ref. 10). In contrast, Fig. 2.4 (top) shows that anti-correlated ground state vibrations create much stronger oscillations on CP12 (with excitation frequency |ωτ| > detection frequency |ωt|) than CP21. Diagrams (Fig. 2.3) show that CP12 involves resonant vibrational-electronic coupling between excited states at each step, while the weaker peak does not. Only beats for CP12, the cross-peak that the non-adiabatic anti-correlated
vibration model predicts to be stronger, have been reported for FMO \(^{18,19}\) and PC645 \(^{30}\). Fig. 3 of Ref. \(^{32}\) shows both cross-peaks for PE545 – they have asymmetric beat amplitudes with the stronger beats as calculated here.

A fourth 2D signature, oscillations of diagonal peaks with phase \(\sim 90^\circ\) behind CP12 oscillations in the rephasing 2D spectrum, \(^{10,18}\) has been interpreted as evidence of QT because electronic coherence alone cannot explain oscillations of the diagonal peaks in a rephasing 2D spectrum. It is not clear what determines the experimental phase relationship in QT models (ref. \(^{18}\) discusses a different phase relationship than shown in Fig.5 of ref. \(^{10}\)). The top panel of Fig. 2.4 shows a 120\(^\circ\) phase relationship between CP12 and DP2 near vibrational-electronic resonance. Ground state vibrational wavepacket oscillations do not require a close resonance between intramolecular vibrational and electronic energy difference frequencies, but are enhanced within a broad maximum (\(~130\ \text{cm}^{-1}\) width) around resonance (Fig. 2A.2). However, away from resonance, where non-adiabatic vibrational-electronic mixing is reduced and energy transfer between the pigments is presumably less efficient, the calculations show a 180\(^\circ\) phase relationship between CP12 and the diagonal beats (Fig. 2A.2). Therefore, a 90\(^\circ\) phase relationship would suggest that electronic energy transfer involves non-adiabatic vibrational-excitonic resonance in FMO.

2.4. Discussion

Although peak overlap in the experimental 2D spectra of antennas can distort the amplitude and phase relationships found here for resolved peaks, the above observations suggest that efficient energy transfer proceeds through non-adiabatic interaction between two excitons that is resonantly enhanced by a FC active vibration of the monomeric pigment. The delocalized,
anti-correlated vibration of the pigments may be the key component of the bath that exchanges energy with the electronic system in QT models. When the resonant mechanism is operative, the average energy gap between excitons should match a pigment vibrational frequency in the fluorescence or resonance Raman spectrum. 7 out of 9 off-diagonal beating frequencies reported for FMO match FC active vibrational frequencies of \textit{BChl} \textit{a} (Tab. 2A.1). The two frequencies that do not match have only been reported in ref. 19. Furthermore, the vibrational frequencies that match off-diagonal beat frequencies in FMO are remarkably stable with respect to structural and isotopic perturbations (Tab. 2A.1). Turner et al. 30 remark that pigment vibrational frequencies are close to frequencies in “rephasing” 2D spectra of PC645.

The calculations above all show the contribution to the 2D spectrum from ground state vibrations. The ground state signal is only driven by initial dynamics on the excited states while still coherent with the ground state, so it can be accurately calculated without including slower relaxation between excited states. Decay of coherence between the ground and excited states is included through damped correlated vibrations and gives rise to the anti-diagonal width of the diagonal peaks (for the real absorptive 2D spectrum of the dimer model, this ~80 cm\textsuperscript{-1} width at waiting time $T=0$ appears roughly comparable to that for the resolved lowest exciton peak in the 2D spectrum of FMO 29). Electronic coherence plays no role in the timescales over which oscillations on the ground electronic state persist; they decay through vibrational dephasing on the ground electronic state.

Given that the key vibrational-electronic resonance between two excitons is established by the electronic absorption spectrum of FMO 26 and the fluorescence spectrum of \textit{BChl} \textit{a} 25, the above signatures of delocalized anti-correlated vibrations should be present in the 2D spectra of FMO 18,31. They also seem to arise in antennas from cryptophyte marine algae, 30,32 which harvest
different wavelengths. The breakdown of the adiabatic framework drastically alters the modelling and interpretation of antenna 2D spectra. Figure 2.5 shows coherence signatures from the total 2D spectrum, which includes excited state contributions (but without any relaxation of population or coherence between excited states). The experimentally reported phase relationships and asymmetrical cross-peak oscillation signatures in Fig. 2.4 are obscured by excited state coherences. DP2 has a dominant oscillation with ~1 ps period arising from the splitting between its nearly degenerate non-adiabatic levels (the 2nd and 3rd levels in Fig. 2.1). DP1 has its deepest oscillations at the excitonic splitting $\Delta_{ex}$ in the non-rephasing 2D spectrum; these arise from electronic coherence and decay within ~300 fs in Fig. 2.5. Comparison to Fig. 2.4 shows that amplitudes for ground and excited state beats at the vibrational-excitonic frequency are comparable after ~300 fs.

Broadly, three types of coherence at a common frequency $\Delta_{ex} \approx \omega$ arise with resonant non-adiabatic coupling, so there are roughly three coherence decay timescales. First, 2D signatures of excited state coherence at $\Delta_{ex}$ (for resonance, $\Delta_{ex} \approx \omega$) disappear on a timescale dictated by anti-correlated inhomogeneity and coupling; these are gone by ~300 fs for the dimer model ($\sigma_{AEV} = 26 \text{ cm}^{-1}$) and disappear by ~200 fs for $\sigma_{AEV} = 34 \text{ cm}^{-1}$ [the anisotropy beat decay for this exciton splitting inhomogeneity is ~180 fs in FMO$^{27}$] even though the model does not include any coherence decay for an individual dimer. Second, relaxation of excited state coherence at the vibrational frequency $\omega$ (for resonance, $\omega \approx \Delta_{ex}$) may have aspects of both vibrational and electronic coherence decay (perhaps this relaxation generates electronic coherence signatures identical to those already present on the ground state - if not, this coherence probably decays before ground state signatures are seen). Third, coherent vibrations on the ground state (and possibly correlated vibrations on the excited state) at $\omega$ should decay with
Figure 2.5. Absolute amplitudes and relative phase relationships between the diagonal and cross-peaks as a function of waiting time $T$ in the total “rephasing” (top) and “non-rephasing” (bottom) 2D spectra for the dimer model, including ground state bleaching, excited state emission, and excited state absorption for zero bi-exciton binding energy. Vertical scales can be compared directly to Fig. 2.4. So long as the irregular excited state oscillations persist, the phase relationships in Fig. 2.4 will be obscured and CP21 beats will have roughly the same amplitude as CP12 beats.
typical vibrational timescales (~ps). As a result, ground state vibrational coherence is likely to survive longest, with frequencies that are non-adiabatically enhanced by vibrational-excitonic resonance generating the most persistent observed signatures. For antennas, the timescale on which the excited state coherence signatures in Fig. 2.5 decay and give way to the ground state coherence signatures in Fig. 2.4 is not yet clear, but it could be as short as ~200 fs for FMO at 80 K.

Signatures found here may not be unique to nonadiabatic vibrational-excitonic resonance; for example, asymmetries across the 2D diagonal can occur for FC excitation of vibrations upon electronic excitation. Models for antennas are needed to quantitatively test how much of the beating can be accounted for by resonant electronic enhancement of ground state vibrations. Polarization sequences may provide additional signatures. By analogy to the two-color pump-probe experiments that Vos et al. used to distinguish between ground state and excited state vibrations, two-color 2D spectra with pulses $a/b$ of one color and pulse $c$ of another could separately probe for ground and excited state beating.

For energy transfer outside the adiabatic framework, the protein has at least four ways to control energy transfer: first, it can control the electronic coupling; second, it can control the excitonic energy gap; third, it can control the vibrational displacement; and fourth, it might control how the coupled vibration dissipates energy. Comparing two related phycocyanins, Womick and Moran have calculated that a vibrational-excitonic resonance in one speeds up energy transfer. Resonance with a FC active vibration (or group of vibrations) may provide a way for the protein to select the energy acceptor for each donor. The first three control mechanisms dictate the strongest non-adiabatic interactions and establish a nested intermolecular funnel; the fourth may dictate how population relaxes down to the lowest, electronically
decoupled \( v=0 \) level, which completes the electronic energy transfer process (so long as \( \hbar \omega_{\text{vb}} > k_B T \)). Vibrational relaxation on the ground electronic state of the isolated pigments is relevant to this completion; in a fully localized basis, energy transfer from the \( v=0 \) level of excited pigment \( B \) can leave pigment \( A \) electronically excited with \( v=0 \) and pigment \( B \) in its ground electronic state with \( v=1 \) (a possibility excluded by approximations in refs. 21,34,35).

2.5. Conclusions

Reported signatures of photosynthetic energy transfer in the 2D electronic spectra of antennas (Fig. 2A.3, Tab. 2A.2) point towards non-adiabatic vibrational-electronic mixing resonantly enhancing the amplitude of delocalized, anti-correlated vibrational wavepacket motion on the ground electronic state, which is likely to outlive electronic and vibrational-electronic coherence. Furthermore, the present mechanism predicts an asymmetry between opposite cross-peak oscillations (found for an antenna from cryptophyte marine algae, PE545), but not explained by electronic coherence or QT. The mechanism also predicts a reduced amplitude cross-peak beating in non-rephasing 2D spectra that is consistent with experiment, but has not yet been reported. Although quantitative models with both non-adiabatic vibrational-electronic resonance, electronic decoherence, and relaxation are needed to develop a deeper picture of and perspective on this energy transfer mechanism, it is remarkable how closely beat frequencies in the 2D spectra of the FMO complex match the frequencies of FC active skeletal vibrations in its bacteriochlorophyll \( a \) pigment. Thus, while additional studies are needed, it seems to us likely that resonant non-adiabatic coupling plays a role in photosynthetic energy transfer and that vibrational-electronic resonances in nested intermolecular funnels are an important design principle.
2.6 Appendix 2A

Methods: Non-adiabatic dynamics are fully incorporated using quantum states of the dimer Hamiltonian in sum over states formulas for the nonlinear optical response. 2D spectra also reflect interactions with the bath, which should be decomposed into correlated and anti-correlated parts. To approximate the anti-correlated component of both the phonon sideband and static inhomogeneities in the protein, the non-adiabatic problem is solved for members of an ensemble with a static Gaussian distribution of electronic site energy differences ($\sigma = 34 \text{ cm}^{-1}$) and the non-linear responses are added. The temperature was fixed at 80 K to match experiments on FMO. The sum over states response was multiplied by the correlated Brownian oscillator response and 2D Fourier transform (FT) spectra were calculated (see Appendix) using a 3D FT algorithm. Calculation of a waiting time series of 2D spectra took approximately 3 hours on two hex-core 2.8 GHz Intel Westmere processors.

Construction of Figure 2.1: In Fig. 2.1, the character of the electronic wavefunction has been mapped onto a color wheel in terms of the two monomeric pigment electronic basis states. Orange indicates pigment A and blue indicates pigment B. The potential energy curves for the isolated pigments, shown as dashed orange and blue curves, intersect and cross at about $q_c \approx 72.4$. (For reference, the classical turning points of the zero point level are located at $q_c = \pm 1$.) The vibrational energy eigenstates of the isolated pigments, which have electronic character independent of the vibrational coordinate, are shown with dashed horizontal lines. (In the language of non-adiabatic theory, the isolated pigment states are a set of diabatic basis states.)

The Born-Oppenheimer adiabatic eigenstates, attached to their respective potential energy curves, are shown as solid lines with coordinate dependent color. The symmetric and
antisymmetric combinations of $A$ and $B$ that arise at the avoided crossing near $q_7 \approx -2.4$ are shown as purple-red and yellow-green, respectively. The curves are generated by neglecting vibrational momentum in the Hamiltonian, treating the vibrational coordinate operator as a parameter, and diagonalizing to obtain the coordinate dependent electronic energies

$$U_\pm(q_\pm) = \frac{1}{2} \omega q_\pm^2 \pm \sqrt{(\Delta_+ / 2 + \omega d_+ / \sqrt{2})^2 + J^2}$$

that form the effective potential energy for the vibrational motion. The coordinate dependence of the color is taken from the expansion coefficients when writing the eigenfunctions as linear combinations of isolated pigment basis functions. Using $\{\beta^+;q_-,\alpha^-;q_-,\}$ and $\{|A\},|B\}$ to indicate the set of electronic wavefunctions in the adiabatic and isolated pigment approximations, we get

$$|\beta^+;q_-\rangle = \frac{1}{\sqrt{N}} \left( J \langle A | + \left( (\Delta_+ / 2 + \omega d_+ / \sqrt{2}) + \sqrt{(\Delta_+ / 2 + \omega d_+ / \sqrt{2})^2 + J^2} \right) |B\rangle \right)$$

$$|\alpha^-;q_-\rangle = \frac{1}{\sqrt{N}} \left( -\left( (\Delta_+ / 2 + \omega d_+ / \sqrt{2}) + \sqrt{(\Delta_+ / 2 + \omega d_+ / \sqrt{2})^2 + J^2} \right) |A\rangle + J |B\rangle \right),$$

where $N$ is a normalization constant. In the language of energy transfer, the Born-Oppenheimer adiabatic electronic states at $q_7 = 0$ are the excitonic eigenstates of the purely electronic Hamiltonian (which neglects anti-correlated vibrational motion). For the parameters of the dimer Hamiltonian, Fig. 2.1 shows that each adiabatic excitonic eigenstate is approximately localized on the corresponding pigment – this picture is misleading.

In the adiabatic approximation, each total eigenfunction is a product of one of the two coordinate dependent electronic eigenfunctions above with a vibrational eigenfunction $\psi_{\nu}^{\alpha-(or \beta+)}(q_-)$ of its potential curve $U_\pm(q_-)$. The adiabatic vibrational-electronic eigenstates shown in Fig. 2.1 are the harmonic oscillator vibrational levels for each potential surface (the potential curves are very nearly harmonic). Based on the analysis of Born and Huang, some
authors distinguish between the Born-Oppenheimer and adiabatic approximations, including the
diagonal energy correction from the non-adiabatic coupling in the Born-Oppenheimer but not
adiabatic energy levels $^{37}$ or vice versa $^{14,38,39}$. The diagonal energy correction from the non-
adiabatic coupling is not included in Fig. 2.1, but is relatively small for any given level (less than
3 cm$^{-1}$). The diagonal energy correction has no effect on the electronic wavefunction $^{39}$. Within
both the Born-Oppenheimer and adiabatic approximations, the electronic character depends only
on the vibrational coordinate, not on the vibrational quantum number, so that each vibrational-
electronic level has a coloring that matches the adiabatic potential energy curve it belongs to.

Starting from the adiabatic states, the non-adiabatic calculation includes couplings (given
by Eq. (VIII.7) of ref. $^{36}$) that depend on wavefunction derivatives $^{16}$. The exact non-adiabatic
energy levels and eigenfunctions are more easily obtained by directly diagonalizing the
Hamiltonian for the dimer model in the diabatic basis. $^{16}$ The energy differences between the
Born-Oppenheimer adiabatic and corresponding exact non-adiabatic levels range up to 15 cm$^{-1}$
for the states shown in Fig. 2.1. In the isolated pigment basis, the exact eigenfunctions are
\[
|\Psi_n\rangle = \sum_v c_{n,v}^A |v\rangle |A\rangle + \sum_v c_{n,v}^B |v\rangle |B\rangle
\]
\[
= \chi_n^A(q_-) |A\rangle + \chi_n^B(q_-) |B\rangle
\]
\[
= \psi_n(q_-) \{ \cos[\theta_n(q_-)] |A\rangle + \sin[\theta_n(q_-)] |B\rangle \} .
\]

$\chi_n^{A(\text{or } B)}(q_-)$ describes the coordinate-dependent amplitude for the $A$ (or $B$) isolated pigment’s
electronic wavefunction in the exact eigenfunction $n$. ($n$ is an index used to order eigenfunctions
according to their energy eigenvalue.) The coordinate-dependent electronic character is given by
the angle $\theta_n(q_-) = \arctan \left( \frac{\chi_n^B(q_-)}{\chi_n^A(q_-)} \right)$. $\theta = 0$ corresponds to $|A\rangle$, $\theta = \pm \pi / 4$ correspond to
$(|A\rangle \pm |B\rangle) / \sqrt{2}$, and $\theta = \pm \pi / 2$ corresponds to $|B\rangle$. The coordinate dependent vibrational
wavefunction amplitude is \( \psi_n(q_-) = \sqrt{\chi_n^A(q_-)^2 + \chi_n^B(q_-)^2} \). The dependence of the coordinate dependent mixing angle \( \theta_n(q_-) \) on \( n \) shown in Fig. 2.1 indicates that the set of wavefunctions cannot be approximately separated into products involving only two coordinate dependent electronic wavefunctions, as in the adiabatic approximation. A fully non-adiabatic picture does not involve a vibrational potential energy curve; the non-adiabatic levels are not connected to any curve. In Fig. 2.1, the length of each line segment representing a non-adiabatic level has been chosen to contain the same percentage of the probability density as is contained between classical turning points for the dominant isolated pigment state.

**Calculation of 2D Spectra:** The calculation is based on a standard two pigment dimer Hamiltonian (as described in the text) in a complete (direct product) diabatic basis of vibrational and electronic states. The direct product basis state notation differs from the more compact notation used above and in the paper. In the direct product basis, the ground electronic state of the dimer is represented by \( |0_A\rangle |0_B\rangle \), the singly excited electronic states of pigments A and B in the dimer are represented by \( |A\rangle |0_B\rangle \) and \( |0_A\rangle |B\rangle \), respectively, and the doubly excited state of the dimer is represented by \( |A\rangle |B\rangle \) (both pigments excited). The harmonic oscillator vibrational basis states in the respective pigments are \( |\nu_A\rangle \) and \( |\nu_B\rangle \), where \( \nu_A \) and \( \nu_B \) specify harmonic oscillator quantum numbers (non-negative integers). Every electronic basis state must be multiplied by \( |\nu_A\rangle |\nu_B\rangle \), to specify the vibrational basis state. Complete vibrational-electronic basis states are thus \( |0_A\rangle |0_B\rangle |\nu_A\rangle |\nu_B\rangle \) for the ground electronic state, \( |A\rangle |0_B\rangle |\nu_A\rangle |\nu_B\rangle \) and \( |0_A\rangle |B\rangle |\nu_A\rangle |\nu_B\rangle \) for excitation of pigments A and B, respectively, and \( |A\rangle |B\rangle |\nu_A\rangle |\nu_B\rangle \) for the
doubly excited state. Unlike the Coherent Exciton Scattering approximation and Vibronic Exciton model, the calculation here includes states with vibrational excitations on electronically unexcited pigments (excited states of the dimer in which one pigment is electronically excited and the other is vibrationally excited are sometimes referred to as “two-particle excitations”). The anti-correlated ground state vibrational wavepackets that give rise to the signatures in Fig. 2.4 occur on the ground electronic state \(|0_A \rangle |0_B \rangle\) of the dimer. As shown in Fig. 2.3, their excitation involves correlated vibration on the singly excited (single-exciton) states of the dimer. The vibrational basis states for all electronic states are identical, chosen as vibrational eigenstates of the ground state pigments. Because they are not displaced with respect to the ground state, they are not vibrational eigenstates on the excited electronic states of the isolated pigments. The displacement is included in the Hamiltonian and treated on an equal footing with the coupling between pigments. This approach simplifies the expression for the transition dipole moments.

In the direct product basis, the Hamiltonian for isolated pigment \(A\) is

\[
\hat{H}_A = \frac{1}{2} \omega_A (\hat{q}_A^2 + \hat{p}_A^2) \hat{I}_A + (E_A - \omega_A d_A \hat{q}_A) |A\rangle \langle A|,
\]

(2A.7)

where \(\hat{I}_A = |0_A \rangle \langle 0_A| + |A\rangle \langle A|\) and all other symbols have the same meaning specified in the main text. The matrix elements for the operator \(\hat{q}_A\) of the dimensionless normal coordinate are given by Appendix E.1 of ref. and couple \(\nu_A\) to \(\nu_A \pm 1\). The Hamiltonian for isolated pigment \(B\) is obtained by replacing \(A\) with \(B\) everywhere. The harmonic oscillator frequencies and displacements with respect to the ground state equilibrium geometry upon electronic excitation are assumed identical for the two pigments. The two singly excited states have a coordinate independent off-diagonal coupling, \(J\), with
in the direct product basis. \( \delta \) is the bi-exciton binding energy for the doubly-excited state. It should be noted that the coupling is not restricted to proceed through the zero point level on the ground electronic state, as in refs. \(^{21,34}\). The Hamiltonian of the interacting dimer is

\[
\hat{H}_{\text{dimer}} = \hat{H}_A + \hat{H}_B + \hat{H}_{\text{coupling}}. \tag{2A.9}
\]

When the bi-exciton binding energy \( \delta \) is zero, \( \hat{H}_{\text{dimer}} \) is the Frenkel-exciton Hamiltonian given by Equations 5 to 7 of ref. \(^{44}\) (with a harmonic approximation to the monomeric pigment’s vibrational potential energy surface). The excited state absorption contribution to the signal is sensitive to the bi-exciton binding energy; however, for a bi-exciton binding energy of 40 cm\(^{-1}\), differences in the coherence signatures are small.

The two singly excited diabatic basis electronic states are distinguished from one another by the directions of their transition dipoles from the ground state to the singly excited state. In the diabatic basis, these are assumed to be coordinate independent, equal in magnitude, and perpendicular, i.e. \( \langle 0_A | \mu | A \rangle = \mu \hat{x} \) and \( \langle 0_B | \mu | B \rangle = \mu \hat{y} \). The coordinate independence of these transition dipoles in the diabatic basis amounts to assuming the Condon approximation\(^{45}\) holds for each isolated pigment, but does not imply that the Condon approximation holds for the coupled dimer. The Condon approximation is formulated within the adiabatic framework, and depends upon the existence of a single (vibrational coordinate dependent) electronic eigenfunction that multiplies every vibrational eigenfunction of the potential surface; two adiabatic electronic eigenfunctions generate a coordinate dependent electronic transition dipole moment function between the two electronic states. The Condon approximation neglects the coordinate dependence of this transition dipole function. Even in the adiabatic approximation,
the coordinate dependent color variation in Fig. 2.1 shows that the Condon approximation fails quantitatively. Once the non-adiabatic singly excited state Hamiltonian is solved, the vibrational level dependent variation in electronic character with coordinate shown in Fig. 2.1 indicates the Condon approximation cannot be meaningfully discussed (except for emission from the lowest level). Each transition dipole from a vibrational level of the ground state to a vibrational-electronic eigenstate on the singly excited state has a direction and magnitude that depend on both the initial and final state. As shown in Fig. 2.3, the breakdown of the Condon approximation plays a role in excitation of anti-correlated vibrational wavepackets. In the context of 2D infrared spectroscopy, a vibrational anti-correlation for two modes coupled to the same bath has been included in the Reduced Hierarchy Equations 46.

The numerical calculation of the 3D time domain nonlinear impulse response function for the dimer Hamiltonian (Eq. (2A.9)) includes 9 quanta of vibration for both $|\psi_1\rangle$ and $|\psi_2\rangle$ on all 4 electronic states. The impulse response function 4 for each diagram (such as shown in Fig. 2.3) is calculated on a 3D time grid with 384 time points in each dimension and time increments of 15 fs. For each diagram, the orientational average (see below) is calculated for an all parallel pulse polarization sequence. Decoherence 47 between electronic states with different numbers of excitons is included using the Brownian oscillator model 48. Eqs. (3) and (4) in ref. 49 (as corrected in refs. 50 and 51) show that correlated and anti-correlated decoherence both act to broaden the anti-diagonal cross-width of 2D peaks. Correlated decoherence is included by multiplying the nonadiabatic 3D time domain impulse response function from each diagram point by point with the 3D time domain impulse response for damped correlated vibrations for the same diagram. The analytic formulas for the nonlinear response from a four-level electronic system with damped harmonic motion at the same frequency on all four surfaces are given by
Eq. (3) of refs. 49-51, which assumes correlated vibrational motion on the singly excited electronic states. The lineshape function is that for a quantum mechanical critically damped Brownian oscillator (given by Eq. (13) of ref. 52), evaluated using the subroutine cdqogoftarrayR2.f90 available in the Electronic Physics Auxiliary Publication Service (EPAPS) for refs. 53,54. The resulting contribution to the 3D time domain impulse response is then added to that obtained from prior diagrams (sum over states). To get the nonlinear polarization for a finite pulse duration, the resulting impulse response is multiplied by a frequency filter 55 corresponding to a transform limited Gaussian laser pulse with a 20 fs full-width at half-maximum (FWHM) of the intensity and a frequency centered at the energy gap between the ground and singly excited states i.e. $\omega_{\text{laser}} = \omega_{eg} = 11,574 \text{ cm}^{-1}$ where $\omega_{eg}$ is defined by $(E_A + E_B)/2$. $E_A$ and $E_B$ are the site energies for the singly excited electronic states corresponding to excitation in pigments $A$ and $B$ respectively. 2D spectra are averaged over a Gaussian distribution of site energy gaps $\Delta = E_B - E_A$ ranging from 80 cm$^{-1}$ to 220 cm$^{-1}$ centered on an average site energy gap $\langle \Delta \rangle = 150 \text{ cm}^{-1}$ with standard deviation $\sigma_\Delta = 34 \text{ cm}^{-1}$. $(E_A + E_B)/2$ is held constant during this average (in other words, the ensemble average uses a purely anti-correlated inhomogeneity). To match the conditions of ref. 29, the calculations were carried out at a temperature of 80 K. To make the sum-over-states scheme for response function calculation computationally less expensive, starting states with fractional population less than $10^{-2}$ relative to the ground state are neglected. The neglect of these states leads to a neglect of < 0.6 % of the thermal population for a vibrational frequency of 200 cm$^{-1}$. Transition dipole matrix elements with magnitudes below $\mu \cdot 10^6$ were neglected as zero.

The 3D impulse response for the dimer does not include anti-correlated decoherence, so each member of the inhomogeneous ensemble retains coherence between the two singly excited
electronic states. However, the anti-correlated inhomogeneity does cause anti-correlated
dehphasing (see Fig. 2.5); based on Eq. (32) of ref. $^56$, $\sigma_{\Delta \mu} = 34 \text{ cm}^{-1}$ will cause beats at $\Delta$ in the
pump-probe polarization anisotropy to decay to half their initial amplitude in $\sim 180$ fs (cf. ref. $^27$),
which is a lower bound for the anti-correlated decoherence timescale in the Fenna-Matthews-
Olson (FMO) complex. Because it enters multiplicatively, the correlated decoherence (which
decays on a $\sim 100$fs timescale at 80K and solely determines the anti-diagonal cross-widths in the
2D spectrum of the dimer model) will, to a good approximation, prevent slower relaxation of the
excited electronic states (e.g. anti-correlated decoherence) from influencing the ground electronic
state contribution to the 2D spectrum. This correlated decoherence which is faster than anti-
correlated dephasing justifies the neglect of excited state relaxation in calculating the ground
state 2D spectra – the results should be reasonably accurate for waiting times less than the
relaxation time of the anti-correlated vibrations on the ground state. Because these ground state
signals resemble those previously expected for the excited state, the excited state 2D spectra
depend strongly on relaxation processes that are not yet characterized. No excited state relaxation
is included in these calculations – the results may be reasonably accurate for waiting times less
than the shortest excited state relaxation time.

**Wave-Mixing Diagram Calculations:** Each wave-mixing diagram, such as those in Fig. 2.3
and Fig. 2A.1, represents one term in the density matrix perturbation theory (sum over states)
calculation of the nonlinear optical impulse response. In the diagrams, time runs from left to
right, and the vertical scale indicates the energy of each level. All arrows must originate from
one initial state and all arrows of the same type (solid or dashed) must connect head to tail. The
type of arrow is connected to the phase matching condition: solid and upward or dashed and
downward for pulses with a positive wave-vector ($b$ and $c$); dashed and upward or solid and
by sequentially applying first order time dependent perturbation theory to the bra/ket indices of the density matrix, the following general rules can be used to write down the contribution from each diagram to the four-wave mixing signal field: 1) A dashed arrow connecting levels \(a\) and \(b\) in a wave-mixing diagram represents a field matter interaction that transforms bra index \(|a\rangle\) into bra index \(|b\rangle\) with amplitude
\[-i\mu_{ab} \cdot \hat{E}(\omega_{ab}) / \hbar.\] 2) A solid arrow represents a field-matter interaction that transforms ket index \(|a\rangle\) into ket index \(|b\rangle\) with amplitude \(i\mu_{ba} \cdot \hat{E}(\omega_{ba}) / \hbar\). In both cases, \(a\) is the state where the arrow originates and \(b\) is the state where the arrow terminates. 3) Between field-matter interactions, the density matrix element \(|m\rangle\langle n|\) with bra index \(|n\rangle\) and ket index \(|m\rangle\) of an isolated system oscillates in time as
\[
\rho_{mn}(t') = \rho_{mn}(t) \exp\left(-i\omega_{mn}(t'-t)\right),
\] where \(\omega_{mn} = (E_m - E_n) / \hbar\) is the Bohr frequency. 4) After pulse \(c\), the oscillating dipole between the exposed bra and ket indices, which are connected by a wavy line in the diagram, radiates a four-wave mixing signal with electric field vector parallel to the oscillating dipole. The signal field interferes with the electric field of the detection pulse, generating intensity interference proportional to their dot product. The frequencies detected in a 2D experiment are given by Eq. (2A.10), with the frequency shown in the diagrams. Thus, the sign of the frequency is positive when the ket level is above the bra level, which means that the solid arrow terminates above the dashed arrow. In the diagrams shown in Fig. 2.3, the first arrow, which gives the excitation frequency in a 2D spectrum, is dashed, so the 2D peak has a negative excitation frequency \(\omega_c\). After pulse \(c\), the wavy line gives the detection frequency in a 2D spectrum. Because the last solid arrow has terminated above the last dashed arrow in the diagrams shown, the 2D peaks
have a positive detection frequency $\omega_t$. If the time interval between pulses is considered to be negative (for example, the $a$-$b$ delay $\tau$ is usually considered negative when pulse $b$ arrives at the sample before pulse $a$ in the “non-rephasing” pulse sequence), this negative time interval reverses the sign of the conjugate frequency recovered by Fourier transformation. Thus, the “non-rephasing” 2D spectrum has peaks at negative $\omega_\tau$ and positive $\omega_t$. For CP12, during the positive waiting time $T$ between pulses $b$ and $c$, the dashed arrow has terminated above the last solid arrow (which is not present, indicating that the ket level is still $v.=0$ of $G$), so the 2D peak oscillates with a negative frequency conjugate to $T$ for CP12, as reported by Turner et al. $^{30}$.

For a diagram to contribute to the four-wave mixing signal measured to record 2D spectra, it must have a non-zero orientational average for the polarization vectors of the pulses. The calculations here have used all parallel polarizations. A polarization sequence originally designed to isolate the antisymmetric component of the Raman scattering tensor in four-wave mixing experiments $^{58}$ and subsequently shown to emphasize cross-peaks in 2D infrared spectra $^{59}$ has been applied to selectively detect coherent contributions to the 2D spectra of photosynthetic antennas $^{28,60}$. The polarization sequence is based on the requirement that, for a given diagram to generate four-wave mixing signal in an isotropic medium, the orientationally averaged transition dipole product must be non-zero. These orientational averages are treated in ref. $^{61,62}$. The orientationally averaged transition dipole product is

$$\left\langle (\mathbf{\mu}_a \cdot \hat{\mathbf{E}}_a)(\mathbf{\mu}_b \cdot \hat{\mathbf{E}}_b)(\mathbf{\mu}_c \cdot \hat{\mathbf{E}}_c)(\mathbf{\mu}_s \cdot \hat{\mathbf{E}}_d) \right\rangle$$

where $\mathbf{\mu}_a$ is the transition dipole moment vector for the transition excited by pulse $a$ with electric field vector $\hat{\mathbf{E}}_a$, $\mathbf{\mu}_b$ is excited by pulse $b$ with field $\hat{\mathbf{E}}_b$, $\mathbf{\mu}_c$ is excited by $\hat{\mathbf{E}}_c$, and the field radiated with transition dipole moment vector $\mathbf{\mu}_s$ is detected by interference with a field $\hat{\mathbf{E}}_d$. 
The brackets indicate an average over all angular orientations of the photosynthetic antenna. So long as two sequences of transition dipole moment vector directions are the same, their orientationally averaged transition dipole products will be proportional for every field polarization sequence. These sequences can be read off the diagrams (Fig. 2A.1).

**Calculation of 2D Peak Oscillations:** The amplitude oscillations with $T$ for the 4 peaks in the 2D spectrum are extracted at the 2D frequency coordinates of their maxima in the real part of the rephasing 2D spectra at waiting time $T = 0$. This use of $T = 0$ maxima appears to be the procedure followed in ref. 32 and 20. Because the oscillations arise from phase-twist beats, the phase of the oscillations found with this approach is quite sensitive to displacement of the 2D frequency coordinates away from the peak maximum. As implemented here, this procedure ignores any changes in frequency of the real maxima as peaks evolve with $T$; such changes in frequency occur with a time dependent Stokes shift. (Using an alternative procedure, ref. 19 plots oscillations of maxima in the absolute value of the rephasing 2D spectra.) Fig. 2.4 shows oscillations with $T$ in the real part of the ground state contribution to the ensemble averaged “rephasing” 2D spectrum (Fig. 2.2). The corresponding 2D peak coordinates (in cm$^{-1}$) are: DP1 ($\omega_\tau$, $\omega_t$) = (-11487, 11476), DP2 ($\omega_\tau$, $\omega_t$) = (-11690, 11678), CP12 ($\omega_\tau$, $\omega_t$) = (-11684, 11499) and CP21 ($\omega_\tau$, $\omega_t$) = (-11510, 11673). It should be noted that the “diagonal” peaks have maxima with $|\omega_\tau|$ slightly greater than $|\omega_t|$, a consequence of the Stokes shift in the Brownian oscillator model. Fig. 2.5 shows oscillations with $T$ in the real part of the total (including ground state, excited state emission, and excited state absorption contributions) ensemble averaged “rephasing” 2D spectrum. The corresponding 2D peak coordinates used are: DP1 ($\omega_\tau$, $\omega_t$) = (-11487, 11476), DP2 ($\omega_\tau$, $\omega_t$) = (-11690, 11678), CP12 ($\omega_\tau$, $\omega_t$) = (-11696, 11481) and CP21 ($\omega_\tau$, $\omega_t$) = (-11522, 11661).
Figure 2A.1 compares wave-mixing diagrams contributing to oscillations of cross-peaks CP12 (top row) and CP21 (bottom row) in the rephasing 2D spectrum. The oscillation frequency of these peaks as a function of waiting time $T$ is given by the coherent superposition oscillation frequency between pulses $b$ and $c$ (see Appendix). Diagrams on the left arise from purely electronic coherence (in the excited state emission (ESE) signal), while those on the right arise from non-adiabatic excitation of anti-correlated vibrations on the ground electronic state (in the ground state bleach (GSB) signal). Levels of the ground electronic state are black, and the excited state levels of the isolated pigments $A$ and $B$ are orange and blue, respectively (as in Fig. 2.1 and Fig. 2.3). The electronic coherence model (left column) does not incorporate vibrations in the system. In the ground state anti-correlated vibration model (right column), the lowest $v_-=0$ vibrational level for each state is shown as a solid horizontal line, $v_-=1$ levels are dashed, and $v_-=2$ levels are dotted. The GSB diagrams on the right are the same ones shown in Fig. 2.3.

For CP12, all frequencies (arrow lengths) and transition dipole directions (arrow colors) are the same for both models, and all transitions are electronically enhanced by resonance. In this resonance, pairs of isolated pigment states with approximately the same energy are strongly non-adiabatically mixed by the dimer Hamiltonian: $v_-=1$ of pigment $A$ is strongly mixed with $v_-=0$ of pigment $B$; $v_-=2$ of pigment $A$ is strongly mixed with $v_-=1$ of pigment $B$; and so on. This resonant mixing accounts for much of the color variation shown in Fig. 2.1. For the purposes of this discussion, we approximate the mixed excited state reached by arrows in the CP12 diagram on
the right of Fig. 2A.1 as \(|A|\psi_1 = 1\rangle - |B|\psi_0 \rangle\) / \(\sqrt{2}\). It has strong transitions (with \(\Delta v = 0\) vibrational character) from \(v = 0\) of the ground state (using the \(B\) electronic character – blue vertical arrows) and to \(v = 1\) of the ground state (using the \(A\) electronic character – orange vertical arrow and orange wavy line).

As a result of this strong non-adiabatic mixing, the GSB diagram on the right has almost the same pattern of transition dipole moments (indicated by color) as in the excited state emission diagram on the left (these differ slightly because of non-resonant coupling and non-zero vibrational overlap integrals for transitions with \(\Delta v \neq 0\)). Thus, for CP12, non-adiabatic mixing on the excited state leads to purely vibrational oscillations on the ground electronic state with signatures nearly identical to those of purely electronic oscillations on the excited state. In particular, ESE and GSB diagrams for CP12 both indicate negative oscillation frequencies during the interval between pulses \(b\) and \(c\) [as shown for ESE in ref. \(^{30}\)], and both survive the coherence specific polarization sequence [used in \(^{28,60}\)].

In contrast, for CP21, the ESE and GSB diagram frequencies are different for pulses \(b\) and \(c\); these transitions are also weakened for the GSB path by a lack of resonance (pulse \(b\)) and a change in \(v\) (pulse \(c\)). These factors underlie the asymmetry between CP12 and CP21 oscillation amplitudes. While simple consideration of the four diagrams shown in Fig. 2.3 happens to indicate relative 2D beat amplitudes in the top panel of Fig. 2.4, full analysis requires a sum over all (possibly interfering) paths, as in ref. \(^{64}\) and \(^{12}\). For CP21, there is a fully electronically enhanced path starting from the thermally excited \(v = 1\) level. Depending on temperature, such paths may contribute more than the one shown above.
Figure 2A.2. (Left) Variation of peak-peak beat amplitude for the cross-peaks CP12 and CP21 with change in vibrational frequency at a constant donor acceptor excitonic energy gap of $\Delta_{ex} = 2\sqrt{(\Delta / 2)^2 + J^2} = 200 \text{ cm}^{-1}$. The calculation is for the ground state contribution to the “rephasing” 2D spectrum (shown for waiting time $T = 0$ in Fig. 2.2). All parameters are the same as for the dimer model except that the anti-correlated inhomogeneous distribution of $\Delta$ is omitted and the vibrational frequency is varied from 130 cm$^{-1}$ to 250 cm$^{-1}$. Calculations for both panels of this figure were carried out on a 3D time grid with 256 time points in each dimension and time increments of 15 fs. For the resonant vibrational frequency of 200 cm$^{-1}$, the 2D peaks are sampled at DP1 ($\omega_\tau, \omega_t$) = (-11487, 11478), DP2 ($\omega_\tau, \omega_t$) = (-11686, 11678), CP12 ($\omega_\tau, \omega_t$) = (-11687, 11496) and CP21 ($\omega_\tau, \omega_t$) = (-11505, 11673). As the vibrational frequency is detuned from resonance by $\pm 70 \text{ cm}^{-1}$, only the coordinates for the real maxima of CP12 change (by 8.7 cm$^{-1}$ along $\omega_t$ and 17.4 cm$^{-1}$ along $\omega_\tau$, on either side of resonance). The real amplitudes of the peaks in the ground electronic state contribution to the “rephasing” 2D spectrum oscillate only at the vibrational frequency. CP21 oscillations do not show a resonant enhancement. Accounting for the additive constant for CP21 oscillations used in composing the figure (see legend), CP12 oscillations are $\sim 14\times$ stronger than CP21 oscillations at their peak. CP12 oscillations are at least $\sim 7\times$ stronger than CP21 oscillations for vibrational frequencies over a range of 130 cm$^{-1}$. This range is wider than the vibrational-excitonic resonance in the excited state. (Right) Peak amplitude beating from the ground state contribution to the “rephasing” 2D spectrum for a mismatch between the excitonic energy gap and the vibrational frequency. The calculation used the dimer model except that the average site energy splitting was increased from $<\Delta> = 150 \text{ cm}^{-1}$ to $<\Delta> = 190 \text{ cm}^{-1}$ (increasing the excitonic energy gap from 200 cm$^{-1}$ to 230 cm$^{-1}$) and the vibrational frequency was reduced from $\omega/2\pi = 200 \text{ cm}^{-1}$ to $\omega/2\pi = 150 \text{ cm}^{-1}$. (The inhomogeneous distribution of $\Delta$ is included here.) The corresponding 2D peak coordinates used are: DP1 ($\omega_\tau, \omega_t$) = (-11470,11461), DP2 ($\omega_\tau, \omega_t$) = (-11703,11696), CP12 ($\omega_\tau, \omega_t$) = (-11687,11496) and CP21 ($\omega_\tau, \omega_t$) = (-11496,11687). The v=1 vibrational level of the pigment with the lower electronic excitation energy lies, on average, 90 cm$^{-1}$ below the v=0 vibrational state of the pigment with higher electronic excitation energy that it is coupled to. Compared to
the resonance case in Fig. 2.4, the phase of CP12 beating changes by 60° relative to the beating of the other peaks so that CP12 beats 180° out of phase with the diagonal peaks here. The phase relation between CP21 and diagonal peak beating does not change across resonance. Note that the oscillation period of ~220 fs corresponds to the vibrational frequency $\omega/2\pi c = 150 \text{ cm}^{-1}$, not the average excitonic energy gap $\langle \Delta_{EX} \rangle = 230 \text{ cm}^{-1}$. 
Figure 2A.3. (Top) Diagonal and anti-diagonal widths for the lowest diagonal peak (DP1) in the ground state contribution to the absorptive 2D spectrum of the dimer model. The FWHM width in each direction is obtained by fitting a slice through the 2D spectrum to a Gaussian. Both widths oscillate at a vibrational frequency of 200 cm$^{-1}$, which is in resonance with the excitonic donor-acceptor energy gap. The relative phase between the widths is $\sim 150^\circ$. A $180^\circ$ phase shift is predicted by both electronic coherence models and models incorporating only Franck-Condon active vibrations. This $180^\circ$ phase shift signature has been experimentally reported for Franck-Condon active vibrations in the dye PERY ($N,N'$-bis(2,6-diethylphenyl)perylene-3,4,9,10-tetracarboxylicdiimide). (Middle) Diagonal amplitude and diagonal widths for the lowest diagonal peak (DP1) in the ground state contribution to the absorptive 2D spectrum of the dimer model. The diagonal width and peak amplitude both oscillate at a vibrational frequency of 200 cm$^{-1}$, which is in resonance with the excitonic donor-acceptor energy gap. The relative phase between the width and amplitude is $\sim 100^\circ$. This phase differs significantly from the $180^\circ$ phase shift predicted by electronic coherence models. The $180^\circ$ phase shift signature originally predicted for electronic coherence has been experimentally reported only for a cyanine dye, where it must arise from Franck-Condon active vibrations, a result verified by computational models. In contrast, a near-zero phase shift arises from Franck-Condon active
vibrations in the dye PERY\textsuperscript{65}; the difference has been attributed to the frequency offset between the molecular spectrum and the excitation pulse spectrum\textsuperscript{66}.

(Bottom) Peak shape beating and amplitude beating for the lower energy diagonal peak, DP1, in a real absorptive (“rephasing” plus “non-rephasing”) 2D spectrum (ground electronic state contributions only) for the dimer model. The shape is quantified by the ratio diagonal FWHM/anti-diagonal FWHM. The shape and amplitude both oscillate at a vibrational frequency of 200 cm\textsuperscript{-1}, which is in resonance with the excitonic donor-acceptor energy gap. Anti-correlation between the width ratio and the amplitude has been used as an experimental signature because the ratio of two positive quantities that oscillate out of phase with each other is always in phase with the numerator; as a result, this signature necessarily follows if both of the two preceding signatures hold. Franck-Condon active vibrations have not as yet reproduced this signature experimentally. Despite the significant disagreement with the predicted phase in the middle panel, the phase between the width ratio and amplitude oscillations is \(~130^\circ\) here. This differs from the theoretical \(180^\circ\) phase shift, but might approach the level of agreement in published experimental data on antennas [compare Fig. 3b of ref. \textsuperscript{29} and Fig. 4c of ref. \textsuperscript{67}].
<table>
<thead>
<tr>
<th>FMO 2D Off-Diagonal Beat Frequency (cm⁻¹)</th>
<th>BCChl a Raman/Franck-Condon active vibrational frequency (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>87 (±1)²⁹,⁶⁸ (CP35)</td>
<td>84(±2)²⁵, 82(±3)⁶⁹, 88⁷⁰</td>
</tr>
<tr>
<td>104(±1)²⁹ (CP35)</td>
<td></td>
</tr>
<tr>
<td>107(±2)²⁹ (CP46)</td>
<td></td>
</tr>
<tr>
<td>147 (±1)²⁹ (CP24)</td>
<td></td>
</tr>
<tr>
<td>161.3(±0.7)¹⁸, 163(±1)¹⁹, 157(±1)⁷¹ for CP12</td>
<td>167(±2)²⁵,²⁷², 173(±2)⁷³, 164(±3)⁶⁹, 161(±3)⁷⁴, 164⁷⁰</td>
</tr>
<tr>
<td>159(±3)²⁹ (CP36)</td>
<td></td>
</tr>
<tr>
<td>198.7(±1.4)¹⁸, 203(±1)¹⁹, 198(±2)⁷⁵ for CP13</td>
<td>191(±2)²⁵,²⁷², 195(±2)⁷³, 196(±3)⁶⁹, 195(±2)⁷⁴, 190⁷⁰</td>
</tr>
<tr>
<td>248²⁹,⁶⁸ (CP13)</td>
<td>243(±2)⁷², 239(±2)²⁵, 237(±2)⁷³, 232(±3)⁶⁹, 235⁷⁰, 238⁷⁴</td>
</tr>
<tr>
<td>263(±2)²⁹ (CP26)</td>
<td></td>
</tr>
<tr>
<td>264(±2)²⁹ (CP37)</td>
<td>263(±2)²⁵, 256(±2)²⁵, 260(±2)⁷³, 260⁷⁴, 257⁷⁰</td>
</tr>
</tbody>
</table>

Table 2A.1. Comparison of off-diagonal beat frequencies from 2D spectra of the Fenna-Matthews-Olson complex with ground state vibrational frequencies measured for $Q_y$ excitation of its pigment, bacteriochlorophyll $a$.

Refs. ¹⁸,²⁹,⁶⁸,⁷¹,⁷⁵, located cross-peaks near maxima or shoulders in the 2D spectra. Ref. ²⁹ used the model exciton Hamiltonian of ref. ⁶⁸ to assign these off-diagonal cross-peaks by their 2D spectral coordinates. In refs. ¹⁸,⁷¹,⁷⁵, the exciton Hamiltonian of ref. ⁷⁶ was used to assign off-diagonal cross-peaks based on their 2D spectral coordinates. In ref. ¹⁹, 3 off-diagonal spectral regions (not necessarily near maxima or shoulders) were sampled to extract multiple beat frequencies, and the beat frequencies were used for exciton cross-peak assignments based on the exciton Hamiltonian of ref. ⁷⁰. Cross-peak labels in Table 2A.1 have not been renumbered to account for the differences in labelling in the original papers.

Ref. ⁷² studied fluorescence from BCChl $a$ in the FMO complex from Chlorobaculum tepidum (the organism formerly known as Chlorobium tepidum ⁷⁷).
(*) Ref. 73 studied fluorescence from $BChl$ $a$ in the FMO complex from *Prosthecochloris Aestuarii*.

Refs. 25,69 studied fluorescence from isolated $BChl$ $a$ molecules in low-temperature glass matrices. Ref. 74 used hole-burning spectroscopy to measure vibrational frequencies of the excited electronic state for isolated $BChl$ $a$ molecules in low-temperature glass matrices. Ref. 70 studied resonance Raman spectra from dried films of $BChl$ $a$ with 750 nm excitation.
<table>
<thead>
<tr>
<th>2D Signature</th>
<th>System</th>
<th>Theory</th>
<th>Computation</th>
<th>NA-GS-vib</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP diagonal and anti-diagonal widths anti-correlated (ABS)</td>
<td>PERY dye</td>
<td>EC $^{44}$ FC-vib $^{33,65}$</td>
<td>EC (2) FC-vib $^{33,65}$</td>
<td>~ (Fig. 2A.3)</td>
</tr>
<tr>
<td>DP amplitude anti-correlated to diagonal width osc. (ABS)</td>
<td>cyanine dye</td>
<td>EC $^{44}$</td>
<td>EC $^{44}$ FC-vib $^{66}$</td>
<td>No (Fig. 2A.3)</td>
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<tr>
<td>DP amplitude anti-correlated to ratio* of diagonal/anti-diagonal widths (ABS)</td>
<td>FMO $^{29}$ polymer $^{67}$</td>
<td></td>
<td></td>
<td>~ (Fig. 2A.3)</td>
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<tr>
<td>DP osc. (NR)</td>
<td>LHCII $^{78}$</td>
<td>EC $^{79}$</td>
<td>EC $^{79}$</td>
<td>Yes (Fig. 2.4)</td>
</tr>
<tr>
<td>180° phase between CP12 and CP21 osc. (R) †</td>
<td>PE545 $^{32}$ PC645 $^{32}$</td>
<td>EC-No $^{20}$</td>
<td>EC-No $^{20}$ FC-vib-No $^{20}$</td>
<td>Yes (Fig. 2.4)</td>
</tr>
<tr>
<td>DP osc. (R)</td>
<td>FMO $^{18}$</td>
<td>EC-No QT $^{10}$</td>
<td>QT $^{10}$</td>
<td>Yes (Fig. 2.4)</td>
</tr>
<tr>
<td>90° phase between CP12 and DP2 osc. (R)</td>
<td>FMO $^{18}$</td>
<td>EC-No QT $^{18}$</td>
<td>QT $^{10}$-No‡</td>
<td>Yes (Fig. 2.4)</td>
</tr>
<tr>
<td>Negative CP12 osc. frequency (R)</td>
<td>PC645 $^{30}$</td>
<td>EC $^{30}$</td>
<td></td>
<td>Yes (see Appendix &amp; Fig. 2A.1)</td>
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<tr>
<td>Polarization sequence (NR)</td>
<td>LHCII $^{28}$</td>
<td></td>
<td></td>
<td>Yes (see Appendix &amp; Fig. 2A.1)</td>
</tr>
<tr>
<td>CP12 osc. stronger than CP21 osc. (R)</td>
<td>PE545 $^{32}$</td>
<td>EC-No QT-No</td>
<td></td>
<td>Yes (Fig. 2.4)</td>
</tr>
<tr>
<td>CP osc. only (R) but not (NR)</td>
<td>PC645 $^{30}$</td>
<td>EC $^{79}$</td>
<td></td>
<td>Yes; Fig. 2.4 consistent with S/N of ref. $^{30}$</td>
</tr>
<tr>
<td>CP12 osc. stronger (R) than (NR)</td>
<td>PC645 $^{30}$</td>
<td>EC-No</td>
<td></td>
<td>Prediction (Fig. 2.4)</td>
</tr>
</tbody>
</table>
Table 2A.2. Status of Photosynthetic Energy Transfer Signatures

* The ratio of two positive quantities that oscillate out of phase with each other always oscillates in phase with the numerator. Thus, if signatures 1 and 2 both hold, then signature 3 necessarily follows. However, the ratio of two quantities that oscillate in phase with each other can be in or out of phase with both; for example, \([(10 + \cos A)/(2 + \cos A)]\) oscillates in phase with \(\cos A\), whereas \([(10 + \cos A)/(11 + \cos A)]\) oscillates out of phase with \(\cos A\). As a result, signature 3 need not imply signatures 1 and 2 (\(\cos A\) is the cosine of the angle \(A\)).

† This phase relationship was proposed for EC based on a brief theoretical discussion in ref. 32. Ref. 20 proves this discussion is incorrect; we have verified the analytic theory and computational results of ref. 20 for EC.

‡ Fig. 5 of ref. 10 shows 2D spectra computed using a QT model. The phase relationship between \(D_2\) (labelled DP2 here) and \(C_1\) (labelled CP12 here) is \(\sim 180^\circ\), not the \(90^\circ\) phase relationship discussed in ref. 18. The factors controlling this phase relationship in QT models are not yet clear.

Table 2A.2 shows proposed 2D signatures of photosynthetic energy transfer (first column), systems for which they have been experimentally reported (second column), theories used to explain them (third column), whether they have been computationally found for a model system (fourth column), and whether they are reproduced by signatures of non-adiabatically excited ground state vibrations (fifth column). Signatures are labelled according to the type of 2D spectrum in which they appear: absorptive (ABS), rephasing (R), and non-rephasing (NR); DP stands for diagonal peak and “oscillations” is abbreviated “osc.” Theories are labelled EC for Electronic Coherence, QT for Quantum Transport, FC-vib for Franck-Condon vibrational excitation on a single excited electronic state, and NA-GS-vib for Non-Adiabatic excitation of vibrations on the Ground electronic state. Thus, the first two signatures do not distinguish between electronic and vibrational coherence. It is not entirely clear what controls these signatures; computations indicate that electronic coherence 44, Franck-Condon active vibrations 33,65,66, phase-twists 63 which are sensitive to coherence transfer 80, and pulse spectra 66 all play a role. Because signature 3 is derived from signatures 1 and 2, the status of signature 3 as an indicator of electronic coherence is not clear. Some theories have not reproduced some signatures; these negative results are indicated by EC-No (not reproduced by Electronic Coherence) and FC-vib-No (not reproduced by Franck-Condon active vibrations). The last column shows that non-adiabatic excitation of ground state vibrations can account for all reported electronic coherence/quantum transport signatures, replicates an asymmetry between opposite cross-peak oscillation amplitudes (third to last row) that is not predicted by electronic coherence or quantum transport models [this symmetry is observed in 2D IR spectra of coupled vibrations – see ref. 63 and 80], and predicts a strength for CP12 oscillations in non-rephasing 2D spectra that is below the experimental signal to noise (S/N) ratio in a case where non-rephasing CP12 oscillations were not detected.
References


CHAPTER 3

NON-ADIABATIC VIBRATIONAL-ELECTRONIC RESONANCE IN A MODEL DIMER

In chapter 2 of this thesis it was shown that vibrational-electronic resonance in a model donor-acceptor system leads to spectroscopic signatures previously attributed to electronic coherence and quantum energy transport in photosynthetic antennas. Such non-adiabatic vibrational-electronic resonance in natural photosynthetic antennas drastically alters the adiabatic framework in which electronic energy transfer has been conventionally studied, and leads to a possibility of exploiting non-adiabatic dynamics for directed energy transfer between molecules. The present chapter considers such a vibrational-electronic resonance and its spectroscopic signatures in greater detail. Starting from a general description of a dimer with electronic states coupled to several vibrational modes, a generalized ‘tuning coordinate’ along which non-adiabatic dynamics occurs is formulated. For the case of identical Franck-Condon active modes on each pigment, it is shown that non-adiabatic dynamics due to vibrational-electronic resonance are coupled only in the sub-space of anti-correlated vibrations which tunes the relative energy gap of the two pigments. Ground state 2D calculations with resolved peakshapes show that vibrational-electronic mixing around resonance strongly affects the 2D peakshapes and that these effects are completely obscured due to correlated broadening. Calculations in the presence of an additional Franck-Condon active vibrational mode which is near-resonant, suggest that non-adiabatic effects on the 2D spectra arising due to the resonant and the near-resonant mode are roughly additive such that two equally coupled modes lead to a ~2.3x increase in the oscillation amplitude of the dominant cross-peak. Due to such effects, consideration of the finite width of non-adiabatic coupling and several near-resonant modes in the FMO antenna complex becomes
important. Using an environmental mode that affects only one pigment, it is shown that ground state signatures of non-adiabatic vibrational-electronic resonance are robust to the protein environment. The previously predicted asymmetry in the 2D cross-peak oscillation amplitude is further enhanced due to such coupling with the protein environment. The role of vibrational relaxation on the ground state of the donor pigment in making the energy transfer permanent is also highlighted.

3.1 Introduction

The fast and efficient nature of electronic energy transfer from natural photosynthetic antennas to the reaction center \(^1,2\) has been an area of significant experimental and theoretical interest in condensed matter physics for several years, although the underlying physics is still controversial. A conceptual understanding of the mechanism exploited by natural molecular assemblies can lead to possible applications in semiconductor devices based on organic assemblies where Frenkel-exciton delocalization is useful for achieving long-range energy and charge transport \(^3,4\) as well as inorganic assemblies such as quantum-dot arrays where inter-dot interactions and surfactants can alter the electronic structure of the dot, allowing for efficient electron or hole delocalization \(^5\).

Over the last several years, experiments on photosynthetic antennas \(^6-13\) and reaction centers \(^14\) using femtosecond two-dimensional (2D) electronic spectroscopy \(^15\) have revealed that energy transfer in these systems is associated with coherences which last for picoseconds \(^7\). The reported signatures of these coherences that suggested purely electronic coherence between different excitons spanning more than one pigment \(^16,17\) and reversible energy flow between the pigments and the protein, termed as quantum energy transport \(^18\). Experiments \(^19\) measuring
fluorescence after exciting single LH2 antennas with phase coherent pulses have also been interpreted in terms of oscillatory energy transfer between the B850-B800 rings. Parallel theoretical works on several different antennas have considered the role of intramolecular vibrations in energy transfer in photosynthetic antennas, with some works suggesting that excited state vibrational-electronic mixing allows excited state coherences to live longer than a picosecond.

Recently, Tiwari et al. have shown that presence of vibrational-electronic resonance in a model dimer loosely based on a pair of excitons in the Fenna-Matthews-Olson antenna protein complex (FMO) can lead to strong non-adiabatic vibrational-electronic mixing on the excited state. This leads to excitation of ground-state wavepackets which mimic many 2D signatures (ref. Table S2) previously attributed to purely electronic coherence and quantum energy transport. Moreover, calculations further show that signatures of electronic coherence between excitons in FMO are short-lived due to the previously measured inhomogeneity in the protein environment, but indicate that coherence in individual protein complexes can live longer than its 2D signatures (in analogy to the way individual dipoles oscillate longer than their collective free induction decay). One important point here is that although 2D spectra can eliminate inhomogeneity in the ground and excited state energy gap by rephasing, 2D spectra based on third order nonlinearities do not have a rephasing pathway that eliminates inhomogeneities in the excitonic energy gap between excited states. A second important point is that these vibrational wavepackets generating the longest lived signatures are in antennas for which no pigments have been electronically excited. This means that, like Franck-Condon (FC) vibrational wavepacket holes, they can persist even after all electronic excitations of the antenna have been quenched. In particular, these Raman vibrational wavepackets can persist after energy transfer on the
excited state of the antenna is stabilized on the final acceptor. However, the vibrations observed on the ground electronic state of the antenna in the aftermath of energy transfer have 2D signatures that reflect the role of non-adiabatic vibrational-electronic resonance in both the energy transfer processes and their resonance Raman excitation process which occurs during the initial stage of the energy transfer process. More precisely, the femtosecond resonance Raman excitation process for vibrational wavepackets is sensitive to forces and dynamics driving energy transfer only while the ground and excited state of the antenna remain coherent. Thus, the 2D signatures of photosynthetic energy transfer illustrate the utility of resonance Raman spectroscopy for elucidating the initial driving forces behind excited state dynamics\textsuperscript{27-29}.

In contrast to prior work in energy transfer, calculations presented in ref. \textsuperscript{23} rely on an exact numerical diagonalization of the full non-adiabatic Hamiltonian. The resulting excited state non-adiabatic dynamics over the first 100 femtoseconds or so is treated exactly, without any approximations regarding the derivative coupling terms\textsuperscript{30} responsible for the non-adiabatic dynamics. In contrast to the adiabatic approximation\textsuperscript{30}, which first solves for electronic states of the dimer for a fixed nuclear frame, the non-adiabatic treatment simultaneously solves for mixed vibrational-electronic states. The presence of vibrational-electronic resonance on the excited state leads to eigenfunctions with rapidly changing electronic character over the whole range of anti-correlated vibrational coordinates. This breakdown of Born-Oppenheimer approximation\textsuperscript{31} over the whole range of vibrational coordinates renders the adiabatic framework, under which electronic energy transfer has been traditionally studied\textsuperscript{32}, invalid. The resulting ‘unavoidable nested funnel’\textsuperscript{23} is different from a conical funnel\textsuperscript{33-35} because a finite Coulomb coupling between the pigments does not allow the excited state potential energy surfaces to intersect. Also, the range of anti-correlated vibrational coordinates where the excited state eigenfunctions
rapidly change their electronic character is greater because the surfaces are nested and therefore
different from the localized curve crossing region in the adiabatic framework of Förster’s energy
transfer theory\textsuperscript{32,36}. The presence of vibrational-electronic resonance in FMO, the antenna protein
most studied so far, and possibly in a number of other photosynthetic antennas, suggests that
resonant non-adiabatic vibrational-electronic mixing is at least one of the energy transfer
mechanisms exploited by Nature. A better understanding of the underlying physics requires a
detailed understanding of the spectroscopic signatures resulting from such non-adiabatic
dynamics and an investigation into the role of several near-resonant FC active vibrational modes
(for e.g. ref.\textsuperscript{23} Table S1) present in such photosynthetic pigments.

Here, the non-adiabatic dynamics of a dimer is considered in greater detail. A dimer
model with two electronically coupled pigments and several FC active modes is considered. The
FC active vibrational modes can be localized intramolecular vibrational modes or belong to the
protein environment and be delocalized over both pigments simultaneously, affecting one or both
of the pigments to varying degrees. The intramolecular FC active vibrational modes\textsuperscript{31} in antenna
pigments are weakly coupled to the electronic states, such that the equilibrium nuclear geometry
in the excited electronic state, along such a vibrational coordinate, is only slightly displaced
relative to the ground state. Starting from this generalized Hamiltonian, a ‘tuning coordinate’ is
formulated for the dimer system, along which non-adiabatic vibrational-electronic mixing
occurs. Simplifying this generalized Hamiltonian, a specific case of a dimer system with one FC
mode on each pigment is considered. For this case, we briefly show that non-adiabatic energy
transfer to the acceptor also involves a pathway where after electronic ‘de-excitation’, the donor
is still left vibrationally excited on its ground state. Coherent exciton scattering approximation
(CES) or ‘one-particle’ approximation\textsuperscript{37,38} fails to capture this essential pathway that is involved
in non-adiabatic enhancement of vibrations on both the ground and excited states of the antenna as a whole.

Ground state 2D calculations for the case of one FC mode on each pigment reveal split peaks where the relative phase of cross-peak (CP) and diagonal peak (DP) oscillations varies over a range of vibrational frequencies across resonance, with a predicted ground state oscillation amplitude asymmetry between the opposite 2D cross-peaks arising due to vibrational-electronic mixing on the excited state. For this case, we also consider the correlated \( \hat{q}_+ \) and anti-correlated \( \hat{q}_- \) modes separately to explicitly show that non-adiabatic enhancement of ground state vibrations only arises due to anti-correlated nuclear motion which tunes the energy gap between the singly-excited states of the two pigments. Apart from the localized intramolecular vibrational modes, we also consider a global environmental mode \( \hat{q}_E \) corresponding to the low-frequency phonon sideband of FMO antenna protein and show that ground state 2D signatures are robust to the presence of such an environmental mode.

Non-adiabatic enhancement of ground state vibrations has a finite width around the resonant vibrational frequency, which makes it essential to also consider the role of near-resonant vibrations in energy transfer. To investigate the role of possible near-resonant vibrations in bacteriochlorophyll a (BChl a), the pigment in the FMO antenna complex, the generalized dimer Hamiltonian is used to consider the specific case of two FC active vibrational modes on each pigment such that, in contrast to the intra-pigment normal modes with vibrational frequencies resonant with the excitonic energy gap, the vibrational frequencies of these additional intra-pigment modes are slightly off-resonant with respect to the excitonic energy gap. 2D calculations suggest that near-resonant modes can lead to roughly additive non-adiabatic
effects causing ~ 2x non-adiabatic enhancement of the 2D signatures of ground state vibrational
wavepackets.

3.2 Models

3.2.1 Dimer with intramolecular and environmental vibrational modes

The essential features of the dimer model used for this study are the same as in ref. 23 and
can be specified in a complete diabatic basis of localized vibrational and electronic states. The
two pigment system has a single electronic ground state \( |0_A\rangle |0_B\rangle \) (no pigments excited), two
singly excited electronic states \( |A\rangle |0_B\rangle \) and \( |0_A\rangle |B\rangle \) (for pigment \( A \) or \( B \) excited respectively),
and a doubly excited electronic state \( |A\rangle |B\rangle \) (both pigments excited). Each excited electronic
state of the dimer supports a bath of vibrational modes represented by harmonic oscillators. The
pigment electronic excitations involved in photosynthesis are typically highly delocalized \( \pi - \pi^* \)
transitions\(^{1,39}\) that cause small changes in equilibrium molecular structure and bonding\(^{40-44}\). As a
result, electronic excitation causes small changes in the equilibrium vibrational coordinates (less
than the root mean square zero point motion) and the vibrational frequencies change only a few
percent upon electronic excitation\(^{45,46}\). Accordingly, the vibrational motion in an isolated
pigment is of small amplitude and, for short times, can be approximated as harmonic, with the
same frequency \( \omega_i \) on all electronic states. The diabatic vibrational Hamiltonian on each
electronic state is represented by functions of the dimensionless position and momentum
operators corresponding to each vibrational mode \( i \). Use of vibrational coordinates and position
operators allows the vibrational Hamiltonian to be expressed without introducing a vibrational
basis set. Using energy in frequency units, the overall Hamiltonian of the dimer in the direct
product basis is
\[ \hat{H}_{\text{dimer}} = \sum_i \frac{1}{2} \omega_i (\hat{q}_i^2 + \hat{p}_i^2) \]

\[ + \left( E_A - \sum_i \omega_i d_i^A \hat{q}_i \right) \left| A \right\rangle \left\langle 0_B \right| + \left| 0_B \right\rangle \left\langle A \right| \]

\[ + \left( E_B - \sum_i \omega_i d_i^B \hat{q}_i \right) \left| 0_A \right\rangle \left\langle B \right| + \left| B \right\rangle \left\langle 0_A \right| \]

\[ + \left( E_A + E_B - \delta - \sum_i \omega_i (d_i^A + d_i^B) \right) \left| A \right\rangle \left\langle B \right| + \left| B \right\rangle \left\langle A \right| \]

(3.1)

where \( \hat{I}_{\text{dimer}} = \left| 0_A \right\rangle \left\langle 0_B \right| + \left| 0_B \right\rangle \left\langle 0_A \right| + \left| A \right\rangle \left\langle 0_B \right| + \left| 0_B \right\rangle \left\langle A \right| + \left| 0_A \right\rangle \left\langle B \right| + \left| B \right\rangle \left\langle 0_A \right| \) is the identity operator for all the electronic states of the dimer system. The dimensionless position and momentum operators of the \( i \)th bath vibrational mode are given by \( \hat{q}_i \) and \( \hat{p}_i \) respectively. \( d_i^A (d_i^B) \) is the FC displacement of the equilibrium vibrational coordinate on the excited electronic state of the dimer when only pigment \( A (B) \) is excited. Modes localized on one pigment have no displacement upon excitation of the other pigment. For example, if mode \( i \) is localized on pigment \( A (B) \), then \( d_i^B = 0 (d_i^A = 0) \). In the isolated pigment, such displacements give rise to vibrational progressions in absorption and emission according to the Franck-Condon principle\(^{47}\).

Protein vibrational modes may have displacements upon electronic excitation of either pigment. An approximation that the change in force on each vibrational mode is additive for each electronic excitation leads to the harmonic oscillator displacement \( d_i^A + d_i^B \) in the doubly excited state \( \left| A \right\rangle \left\langle B \right| \). \( E_A (E_B) \) is the vertical electronic excitation energy from the ground electronic state of the dimer to the singly excited electronic state in which only pigment \( A (B) \) is excited. Due to differences in the local protein environment around each pigment, pigment electronic excitation energies can differ from each other by as much as a vibrational quantum of energy. This Hamiltonian differs from that used in ref.\(^{23}\) in one key respect: vibrational modes of
the pigments and the protein are all included on an equal footing in the summation over vibrational modes $i$.

If either pigment is excited, the two pigments interact through a Coulombic coupling, which at long range can be calculated by Förster’s transition dipole approximation. The dimer Hamiltonian $\hat{H}_{\text{coupling}}$ couples the singly excited electronic states of the dimer through the Coulomb coupling so that in the direct product basis

$$
\hat{H}_{\text{coupling}} = J \left| 0_A \right> \left< 0_B \right| + J \left| B \right> \left< A \right| + J \left| B \right> \left< 0_B \right|
$$

where $J$ is the Coulombic coupling between the two pigments. $\delta$ is the bi-exciton binding energy in the doubly-excited electronic state of the dimer. The bi-exciton binding energy also has a Coulombic origin. The delocalized electronic states of such coupled pigment systems are referred to as (Frenkel) excitons.

In a rigid protein scaffold, fluctuations in the pigment orientations around the equilibrium position are only a small fraction of the center-to-center inter-pigment separations (~12-15 Å in a FMO monomer, or ~21 Å between $BChl$ a pigments of adjacent B800 and B850 rings in LH2), such that the fluctuations in Coulombic coupling are small compared to the magnitude of coupling. Thus, $J$ is approximated to be independent of both vibrational coordinates of the protein and intramolecular vibrational coordinates. The above Hamiltonian is standard in describing electronic energy transfer within a network of chromophores which are linearly coupled to the vibrational degrees of freedom in their excited states. For a pair of chromophores, the Hamiltonian described by Eqns. 1 and 3 of ref. can be reduced to the dimer Hamiltonian described by Eqn. (3.1). In a quasi-particle representation, the Hamiltonian in Eqn. (3.1) can also be described in terms of the creation and annihilation operators for excitons and phonons.
The model assumes that, in the absence of the Coulomb coupling $J$ and bi-exciton binding energy $\delta$, the two electronic excitations are coupled linearly to a bath of harmonic vibrational modes, that the two electronic excitations have an additive effect on the bath, and that the bath vibrations do not alter the coupling $J$ or bi-exciton binding energy $\delta$. The additive assumption is appropriate for electronic basis states localized on different pigments in the absence of the coupling. The normal vibrational modes of the bath may be intramolecular vibrations localized on one pigment or vibrational modes of the protein environment. Protein modes can affect the pigments equally or unequally.

The diabatic vibrational tuning coordinate that adjusts the energy gap between the excited states of pigments $A$ and $B$ is

$$\hat{g}_{D}^{AB} = \sum_{i} \omega_{i} (d_{i}^{A} - d_{i}^{B}) \hat{q}_{i} \quad (3.3)$$

More generally, the diabatic tuning coordinate is the gradient of the energy difference $E^{A}(q) - E^{B}(q)$ between the excited electronic states with pigment $A$ or $B$ excited, and can be written as

$$\hat{g}_{D}^{AB} = \nabla_{q} \left( E^{A}(q) - E^{B}(q) \right), \quad (3.4)$$

where $q$ represents the vibrational subspace of the dimer system. Appendix 3A describes the electronic energies $E^{A}(q)$ and $E^{B}(q)$ in terms of the Hamiltonian defined in Eqns. (3.1) and(3.2). The diabatic vibrational coordinate $\hat{g}_{D}^{AB-G}$ causes correlated changes in the energies of the electronically excited pigments that do not affect the energy gap between them. It can be described in terms the excited electronic state energies, $E^{A}(q)$ and $E^{B}(q)$, and the ground electronic state energy $E^{G}(q)$ as
\[ \hat{c}_{D}^{AB-G} = \nabla_q \left( E^A(q) + E^B(q) - 2E^G(q) \right). \]  

(3.5)

In terms of the vibrational coordinates and the corresponding FC displacements, the correlated coordinate \( \hat{c}_{D}^{AB-G} \) can be written as

\[ \hat{c}_{D}^{AB-G} = \sum_i \omega_i \left( \hat{d}^A_i + \hat{d}^B_i \right) \hat{q}_i \]  

(3.6)

In terms of these coordinates, the dimer Hamiltonian becomes

\[
\hat{H}_{dimer} = \sum_i \frac{1}{2} \omega_i (\hat{q}_i^2 + \hat{\rho}_i^2) \hat{I}_{dimer} + \left( E_A - \frac{1}{2}(\hat{c}_{D}^{AB-G} + \hat{g}_{D}^{AB}) \right) |0_A\rangle \langle 0_A| + \left( E_B - \frac{1}{2}(\hat{c}_{D}^{AB-G} - \hat{g}_{D}^{AB}) \right) |0_B\rangle \langle 0_B| + \left( E_A + E_B - \delta - \hat{c}_{D}^{AB-G} \right) |A\rangle \langle B| + |B\rangle \langle A| + \hat{H}_{coupling} \]  

(3.7)

Only the correlated coordinate \( \hat{c}_{D}^{AB-G} \) affects the energy gap between the ground state and the doubly excited state. Coordinates orthogonal to \( \hat{c}_{D}^{AB-G} \) have no effect on this gap. Similarly, only the tuning coordinate \( \hat{g}_{D}^{AB} \) causes anti-correlated changes in the energies of the two singly-excited states; coordinates orthogonal to \( \hat{g}_{D}^{AB} \) have no effect on this gap. Note however, that the correlated and tuning coordinates are not, in general, orthogonal. Such an anti-correlated tuning coordinate \( \hat{g}_{D}^{AB} \), is akin to the ‘tuning coordinate \( \hat{g} \)’ in the context of conical intersections.\(^{35}\)

Although due to a constant off-diagonal Coulomb coupling \( J \), there is no ‘coupling coordinate \( \hat{h} \)’ in this Hamiltonian, which makes it different from a conical funnel problem. The motions along \( \hat{c}_{D}^{AB-G} \) and \( \hat{g}_{D}^{AB} \), which are driven by electronic excitation and energy transfer between
pigments, can be coupled by the harmonic oscillator Hamiltonian. In general, each normal mode of vibration may have some effect on both $\hat{c}^{A\rightarrow G}_{D}$ and $\hat{G}^{A\rightarrow B}_{D}$.

If two pigments are the same and have identical frequencies, but unequal FC displacements $d$, the degeneracy $\omega_{j}^{A} = \omega_{j}^{B}$ allows a choice of new, delocalized pigment normal coordinates that simultaneously separate their harmonic oscillator and electronic Hamiltonians on the first excited state of the dimer:

$$\hat{q}_{j}^{+} = \frac{d_{j}^{B} \hat{q}_{j}^{A} + d_{j}^{A} \hat{q}_{j}^{B}}{n_{j}}$$  \hspace{1cm} (3.8)$$

$$\hat{q}_{j}^{-} = \frac{d_{j}^{A} \hat{q}_{j}^{A} - d_{j}^{B} \hat{q}_{j}^{B}}{n_{j}}$$  \hspace{1cm} (3.9)$$

where $n_{j} = \sqrt{(d_{j}^{A})^{2} + (d_{j}^{B})^{2}}$. In a vibrational basis delocalized over both pigments, along a correlated vibrational normal mode coordinate, every bond length and angle vibrates with exactly the same phase on both molecules. In contrast, along an anti-correlated vibrational normal mode coordinate, every bond in one molecule contracts while it expands in the other. Similarly, along an anti-correlated vibrational coordinate, the corresponding bond angles on the two pigments bend in the opposite sense. Since proteins can alter pigment vibrations, the two pigments might not have identical sets of frequencies or FC displacements. In the most general case of unequal frequencies and FC displacements, the correlated and anti-correlated coordinates do not project with equal magnitude on the two coupled pigments.

The transformation between $(\hat{q}_{j}^{A}, \hat{q}_{j}^{B})$ and $(\hat{q}_{j}^{+}, \hat{q}_{j}^{-})$ is orthogonal and the correlated and anti-correlated normal coordinates are orthogonal. Inverting Eqns. (3.8) and (3.9), Eqns. (3.3) and (3.6) become
\[ \hat{g}_{ij}^{AB} = \sum_j \omega_j n_j \hat{q}_{j+} + \sum_k \omega_k (d_k^A - d_k^B) \hat{q}_k \]  

(3.10)

\[ \hat{c}_{D}^{AB-G} = \sum_j \omega_j \left[ \frac{2(d_j^A d_j^B)}{n_j} \hat{q}_{j+} + \frac{(d_j^A)^2 - (d_j^B)^2}{n_j} \hat{q}_{j-} \right] + \sum_k \omega_k (d_k^A + d_k^B) \hat{q}_k \]  

(3.11)

Here summation over \( j \) represents the sum over all the vibrational modes that arise due to intramolecular vibrational modes of the pigments. Summation over \( k \) represents all the environmental modes which may be delocalized over the pigments. While motion along the generalized anti-correlated coordinate \( \hat{g}_{ij}^{AB} \) is a weighted linear combination of only the anti-correlated motions along corresponding FC modes on the two pigments, motion along the generalized correlated coordinate \( \hat{c}_{D}^{AB-G} \) is more complicated, with projections along both the correlated and anti-correlated motions of the two pigments.

If no environmental modes \( k \) are coupled (all \( d_k = 0 \)) then the Hamiltonian \( \hat{H}_1 \) for the singly-excited electronic states is separable,

\[ \hat{H}_1 = \hat{H}_+ (\hat{q}_{j+}, \hat{p}_{j+}) + \hat{H}_- (\hat{q}_{j-}, \hat{p}_{j-}) \]  

(3.12)

where

\[ \hat{H}_+ = \left[ \left( \frac{E_A + E_B}{2} \right) + \sum_j \left( \frac{1}{2} \omega_j (\hat{p}_{j+}^2 + \hat{q}_{j+}^2) - \omega_j d_j^A \hat{q}_{j+} \right) \right] \hat{I}_1 \]  

(3.13)

with

\[ d_j^+ = \frac{d_j^A d_j^B}{n_j} \]  

(3.14)
\[
\hat{H}_- = \sum_j \left( \frac{1}{2} \omega_j \left( \hat{p}_{j-}^2 + \hat{q}_{j-}^2 \right) - \omega_j \left( \frac{(d_j^A)^2 - (d_j^B)^2}{n_j} \right) \hat{q}_{j-} \right) \hat{I}_1
\]

and
\[
\left[ \left( -\frac{\Delta}{2} - \frac{\hat{g}_{AB}^{AB}}{2} \right) A \right] |0_A\rangle |0_B\rangle \langle A| + J |0_A\rangle |0_B\rangle \langle A| + \left( \frac{\Delta}{2} + \frac{\hat{g}_{AB}^{AB}}{2} \right) |0_A\rangle |B\rangle \langle B|0_A\rangle .
\]

(3.15)

where \( \hat{I}_1 = |A\rangle \langle 0_B| + |0_A\rangle \langle B| \).

From Eqns. (3.13) and (3.15), it is seen that in case of identical frequencies, correlated and anti-correlated motions delocalized over the corresponding normal coordinates on the two pigments are completely separable. \( \hat{H}_+ \) involves only correlated motions (\( \hat{q}_{j+} \)) which do not change the relative energy gap \( \Delta \) between the excited pigments. Even though \( \hat{H}_- \) only involves anti-correlated motions (\( \hat{q}_{j-} \)), it can simultaneously increase (or decrease) the absolute energies of the two excited pigments, so long as the pigment displacements are unequal (\( d_j^A \neq d_j^B \)). In \( \hat{H}_- \), the individual anti-correlated motions add up according to Eqn. (3.10) to give rise to the generalized anti-correlated tuning coordinate \( \hat{g}_{AB}^{AB} \), which is solely responsible for linear vibrational coordinate dependent tuning of the relative energy gap between the two pigments. In this case of unequal displacements, the correlated and anti-correlated coordinates are asymmetric (neither symmetric nor anti-symmetric). Since the correlated vibrational motions along \( \hat{q}_{j+} \) do not alter the relative potential energy difference between the two singly-excited states, the relative quantum phase relationship between these two states also remains unaffected. The correlated Hamiltonian in Eqn. (3.13) can only cause adiabatic changes in vibrational and electronic wavefunctions. Correlated vibrations are therefore expected to oscillate coherently throughout the process of energy transfer between the pigments, as observed in refs 54 and 55. In
contrast, the anti-correlated Hamiltonian $\hat{H}_-(\hat{q}_j, \hat{p}_j)$ in Eqn. (3.15) shows that anti-correlated vibrational motions between the two pigments along $\hat{q}_j$ can affect the relative energy gap, and therefore the relative quantum phase between the two singly-excited states. Thus, anti-correlated motions strongly affect the vibrational and electronic motions, couple them together and drive non-adiabatic dynamics. Because anti-correlated vibrational motions occur between two pigments, such non-adiabatic dynamics cannot arise in an isolated pigment or in a single pigment protein $^{17}$.

In the case of identical FC displacements as well as identical frequencies, that is $d^A_j = d^B_j = d_j$, no projection of anti-correlated motions along $\hat{q}_j$'s contributes towards the generalized correlated coordinate $\hat{c}_D^{AB}$, and $\hat{H}_+$ as defined in Eqn. (3.13) becomes

$$\hat{H}_+ = \left[ \frac{E_A + E_B}{2} + \sum_j \left( \frac{1}{2} \omega_j \left( \hat{p}_j^2 + \hat{q}_j^2 \right) - \frac{\omega_j d_j \hat{q}_j^+}{\sqrt{2}} \right) \right] \hat{l}_1$$  \hspace{1cm} (3.16)

Eqn. (3.16) is a sum of terms identical to those defined in the correlated Hamiltonian $\hat{H}_{\text{cor}}$ in Eqn. (2) of ref. $^{23}$. In this case, every correlated coordinate is symmetric (equal in magnitude and sign) under interchange of the two identical pigments $^{56}$. Similarly, $\hat{H}_-$ as defined in Eqn. (3.15) becomes

$$\hat{H}_- = \left[ \sum_j \frac{1}{2} \omega_j \left( \hat{p}_j^2 + \hat{q}_j^2 \right) \right] \hat{l}_1$$

$$+ \left[ \left( -\frac{\Delta}{2} - \frac{\hat{\mathbf{g}}_{DB}^{AB}}{2} \right) A \rangle \langle 0_B | \langle A \right| J \right| A \rangle \langle 0_B | \langle B \right| \langle 0_A | J \right| 0_A \rangle \langle B | \langle 0_A | J \right| 0_A \rangle \langle B | \langle 0_A | \right]$$ \hspace{1cm} (3.17)
Eqn. (3.17) extends the interaction Hamiltonian \( \hat{H}_{int} \) defined in Eqn. [3] of ref. \(^{23}\) to a sum over vibrations. The symmetric vibrational coordinate \( \hat{q}_{j+} = (\hat{q}_A^j + \hat{q}_B^j)/2^{1/2} \) and the anti-symmetric vibrational coordinate \( \hat{q}_{j-} = (\hat{q}_A^j - \hat{q}_B^j)/2^{1/2} \) are special cases of the delocalized correlated and anti-correlated vibrational coordinates defined in Eqn. (3.8) and Eqn. (3.9), respectively. Every anti-correlated coordinate has anti-symmetric displacements (equal in magnitude and opposite in sign under interchange of the two identical pigments) on the two singly-excited electronic states. As in Eqns. (2) and (3) of ref. \(^{23}\), the single exciton Hamiltonian can be separated as
\[
\hat{H}_1 = \hat{H}_{corr} + \hat{H}_{int},
\]
with \( \hat{H}_{corr} = \hat{H}_+ \) as defined in Eqn. (3.16), and \( \hat{H}_{int} = \hat{H}_- \) as defined in Eqn. (3.17). The difference between the anti-correlated Hamiltonian \( \hat{H}_- \) in Eqn. (3.15) and Eqn. (3.17) is that, in case of Eqn. (3.15), unequal FC displacements on the corresponding FC modes of the two pigments cause the anti-correlated coordinates \( \hat{q}_{j-} \) to affect the energies of the excited pigments in a correlated manner as well. Thus, in the case of unequal FC displacements \( \hat{H}_- \) contributes to both adiabatic as well as non-adiabatic dynamics, coupling them.

### 3.2.1.1 One or two intramolecular vibrations on each pigment

The special case of one FC-active vibrational mode on each pigment, with identical frequencies \( \omega \) and FC displacements \( d \), has been treated in ref. \(^{23}\). The total single exciton Hamiltonian \( \hat{H}_1 \) is a sum of the coorelated Hamiltonian \( \hat{H}_+ \) in Eqns. (3.16) (\( \hat{H}_+ = \hat{H}_{corr} \) of ref. \(^{23}\)) and the interaction Hamiltonian \( \hat{H}_- \) in Eqn. (3.17) (\( \hat{H}_- = \hat{H}_{int} \) of ref. \(^{23}\)). The generalized tuning coordinate \( \hat{g}^{AB}_D \) derived using Eqn.(3.10) is \( \hat{g}^{AB}_D = \sqrt{2\omega d}\hat{q}_- \). Since there is only one vibrational mode on each pigment, the subscript \( j \) which represents intramolecular vibrational modes is not
needed. Tiwari et al.\textsuperscript{23} have shown that a single FC-active vibrational mode resonant with the excitonic energy gap of the dimer system can lead to strongly coupled nuclear and electronic motion on the excited state of the dimer. In their model dimer, based on the FMO protein, the non-adiabatic coupling had an energetic range of 34 cm\textsuperscript{-1} around resonance.

Several fluorescence line narrowing studies on the \textit{BChl a} chromophore of the FMO antenna complex, both isolated and in the FMO protein environment, show a series of 4 broad peaks in the 160 – 200 cm\textsuperscript{-1} vibrational frequency range. It is not clear that these are each single FC active vibrational modes – it could be that each peak represents a FC bright state coupled to more than one vibrational eigenstate\textsuperscript{57}. This range of vibrational frequencies sits close to some of the excitonic energy gaps in the FMO protein (See Table 3.1). It is clear from ref.\textsuperscript{23} that, even if the vibrational peaks represent vibrational eigenstates, more than one lies within the energetic range of the non-adiabatic coupling. To study the effect of several such closely lying FC-active vibrational modes on the dynamics, the case of two FC-active modes on each pigment is treated here under the assumption that the corresponding frequencies and FC displacements on each pigment are identical. From Eqn.(3.10), the generalized tuning coordinate is

\[ \hat{g}_{D}^{\text{AB}} = \sqrt{2} \omega_{1}d_{1}\hat{q}_{1-} + \sqrt{2} \omega_{2}d_{2}\hat{q}_{2-}, \]

where the subscripts 1 and 2 correspond to the two intramolecular FC active vibrational modes. The total single exciton Hamiltonian \( \hat{H}_i \) is a sum of the correlated and interaction Hamiltonians in Eqns. (3.16) and (3.17) respectively.

\textbf{3.2.1.2 One intramolecular vibration on each pigment with a global protein mode}

We now consider the effect of one environmental vibrational mode belonging to the protein that surrounds the pigments along with one identical FC-active vibrational mode on each pigment. The position and momentum operators corresponding to the environmental mode are \( \hat{q}_{e} \) and \( \hat{p}_{e} \), respectively.
Table 3.1. Comparison of reported off-diagonal beat frequencies from 2D spectra of the Fenna-Matthews-Olson complex with vibrational frequencies below 270 cm\(^{-1}\) measured for \(Q_y\) excitation of its pigment, bacteriochlorophyll \(a\). Single (double) prime denotes vibrational frequency on the excited (ground) electronic state. All the beating frequencies extracted from a 2D peak or a shoulder match ground state vibrational frequencies of the \(BChl\ a\) chromophore. The two frequencies (104 cm\(^{-1}\) and 147 cm\(^{-1}\)) that were not sampled from a peak or a shoulder are reported in only one study and do not match any ground state vibrational frequency.

<table>
<thead>
<tr>
<th>Resonance Raman (\tilde{\nu}'') (cm(^{-1}))</th>
<th>(\Delta)FLN (\tilde{\nu}'') (cm(^{-1})) in FMO from (C.\ tepidum)</th>
<th>(\Delta)FLN (\tilde{\nu}'') (cm(^{-1})) in FMO from (Pc.\ Aestuarii)</th>
<th>Fluorescence Excitation (\tilde{\nu}'') (cm(^{-1}))</th>
<th>Hole Burning (\tilde{\nu}') (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(87,^{6,13})</td>
<td>88</td>
<td>84</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>(104(\pm 1),^{60}) , (107(\pm 2),^{60})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(147(\pm 1),^{60})</td>
<td>117</td>
<td>117</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(161.3(\pm 0.7),^{8}) , (163(\pm 1),^{60}) , (157(\pm 1),^{61}) , (159(\pm 1),^{60})</td>
<td>164</td>
<td>167</td>
<td>167</td>
<td>173</td>
</tr>
<tr>
<td>(198.7(\pm 1.4),^{8}) , (203(\pm 1),^{60}) , (198(\pm 2),^{62})</td>
<td>190</td>
<td>191</td>
<td>191,202</td>
<td>195</td>
</tr>
<tr>
<td>(248,^{6,13})</td>
<td>235</td>
<td>239</td>
<td>243</td>
<td>237</td>
</tr>
<tr>
<td>(263(\pm 2),^{60}) , (264(\pm 2),^{60})</td>
<td>257</td>
<td>256</td>
<td>263</td>
<td>260</td>
</tr>
</tbody>
</table>

Reported 2D off-diagonal beating frequencies for FMO antenna complex from \(C.\ tepidum\) (cm\(^{-1}\))

Franck-Condon active vibrational frequencies for \textit{Bacteriochlorophyll a}
An environmental mode with frequency $\omega_e$ will in general experience a displacement in equilibrium coordinate upon electronic excitation of either pigment, but the displacements are not simply related. Upon excitation of pigment $A$, let us call the displacement in Eqn.(3.1) $d_e^A$; upon excitation of pigment $B$, the displacement along the same environmental mode is $d_e^B$. In the dimer Hamiltonian of Eqn. (3.1) with only one intramolecular FC-active mode on each pigment, an environmental mode adds a harmonic oscillator energy corresponding to $\hat{q}_e$ and $\hat{p}_e$ on each of the four electronic states, with a corresponding FC displacement of $d_e^A$, $d_e^B$ or $(d_e^A + d_e^B)$ depending upon the excitation of one pigment (singly-excited states) or both pigments (doubly-excited state). In general, low-frequency protein modes are anharmonic in nature \(^63\) and the assumption of a harmonic protein mode made here may not describe the dynamics for very long. The purpose of this simple model is to explore the consequences of reduced symmetry. If the environment has identical sites for two identical pigments, environmental vibrations may be classified as symmetric vibrations with $d_e^A = d_e^B$ or anti-symmetric vibrations with $d_e^A = -d_e^B$. Environmental modes may not have such a symmetry (e.g. the special pair in the bacterial reaction center \(^64\)).

With a single mode affecting both pigments unequally, the separation into $\hat{H}_+$ and $\hat{H}_-$ in Eqn. (3.12) no longer applies and we must revert to Eqn. (3.7). Thus, adiabatic and non-adiabatic dynamics that were completely separable into correlated and interaction parts of the Hamiltonian ($\hat{H}_{\text{corr}}$ and $\hat{H}_{\text{int}}$ respectively, in ref.\(^{23}\)) are no longer separable. Apart from the anti-correlated vibrations $\hat{q}_{j-}$ which tune the relative energy gap between the singly-excited states and allow non-adiabatic energy transfer between them, additional contributions from the environmental
mode \( \hat{q}_r \) in tuning the relative energy gap (Eqn. (3.10)) can affect the non-adiabatic energy transfer between the pigments. Recently\textsuperscript{51}, such delocalized environmental vibrational modes in the FMO antenna complex have been implicated in the fast dephasing of coherences and fast energy transfer. When the environmental vibrational mode is symmetric, that is, when the FC displacements on the excited state of the two pigments due to the environmental mode become equal, the contribution of the environmental mode towards the tuning coordinate \( \hat{g} \) vanishes (Eqn. (3.10)), and energy transfer between the pigments due to the anti-correlated vibrations is not affected.

3.3 Results

3.3.1 Dimer with one intramolecular vibrational mode per pigment

The purpose of this model is to explore the effect of one FC active vibration that is resonant with the excitonic energy gap. Excitons are delocalized electronic states that arise from the Coulombic coupling between pigments\textsuperscript{32}. The vibrationally unresolved electronic absorption spectra of photosynthetic antenna reflects the dipole strength and frequencies of transitions to excitonic states\textsuperscript{24}. For FMO, the low temperature absorption spectrum\textsuperscript{24} clearly shows that there is an exciton \( \sim 200 \text{ cm}^{-1} \) above the transition to the lowest observed exciton. A pump-probe polarization anisotropy experiment\textsuperscript{25} also establishes the distribution of excitonic energy gaps, caused by the distribution of protein environments around individual \( BChl \alpha \) pigments, for a different pair of excitons (with \( \sim 150 \text{ cm}^{-1} \) energy gap) in FMO. Resonance Raman\textsuperscript{58} and fluorescence line-narrowing\textsuperscript{42,43,59} experiments, carried out independently of, and in some cases prior to, the 2D experiments on FMO\textsuperscript{6,7,13}, have shown a FC active vibration of the \( BChl \alpha \) pigment with \( \omega / 2\pi c \sim 200 \text{ cm}^{-1} \) and \( d \sim 0.2 \). As a result, the \( v=1 \) level of this vibrational on
the lowest observed exciton is resonant with the v=0 level of the higher exciton; there can be no doubt that these experiments establish a vibrational-excitonic resonance in FMO\textsuperscript{23}.

3.3.1.1 Parameters

For calculating the excited state eigenvectors for the special case of one identical FC-active vibrational mode on each pigment, the single-exciton Hamiltonian \( \hat{H}_1 = \hat{H}_{\text{corr}} + \hat{H}_{\text{int}} \) is used, with \( \hat{H}_{\text{corr}} = \hat{H}_+ \) and \( \hat{H}_{\text{int}} = \hat{H}_- \). The Hamiltonians \( \hat{H}_+ \) and \( \hat{H}_- \) are given by Eqns. (3.16) and (3.17) respectively, for \( j_{\text{max}} = 1 \). Model parameters are roughly based on one pair of excitons in the Fenna-Matthews-Olson (FMO) complex from green sulfur bacteria\textsuperscript{24} and are identical to those used in ref.\textsuperscript{23}. In the dimer model, the transition dipoles of the two pigments are perpendicular and are assumed to be of equal strength. Based on various fluorescence and absorption based studies mentioned in Table 3.1, a FC active vibrational frequency \( \omega \approx 200 \text{ cm}^{-1} \) and stabilization energy of \( \lambda = (1/2)\omega d^2 \approx 5 \text{ cm}^{-1} \) is chosen for the pigments. Neither the pigment site energies or Coulombic couplings are directly known, only the excitonic energy gap.

An average site energy gap \( \langle \Delta \rangle = 150 \text{ cm}^{-1} \textsuperscript{25} \) and Coulombic coupling \( J = 66 \text{ cm}^{-1} \) are chosen. These values are typical for FMO\textsuperscript{24}, and chosen to reproduce an average energy gap between the excitons \( \langle \Delta_{\text{EX}} \rangle = \sqrt{2[(\Delta/2)^2 + J^2]^{1/2}} = 200 \text{ cm}^{-1} \), matching the pigment vibrational frequency. The standard deviation of excitonic energy gaps is not known for this pair of excitons at 77 K, so the standard deviation \( \sigma_\Delta = 34 \text{ cm}^{-1} \) for the pigment energy gaps was chosen to roughly match the measured standard deviation of the 155 cm\textsuperscript{-1} excitonic energy gap. For the dimer, the assumption that this gap is static in each antenna protein means that there is no decoherence between excitons in each dimer; however ensemble dephasing caused by the inhomogeneous distribution of excitonic energy gaps for an ensemble of antenna proteins (and
dimers), eliminates 2D signatures of excitonic coherence on a timescale given by the Fourier transform of the excitonic energy gap distribution\textsuperscript{23}. No published model has reconciled the measured 140-180 fs damping timescale for this inhomogeneous dephasing of excitonic coherence at \(\sim 150 \text{ cm}^{-1}\), with the frequently proposed assignment of the 2D cross-peak beating at \(\sim 160 \text{ cm}^{-1}\) to electronic coherence or vibronic coherence on the excited state.

Table 3.1 compares the oscillation frequencies reported in various 2D experiments on the FMO antenna complex with the ground state vibrational frequencies of \(BChl\ a\), the chromophore in the FMO antenna. Apart from the \(\sim 200 \text{ cm}^{-1}\) oscillation frequency that shows up in the 2D spectra, other reported 2D cross-peak oscillation frequencies match with \(BChl\ a\) ground state FC-active vibrations, suggesting either that additional vibrational-electronic resonances are likely (ref.\textsuperscript{23} Table S1) or that the 2D spectra are measuring primarily vibrational quantum beats, or both.

To model the broadened 2D peakshapes observed in the experiments\textsuperscript{6,13}, broadening due to a correlated component of the low-frequency phonon sideband of FMO protein is incorporated using a Brownian oscillator that introduces an effective damping of the coherence between the ground and singly-excited state, and therefore broadens the 2D peakshapes. In general, all FC active vibrations of the system and bath (correlated and anti-correlated) contribute to homogeneous decoherence between the ground state and a singly-excited state of the system. However, this Brownian oscillator lineshape only takes decoherence due to correlated vibrations into account. A critically damped Brownian oscillator with a frequency of 70 cm\(^{-1}\) and a stabilization energy of 30 cm\(^{-1}\), which reproduces the Stokes’ shift in FMO\textsuperscript{2}, is used. The calculations were carried out at a temperature \(T = 80\ K\) used for many experiments. This Brownian oscillator broadening is just an effective lineshape. It gives a time-dependent
peak shape that probably arises from a great many modes because it has roughly the same spectral density even if the dynamics are over-simplified. The timescale for damping of homogeneous coherence between the ground and a single exciton state (of a single antenna protein) can be inferred from the anti-diagonal linewidth, or the echo slice, of an isolated diagonal peak in a 2D correlation spectrum. Any static inhomogeneity in the ground to singly-excited state energy gap is rephased in a photon echo and does not affect the decay of the echo slice.

In contrast to the homogeneous decoherence between the ground state and a single exciton state, homogeneous decoherence between the two single exciton states is only caused by the anti-correlated FC-active vibrations. Unlike the homogeneous decoherence between the ground and a single-excited state that is reflected in the anti-diagonal linewidth of an isolated 2D diagonal peak, the timescale of homogeneous decoherence between the two single exciton states that shuts down coherent energy transfer between them has been observed by inhomogeneous dephasing in 2D experiments (see above) and has not been probed by any experiment reported to date. Homogeneous decoherence between the two singly-excited states due to anti-correlated vibrations is not included in the present model. However, inhomogeneous dephasing between the two single exciton states is included by a static inhomogeneous distribution of excited state energy gaps with a standard deviation $\sigma_\Delta = 34 \text{ cm}^{-1}$. This only introduces ensemble dephasing of 2D cross-peak oscillations without introducing homogeneous decoherence between the two single-excited states. Unlike the inhomogeneity in the ground to singly-excited state energy gaps, an inhomogeneity in the relative energy gaps between singly-excited states is not rephased in a photon echo and can affect the decay of the 2D echo slice.
3.3.1.2 Linear Absorption Spectrum

To roughly understand the absorption spectrum of the simplest case of a dimer with one identical FC-active mode per pigment described in Section 3.2.1.1 and the above mentioned parameters, it is instructive to see how the localized undisplaced vibrational basis kets $|\nu_a\rangle|\nu_b\rangle$ transform into the delocalized vibrational basis kets $|\nu_+\rangle|\nu_-\rangle$. The undisplaced vibrational-site basis is used for these calculations. In this basis, all the electronic states have a common set of vibrational basis kets that are vibrational eigenstates of the ground electronic state. The site electronic states $|A\rangle|0_b\rangle$ and $|0_a\rangle|B\rangle$ are coupled via electronically off-diagonal but vibrationally diagonal Coulombic coupling $J$ such that only the basis states with identical vibrational quantum numbers on both pigments are mixed together by it. The excitonic states $|\alpha\rangle$ and $|\beta\rangle$ are obtained from the electronic states in the site basis by diagonalizing the Coulomb coupling $J$ using a 2x2 matrix transformation, such that

$$
|\alpha\rangle = \cos \theta |A\rangle |0_b\rangle - \sin \theta |0_a\rangle |B\rangle \\
|\beta\rangle = \sin \theta |A\rangle |0_b\rangle + \cos \theta |0_a\rangle |B\rangle
$$

(3.1)

where $\theta$ is the mixing angle given by $\theta = \arctan \left(\frac{J}{\sqrt{\left(\Delta/2 + \sqrt{\left(\Delta/2\right)^2 + J^2}\right)}}\right)$. For the one-mode dimer parameters, with site energy gap $\Delta = 150$ cm$^{-1}$ and Coulomb coupling $J = 66.14$ cm$^{-1}$ (described above in Section 3.1.1), the mixing angle $\theta$ between the excited pigments $A$ and $B$ is $\sim 21^\circ$ such that electronic character of exciton $|\alpha\rangle$ is primarily that of pigment $A$ and electronic character of exciton $|\beta\rangle$ is primarily that of pigment $B$. In the vibrational-excitonic basis states $|\alpha\rangle|v_A = m\rangle|v_B = n\rangle$ and $|\beta\rangle|v_A = m\rangle|v_B = n\rangle$, the electronic basis is delocalized while the vibrational basis is still localized. Under the above transformation from site to excitonic basis,
only a part of the vibrationally off-diagonal coupling terms $\hat{g}_{D}^{AB} / 2$ in Eqn.(3.17) stays
electronically diagonal. A part of the vibrationally off-diagonal coupling $\hat{g}_{D}^{AB} / 2$ becomes
electronically off-diagonal as well, and therefore can mix different excitons. The part of the
linear vibrational coupling that remains electronically diagonal does not mix two different
excitons and is ignored for the purpose of this simplified illustration. For an excitonic energy
gap resonant with the vibrational frequency of the FC active mode, the states involved in the
splitting seen in the absorption spectrum can be roughly understood from the isoenergetic
vibrational-excitonic states and their coupling. In the vibrational-excitonic basis there are two
states corresponding to the lower energy exciton $|\alpha\rangle$, each with one quantum of vibrational
excitation, that are isoenergetic with the higher exciton $|\beta\rangle$ with no vibrational excitation. The
three isoenergetic basis states, with basis energy $\varepsilon$ are:

$$
\begin{align*}
|\alpha\rangle_{v_A=1} |v_B=0\rangle \\
|\alpha\rangle_{v_A=0} |v_B=1\rangle \\
|\beta\rangle_{v_A=0} |v_B=0\rangle \\
\end{align*}
$$

(3.2)

$|\beta\rangle_{v_A=0} |v_B=0\rangle$ is coupled to $|\alpha\rangle_{v_A=1} |v_B=0\rangle$ and $|\alpha\rangle_{v_A=0} |v_B=1\rangle$ through the
excitonically and vibrationally off-diagonal part of the linear vibrational coupling given by
$\hat{g}_{D}^{AB} \sin 2\theta / 2$. The first basis two states in Eqn. (3.2) are not coupled to each other because there
is a simultaneous change in both vibrational quantum numbers between them. Thus, the
Hamiltonian matrix $\hat{H}$ coupling the three isoenergetic states in the vibrationally localized
vibrational-exciton basis becomes:
\[
\hat{H} = \begin{pmatrix}
\varepsilon & 0 & -\frac{\hat{g}_{D}^{AB} \sin 2\theta}{2} \\
0 & \varepsilon & -\frac{\hat{g}_{D}^{AB} \sin 2\theta}{2} \\
-\frac{\hat{g}_{D}^{AB} \sin 2\theta}{2} & -\frac{\hat{g}_{D}^{AB} \sin 2\theta}{2} & \varepsilon
\end{pmatrix}.
\] (3.3)

Under the transformation \(U\)

\[
U = \begin{pmatrix}
\frac{1}{\sqrt{2}} & -\frac{1}{\sqrt{2}} & 0 \\
\frac{1}{\sqrt{2}} & \frac{1}{\sqrt{2}} & 0 \\
0 & 0 & 1
\end{pmatrix}
\] (3.4)

the matrix in Eqn. (3.3) transforms to

\[
\begin{pmatrix}
\varepsilon & 0 & 0 \\
0 & \varepsilon & -\frac{\hat{g}_{D}^{AB} \sin 2\theta}{\sqrt{2}} \\
0 & -\frac{\hat{g}_{D}^{AB} \sin 2\theta}{\sqrt{2}} & \varepsilon
\end{pmatrix}.
\] (3.5)

The coupling \(\hat{g}_{D}^{AB} \sin 2\theta/\sqrt{2}\) in Eqn. (3.5) couples together states with different excitonic character such that the vibrational-electronic state \(|\beta\rangle|v_A = 0\rangle|v_B = 0\rangle\) only couples with \(-|\alpha\rangle(|v_A = 1\rangle|v_B = 0\rangle-|v_A = 0\rangle|v_B = 1\rangle)/\sqrt{2}\). The vibrational part of these vibrational-electronic states can be understood from the viewpoint of delocalized vibrational modes. Using the vibrational wavefunctions \(\langle q_j |v_j \rangle\) where \(|q_j \rangle\) is the position ket corresponding to a vibration on pigment \(j\), and Eqns. (3.8) and (3.9) one can show that
$$|\alpha\rangle|v_+ = 1\rangle|v_- = 0\rangle = \frac{1}{\sqrt{2}} (|\alpha\rangle|v_A = 1\rangle|v_B = 0\rangle + |\alpha\rangle|v_A = 0\rangle|v_B = 1\rangle)$$

$$|\alpha\rangle|v_+ = 0\rangle|v_- = 1\rangle = -\frac{1}{\sqrt{2}} (|\alpha\rangle|v_A = 1\rangle|v_B = 0\rangle - |\alpha\rangle|v_A = 0\rangle|v_B = 1\rangle)$$

$$|\beta\rangle|v_+ = 0\rangle|v_- = 0\rangle = |\beta\rangle|v_A = 0\rangle|v_B = 0\rangle.$$ (3.6)

The transformed matrix in Eqn. (3.5) shows how the first state in Eqn. (3.6) with a quantum of excitation along the correlated vibrational coordinate $\hat{q}_+$ is decoupled from the other two isoenergetic states. Only a quantum of excitation along the anti-correlated vibrational coordinate $\hat{q}_-$ on exciton $\alpha$ is coupled to the higher energy exciton $\beta$. With vibrational-electronic resonance, this mixing gives rise to mixed vibrational-electronic eigenvectors with electronic character strongly dependent on the anti-correlated nuclear coordinate. Thus, transition dipoles from the ground to the excited state of the dimer acquire a strong nuclear coordinate dependence. Using a Condon approximation in such a scenario neglects these effects. Such vibrational-electronic mixing has also been shown to give rise to an ‘unavoidable nested funnel’ on the excited state that allows non-adiabatic energy transfer between the excited states, and leads to enhanced Raman excitation of the anti-correlated vibrational wavepackets on the ground electronic state of the dimer.

Fig. 3.1a shows the absorption cross-section for a one-mode dimer calculated using the one-mode dimer parameters at vibrational-electronic resonance in Section 3.3.1.1.
Figure 3.1a. Absorption cross-section corresponding to a dimer with one identical FC active vibrational mode per pigment, discussed in Section 3.2.1 of the text. The site energy gap between the pigments $A$ and $B$ is 150 cm$^{-1}$ with a coupling of 66.14 cm$^{-1}$ such that the excitonic energy gap of 200 cm$^{-1}$ is resonant with the FC-active vibrational frequency. The stabilization energy $\lambda$ for the vibration is 5 cm$^{-1}$. The calculation assumes equal ground to first excited state transition dipole strengths for both the pigments. The spectrum is calculated at a temperature $T = 80$ K.
The higher energy exciton peak is split into peaks marked as 2a and 2b on the plot and lie at 11655 cm$^{-1}$ and 11684 cm$^{-1}$ respectively. Peaks 2a and 2b are separated by $\sim$29 cm$^{-1}$ due to vibronic coupling between the states $|\beta\rangle|v_+ = 0\rangle|v_- = 0\rangle$ and $|\alpha\rangle|v_+ = 0\rangle|v_- = 1\rangle$, as described in Section 3.3.1.2 of the text. The lower energy exciton peak 1 lies at 11470 cm$^{-1}$. The peak intensities are shown relative to peak 1 which has a normalized intensity of one. A small peak at 11670 cm$^{-1}$, between peaks 2a and 2b, with intensity about 100x smaller than peak 1 is due to the state $|\alpha\rangle|v_+ = 1\rangle|v_- = 0\rangle$, with a vibrational excitation into the correlated vibration. As described in Section 3.3.1.2, this state does not mix with the higher energy exciton $|\beta\rangle|v_+ = 0\rangle|v_- = 0\rangle$ and therefore has a weak FC intensity. Very weak peaks between 11850 – 11890 cm$^{-1}$ with intensities $\sim$200x less than peak 1 are higher FC progressions involving transitions from ground electronic and vibrational state $|0\rangle|v_+ = 0\rangle|v_- = 0\rangle$ to singly-excited states $|\beta\rangle|v_+ = 0\rangle|v_- = 1\rangle$ and $|\alpha\rangle|v_+ = 0\rangle|v_- = 2\rangle$. Despite non-adiabatic vibrational electronic mixing between these states, a weak $\Delta v_\pm = \pm 1$FC factor involved in making a transition from the ground state renders these peaks very weak. A critically damped Brownian oscillator with frequency 70 cm$^{-1}$ and stabilization energy 30 cm$^{-1}$ is used to model the correlated line broadening in the FMO antenna complex. The corresponding absorption cross-section is shown in red. The vibronic splitting of the higher energy exciton is obscured due to correlated line broadening, which amounts to convolution of the stick spectrum with the correlated Brownian oscillator lineshape.

**Figure 3.1b.** The line strength of peaks 2a and 2b in Fig. 3.1a as a function of vibrational frequency. At the resonant vibrational frequency of 200 cm$^{-1}$, the two split peaks have equal line strengths. The line strengths not being exactly equal at the vibrational frequency of 200 cm$^{-1}$ indicates that the vibrational frequency of the two adiabatic excitonic curves, obtained from the site basis by neglecting the nuclear momentum and diagonalizing the Coulomb coupling $J$, are not identical. Because of different vibrational frequencies of the two curves, the excitonic energy gap of 200 cm$^{-1}$ is no longer resonant with the vibrational frequency, such that the amplitudes of the two split peaks become unequal at the 200 cm$^{-1}$ vibrational frequency. The FWHM width of the non-adiabatic coupling is defined based on the range of the vibrational frequencies with peak amplitude atleast half of the amplitude at resonance; the peak amplitudes 2a and 2b are in a 1:2 (or 2:1) ratio at the half maxima.
For the stick spectrum (shown in black), the transition to the lower energy exciton has an essentially unmodified \( |0\rangle |v_+ = 0\rangle |v_- = 0\rangle \) to \( |\alpha\rangle |v_+ = 0\rangle |v_- = 0\rangle \) band origin (peak 1a). The higher energy peak is split by \( \sim 29 \text{ cm}^{-1} \) into two lines (2a and 2b) with nearly equal intensity due to vibronic coupling between the states \( |\beta\rangle |v_+ = 0\rangle |v_- = 0\rangle \) and \( |\alpha\rangle |v_+ = 0\rangle |v_- = 1\rangle \) in Eqn. (3.6). For a purely electronically coupled dimer (vibrational stabilization energy \( \lambda = 0 \text{ cm}^{-1} \)), the excitonic state \( |\beta\rangle |v_+ = 0\rangle |v_- = 0\rangle \) has all the intensity. In the presence of FC vibrational displacement upon electronic excitation, this intensity is partially borrowed by the isoenergetic state \( |\alpha\rangle |v_+ = 0\rangle |v_- = 1\rangle \). A small peak between the peaks 2a and 2b is due to the state \( |\alpha\rangle |v_+ = 1\rangle |v_- = 0\rangle \), with a quantum of excitation along the correlated vibrational coordinate \( \hat{q}_+ \). Since this state does not borrow intensity from the isoenergetic state \( |\beta\rangle |v_+ = 0\rangle |v_- = 0\rangle \), a weak \( \Delta v_+ = \pm 1\text{FC} \) factor renders transition intensity from the ground electronic and vibrational state \( |0\rangle |v_+ = 0\rangle |v_- = 0\rangle \) to the state \( |\alpha\rangle |v_+ = 1\rangle |v_- = 0\rangle \) ~100x less than peak 1. Higher FC progressions involving transitions from the ground state \( |0\rangle |v_+ = 0\rangle |v_- = 0\rangle \) to higher lying vibrational-electronic states with mixed \( |\alpha\rangle |v_+ = 0\rangle |v_- = 2\rangle \) and \( |\beta\rangle |v_+ = 0\rangle |v_- = 1\rangle \) character are weak due to a \( \Delta v_- = \pm 1\text{FC} \) factor and lead to very weak peaks in the frequency range 11850 – 11890 cm\(^{-1}\) with intensities ~200x less than peak 1. There are also weak hot band transitions from \( |0\rangle |v_+ = 0\rangle |v_- = 1\rangle \) to the resonant pair \( |\alpha\rangle |v_+ = 0\rangle |v_- = 1\rangle \) and \( |\beta\rangle |v_+ = 0\rangle |v_- = 0\rangle \) near the band origin peak 1a. Similarly, weak hot band transition from \( |0\rangle |v_+ = 1\rangle |v_- = 0\rangle \) to \( |\alpha\rangle |v_+ = 1\rangle |v_- = 0\rangle \) are also present near peak 1a. These transitions derive their strength from
\[ \Delta v_\omega = 0 \text{ and } \Delta v_\nu = 0 \] FC factors, but are weak because only \( \sim 5\% \) of the total ground state population is on the vibrationally hot ground state at \( T = 80 \text{ K} \).

When a Brownian oscillator lineshape is used to model the peak broadening due to the correlated part of the low-frequency phonon sideband in FMO, the absorption linewidths (shown in red) completely obscure the splitting seen in the stick spectrum even at 80 K. The Brownian oscillator lineshape used here does not include the Gaussian inhomogeneous broadening of the excited pigment energy gaps (Section 3.3.1.1). In linear absorption and 2D spectra of the actual antenna protein, with overlapping peaks and both homogeneous and inhomogeneous broadening present, the peak locations in a 2D spectrum match a purely electronic model Hamiltonian even though the underlying vibronic structure may generate different dynamics.

Fig. 3.1b shows the amount of intensity borrowing, or the vibrational-electronic mixing, between the second and the third state in Eqn.(3.6), as a function of vibrational frequency for a fixed excitonic energy gap of 200 cm\(^{-1}\). The amplitudes of the split peaks are equal at resonance, that is, the intensity borrowing is maximum at resonance (\( \omega_{\nu b} = 200 \text{ cm}^{-1} \)), with a single dominant peak for much lower or much higher vibrational frequencies. A width with an approximate full-width at half maximum (FWHM) of 34 cm\(^{-1}\) is calculated based on how off-resonant the vibrational frequency can be on either side of the resonance, so that peak amplitudes in the absorption spectrum are split in a 1:2 (or 2:1) ratio. The width of such a vibrational-electronic resonance is dictated by the strength of the the dimensionless FC displacement \( d \) and the electronic coupling \( J \). As mentioned in Section 3.3.1.1, for a vibrational frequency of 200 cm\(^{-1}\), the dimensionless displacement \( d = 0.22 \) corresponds to a stabilization energy of 5 cm\(^{-1}\). This stabilization energy is roughly based on the observed \( \sim 3-5 \text{ cm}^{-1} \) vibrational stabilization energy (\( \lambda = \frac{1}{2} \omega d^2 \)) from various fluorescence line-narrowing studies on \( \text{BChl a}^{42,43,59} \). Electronic
coupling between different pigment sites in the FMO antenna protein can range from \(~30-110\) cm\(^{-1}\) \(^{24,51,67,68}\) and is chosen to be 66.14 cm\(^{-1}\) for this study. A different choice of \(J\) will change the 34 cm\(^{-1}\) FWHM of this resonance, as would a different vibrational stabilization energy. Due to a finite width of the resonance, all FC-active modes lying close to the excitonic energy gap contribute to vibrational-electronic mixing.

3.3.1.3 2D Spectra.

In a 2D experiment \(^{69}\), three noncollinear femtosecond pulses (wavevectors \(k_a\), \(k_b\) and \(k_c\)) are crossed inside a sample and the four-wave mixing signal radiated in the phase matching direction \(k_s = k_c + k_b - k_a\) is measured as a function of \(\tau\), the time delay between the pulses \(a\) and \(b\), and \(t\), the time after pulse \(c\), while the time delay \(T\), between the second and third pulses, is kept constant. The time axes \(\tau\) and \(t\) are Fourier-transformed to give an excitation frequency axis \(\omega_\tau\) and a detection frequency axis \(\omega_t\), respectively. Some coherence signatures in 2D spectroscopy depend on relative pulse ordering of pulses \(a\) and \(b\). If pulse \(a\) arrives before pulse \(b\), \(\tau\) is positive and an N-type 2D spectrum is obtained. This time-ordering allows photon-echo rephasing of inhomogeneity for a two-level system, so that N-type 2D spectra are often called rephasing 2D spectra. When \(\tau\) is negative, a P-type (often called non-rephasing) 2D spectrum is obtained. A scan over both positive and negative \(\tau\) generates, a 2D spectrum equal to the sum of N-type and P-type 2D spectra, which is called a 2D correlation \((T = 0)\) or relaxation \((T > 0)\) spectrum; the real part of 2D relaxation spectra reveals net changes in sample absorption as a function of excitation and detection frequencies. Peak oscillations in a series 2D spectra as a function of \(T\) indicate quantum beats which can arise due to quantum coherence between two
vibrational or electronic levels on either the ground or the excited electronic state. For small FC
displacements as in photosynthetic chromopores, modulations in a 2D spectrum due to purely
vibrational coherences were initially expected to be weak and are weak in isolated
photosynthetic pigments and one-pigment proteins. Ref. has shown that non-adiabatic
vibrational-electronic mixing on the excited states of multi-pigment proteins can lead to
electronically enhanced Raman excitation of vibrational wavepackets on the ground electronic
state of the antenna (that is, the electronic state in which no pigments are excited). The
modulation amplitude from ground state vibrations in pigments that were not electronically
excited is expected to dominate at long times in a real rephasing 2D spectrum. Such signatures
arising from ground state vibrational wavepackets match many 2D signatures of photosynthetic
energy transfer (See Table S2 of Ref. 23).

Fig. 3.2a shows the ground-state real rephasing 2D spectrum calculated for a dimer with
one identical FC-active vibrational mode per pigment. The parameters are mentioned in Section
3.3.1.1, and are identical to the parameters used for calculating the absorption spectrum in Fig.
3.1a. The vibrational frequency is resonant with the excitonic energy gap of 200 cm\(^{-1}\). Peak
broadening due to the correlated component of the phonon sideband is introduced by a critically
damped Brownian oscillator with a 30 cm\(^{-1}\) Stokes’ shift. Unlike the 2D calculation in Fig. 2 of
ref. 23, this calculation does not include the Gaussian inhomogeneous broadening due to a
distribution of excited-state energy gaps. The maximum of the plotted spectrum is normalized to
one. The structure of the four prominent peaks in the 2D spectrum is similar to that in Fig. 2 of
ref. 23 and is almost not affected by the exclusion of excited-state inhomogeneous broadening.
Figure 3.2a. Real part of the ground electronic state contribution to the “rephasing” 2D electronic spectrum for a dimer with one identical FC active vibrational mode per pigment. The ground to excited state energy gap \((E_A + E_B)/2 = 11,574 \text{ cm}^{-1}\). The spectrum is calculated at a temperature of 80K. Here, the vertical – \(\omega_\tau\) frequency axis is the excitation axis and the horizontal \(\omega_t\) frequency axis is the detection axis. The calculation does not include the inhomogeneous distribution of excited state energy gaps between the pigments. The amplitude of the 2D spectrum for each frequency pair is indicated by color and via contours at the 0, 2, 4, 6, 8, 10-90 % levels. Positive and negative contours are solid and dashed, respectively. The waiting time is \(T = 0\) fs. The 2D spectra are dominated by 4 resolved peaks which oscillate in amplitude and shape with \(T\). Due to correlated line broadening introduced by a critically damped Brownian oscillator with frequency 70 cm\(^{-1}\) and stabilization energy 30 cm\(^{-1}\), the ~29 cm\(^{-1}\) peak splitting under the higher exciton peak in Fig. 3.1a is completely obscured. Diagonal peak maxima are marked with an o (lower left - DP1, upper right - DP2); cross-peak maxima are marked with an x (upper left - CP12, lower right - CP21). The corresponding 2D peak coordinates \((\omega_t, \omega_\tau)\) in centimeters\(^{-1}\) are: diagonal peak DP1 (-11,487, 11,479), diagonal peak DP2 (-11,687, 11,678), cross-peak CP12 (-11,687, 11,496) and cross-peak CP21 (-11,505, 11,670).

Figure 3.2b. Variation of CP12 and CP21 oscillation amplitudes and the relative phase of DP1 and CP12, as a function of vibrational frequency of the FC-active mode, over a range of 130 cm\(^{-1}\) to 270 cm\(^{-1}\), corresponding to the 2D spectra calculated above. The stabilization energy \(\lambda = (1/2)\omega d^2\) for the FC-active vibration is kept constant at 5 cm\(^{-1}\) throughout the range of vibrational frequencies. Thus, lower vibrational frequencies have a larger dimensionless displacement \(d\) compared to vibrational frequencies above resonance. This is manifested in
asymmetric amplitudes of CP12 and CP21 across the resonant vibrational frequency of 200 cm$^{-1}$. As predicted by resonant non-adiabatic vibrational-electronic mixing model, CP12 oscillation amplitude is enhanced at resonance whereas CP21 oscillation amplitude stays approximately constant. In the presence of correlated broadening giving rise to unresolved peaks the CP12/CP21 oscillation amplitude asymmetry is ~ 14x at resonance. The relative phase of oscillation of DP1 versus CP12 over the range of vibrational frequencies is ~115°. c) Peak amplitudes of diagonal peaks, DP1 in black and DP2 in gray, and cross-peaks, CP12 in red and CP21 in green, as a function of population time $T$ in the “rephasing” 2D spectrum with contributions from ground and excited states, calculated at a temperature of 100 K. The average site energy gap is 200 cm$^{-1}$ with a Coulomb coupling of 66.14 cm$^{-1}$, yielding an excitonic energy gap of 240 cm$^{-1}$. The vibrational frequency is 100 cm$^{-1}$, which is non-resonant with the excitonic energy gap. The vibrational stabilization energy is 15 cm$^{-1}$. The cross-peak oscillations at the donor-acceptor electronic energy gap frequency of ~240 cm$^{-1}$ are rapidly suppressed by the presence of an inhomogeneous distribution of excited state energy gaps corresponding to a standard deviation $\sigma_\Delta = 34$ cm$^{-1}$, revealing longer-lived oscillations at the vibrational frequency of 100 cm$^{-1}$.

**Figure 3.2 c.** Peak amplitudes of diagonal (DP1 and DP2) and cross-peaks (CP12 and CP21) as a function of population time $T$ in the “rephasing” 2D spectrum with contributions from ground and excited states, calculated at a temperature of 100 K. The average site energy gap is 200 cm$^{-1}$ with a Coulomb coupling of 66.14 cm$^{-1}$. The vibrational frequency is 100 cm$^{-1}$, which is non-resonant with the excitonic energy gap of ~240 cm$^{-1}$. The vibrational stabilization energy is 15 cm$^{-1}$. The cross-peak oscillations at the donor-acceptor electronic energy gap frequency of ~240 cm$^{-1}$ are rapidly suppressed by the presence of an inhomogeneous distribution of excited state energy gaps corresponding to a standard deviation $\sigma_\Delta = 34$ cm$^{-1}$, leading to longer-lived oscillations at the vibrational frequency of 100 cm$^{-1}$. 
The 2D peak coordinates \((\omega_1, \omega_2)\) in cm\(^{-1}\) (corresponding to the nearest grid points) are: diagonal peak DP1 (-11,477, 11,465), diagonal peak DP2 (-11,678, 11,667), cross-peak CP12 (-11,671, 11,486) and cross-peak CP21 (-11,500, 11,658). Note that the cross-peak frequency coordinates do not exactly match the frequency coordinates of the diagonal peaks they connect and are not precisely opposite. This is a consequence of peak broadening, shifts, and overlap in the Brownian oscillator lineshapes. Experimental 2D spectra show similar effects on the 2D frequency coordinates. Broadened 2D peakshapes completely obscure the \(\sim 29\) cm\(^{-1}\) splitting under the higher energy diagonal peak seen in the stick absorption spectrum (Fig. 3.1a).

Fig. 3.2b shows the variation of the opposite cross-peak oscillation amplitudes with \(T\) as the vibrational frequency is tuned across resonance, while keeping the excitonic energy gap fixed at 200 cm\(^{-1}\) and the stabilization energy, \(\lambda = (1/2) \omega d^2\), fixed at 5 cm\(^{-1}\) (thus \(d\) varies with \(\lambda\)). The peaks are sampled at positions corresponding to their maxima at \(T = 0\), at the 2D coordinates mentioned above. The cross-peak closer to the excitation axis, CP12, experiences an enhancement in oscillation amplitude near the resonant frequency with a \(\sim 130\) cm\(^{-1}\) width. This approximate width is based on the vibrational frequencies (on either side of the resonant vibrational frequency) for which the corresponding oscillation amplitude is 50% of the oscillation amplitude at resonance. Ref.\(^{23}\) shows that such an increase in oscillation amplitude near resonance is a consequence of non-adiabatic enhancement of ground state vibrational wavepackets which is maximum at resonance. CP21 oscillations are not enhanced because of weak \(\Delta \nu = \pm 1\) optical transitions involved in the Feynman pathway giving rise to CP21 oscillations in the ground state real rephasing 2D spectrum (See Fig. 3 of ref.\(^{23}\)). At resonance, ground state CP12 oscillation amplitude is \(\sim 14\)x more than CP21 oscillations.
Fig. 3.2b also shows the variation for relative phase of oscillation between the lower energy diagonal peak DP1 and the cross-peak CP12 as the vibrational frequency is tuned across resonance. The relative phase varies by $\sim 115^\circ$ over the range of vibrational frequencies sampled.

The above 2D calculations only include the ground state contributions for the case of resonant vibrational and electronic frequencies. In a 2D calculation including both ground and excited state contributions and resonant vibrational and electronic frequencies, a frequency based distinction between electronically enhanced ground state vibrational oscillations and purely electronic oscillations on the excited state is not possible. Ref. 23 has shown that an inhomogeneous distribution of excited state energy gaps wipes out 2D signatures of excited state electronic coherence within $\sim 300$ fs. The effect of excited state static inhomogeneity on the purely electronic oscillations can be clearly seen when a frequency distinction between vibrational and electronic coherence is possible. Fig. 3.2c shows 2D peak oscillations corresponding to a real rephasing calculation where the excitonic energy gap of $\sim 240$ cm$^{-1}$ (average site energy gap $\Delta = 200$ cm$^{-1}$, Coulomb coupling $J = 66.14$ cm$^{-1}$) is not resonant with the vibrational frequency of 100 cm$^{-1}$. The calculation includes contributions from the ground as well as the excited states and is averaged over the excited state inhomogeneous distribution of energy gaps. In contrast to Fig. 5 of ref. 23, where excitonic and vibrational frequencies are the same, a frequency distinction between the excitonic energy gap and the vibrational frequency in Fig. 3.2c makes it easy to recognize that the initial fast oscillations, easily seen on the cross-peaks (as predicted for purely electronic coherence$^2$), with period $\sim 140$ fs corresponding to the 240 cm$^{-1}$ excitonic energy gap, dephase within $\sim 300$ fs to give way to longer lived oscillations at the vibrational frequency of 100 cm$^{-1}$, that are present on all 4 peaks and larger on the diagonal peaks (as predicted for FC active vibrations$^{70,71}$). Thus, inhomogeneous ensemble dephasing
rapidly wipes out 2D signatures of excited state electronic coherence leaving vibrational and vibrational-electronic beats \(^{23}\). The ground state signatures are not damped by such inhomogeneity on the excited electronic state.

To understand the enhancement of ground state vibrations due to non-adiabatic vibrational-electronic mixing on the excited state, and how it shows up in the 2D spectrum, it is instructive to use the resolved lineshapes of a Bloch model with slower dephasing. A Bloch dephasing model has exponential decay of density matrix populations and coherences and 2D Lorentzian peakshapes (which may be phase-twisted). Even though all the effects of bath memory are neglected in a Bloch model \(^{72}\), resolved peaks help connect the stick absorption spectrum of Fig. 3.1a to the 2D spectrum. For the Bloch model, a population lifetime of \(T_1 = 250\) fs is used, with a lifetime limited dephasing time \(T_2 = 500\) fs for the decay of the coherence created during the first and the third time intervals in a 2D experiment. Figs. 3.3(a-c) shows ground state real rephasing 2D spectra calculated for a dimer with one identical Franck-Condon vibrational mode on each pigment. The site energy gap is 150 cm\(^{-1}\) and the Coulomb coupling \(J\) is 66.14 cm\(^{-1}\) such that the excitonic energy gap is 200 cm\(^{-1}\). The vibrational frequency of 200 cm\(^{-1}\), with a stabilization energy \(\lambda = 5\) cm\(^{-1}\), is resonant with the excitonic energy gap. This set of parameters is described in Section 3.3.1.1 and is the same as that used in Fig. 3.2a, except that the present calculation uses the Bloch dephasing model for resolved 2D peakshapes.
Figure 3.3(a-c). Effect of vibrational frequency tuning on vibrationally resolved 2D electronic spectra of a dimer. The real part of the ground state “rephasing” 2D spectrum is shown Bloch model dephasing (exponential decay of population and coherence) with population lifetime $T_1 = 250$ fs in order to partly resolve the peak splitting observed in the absorption spectrum of Fig. 3.1a under DP2. Spectra in all three panels are calculated for a waiting time $T=0$ fs. Compared to the excitonic energy gap of 200 cm$^{-1}$, the frequency of the Franck-Condon active vibration is below resonance in Fig. 3.3a ($\omega/(2\pi c) = 130$ cm$^{-1}$), at resonance in Fig. 3.3b ($\omega/(2\pi c) = 200$ cm$^{-1}$), and above resonance in Fig. 3.3c ($\omega/(2\pi c) = 270$ cm$^{-1}$). In each of the three panels, labels on the horizontal (vertical) dashed lines indicate the excitation (detection or radiation) frequencies, respectively, at which the lines are drawn. ‘E1’ and ‘E2’ correspond to the excitation frequencies for the first and second excitons, respectively, while ‘v’ corresponds to the vibrational frequency used in each calculation. The 2D peaks, which have a vibrational, electronic or a mixed vibrational-electronic origin, fall near the intersections between dashed lines. All the other parameters - vibrational stabilization energy of 5 cm$^{-1}$, temperature of 80 K and ground to excited state energy gap of 11574 cm$^{-1}$, are the same as in Fig. 3.2a. For the off-resonant cases, Figs. 3.3a and 3.3c, the vibrational peaks are separated from the purely excitonic peaks, which are at approximately 200 cm$^{-1}$ energy gap from DP1 along each axis. 3.3a.) When the vibrational frequency is off-resonant at 130 cm$^{-1}$, ‘E1’ corresponds to an energy of 11,473.8 cm$^{-1}$ along each axis and ‘E2’ corresponds to an energy of 11,674.2 cm$^{-1}$ along each axis. The vibrational energy corresponding to the label ‘v’ is 130 cm$^{-1}$. 3.3c.) When the vibrational frequency is off-resonant at 270 cm$^{-1}$, ‘E1’ corresponds to an energy of 11,467.5 cm$^{-1}$ along each axis. ‘E2’ corresponds to an energy of 11,661.7 cm$^{-1}$ along each axis. The vibrational energy corresponding to the label ‘v’ is 270 cm$^{-1}$. 3.3b.) At vibrational-electronic resonance, peaks in the diagonal and cross-peak regions arise from wave-mixing pathways involving eigenstates with a mixed $|\alpha\rangle$ and $|\beta\rangle$ excitonic character, and cannot be separated as having a purely electronic or vibrational origin. The Bloch dephasing model used only partly resolves the 2D peaks at resonance. The ~29 cm$^{-1}$ splitting under the higher energy peak in Fig. 3.1a shows up in the 2D spectrum in Fig. 3.3b such that DP2, CP12 and CP21 are split. ‘E1’ corresponds to an energy of 11,467.5 cm$^{-1}$ along each axis. ‘E2’ corresponds to an energy of 11,668 cm$^{-1}$ along each axis, but the intersection of the E2 lines lies midway between the 4 resolved diagonal peaks. The vibrational energy corresponding to the label ‘v’ is 200 cm$^{-1}$. For the resonant case, the dashed lines corresponding to ‘E2’ coincide with ‘E1+v’, and the lines ‘E1’ coincide with ‘E2-v’.
The 2D spectra in Fig. 3.3 show several resolved peaks, arising due to multiple wavemixing pathways involving all three eigenstates in Eqn. (3.2); these are not resolved in the presence of correlated broadening in Fig. 3.2 where all contribute to a single diagonal peak (DP2) connected to the lower diagonal peak (DP1) by the two opposite cross-peaks (CP12 and CP21). The three different spectra in Figs. 3.3(a-c) correspond to vibrational frequencies of 130 cm\(^{-1}\), 200 cm\(^{-1}\) and 270 cm\(^{-1}\) respectively, while the excitonic energy gap is fixed at 200 cm\(^{-1}\). In each of the three panels, labels on the horizontal (vertical) dashed lines indicate the excitation (detection or radiation) frequencies, respectively, at which the lines are drawn. ‘E1’ and ‘E2’ correspond to the excitation frequencies for the first and second excitons, respectively. ‘v’ corresponds to the vibrational frequency used in each calculation. The 2D peaks approximately fall near the intersections between dashed lines, and can have a vibrational, electronic or a mixed vibrational-electronic origin. For the non-resonant cases (Figs. 3.3a and 3.3c), the vibrational peaks are well separated from the purely excitonic peaks which lie at frequencies ‘E1’ and ‘E2’. For the case of vibrational-electronic resonance (Fig. 3.3b), peaks arise from wave-mixing pathways that involve states that have a mixed vibrational-electronic character. Thus, 2D peaks in Fig. 3.3b cannot be classified as purely vibrational or excitonic peaks.

At off-resonant vibrational frequencies of 130 cm\(^{-1}\) or 270 cm\(^{-1}\) respectively (Fig. 3.3a or 3.3c), the basis states in Eqn. (3.2) are not isoenergetic, with the first two basis states with \(|\alpha\rangle\) exciton character lying 70 cm\(^{-1}\) below (or above) in energy compared to the third basis state with \(|\beta\rangle\) exciton character. Thus, intensity borrowing due to vibrational-electronic mixing between excitons with different electronic character is weak, and the 2D peaks can be differentiated as either purely electronic cross-peaks which do not appear in case of an isolated chromophore, or almost purely vibrational peaks which correspond to an isolated chromophore. However, at
resonance (Fig. 3.3b), the three basis states in Eqn. (3.2) become isoenergetic and the
vibrational-electronic mixing between them is strong, giving rise to split peaks in multiple
regions of the 2D spectra which cannot be distinguished as purely vibrational or purely electronic
peaks. In Fig. 3.3b, the frequencies corresponding to ‘E1’ and ‘E2-v’, and ‘E1+v’ and ‘E2’
coincide at resonance. Although 4 peaks are present in the CP12 region of Figs. 3.3a and 3.3c,
only two such CP12 peaks are resolved at resonance (Fig. 3.3b). Similarly, for off-resonant
frequencies of 130 cm$^{-1}$ or 270 cm$^{-1}$ (Figs. 3.3a and 3.3c), CP21 region has a purely vibrational
peak at the intersection of ‘E1’ and ‘E1+v’ and an excitonic cross-peak at ‘E2’. At resonance
(Fig. 3.3b), strongly mixed vibrational-electronic states give rise to two resolved CP21 peaks. In
the DP2 region (gray circles), for off-resonant vibrational frequencies (Figs. 3.3a and 3.3c), there
are 4 resolved peaks (or shoulders) which merge together at resonance (Fig. 3.3b) due to
vibrational-electronic mixing, to give rise to a splitting of DP2 both along and perpendicular to
the diagonal. This splitting corresponds to the one seen in the absorption spectrum in Fig. 3.1a,
due to intensity borrowing from the second to the third state in Eqn. (3.6). Even though the peaks
in Fig. 3.3b arise from mixed vibrational-electronic states, the weak 2D peaks lying at the
intersections of ‘E1-v’ and ‘E2+v’ lines arise from interactions in the wave-mixing pathways
that are weak because of $\Delta v_\perp = \pm 1$ or $\Delta v_\parallel = \pm 1$ FC factors. This makes their amplitudes similar
to vibrational transitions, resulting in weak 2D peaks.

Fig. 3.4 shows variation of CP12 and CP21 oscillation amplitudes with $T$ as a function of
vibrational frequency for Bloch 2D spectra calculated in Figs. 3.3(a-c). Unlike in the case of
Brownian oscillator lineshapes shown in Fig. 3.2b, the dimensionless FC displacement $d$ is held
constant for all the vibrations. Thus, the vibrational overlap factors involved in the wave-mixing
pathways$^{73}$ are same for all the vibrational frequencies.
Figure 3.4. Variation of CP12 and CP21 oscillation amplitudes as a function of vibrational frequency of the FC-active mode, over a range of 130 cm\(^{-1}\) to 270 cm\(^{-1}\), corresponding to the real “rephasing” 2D spectra calculated using a Bloch dephasing model and shown in Fig. 3.3. The Huang-Rhys factor \(S = (1/2)\omega d^2\), where \(d\) is the FC vibrational displacement on the excited electronic state, is kept constant at 0.025 over the range of vibrational frequencies sampled. CP12 oscillation amplitude is enhanced by \(~35\)x at resonance with a \(~60\) cm\(^{-1}\) FWHM, whereas CP21 oscillation amplitude stays approximately constant. For all the vibrational frequencies, the CP amplitudes are extracted from the intersections of ‘E1’ and ‘E2’ lines corresponding to the 2D spectrum for a vibrational frequency of 270 cm\(^{-1}\) (Fig. 3.3c). For a vibrational frequency far away from resonance, the vibrational peaks do not affect the shape of the cross-peaks and allow for a better peak-center determination. A similar analysis with peak positions corresponding to a vibrational frequency of 130 cm\(^{-1}\) is shown in Fig. 3A1. The sampled 2D peak coordinates \((\omega_r, \omega_i)\) in centimeters\(^{-1}\) are: CP12 (-11,661.7, 11,467.5) and CP21 (-11,467.5, 11,661.7).
As seen in Figs. 3.3(a-c), due to strong vibrational-electronic mixing around resonance, peaks around resonance change their position and shape as a function of vibrational frequency. For a vibrational frequency sufficiently away from resonance, the four resolved peaks in the CP12 region are well separated and their effect on the position and shape of the purely excitonic cross-peaks (that lie at the intersections of lines ‘E1’ and ‘E2’) is minimized. In order to sample a fixed position on the 2D spectrum, the cross-peak oscillation amplitudes are extracted by sampling the 2D cross-peaks at coordinates which correspond to the excitonic CP12 and CP21 peak positions of the $T = 0$ real rephasing 2D spectrum for the case of 270 cm$^{-1}$ vibrational frequency (Fig. 3.3c). The frequencies corresponding to ‘E1’ and ‘E2’ are 11,467.5 cm$^{-1}$ and 11,661.7 cm$^{-1}$, respectively. Because the cross-peaks change positions as a function of the relative difference between the electronic energy gap and the vibrational frequency, sampling the cross-peaks at a fixed position on the 2D spectra will lead to progressively off-centered peak sampling as the vibrational frequency changes towards 130 cm$^{-1}$. To see the effect of off-centered peak sampling, a similar analysis was done by keeping the sampling positions fixed at the corresponding cross-peaks for the other extreme of the vibrational frequency range, that is a vibrational frequency of 130 cm$^{-1}$ (Fig. 3.3a). The amplitude enhancement is plotted in Fig. 3A1. These results suggest that the amplitude enhancement close to the resonant vibrational frequency is not affected by off-centered peak-sampling. As shown in Fig. 3.4, in the absense of correlated broadening, the non-adiabatic resonant enhancement for oscillation amplitude of CP12 versus CP21 increases significantly from $\sim$14x to $\sim$35x, with a smaller width of $\sim$60 cm$^{-1}$ compared to $\sim$130 cm$^{-1}$ in the presence of correlated broadening (Fig. 3.2b). As noted in ref. 23 SI Table S2, a CP12/CP21 asymmetry can arise solely due to FC active vibrations, although, unlike in the present case, such an asymmetry is independent of the vibrational-electronic resonance. From Fig. 3.4, the CP12
versus CP21 oscillation asymmetry at vibrational frequencies of 130 cm$^{-1}$ and 270 cm$^{-1}$, which primarily arises due to FC-active vibrations, is $\sim 12x$ on average compared to the $\sim 35x$ asymmetry arising due to vibrational-electronic mixing at resonance. Since the CP12 peak-to-peak oscillation amplitude curve is still decaying at the extremes of vibrational frequency range sampled, CP12/CP21 oscillation amplitude asymmetry due to FC active vibrations alone will be less than $\sim 12x$.

Thus, vibrational-electronic resonance on the excited state is expected to enhance$^{23}$ the 2D CP12 oscillations coming from the ground state of the system with no chromophore excited. At vibrational-electronic resonance in Fig. 3.3b, even the absolute amplitude offset of CP12 peaks are at least 20x higher than peaks involving wave-mixing pathways with a weak vibrational overlap factors $\Delta v_\pm = \pm 1$. Therefore, peaks arising due to mixed vibrational-electronic states that have perfect vibrational overlap factors in the wave-mixing pathways are expected to dominate both the offset as well as the oscillation amplitude of the peaks in the 2D spectrum. In the presence of strong vibrational-electronic mixing on the excited state, ground state vibrational wavepackets$^{26,74}$ are expected to survive longer than the excited state vibrational-electronic wavepackets$^{75,76}$. Thus, enhanced CP12 ground state oscillations are expected to dominate the long-lived signatures in an antenna 2D spectrum (See Tab S2 of ref. $^{23}$).

3.3.2 Separability of adiabatic dynamics from non-adiabatic dynamics

As discussed in Section 3.2.1.1, for the simplest case of a dimer with one identical FC-active vibrational mode on each pigment, the dynamics along the correlated and the anti-correlated vibrational coordinate become completely separable into a correlated part $\hat{H}_{\text{corr}}$ given
by Eqns. (3.16), and an interaction part $\hat{H}_{int}$ given by Eqn. (3.17), respectively. The anti-correlated vibrational coordinate $\hat{q}_- = (\hat{q}_A - \hat{q}_B)/\sqrt{2}$, tunes the relative energy gap between the excited pigments and drives non-adiabatic dynamics on the excited state by strongly coupling nuclear and electronic motions together. In contrast, the correlated coordinate, $\hat{q}_+ = (\hat{q}_A + \hat{q}_B)/\sqrt{2}$ only drives adiabatic dynamics for which the associated electronic motion always keeps up with vibrational motion. Both delocalized vibrational coordinates have equal amplitude projections on the localized vibrational coordinates $\hat{q}_A$ and $\hat{q}_B$. Because only non-adiabatic dynamics is responsible for enhancement of ground state vibrational wavepackets, it is useful to compare the 2D signatures arising from the individual parts of the total single exciton Hamiltonian.

Fig. 3.5 (upper panel) compares ground state real rephasing 2D cross-peak oscillations due to the interaction part of the Hamiltonian with 2D oscillations due to the correlated part of the Hamiltonian. The parameters used in this calculation are described in Section 3.3.1.1, and are based on a dimer with one identical FC-active mode per pigment and excitonic energy gap resonant with the vibrational frequency. The average excited state site energy gap between uncoupled pigments is 150 cm$^{-1}$ with a standard deviation of 34 cm$^{-1}$. The Coulombic coupling between the pigments is 66.14 cm$^{-1}$ and the vibrational frequency is 200 cm$^{-1}$, with a stabilization energy of 5 cm$^{-1}$. A critically damped Brownian oscillator with a 30 cm$^{-1}$ Stokes’ shift is used to introduce peak broadening due to the correlated part of the phonon sideband. While peak-to-peak oscillation amplitude of CP21 oscillations is nearly the same for both cases, peak-to-peak oscillations in the CP12 2D peak due to anti-correlated vibrations are more than two times as large as those from correlated vibrations.
Figure 3.5 (Upper panel): Absolute amplitudes of the cross-peaks (CP12 and CP21) as a function of waiting time $T$ in the real “rephasing” ground electronic state 2D spectra for contributions coming from correlated ($\hat{q}_+$, solid line) versus anti-correlated ($\hat{q}_-$, dashed line) vibrations in a dimer model with one identical FC active vibrational mode per pigment. The 2D spectra are calculated with correlated broadening from a critically damped Brownian oscillator with frequency 70 cm$^{-1}$ and stabilization energy 30 cm$^{-1}$ at a temperature $T = 80$ K. The calculation is averaged over a static Gaussian distribution of site energy gaps with $\sigma_\Delta = 34$ cm$^{-1}$. The peaks oscillate at a vibrational frequency of 200 cm$^{-1}$ which is in resonance with the excitonic energy gap. Due to non-adiabatic vibrational-electronic mixing along the anti-correlated coordinate, the corresponding CP12 oscillations are enhanced by ~2.1x compared to CP12 oscillations due to adiabatic dynamics along the correlated coordinate. (Lower panel) CP oscillations in the 2D spectrum corresponding to the total single exciton Hamiltonian of a dimer with one FC-active mode per pigment. The calculation parameters are the same as described above. Total 2D slices with $T$ are approximately a sum of 2D slices corresponding to the interaction and correlated parts of the Hamiltonian (Eqns. (3.16) and (3.17)) individually.
The CP12/CP21 peak-to-peak oscillation amplitude asymmetry for non-adiabatically enhanced anti-correlated vibrations is $\sim2.3x$ greater than that arising from the adiabatic excitation of FC active correlated vibrations.

Fig. 3.5 (lower panel) shows the ground state CP oscillations from the 2D calculation based on the total single exciton Hamiltonian in Eqn.(3.12) with one identical FC active vibrational mode per pigment. This 2D calculation for the total single exciton Hamiltonian is based on the same parameters as the above calculations for correlated and interaction parts of the total single exciton Hamiltonian. The total cross-peak oscillation amplitude with $T$ (shown in the lower panel) is approximately a sum of the cross-peak oscillation amplitudes corresponding to correlated and interaction Hamiltonians individually. For a given $T$, the offset is defined as the average around which the peak maximum oscillates. The offsets on the total cross-peaks are approximately 40-50% less than the sum of offsets resulting from correlated and interaction Hamiltonians individually. In each case the peak maxima stay roughly constant. CP12 oscillations corresponding to $\hat{H}_{\text{corr}}$ and $\hat{H}_{\text{int}}$ oscillate in phase with respect to each other and lead to an increase in the modulation depth, defined as the ratio of peak-to-peak oscillation amplitude divided by the offset, from $\sim21\%$ for anti-correlated vibrations alone to $\sim26\%$ in the presence of correlated and anti-correlated vibrations in the total single exciton Hamiltonian. CP21 amplitude oscillations corresponding to $\hat{H}_{\text{corr}}$ and $\hat{H}_{\text{int}}$ are $\sim90^\circ$ out of phase relative to each other, leading to a smaller increase of $\sim1.5\%$ to $\sim1.8\%$ in the modulation depth of CP21 oscillations corresponding to the total single exciton Hamiltonian.

As seen in Eqn.(3.6), in the presence of vibrational-electronic resonance, lower energy exciton $\ket{\alpha}$ with a quantum of excitation in the anti-correlated vibration mixes with the higher energy exciton $\ket{\beta}$ with no quantum of excitation along the correlated or the anti-correlated
coordinate. Roughly these two states are responsible for the non-adiabatic dynamics \(^{23}\) along the anti-correlated vibrational coordinate. The first state in Eqn. (3.6) with a quantum of excitation in the correlated vibration contributes towards the adiabatic dynamics along the correlated vibrational coordinate. In a 2D experiment, due to contributions from wave-mixing pathways involving all the three states in Eqn. (3.6), the signatures of non-adiabatically enhanced ground state anti-correlated vibrational wavepackets are roughly averaged with those coming from weak correlated vibrational wavepackets. The long-lived signatures (See Table S2 of ref.\(^ {23}\)) will be dominated by non-adiabatically enhanced ground state anti-correlated vibrational wavepackets.

### 3.3.3 Dimer with one FC-active vibrational mode per pigment and a global environmental mode

As shown above and discussed in Section 3.2.1.1, for the case of a dimer with one identical FC active vibrational mode per pigment, the non-adiabatic dynamics along \(\hat{q}_-\) is completely separable from the adiabatic dynamics along \(\hat{q}_+\) in all 4 electronic states of the dimer. Eqn. (3.10) shows that apart from the intramolecular FC-active vibrations, a global environmental mode also has a projection on the tuning coordinate and is responsible for changing the relative energy gap between the excited pigments. As discussed in Section 3.2.1.2, in the presence of such a protein vibrational mode, the separability of adiabatic and non-adiabatic dynamics in terms of correlated and anti-correlated vibrational coordinates is not possible. The relative energy gap tuning caused by the environmental mode is expected to affect the energy transfer between the two single-exciton states caused by the anti-correlated vibrations. This section studies the effect of such an environmental vibrational mode on the ground state 2D
signatures of the dimer system modeled on the first and third exciton of the FMO antenna complex.

3.3.3.1 Parameters

The low-frequency phonon sideband of $BChl\ a$ in the FMO complex peaks at around 20 cm$^{-1}$ and has a Huang-Rhys factor, $S = (1/2)d^2$ of 0.3 when measured at a fluorescence excitation wavelength$^{43,59}$ of $\sim 829$ nm, where $d$ represents the corresponding FC displacement. Thus the total stabilization energy, $\lambda = S\omega$ due to the phonon sideband is $\sim 7$ cm$^{-1}$. To introduce the global environmental mode $\hat{q}_e$ described in Section 3.2.1.2, in a dimer with one FC-active vibrational mode per pigment, the frequency of the environmental vibrational mode as deduced from the fluorescence line-narrowing experiments is taken to be 20 cm$^{-1}$. As seen in Eqn. (3.10), when the FC displacements $d^A_k$ and $d^B_k$ on the excited state of the two pigments due to an environmental mode $\hat{q}_k$ become equal, the projection of the environmental mode towards the tuning coordinate vanishes. Therefore, non-adiabatic energy transfer between the two single exciton states can only be affected by the environmental mode when the two pigments are coupled differently to it.

Fluorescence line-narrowing measurements$^{43,59}$ on the $Q_y$ band of the $BChl\ a$ chromophore in the FMO complex have revealed a strong wavelength dependence of the coupling of electronic transitions to the phonon environment, with the measured Huang-Rhys factor for electron-phonon coupling being $\sim 0.6-0.7$ when the 826 nm absorption band is excited at its blue edge, and rapidly decreasing around the red edge to $\sim 0.3$. This change indicates some sort of breakdown of the Condon approximation used to determine $d$. Energy transfer coupling between the chromophores at blue edge excitation has been speculated to be the reason behind
the strong wavelength dependence of the electron-phonon coupling. Such studies are based on
the emission from the lowest energy band in the FMO absorption spectrum. The blue edge of the
lowest energy FMO exciton overlaps with the red edge of higher energy excitons due to static
disorder in the energy gaps. Thus, in the model considered here, at the blue edge excitation, static
energetic disorder along with the finite width of non-adiabatic vibrational-electronic resonance
(Fig. 3.1b) and the presence of several low-frequency intramolecular vibrational modes, can still
lead to delocalized anti-correlated vibrational wavepackets, which drive energy transfer between
the chromophores. The experiments do not make a distinction between delocalized
intermolecular vibrations, such as delocalized anti-correlated vibrations, and the vibrations in the
protein environment. Therefore, a stronger electron-phonon coupling towards the blue edge
excitation could also imply that the higher energy pigments are more strongly coupled to the
protein vibrational modes than the lower energy pigments. This stronger coupling of higher
energy pigments could then generate stronger coupling for higher energy excitons.

Based on the above discussion, in order to consider the effect of the protein environment
on the interaction Hamiltonian $\hat{H}_{\text{int}}$, both excited pigments must be coupled differently to the
protein vibrational mode. Here we assume that only the higher energy pigment, $B$, is coupled to
the environmental mode, with a stabilization energy of 3 cm$^{-1}$. This is about half of the total
stabilization energy of the pigment due to the environment. The Huang-Rhys factor of 0.3 for the
FMO phonon sideband at ~829 nm excitation$^{43,59}$ is based upon low temperature emission from
the lowest energy band in the FMO absorption spectrum. The stabilization energy of 3 cm$^{-1}$ for
the higher energy pigment due to its coupling to the environment does not contribute towards the
total Stokes’ shift determined from emission by the lowest energy exciton. Only the
intramolecular vibrational stabilization energy of 5 cm$^{-1}$ for the lowest pigment contributes
towards the total Stokes’ shift of 35 cm$^{-1}$ such that the Stokes’ shift needed for introducing correlated broadening of 2D lineshapes remains 30 cm$^{-1}$. All the other parameters - average site energy gap of 150 cm$^{-1}$ with a standard deviation of 34 cm$^{-1}$ and Coulomb coupling 66.14 cm$^{-1}$, vibrational frequency of 200 cm$^{-1}$ with stabilization energy 5 cm$^{-1}$, are the same as those described for the one-mode dimer case of Section 3.3.1.1.

### 3.3.3.2 2D spectra

Using the parameters discussed above, ground-state rephasing 2D peak oscillations in Fig. 3.6 show that 2D signatures arising due to non-adiabatic vibrational-electronic mixing$^{23}$, such as oscillatory DPs, asymmetry between CP12 and CP21 oscillation amplitudes and specific phase relationships between the CPs and between CP12 and DP1 are not affected by the environmental coupling to the donor. Because the higher energy pigment is coupled to the environment, DP2, which corresponds to the higher energy exciton, has a slow 20 cm$^{-1}$ environmental modulation (~1.7 ps time period). Since the low frequency phonon sideband can be modelled as critically damped, the oscillation of DP2 in Fig. 3.6, which arises from an undamped environmental mode, might not be observed (See section 4 below). However, an environmental mode does affect the resonant oscillations of other peaks. To correctly capture such a slow modulation due to the phonon sideband of the protein environment in a 2D experiment, waiting time $T$ spanning multiple vibrational periods of the phonon sideband might be necessary.

Comparison with Fig. 3.5 (lower panel) shows that in the presence of a global environmental mode, the modulation depth of CP21 at 200 cm$^{-1}$ vibrational frequency decreases from $\sim$1.8 % to $\sim$ 0.9%, while the modulation depth of CP12 only goes down from $\sim$26% to
Figure 3.6. Absolute amplitudes and relative phase relationships between the diagonal (DP1 and DP2) and cross-peaks (CP12 and CP21) as a function of waiting time $T$ in the “rephasing” ground electronic state contributions to the 2D spectra for a dimer model with one identical intramolecular FC active vibration per pigment and an additional environmental mode $\hat{q}_e$ (Section 3.2.1.2 in the text) with a frequency of 20 cm$^{-1}$ and a stabilization energy of 3 cm$^{-1}$, such that only the higher energy pigment $B$ is directly coupled to the environment. The 2D spectra are calculated with correlated broadening from a critically damped Brownian oscillator with frequency 70 cm$^{-1}$ and stabilization energy 30 cm$^{-1}$ at a temperature $T = 80$ K, and averaged over a static Gaussian distribution of site energy gaps with $\sigma_\Delta = 34$ cm$^{-1}$. The peaks oscillate at a vibrational frequency of 200 cm$^{-1}$ which is in resonance with the excitonic energy gap. The intramolecular vibration has a stabilization energy of 5 cm$^{-1}$. The transients are offset (additive constants in boxes) to show phase relationships, but not multiplicatively scaled. The ground state signatures are similar to those reported in ref.$^{23}$. The cross peak beats are $\sim$160° out of phase with each other while CP12 beats are $\sim$120° ahead of the diagonal peaks. Compared to $\sim$14x asymmetry without the environment, CP12 beats are $\sim$26x stronger than CP21 in the presence of a global environmental mode. The additional slow modulation on DP2, diagonal peak corresponding to the higher energy exciton, is due to the asymmetric environmental mode.
~24%. Thus, the vibrational modulation of non-adiabatically enhanced ground state CP12 peak is nearly unaffected whereas CP21 oscillations are further weakened by almost a factor of 2. In the presence of an environmental mode, ground state CP12 beats are ~ 26x stronger than CP21 beats, compared to ~14x without any environment. Thus, even though an asymmetric environmental mode can tune the relative energy gap between the single exciton states, non-adiabatically enhanced anti-correlated ground state vibrational wavepackets are expected to be robust to the protein environment and the previously predicted ground state CP12/CP21 oscillation amplitude asymmetry (See Table S2 of ref.\textsuperscript{23} is expected to further increase due to coupling with the protein environment.

A similar calculation at a lower temperature of T = 40 K (See Fig. 3A2) shows that CP12 modulation depth actually increases from ~ 24% to ~ 44%, while CP21 modulation depth stays constant at ~0.9%. Modulation depth of DPs is unaffected by lowering the temperature to 40 K. Thus at lower temperature, signatures of non-adiabatically enhanced ground state anti-correlated vibrational wavepackets in a 2D spectrum are predicted here to become more pronounced.

### 3.3.4 Dimer with two FC-active modes per pigment

#### 3.3.4.1 Parameters

Fluorescence line-narrowing (See Fig. 5 of refs.\textsuperscript{42,43,59}) and resonance Raman studies on the BChl a chromophore (See Fig. 1 of ref.\textsuperscript{58} and Fig. 5 of ref.\textsuperscript{53}) that were conducted independently of the 2D experiments on FMO have revealed a broad feature around 160 – 200 cm\(^{-1}\) vibrational frequency range which points to the presence of closely lying FC-active modes in the BChl a chromophore. Features near 161 cm\(^{-1}\) and 195 cm\(^{-1}\) (Table 3.1) are consistently reported and have been assigned to vibrations with highly mixed in-plane deformations of
substituent groups, and highly mixed magnesium doming, macrocycle deformation and substituent group motions, respectively. Four sharper vibrational features at 167 cm$^{-1}$, 180 cm$^{-1}$, 191 cm$^{-1}$ and 202 cm$^{-1}$, superposed on top of the broad pedestal, have been reported for FMO in ref. Superposed on top of the broad pedestal, have been reported for FMO in ref. All the vibrational frequencies mentioned above have weak stabilization energies and lie close to an excitonic energy gap of ~200 cm$^{-1}$ visible in the low temperature absorption spectra of the FMO antenna. Fig. 3.2b shows that in the presence of correlated broadening due to the environment, the width of non-adiabatic enhancement of ground state vibrational wavepackets is ~130 cm$^{-1}$ around the resonant vibrational frequency of 200 cm$^{-1}$. A wide enhancement width around resonance suggests that apart from the resonant vibrational mode, near-resonant vibrational frequencies are also non-adiabatically enhanced and may show up in the 2D spectrum. The effect of non-adiabatic enhancement of such near-resonant vibrational modes on the resonant vibrational mode was not considered in the dimer model of ref. 23.

To explore the effect of a near-resonant mode in a dimer with identical resonant FC active vibrational modes of frequency 200 cm$^{-1}$ on each pigment (Section 3.3.1), we add a second identical FC-active mode on each pigment with frequency 180 cm$^{-1}$ and stabilization energy $\lambda = 5$ cm$^{-1}$. The Stokes’ shift needed to introduce correlated broadening of 2D lineshapes now becomes 25 cm$^{-1}$ (instead of 30 cm$^{-1}$), such that the total Stokes’ shift is constant at 35 cm$^{-1}$, as determined experimentally. All the other parameters are the same as used for the one-mode dimer case (Section 3.3.1.1).

### 3.3.4.2 Linear Absorption Spectrum

Fig. 3.7 shows the calculated absorption cross-section for a dimer with 2 FC active vibrational modes per pigment using the parameters discussed above. Due to a near-resonant
Figure 3.7. Absorption cross-section corresponding to a dimer with 2 FC active vibrational modes per pigment, discussed in Section 3.2.1.1 of the text. The site energy gap between the pigments A and B is 150 cm\(^{-1}\) with a coupling of 66.14 cm\(^{-1}\) such that the excitonic energy gap of 200 cm\(^{-1}\) is resonant with one of the FC-active vibrational frequencies. The near-resonant vibrational frequency is 180 cm\(^{-1}\) and is based on the discussion in Section 3.3.3.1 of the text. The stabilization energy for both vibrations is 5 cm\(^{-1}\). The calculation assumes equal ground to first excited state transition dipole strengths for both the pigments. The spectrum is calculated at a temperature T = 80 K. A critically damped Brownian oscillator with frequency 70 cm\(^{-1}\) and stabilization energy 25 cm\(^{-1}\) is used to model the line broadening due to the correlated part of the low frequency phonon sideband in the FMO antenna complex. The corresponding absorption cross-section is shown in red. With an addition of an additional FC-active mode with a near-resonant vibrational frequency, the higher energy exciton peak is now split into three peaks of different intensities lying at 11636 cm\(^{-1}\), 11657 cm\(^{-1}\) and 11682 cm\(^{-1}\) frequencies. The lower energy exciton lies at 11465 cm\(^{-1}\). As in Fig. 3.1a, broadened lineshapes completely hide the splittings under the higher exciton peak. Very weak peaks between the three split peaks under the higher energy exciton at frequencies 11645 cm\(^{-1}\) and 11665 cm\(^{-1}\) correspond to transitions from the ground electronic and vibrational state to the lower energy exciton with a quanta of excitation along the correlated vibrations 180 cm\(^{-1}\) and 200 cm\(^{-1}\) respectively. The intensities of these peaks are \(\sim 100\)x smaller than the lower energy exciton peak at 11465 cm\(^{-1}\). \(\sim 7\%\) of the total ground state equilibrium population is in the vibrationally excited ground state (one quantum of vibrational excitation) at T = 80 K. The very weak peaks near the lower energy exciton could correspond to transitions starting from a vibrationally hot ground electronic state to a vibrationally excited lower energy exciton.
mode at 180 cm\(^{-1}\), apart from the three isoenergetic states similar to those in Eqn.(3.6), states with a quantum of excitation along the anti-correlated coordinate corresponding to the near-resonant mode also lie close in energy. In contrast to the case of a dimer with one FC active mode per pigment (Section 3.3.1), the higher exciton peak gets split into three (instead of two) peaks that can have roughly equal intensities depending on the choice of parameters for the individual FC-active modes. Thus, with \( n \) vibrational modes lying close to the excitonic energy gap, the oscillator strength of the higher energy exciton gets further distributed to \( n \) dark basis states of excited vibrational levels on the lower energy exciton, yielding \( n + 1 \) peaks with bright higher energy exciton character. At 80 K in FMO, the width of the 0-0 bands and the width of the vibrational-excitonic resonance are such that the nominal higher-exciton band is hardly affected by such mixing and the peak splittings are completely hidden under the broadened lineshapes. However, in a 2D spectrum, sampling peak amplitude oscillations with waiting time \( T \) may reveal whether the near-resonant vibrational frequencies contribute towards non-adiabatic vibrational-electronic mixing, and illuminate their effect on the ground state 2D signatures arising due to the resonant vibrational mode.

3.3.4.3 2D Spectra

Fig. 3.8 shows a \( T=0 \) rephasing 2D spectrum for the ground state of a dimer with 2 FC active vibrational modes per pigment calculated using the parameters discussed above in Section 3.4.1. This calculation, unlike that in Fig. 3.2a, is averaged over the inhomogeneous distribution of excited state energy gaps, and differs from that in Fig. 2 of ref. \(^{23}\) in the inclusion of the additional FC-active mode on each pigment. The maximum of the plotted spectrum is normalized to one. The stick absorption cross-section shown in black in Fig. 3.7 shows that the
Figure 3.8: Real part of the “rephasing” 2D spectrum corresponding to the ground electronic state of a dimer with two FC-active vibrational modes per pigment. The two FC-active vibrational modes have frequencies of 180 cm\(^{-1}\) and 200 cm\(^{-1}\). Both FC-active modes have a stabilization energy of 5 cm\(^{-1}\). The site energy gap between the excited pigments is 150 cm\(^{-1}\) which is averaged over a static Gaussian distribution with \(\sigma_A = 34\) cm\(^{-1}\). The pigments are coupled by a Coulomb coupling of 66.14 cm\(^{-1}\) such that the excitonic energy gap of 200 cm\(^{-1}\) is resonant with one of the FC vibrations. The 2D spectra are calculated with correlated broadening from a critically damped Brownian oscillator with frequency 70 cm\(^{-1}\) and stabilization energy 25 cm\(^{-1}\) at a temperature \(T = 80\) K. Positions ‘x’ and ‘o’ correspond to the maxima of the cross-peaks (CPs) and the diagonal peaks (DPs) respectively. The corresponding 2D peak coordinates \((\omega_1, \omega_2)\) in centimeters\(^{-1}\) are: DP1 (-11,481, 11,470), DP2 (-11,690, 11,684), CP12 (-11,678, 11,487) and CP21 (-11,499, 11,667). The amplitude of the 2D spectrum for each frequency pair is indicated by color and via contours at the 0, 2, 4, 6, 8, 10-90 % levels. Positive and negative contours are solid and dashed, respectively. The waiting time is \(T = 0\) fs. The 2D spectra are dominated by 4 resolved peaks which oscillate in amplitude and shape with \(T\). Due to correlated broadening the split peaks in the absorption spectrum of Fig. 3.7 are completely obscured.
higher energy peak splits into three weaker peaks lying within a ~46 cm\(^{-1}\) interval. Due to correlated broadening, the three split peaks seen in the stick spectrum of Fig. 3.7 are completely obscured under the higher energy absorption peak, as is also seen in the corresponding to the higher energy diagonal peak (DP2) of the 2D spectrum in Fig. 3.8. Compared to the one-mode case (Fig. 2 of ref.\(^2\))\(^3\)), the diagonal linewidth of DP2 increased by ~7\% and the peak amplitude of DP2 decreased by 7\%.

Figs. 3.9(a-c) compare the cross-peak oscillations with \(T\) corresponding to the 2D spectrum of a dimer with 2 FC modes per pigment (Fig. 3.9a), one resonant FC mode per pigment (Fig. 3.9b) with a vibrational frequency of 200 cm\(^{-1}\), and one FC active mode per pigment with a near-resonant vibrational frequency of 180 cm\(^{-1}\) (Fig. 3.9c). On comparison of Fig. 3.9a with Figs. 3.9b and 3.9c, the ground state oscillation amplitudes of the cross-peaks in the presence of two modes are approximately a sum of the cross-peak oscillations amplitudes when individual modes are present. In contrast to the additive oscillation amplitudes when both modes are present simultaneously, the constant offset on the oscillations are not additive and increase slightly compared to when only one mode is present. Therefore, the modulation depth of these ground state CP12 oscillations increases from ~26\% to 59\% when both vibrations are simultaneously present. In comparison, the modulation depth of CP21 oscillations only increases from ~1.8\% to ~2.7\%. Thus, in the presence of several vibrational modes with frequencies around the excitonic energy gap, CP12 modulations can dominate within the signal to noise ratio of an experiment.

Such an enhancement of ground state CP12 oscillations in the presence of near-resonant modes suggests that non-adiabatic vibrational-electronic mixing along the anti-correlated
Figure 3.9 (upper panel): Absolute amplitudes and relative phase relationships between the cross-peaks (CP12 and CP21) as a function of waiting time $T$ corresponding to the 2D calculation of Fig. 3.8. The slices are extracted from coordinates corresponding to ‘x’ in the $T=0$ 2D calculation of Fig. 3.8. In the presence of an additional mode, ground state CP12 oscillations are further enhanced by a factor of $\sim 2.3x$ compared to when there is only one mode present (Fig. 3.5(lower panel)). This suggests that non-adiabatic coupling, both due to resonant as well as near-resonant modes, is additive such that ground state CP12 oscillations would dominate in signal-to-noise ratio of the experiment. The slow modulations correspond to the difference frequency of $20 \text{ cm}^{-1}$ and the higher frequency beats correspond approximately to $190 \text{ cm}^{-1}$, the average frequency of the two FC-active modes.
(middle panel) Absolute amplitudes and relative phase relationships between the cross-peaks (CP12 and CP21) as a function of waiting time $T$ corresponding to the one FC mode per pigment case of Section 3.2.1.1. The vibrational frequency is chosen to be resonant with the excitonic energy gap of 200 cm$^{-1}$ with a stabilization energy of 5 cm$^{-1}$. The stabilization energy of the critically damped Brownian oscillator is 30 cm$^{-1}$, such that the total Stokes’ shift is 35 cm$^{-1}$. All the other parameters are the same as Fig. 3.9a. The slices are extracted from coordinates corresponding to ‘x’ in the $T$=0 2D calculation of Fig. 3.8.

(lower panel) Absolute amplitudes and relative phase relationships between the cross-peaks (CP12 and CP21) as a function of waiting time $T$ corresponding to the one FC mode per pigment case of Section 3.2.1.1. The vibrational frequency of 180 cm$^{-1}$ with a stabilization energy of 5 cm$^{-1}$ is near-resonant with the excitonic energy gap of 200 cm$^{-1}$. The stabilization energy of the critically damped Brownian oscillator is 30 cm$^{-1}$, such that the total Stokes’ shift is 35 cm$^{-1}$. All the other parameters are the same as Fig. 3.9a. The slices are extracted from coordinates corresponding to ‘x’ in the $T$=0 2D calculation of Fig. 3.8.
coordinate is roughly additive. Eqn. (3.10) indicates that the generalized tuning coordinate $\hat{g}$ is a sum of FC active anti-correlated vibrations delocalized over the two pigments. Due to additive anti-correlated motions between FC active modes with frequencies around the excitonic energy gap, vibrational-electronic mixing on the excited state is more pronounced. This also involves the finite width for non-adiabatic coupling around vibrational-electronic resonance (Fig. 3.1b). A finite width allows distribution of the intensity for a single higher energy purely excitonic basis state into $n + 1$ peaks of different intensities in the presence of $n$ near-resonant modes (Fig. 3.7).

The overall result will be cumulative non-adiabatic enhancement of anti-correlated ground state vibrational wavepackets along all FC-active vibrations within the range of the coupling.

3.4. Discussion

3.4.1 Role of vibrational relaxation on the ground electronic state of the donor

As shown by the transformed matrix in Eqn. (3.5) and the basis states in Eqn. (3.6), basis states of $|\alpha\rangle$ and $|\beta\rangle$ excitonic character are coupled together by the vibrationally and electronically off-diagonal coupling $V$. The resulting eigenstates obtained after numerically diagonalizing the exact Hamiltonian have a strongly mixed vibrational-electronic character from both the donor and acceptor pigments which allows non-adiabatic energy transfer from the donor to the acceptor. The nature of states on the acceptor pigment $A$ that receive the electronic energy from the donor pigment $B$, can be better understood by considering the second state in Eqn. (3.6), with $|\alpha\rangle$ excitonic character and a quantum of excitation along the anti-correlated vibrational coordinate, more carefully. The electronic character of this exciton is primarily composed of the electronically excited acceptor pigment $A$ and the ground electronic state of the donor pigment $B$. The vibrational wavefunction has equal non-zero projections onto two localized vibrational
states: one in which the acceptor pigment is vibrationally excited and one in which the donor pigment is left vibrationally excited. This means that the excess energy of the donor pigment can ultimately either be converted into a vibrational quantum on the excited electronic state of the acceptor (as assumed in the vibronic exciton or the coherent exciton scattering approximation \cite{37,38}), or be left behind as a vibrational quantum on the ground electronic state of the donor. This has an interesting consequence. The electronic energy transfer process is completed and made permanent by energetic relaxation down to the lowest vibrational state of the lowest exciton, the state written as $|A\rangle_0|v_A = 0\rangle|v_B = 0\rangle$ in the localized vibrational-site basis. Thus, vibrational relaxation on the electronically excited acceptor and the ground electronic state donor are equally important in completing energy transfer. It is important to point out that, from the excitonic point of view of the dimer as a whole, this latter relaxation path is still an electronic excited state relaxation process in that both states involved have an electronically excited acceptor and belong to the one-exciton manifold. Experimentally determining how this vibrational relaxation on the electronic ground state of the isolated pigment transforms into relaxation on the one-exciton manifold is essential for understanding whether the ground electronic state vibrational relaxation processes of the uncoupled pigments make a significant contribution to stabilizing the final energy transfer product.

Some vibronic exciton models of photosynthetic energy transfer \cite{20,21} based on a Holstein-like Hamiltonian \cite{78}, explicitly exclude electronic energy transfer pathways that leave the ground state of the donor vibrationally excited (as discussed above). In such treatments, the coupling is restricted to proceed through the zero point level on the ground electronic state of the donor. Such a restriction also occurs based upon the Coherent Exciton Scattering (CES) approximation \cite{38} (also called ‘one particle approximation’ \textsuperscript{79}), which assumes that electronic excitation transfer
from one monomer to the other is always accompanied by vibrational de-excitation of the donor. Davydov and Serikov’s treatment of electronic energy transfer in the presence of a resonant vibration with exponential vibrational energy relaxation on the acceptor, and exponential donor relaxation also makes the identical assumption that vibrational excitation strictly accompanies electronic excitation. The coupling between the exciton in one unit cell and the intramolecular vibrations in another unit cell is discarded, that is, “two-particle” states where at least one quantum of vibrational excitation resides in an electronically unexcited molecule are ignored. The analysis of ref. shows that this neglect reduces the energy transfer coupling matrix element by a factor of $\sqrt{2}$ (the ratio between the coupling to the anti-correlated vibration and the acceptor vibration). In the rate equation limit, the factor of $\sqrt{2}$ in the coupling increases the energy transfer rate by a factor of 2. Apart from energy transfer, wavemixing diagrams in ref. show that, to fully capture the 2D signatures on the ground as well as on the excited state resulting from excited state non-adiabatic dynamics, pathways involving a quantum of excitation along the anti-correlated vibration $|\nu_-\rangle$ should be included. Thus, making a ‘one particle’ approximation throws away half of the non-adiabatic effects calculated in ref. The nature of the anti-correlated vibration is essential for a correct treatment of energy transfer and comparison with the 2D experiments on photosynthetic antennas.

Considering the case of energy transfer between a donor and acceptor with the same energy gap ($E_d = E_b$) provides additional insight into the role of vibrations with small Franck-Condon displacements in energy transfer. If a donor pigment is excited to $\nu = 1$ in the excited electronic state, it will have its strongest emission transition (with $\Delta \nu = 0$) to $\nu = 1$ in the ground electronic state. This emitted frequency overlaps with the strongest absorption transition of the acceptor, from $\nu = 0$ in the electronic ground state to $\nu = 0$ in the electronic excited state. This
strongest actual emission/absorption path at large distance becomes the strongest virtual emission/absorption path in Förster’s resonant energy transfer at short distances. Note that leaving the vibration behind on the donor is the overwhelmingly dominant path (which is completely neglected by the “one particle approximation”). The ‘one particle’ basis set has also been shown to be a bad approximation \(^3,^8\) for organic semiconductors where significant nuclear rearrangements associated with charge or energy transport leave a ‘wake’ of vibrational excitations behind.

### 3.4.2 Asymmetry in cross-peak oscillation amplitude

When adiabatic and non-adiabatic dynamics are separable in terms of correlated and anti-correlated vibrations respectively, it is seen that the CP12/CP21 oscillation amplitude asymmetry on the ground electronic state of the dimer, due to non-adiabatically enhanced anti-correlated vibrational wavepackets, is \(~2.3x\) greater than that arising due to adiabatically excited correlated FC vibrational wavepackets (See Fig. 3.5). Even though correlated vibrations play no role in non-adiabatic energy transfer on the excited state, their effect on the non-adiabatically enhanced ground state 2D CP12 oscillations is roughly additive and leads to an increase in CP12 modulation depth from \(~21\%\) due to anti-correlated vibrations alone, to \(~26\%\) in the presence of correlated and anti-correlated vibrations.

An environmental mode that couples the two excited pigments differently couples the adiabatic and non-adiabatic dynamics. Apart from the anti-correlated vibrations, a protein vibrational mode can also tune the relative energy gap between the excited pigments, and therefore can affect the non-adiabatic energy transfer between them. Fig. 3.6 shows that long-lived ground state 2D signatures are persistent even in the presence of such a protein vibration.
The predicted\textsuperscript{23} 2D CP12/CP21 oscillation amplitude asymmetry for ground state anti-correlated vibrational wavepackets is actually enhanced by \( \sim 2x \) in the presence of the protein environmental mode considered here. This represents our best guess, based on independent experiments\textsuperscript{43,59}, at how the protein couples to the pigments in FMO.

A finite width of excited state vibrational-electronic resonance allows for roughly additive non-adiabatic effects. If there is more than one near-resonant vibrational mode, all show up as enhanced CP12 oscillations in a ground state rephasing 2D spectrum. A finite width of excited state vibrational-electronic resonance also renders the excited state energy transfer through the ‘unnecessary nested funnel’\textsuperscript{23} robust to small changes in the electronic energy gap and the vibrational frequency due to naturally occurring mutations or inhomogeneity in the protein environment. Figs. 3.8(a-c) show that, similar to the presence of a protein environment, an additional near-resonant FC vibrational mode leads to \( \sim 1.5x \) increase in the predicted CP12/CP21 ground state oscillation amplitude asymmetry.

Thus, in the FMO protein environment with several possible near-resonant modes in \textit{BChl a}, 2D CP12 oscillations coming from the ground state of the antenna are expected to dominate the signal to noise ratio of the experiment. Only oscillations of CP12 type (the type of cross-peak closer to the excitation axis), the type of cross-peak oscillations that non-adiabatic vibrational-electronic mixing predicts to be stronger\textsuperscript{23}, have been reported in the literature for FMO\textsuperscript{6,8}, PC645\textsuperscript{10} and the bacterial reaction center\textsuperscript{14}. Recently, opposite cross-peak CP21 oscillations have been reported\textsuperscript{11} for the light harvesting complex of purple bacteria, LH2\textsuperscript{1}; the authors have also reported CP12 oscillations in Figs. S1 and S3 of the supplementary information. From those plots it is hard to determine the relative beating amplitudes for the two cross-peaks. In LH2, CP12 oscillations maybe obscured by B800 to B850 energy transfer.
Excited-state asymmetric vibrational wavepackets dephase rapidly due to strong vibrational-electronic mixing on the excited state. A similar mechanism is expected to rapidly dephase excited state anti-correlated vibrational-electronic wavepackets although the exact mechanism is unclear. In contrast, the long-lived 2D signatures proposed in ref. are expected from the anti-correlated vibrational wavepackets on the ground electronic state of the antenna and are expected to last on picosecond timescales instead. The anti-correlated ground state vibrational wavepackets might be understood in terms of a hole created on the ground state after the initial excitation, that continues to oscillate on the ground state without affecting excited state energy transfer. Their 2D signatures will persist even if all the excited states of the antenna were quenched after initial excitation.

Table 3.1 compares all reported FMO 2D beating frequencies with the FC active and Raman active ground state vibrational frequencies for its chromophore, BChl a, from various experiments conducted by different groups independent of the 2D experiments. All seven reported cross-peak beating frequencies found by sampling on a peak or shoulder in the 2D spectra for FMO match one of the five FC active ground state vibrational frequencies of BChl a in this frequency range within experimental error. Another two reported 2D beat frequencies were not sampled on a 2D peak or shoulder and do not match isolated BChl a ground state vibrations but are close to BChl a vibrational frequencies in the special pair of the reaction center.

3.4.3 Relative phase relationships between 2D peak oscillations

Certain phase relationships between peak amplitude oscillations with waiting time $T$ in rephasing 2D spectra have been proposed as indicative of electronic coherence and quantum transport. The proposed phase relationships for electronic coherence were not calculated for
isolated 2D peaks \(^{82}\). When the wave-mixing pathways responsible for rephasing contributions in a 2D spectrum are not balanced by the non-rephasing pathways contributing to the same 2D location, the resulting 2D peaks (rephasing or non-rephasing spectrum alone) have phase-twists \(^{83,84}\) or mixed absorptive and dispersive peakshapes. Thus, the absolute oscillatory phase of an isolated 2D peak oscillating with \(T\) acquires an additional phase due to mixing with a dispersive peakshape. For an isolated peak, this additional phase is zero at the peak center. In multi-level systems, multiple wave-mixing pathways give rise to overlapping peaks with individual features hidden due to peakshape broadening (compare Fig. 3.2a and Fig. 3.3b). The real part of the rephasing 2D peakshape has wide dispersive wings in both frequency dimensions. Thus, a rephasing 2D spectrum of a multi-level system becomes further complicated because the locations of the peak centers are uncertain. For instance, in the 2D spectrum of the FMO antenna protein, with overlapping peaks due to at least 7 different excitons \(^{85}\), inhomogeneities in the protein environment \(^{51,86,87}\) and pulse propagation effects in the sample \(^{83}\), locating peak centers and determining relative phase relationships \(^{8,10}\) based on an excitonic Hamiltonian \(^{6,60}\) becomes challenging. In such condensed phase systems, sampling off-center peak locations or peaks locations with overlapping features due to the underlying peak splitting introduces phase-twists \(^{83}\) in a 2D spectrum which lead to an additional sampling location dependent phase \(^{82}\) in the relative oscillations of 2D peaks.

Recently, a near 90° relative phase relationship has been reported by ref. \(^{8}\) for the FMO antenna complex. Ref. \(^{8}\) suggests an explanation for this relative phase relationship using a quantum transport model \(^{18}\). In a quantum transport model, the phase relation between the oscillations of an off-diagonal density matrix element (coherences) and a diagonal density matrix element (populations) is 90° independent of any vibrational-electronic resonance. Ref. \(^{18}\)
calculates the 2D spectrum using a quantum transport model but the oscillations of diagonal and CP12 type cross-peak are approximately out of phase (See Fig. 5 of ref. 18). This phase relationship in ref.18 indicates that the reasoning in ref. 8, which also applies to the calculation in ref. 18, must be incorrect. Using this reasoning to explain the phase relationships in a 2D spectrum is invalid because diagonal and off-diagonal elements in the density matrix do not map one to one onto diagonal and off-diagonal 2D peaks. For instance, wave-mixing diagrams for oscillatory diagonal peaks in a ground state rephasing 2D spectrum, shown in Fig. 3 of ref. 23, will arise from off-diagonal density matrix elements. Similarly, non-oscillatory wave-mixing diagrams that arise from diagonal density matrix elements can be drawn for 2D cross-peaks (see Fig. 6 of ref. 70). It is not clear what controls the phase relationship for quantum transport. Ref. 23 notes that the two frequencies for which such a relationship has been reported correspond to CP12 type beating frequencies and match with several independent measurements of ground state vibrational frequencies for BChl a (frequencies ~160 cm\(^{-1}\) and ~ 200 cm\(^{-1}\) in Table 3.1) and that a similar phase relationship is reproduced by rephasing 2D spectrum of non-adiabatically enhanced ground state wavepackets in the presence of vibrational-electronic resonance.

3.5. Conclusions

We have extended the theory and calculations of long-lived ground-state 2D signatures due to non-adiabatic vibrational-electronic resonance on the excited state. Analysing the basis transformation from localized vibrational coordinates \(q_A, q_B\), to delocalized vibrational coordinates \(\hat{q}_+, \hat{q}_-\), it is shown that vibrational-electronic mixing near resonance allows for pathways where the electronic excitation from the donor is transferred to the acceptor so that the acceptor is both electronically and vibrationally excited, as well as pathways where an
electronically de-excited donor is still vibrationally excited in its ground electronic state and the acceptor is electronically excited with no excess vibrational energy. Both these pathways are treated on equal footing in the non-adiabatic energy transfer calculations here. Such pathways where vibrational excitation is not strictly accompanied with an electronic excitation render the coherent exciton scattering approximation, made in a vibronic exciton model, invalid. A ‘one-particle’ basis set neglects such pathways and therefore is unable to fully capture the excited state non-adiabatic dynamics and its consequences on the ground state of the antenna. Even under the adiabatic framework of Förster resonance energy transfer\(^{32}\), the pathway that leaves the ground electronic state of the donor vibrationally excited can become dominant in the very weak vibrational displacement limit.

Non-Condon effects are known to arise in individual chromophores based on porphyrins\(^ {88}\) and chlorophylls\(^ {42}\). In the dimer model presented here, it is assumed that the individual chromophores obey the Condon approximation. Previous studies assumed the validity of Condon approximation for both individual pigments and antennas. In contrast to those studies, we have shown that for electronically coupled chromophores, the excited-state vibrational-electronic resonance leads to mixing of states with different excitonic character such that transition dipole from the ground state to the resulting excited eigenstates rapidly changes its electronic character as a function of anti-correlated nuclear coordinate, and the Condon approximation becomes invalid in the dimer even if it holds for individual pigments. Thus, even simplifying assumptions about the validity of the Condon approximation in individual chromophores lead to complicated vibrational-electronic dynamics in the dimer.

In a dimer with identical FC-active intramolecular vibrational modes on both pigments and no coupling to environmental modes, the excited state adiabatic and non-adiabatic dynamics
are completely separable. We have shown that apart from the anti-correlated vibrational coordinate, a global environmental mode that is coupled to both the pigments differently can also contribute towards tuning the relative energy gap between the excited pigments, and couple the adiabatic and non-adiabatic dynamics on the excited electronic state of the dimer. However, many ground electronic state 2D signatures of excited state non-adiabatic vibrational-electronic mixing along the anti-correlated coordinate are not affected by such an environmental mode. Instead, a predicted signature of excited state vibrational-electronic resonance, ground state CP12/CP21 oscillation amplitude asymmetry, is shown to be enhanced in the presence of an environmental mode.

By including an additional near-resonant mode present in BChl a, we have shown that FC-active modes with vibrational frequencies close to the excitonic energy gap can have an additive effect on the non-adiabatic vibrational-electronic mixing on the excited state. This results in further enhancement of ground state anti-correlated vibrational wavepackets, which are experimentally manifested as enhanced CP12 oscillations in the ground state rephasing 2D spectrum. A finite width of non-adiabatic coupling around resonance relaxes the condition for exact resonance and might render such a non-adiabatic mechanism robust to changes in the protein environment. It also offers design principles where structured environments can be engineered to exploit the width of non-adiabatic coupling operative in the presence of such modes.
3.6. Appendix 3A

Methods:

The calculations presented in this work are based on the direct product basis. In the direct product basis with two pigments $A$ and $B$, the ground electronic state of the dimer is represented by $|0_A 0_B\rangle$, the first excited electronic states with either pigment $A$ or $B$ excited are given by $|A\rangle |0_B\rangle$ and $|0_A\rangle |B\rangle$, respectively. The doubly excited state with both pigments excited is given by $|A\rangle |B\rangle$. The harmonic oscillator vibrational basis state corresponding to the $i^{th}$ bath vibrational mode is $|ν_i\rangle$ with vibrational quantum number $ν_i$. An undisplaced vibrational basis is used for the non-adiabatic calculations presented here. In this basis, the undisplaced vibrational basis states of the ground electronic state of the dimer are also used for describing the vibrational manifold on all the excited electronic states. The matrix elements for the operator $\hat{q}_i$ of the dimensionless normal coordinate (Appendix E.1 of ref. 90) are given by

$$
\langle ν_i | q_i | ν'_i \rangle = \left[ \frac{ν_i}{2} \delta_{ν_i,ν'_i+1} + \frac{ν'_i}{2} \delta_{ν_i,ν'_i-1} \right]
$$

where the harmonic oscillator vibrational basis state of the $i^{th}$ normal vibrational coordinate $|ν_i\rangle$ is coupled to $|ν_i \pm 1\rangle$ in the same electronic state. In the undisplaced vibrational basis all the electronic states have a common set of vibrational basis states, such that only the $Δν_{A,B} = 0$ electronic transitions are allowed. The coupling $J_{AB}$ between the singly excited electronic states is given by $\langle 0_A | \langle B | J | A \rangle | 0_B \rangle \delta_{ν_A,ν'_A} \delta_{ν_B,ν'_B}$, $J_{AB} = 66.14$ cm$^{-1}$ for the vibrational-electronic resonance model presented here.
Using the above definitions, the complete vibrational-electronic basis, each dimer electronic state is multiplied by the vibrational basis states \( |\nu_1\rangle |\nu_2\rangle ... |\nu_i\rangle \) such that complete vibrational electronic basis states are \( |0_A\rangle |0_B\rangle |\nu_1\rangle |\nu_2\rangle ... |\nu_i\rangle \) for the ground electronic state of the dimer, \( |A\rangle |0_B\rangle |\nu_1\rangle |\nu_2\rangle ... |\nu_i\rangle \) and \( |0_A\rangle |B\rangle |\nu_1\rangle |\nu_2\rangle ... |\nu_i\rangle \) for the singly excited electronic states and \( |A\rangle |B\rangle |\nu_1\rangle |\nu_2\rangle ... |\nu_i\rangle \) for the doubly excited electronic state of the dimer. The diabatic electronic potential energy of the singly excited electronic state \( |A\rangle |0_B\rangle \) due to the bath vibrations is given by \( E^A(q) = \langle 0_B | \hat{H}_{\text{dimer}} - \hat{H}_{\text{coupling}} | A \rangle |0_B \rangle \). \( q \) is the vibrational subspace of the dimer system and is treated as a parameter in describing the electronic potential energy. The dimer Hamiltonian, \( \hat{H}_{\text{dimer}} \), and the coupling Hamiltonian, \( \hat{H}_{\text{coupling}} \), are defined in Eqns. (3.1) and (3.2), respectively.

The exact eigenvectors and eigenenergies are obtained by numerical diagonalization of the dimer Hamiltonian for the respective parameters mentioned in the text. For the case of one FC vibrational mode per pigment, both with and without an environmental mode, 9 quanta of vibrations are included along each vibrational degree of freedom. For dimer calculations with two FC active modes per pigment, 6 quanta of vibration are included for each vibrational degree of freedom. Starting from these eigenvectors and eigenenergies, the sum over states formulas for the nonlinear optical response functions \(^{15}\) were used to calculate the 2D spectra. Decoherence due to interactions with the bath can be included using a Brownian oscillator model. Interactions with the bath are decomposed into correlated and anti-correlated parts. Correlated decoherence between electronic states with different number of excitons is caused by the interaction of the two electronic states with the correlated part of the low frequency phonon sideband of the protein. It is included by multiplying the non-linear response by the 3D time domain correlated
Brownian oscillator non-linear response function. For calculations using a Bloch dephasing model, correlated decoherence caused by the correlated part of the low frequency phonon sideband is neglected such that the sum over states non-linear response is multiplied at each time point by a Heaviside step function and an exponential relaxation factor. The relaxation time constant for population decay is $T_1 = 250$ fs, while coherence decay time constant $T_2 = 500$ fs. These were chosen in order to obtain better resolved peaks than the 2D spectrum with Brownian oscillator peakshapes.

The anti-correlated components of the low frequency phonon sideband is responsible for anti-correlated decoherence between electronic states with same number of excitons and is neglected in the calculations. However, the overall anti-correlated dephasing between the singly excited electronic states also includes contributions from static energetic inhomogeneities in the protein environment that cause ensemble dephasing of the coherence between the two singly excited electronic states. This contribution is included by calculating the sum over states non-linear response for each member of an ensemble with a static Gaussian distribution of electronic site energy differences ($\sigma_\Delta = 34$ cm$^{-1}$) and adding the response with respective Gaussian weights. This inhomogeneity is slightly smaller than the one determined$^{25}$ for FMO at 19 K from the anisotropy for a pair of excitons separated by $\sim 150$ cm$^{-1}$ which decays to about half the initial amplitude by $\sim 180$ fs. This experimentally measured 180 fs timescale includes the anti-correlated decoherence as well as ensemble dephasing, and puts a lower bound on the anti-correlated decoherence timescale in the FMO complex. The anti-correlated decoherence timescale has not been probed directly by any experiment till date. Although this is not included in the calculations, the ground to excited state correlated decoherence which decays on a $\sim 100$ fs timescale at 80 K will prevent the slower anti-correlated decoherence between the singly excited
electronic states from influencing the ground state signatures. The temperature was fixed at 80 K to match the 2D experiments on the FMO antenna protein.

The 3D impulsive response is multiplied by a frequency filter corresponding to 20 fs FWHM laser intensity pulse duration to give the non-linear polarization. The frequency filter is centered on the ground to excited state energy gap, i.e. $\omega_{laser} = \omega_{eg} = 11574$ cm$^{-1}$. The energy gap $\omega_{eg} = (E_A + E_B)/2$ is defined with respect to the center of the two singly excited states with energies $E_A$ and $E_B$. A 3D FT algorithm$^{91}$ was used to calculate the 2D Fourier transform (FT) spectra. To make the sum over states calculation computationally less expensive, ground vibrational states with fractional population less than $10^{-2}$ with respect to the ground vibrational state at 80 K are neglected. The neglect of these states leads to a neglect of ~0.6 % population for the case of one FC active vibrational mode per pigment with 200 cm$^{-1}$ frequency, ~1.8% of the population for the case of one FC active 200 cm$^{-1}$ vibrational mode per pigment with a 20 cm$^{-1}$ environmental mode, and ~1.0% of the population for case of 2 FC active vibrational modes per pigment with frequencies 200 cm$^{-1}$ and 180 cm$^{-1}$. Transition dipole matrix elements with magnitudes below $\mu.10^{-5}$ are neglected as zero. The calculations were done on two hex-core 2.8 GHz Intel Westmere processors and took approximately 3 hours for the dimer model with one FC active vibrational mode per pigment, 15 hours for the calculation with two FC active vibrational modes per pigment and 18 hours for the calculation with one FC active vibrational mode per pigment and an additional environmental mode.
Figure 3A1. Variation of CP12 and CP21 oscillation amplitudes as a function of vibrational frequency of the FC-active mode, over a range of 130 cm$^{-1}$ to 270 cm$^{-1}$, corresponding to the real “rephasing” 2D spectra calculated using a Bloch dephasing model and shown in Fig. 3.3. The Huang-Rhys factor $S = (1/2)\omega d^2$, where $d$ is the FC vibrational displacement on the excited electronic state, is kept constant at 0.025 over the range of vibrational frequencies sampled. CP12 oscillation amplitude is enhanced by ~35x at resonance with a ~60 cm$^{-1}$ FWHM, whereas CP21 oscillation amplitude stays approximately constant. For all the vibrational frequencies, the CP amplitudes are extracted from the intersections of ‘E1’ and ‘E2’ lines corresponding to the 2D spectrum for a vibrational frequency of 130 cm$^{-1}$ (Fig. 3.3a). For a vibrational frequency far away from resonance, the vibrational peaks do not affect the shape of the cross-peaks and allow for a better peak-center determination. A similar analysis with peak positions corresponding to a vibrational frequency of 270 cm$^{-1}$ is shown in Fig. 3.4. The sampled 2D peak coordinates $(\omega_z, \omega_t)$ in centimeters$^{-1}$ are: CP12 (-11,674.2, 11,473.8) and CP21 (-11,473.8, 11,674.2).
Figure 3A2

**Figure 3A2.** Absolute amplitudes and relative phase relationships between the diagonal (DP1 and DP2) and cross-peaks (CP12 and CP21) as a function of waiting time $T$ in the “rephasing” ground electronic state contributions to the 2D spectra for a dimer model with one identical intramolecular FC active vibration per pigment and an additional environmental mode $\hat{q}_e$ (Section 3.2.1.2 in the text) with a frequency of 20 cm$^{-1}$ and a stabilization energy of 3 cm$^{-1}$, such that only the higher energy pigment $B$ is directly coupled to the environment. The 2D spectra are calculated with correlated broadening from a critically damped Brownian oscillator with frequency 70 cm$^{-1}$ and stabilization energy 30 cm$^{-1}$ at a temperature $T = 40$ K, and averaged over a static Gaussian distribution of site energy gaps with $\sigma_\Delta = 34$ cm$^{-1}$. The peaks oscillate at a vibrational frequency of 200 cm$^{-1}$ which is in resonance with the excitonic energy gap. The intramolecular vibration has a stabilization energy of 5 cm$^{-1}$. The transients are offset (additive constants in boxes) to show phase relationships, but not multiplicatively scaled. The ground state signatures are similar to those reported in ref. 23. Compared to the $\sim 24\%$ CP12 modulation depth at $T = 80$ K, the modulation depth actually increases to $\sim 44\%$ at 40 K, while the CP21 modulation depth stays constant at $\sim 0.9\%$. The additional slow modulation on DP2, diagonal peak corresponding to the higher energy exciton, is due to the asymmetric environmental mode.
References


CHAPTER 4

ABSOLUTE MEASUREMENT OF FEMTOSECOND PUMP – PROBE SIGNAL STRENGTH

The absolute femtosecond pump-probe signal strength of deprotonated fluorescein in basic methanol is measured. Calculations of the absolute pump-probe signal based on the steady state absorption and emission spectrum that use only independently measured experimental parameters are carried out. The calculation of the pump-probe signal strength assumes the pump and probe fields are both weak and includes the following factors: the transverse spatial profile of the laser beams; the pulse spectra; attenuation of the propagating pulses with depth in the sample; the anisotropic transition probability for polarized light; and time dependent electronic population relaxation. After vibrational and solvent relaxations are complete, the calculation matches the measurement to within 10% error without any adjustable parameters. This demonstrates quantitative measurement of absolute excited state population.

This chapter is adapted from the paper titled “Absolute Measurement of Femtosecond Pump Probe Signal Strength”, published in May 2013 in the J. Phys. Chem. A. I worked on this project as the lead graduate student along with our then post-doctorate fellow Dr. Byungmoon Cho. In the introduction, the chapter discusses the importance of absolute optical characterization of pump-probe signal strength in certain systems. A general theoretical framework for calculating the pump-probe signal in terms of the absolute change in the number of probe photons due to the pump beam is developed. This framework is valid for a variety of radiative and non-radiative processes in a multi-level system. The framework is then simplified for a laser dye fluorescein, an approximate two-level system for which the Condon approximation is valid. This is followed by a description of the experimental methods used and the results obtained from
absolute pump-probe measurements on fluorescein. The calculated and measured signals are compared in the discussion section.

4.1 Introduction

Absolute quantification of the concentration of excited electronic states is important for mechanistic and analytical investigations in chemistry. With flash photolysis, transient chemical species were generated and detected with a degree of control and accuracy,\textsuperscript{1,2} enabling time-resolved investigation of radicals and other transient chemical species. Later, using actinometry, Porter and co-workers extended flash photolysis to measure the triplet yield of chlorophylls after photoexcitation;\textsuperscript{3} chemical actinometry determined the fluence of the excitation flash and thus the absolute number of absorbed photons necessary for determination of the yield (for a recent description of actinometry, see ref. \textsuperscript{4}). With the advent of pulsed lasers, flash photolysis has been superseded by the pump – probe technique.\textsuperscript{5} Similar approaches work for pump-probe spectroscopy with nanosecond time resolution.\textsuperscript{6} For picosecond pulses, time dependent rotational alignment\textsuperscript{5} must be considered for molecules in liquids.\textsuperscript{7} For femtosecond pulses, the frequency dependence of the electronic transition probability becomes important.\textsuperscript{8} The Z-scan technique can be used to absolutely determine spectrally averaged optical nonlinearities.\textsuperscript{9-11} As in flash photolysis, relative absorption cross-sections and relative yields can be determined by global analysis of transient changes in the transmitted probe spectrum.\textsuperscript{12} Estimates of signal strength can achieve factor of 2 accuracy with rough approximations for spatial intensity variation and laser bandwidth.\textsuperscript{13} However, a factor of 2 lies at the heart of phenomena such as multiple exciton generation\textsuperscript{14} and singlet fission.\textsuperscript{15} Here, calculation and measurement of the absolute pump-probe signal strength are described for a molecular system with well characterized photophysics (the fluorescein dianion in basic methanol). Like the measurement of
absolute triplet yields, measurement of pump and probe photon numbers and consideration of their attenuation as they propagate in the sample are required; however, treatments of the transverse spatial profiles of the laser beams, the polarization and frequency dependence of the excitation probabilities, and consideration of relaxation dynamics are also required.

In pump-probe experiments, the pump pulse excites a fraction of the ground state molecules into the excited state and a weak, time-delayed probe pulse interrogates the subsequent population change; the measured transient absorption signal discussed here is the pump-induced change in number of transmitted probe photons (or energy, depending on the detector) caused by the presence of the pump. An increase in the transmitted probe photon number is caused by depletion of the ground state population ("ground state bleaching" reduces probe attenuation) and stimulated emission from the excited state population ("excited state emission" amplifies the probe). If the excited state can make a transition to a higher excited state by absorbing a probe photon, this contribution reduces the transmitted probe intensity ("excited state absorption"). The net pump-induced change in the transmitted probe photon number from all three contributions is the pump-probe signal.

At early pump-probe delay, coherent electronic effects (typically lasting ~100 fs in solution), coherent vibrational dynamics (wavepacket motion, typically lasting up to 10 picoseconds in solution) and rotational coherence (typically lasting a few hundred ps in solution) complicate the signal strength. To enable quantitative predictions with the fewest measured parameters, we focus on the pump-probe signal after enough time has elapsed so that electronic and vibrational coherence can be neglected. After vibrational, rotational and solvent relaxation, the pump-probe signal [the change in the transmitted photon number (probe energy) due to pump excitation] is directly related to $\Delta n_e$, the ground state population number density change and
\( \Delta n_e \), the excited state population number density change (initially, \( \Delta n_g = -\Delta n_e \)). For weak excitation of dipolar transitions, non-equilibrium molecular rotational alignment can be phenomenologically incorporated without adjustable parameters via the polarization anisotropy.

The effect of incomplete vibrational relaxation is more complicated and is not treated here, restricting the calculation to pump-probe delays large enough so that vibrational and solvent relaxation are complete (~ 20 ps for deprotonated fluorescein in basic methanol). The calculations are fundamentally based on Einstein’s analysis of the kinetics of absorption and stimulated emission (\( B \) coefficients)\textsuperscript{16-19} but mostly expressed in terms of experimentally accessible cross sections. In the following section, we derive an expression for the pump-probe signal that is an explicit function of the cross sections, which are functions of frequency.

4.2 Theory

Femtosecond pump-probe spectroscopy involves spatially varying, time dependent, non-equilibrium level populations and angular alignment. The approach described here extends Beer’s law in the differential form

\[
\frac{\partial I(R,\nu)}{\partial Z} = -n^0 \sigma^0(\nu)I(R,\nu) = -\alpha^0(\nu)I(R,\nu)
\]

(4.7)

where \( n^0 \) is the total equilibrium molecular number density, \( \sigma^0(\nu) \) is the absorption cross section of the equilibrium state, \( \alpha^0(\nu) \) is the equilibrium absorption coefficient for the intensity\textsuperscript{20} (to avoid confusion, it should be noted that an \( \alpha \) a factor of 2 smaller is sometimes used to quantify attenuation of the field\textsuperscript{21,22}), and \( I(R,\nu) \) is the spectral intensity (or irradiance\textsuperscript{23}) distribution of the light source, \( R \) is the spatial coordinate vector, and \( Z \) is the propagation direction. The beams are approximated as propagating with fixed transverse profiles (similar to
the approximation of collimated Gaussian beams\textsuperscript{24}. This requires a sample pathlength shorter than Rayleigh range over which a Gaussian beam is focused.

The absorption coefficient (dimensions: 1/ length) is

\[ \alpha(v) = \sum_{i,j} n_i \sigma_{ij}(v), \]  

(4.8)

where \( n_i \) is the molecular number density in level \( i \) and \( \sigma_{ij}(v) \) is the absorption or stimulated emission cross section for the transition from level \( i \) to level \( j \). In vacuum, the integrated cross section, in SI units, is

\[ \int \sigma_{ij}(v)dv = \frac{2\pi^2}{3\epsilon_0hc}(\mu_{ij} \cdot \mu_{ji})\nu_{ji} \]

which is proportional to the squared magnitude of the transition dipole \( \mu_{ij} \) and to the Bohr frequency \( \nu_{ji} = (E_j - E_i)/h \), where \( E_j \) and \( E_i \) are level energies,\textsuperscript{19} \( h \) is the Planck constant, \( c \) is the speed of light in vacuum, and \( \epsilon_0 \) is the permittivity of free space. Note that the proportionality to \( \nu_{ji} \) implies that absorption cross sections (\( E_j > E_i \)) are positive and stimulated emission cross sections (\( E_j < E_i \)) are negative. This sign convention is consistent with the sign convention for oscillator strength (positive for absorption, negative for emission)\textsuperscript{25,26} and simplifies some formulas below. The factor of 1/3 arises from isotropic angular averaging of \( \cos^2 \theta \) (\( \theta \) is the angle between transition dipole and optical field). Very roughly, only 1/3 of the molecules (those with transition dipoles aligned more or less parallel to the field) can effectively absorb a given polarization of light. Thus, the pump excites an anisotropic angular distribution.

At equilibrium,

\[ \alpha^0(v) = \sum_{i,j} n_i^0 \sigma_{ij}(v), \]  

(4.9)
where $\alpha^0(\nu)$ is the equilibrium absorption coefficient and $n_i^0$ is the equilibrium number density of level $i$ (dimensions: 1/volume) just before pump arrival at $T = 0$. The total equilibrium molecular number density, $n^0$ is

$$n^0 = \sum_i n_i^0 = \sum_i n_i$$

(4.10)

where the last equality holds in the absence of photodissociation.

The spectral intensity distribution, $I(R, \nu)$ is related to the pulse energy

$$U(Z) = \int_0^\infty U(\nu, Z)d\nu$$

by

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} I(R, \nu)dXdY = U(\nu, Z)k_{rep}$$

(4.11)

where $k_{rep}$ is the repetition rate of the laser (pulses/time) and $U(\nu, Z)$ is the spectral energy distribution per pulse (dimensions: (energy/pulse)/frequency). The spectral energy distribution per pulse changes as the pulse propagates along $Z$. At each $Z$, the spectral energy distribution per pulse is the integral $U(\nu, Z) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} u(R, \nu)dXdY$ of the energy fluence per unit frequency [dimensions: (energy/pulse)/(area·frequency)] over the transverse beam dimensions of the spatial coordinate $R$. The energy fluence per unit frequency is written in terms of photon fluence per unit frequency, $p(R, \nu)$ [dimensions: (photons/pulse)/(area·frequency)]

$$u(R, \nu) = h\nu p(R, \nu).$$

(4.12)

The pump is assumed to propagate linearly along $Z$, perturb level populations, and thus alter the linear propagation of the probe in the perturbed sample. The simplifying assumption of collinear pump and probe propagating along $Z$ neglects any crossing angle between pump and probe; this
neglect requires a sample pathlength short enough that spatial walk-off between the two beams can be neglected.

The total number of probe photons transmitted through the sample depends on the pump-probe delay $T$ and the relative polarization of the pump and probe. For linearly polarized pulses, the relative polarization is quantified by $f = 3\cos^2 \phi - 1$, where $\phi$ is the angle between pump and probe optical electric field vectors. For small beam crossing angles, the optical pathlength is negligibly longer than the sample thickness at normal incidence. The total number of transmitted probe photons (unitless) is obtained by integrating the transmitted probe fluence over frequency and the lateral beam dimensions at $Z=l$, where $l$ is the pathlength of the sample,

$$P_r(f,T) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} \int_{0}^{\infty} [p_r(R,v_r,f,T)|_{z=l}][d\nu_r]dXdY.$$  (4.13)

In general, anisotropic molecular excitation makes the sample dichroic and birefringent so that it can alter the polarization of the probe as it propagates (in general, fields, rather than the intensities must be propagated). When the probe polarization is parallel or perpendicular to the pump, its polarization is not affected by propagation so that Beer’s law can be used to describe probe propagation. With the assumptions above, the differential form of Beer’s law becomes

$$\frac{\partial p_r^{\text{on}}(R,v_r,f,T)}{\partial Z} = -\left[\alpha^0(v_r) + \Delta\alpha(R,v_r,f,T)\right]p_r^{\text{on}}(R,v_r,f,T)$$  (4.14)

where the superscript “on” indicates the probe field with pump excitation.

The formal solution of Eq.(4.14) is obtained by separation of variables,

$$p_r^{\text{on}}(R,v_r,f,T) = p_r(R,v_r)|_{z=0} \exp[-\alpha^0(v_r)Z] \cdot \exp[-\int_0^Z \Delta\alpha(R,v_r,f,T)dZ']$$  (4.15)
where \( p_r(R, \nu_r) \big|_{z=0} \) is the incident probe photon fluence at the front of the sample (which is independent of \( f, T \) and whether the pump is on or off). The integral inside the exponential is proportional to an effective change in absorbance that varies as a function of the transverse spatial coordinates \( X \) and \( Y \); a transverse spatial variation of \( \Delta \alpha \) can generate non-exponential attenuation of the spatially integrated fluence, so a spatially averaged absorbance change may not exist, but a total change in transmission \( \Delta T \) remains.

Without the pump, \( \Delta \alpha(R, \nu_r, f, T) = 0 \), and exponential Beer’s law attenuation of the probe fluence through the length of the sample is recovered:

\[
p_{r}^{\text{off}}(R, \nu_r) = p_r(R, \nu_r) \big|_{z=0} \exp[-\alpha^0(\nu_r)Z]. \tag{4.16}
\]

Since the excited state levels will have initially pumped populations that decay exponentially with \( Z \) due to pump attenuation, \( \Delta \alpha \) will be a sum of terms that decay exponentially with \( Z \). The appendix derives \( \Delta \alpha \) by equating pump photon number depletion to initial molecular excitation, allowing for subsequent relaxation, and including transitions from excited levels, depleted levels, and levels connected by relaxation; the result obtained there is

\[
\Delta \alpha(R, \nu_r, f, T) = \sum_{i,j,k,l} \left[ \int_0^\infty \Delta n_{ijkl}(R, \nu_u, f, T) \sigma_{kl}(\nu_r) d\nu_u \right] \tag{4.17}
\]

where \( \sigma_{kl}(\nu_r) \) is the cross section for the probe transition from level \( k \) to level \( l \) and

\[
\Delta n_{ijkl}(R, \nu_u, f, T) = \Delta n_{ijk}(R, \nu_u, T)[1 + \tilde{r}_{ijkl}(T)]. \tag{4.18}
\]

If the levels \( i, j, k, \) and \( l \) are non-degenerate, the initial anisotropy for a probe transition from level \( k \) to level \( l \) after pump excitation from level \( i \) to level \( j \) is

\[
r_{ijkl}(T = 0) = \frac{1}{5} \left[ \frac{3(\tilde{\mu}_{ij} \cdot \tilde{\mu}_{kl})^2}{(\tilde{\mu}_{ij} \cdot \tilde{\mu}_{ji})(\tilde{\mu}_{ik} \cdot \tilde{\mu}_{ji})} - 1 \right]^5. \tag{5}
\]

The more general theory of the anisotropy need not be
a concern here as the anisotropy is simply used to relate the parallel and perpendicular pump-probe signals to level population change. At a point $\mathbf{R}$ in the sample, $\Delta n_{ij}(\mathbf{R}, \nu, T)$ is the total population density change in level $k$ arising from excitation of the transition from $i$ to $j$ by pump frequency $\nu$ at a time $T$ in the past. As shown in the appendix [Eq.(4A.4)], the molecular number density transferred from $i$ to $j$ by the pump through either absorption or stimulated emission is

$$\Delta n_{ij}(\mathbf{R}, \nu) = p_u(\mathbf{R}, \nu) | n_i^0 | \sigma_g(\nu) | \exp[-\alpha^0(\nu)Z].$$

(4.19)

At time $T = 0$,

$$\Delta n_{ij}(\mathbf{R}, \nu, T = 0) = \Delta n_{ij}(\mathbf{R}, \nu)$$

(4.20a)

the initial change in number density in the level $j$ is equal to the molecular number density excited, and

$$\Delta n_{ji}(\mathbf{R}, \nu, T = 0) = -\Delta n_{ij}(\mathbf{R}, \nu),$$

(4.14b)

the molecules excited to $j$ disappear from the initial state $i$. If spatial migration of molecules and excitation are absent, then changes in population over time can be quantified by conditional probabilities that are independent of the spatial coordinates. Then, at later times,

$$\Delta n_{ijk}(\mathbf{R}, \nu, T) = \Delta n_{ij}(\mathbf{R}, \nu, T = 0) c_{ij}(T) + \Delta n_{ji}(\mathbf{R}, \nu, T = 0) c_{ji}(T)$$

(4.21)

where $c_{ik}(T)$ is the conditional probability that a molecule in level $i$ at $T = 0$ is found in level $k$ after time $T$. The conditional probabilities $c_{ik}(T)$ may be calculated from chemical kinetics and will obey the initial condition $c_{ii}(T = 0) = 1$. In the absence of photodissociation, the conditional
probabilities obey a sum rule, \( \sum_i c_{ik}(T) = 1 \), and ultimately reach thermal equilibrium,

\[
c_{ik}(T = \infty) = g_k \exp(-\beta E_k)/q,
\]

where \( g_k \) is the degeneracy of level \( k \) with energy \( E_k \), \( \beta \) is the inverse temperature, and \( q \) is the molecular partition function.\(^{27}\) With the equilibrium conditional probabilities, Eqs. (14) and (4.21) yield \( \Delta n_{ijk} = 0 \), and the pump-probe signal is zero.

If transitions between levels have unresolved structure, sums over sub-level relaxation kinetics can give rise to an effective cross section for the probe transition that depends on the pump frequency and pump-probe delay (e.g., the narrowing of an electronic band upon vibrational cooling) necessitating the use of \( \sigma_{kl}(\nu_r, T, \nu_u) \) in place of \( \sigma_{kl}(\nu_r) \) in Eq. (4.17) then \( r_{ijkl}(T, \nu_u, \nu_r) \) may be needed in place of \( r_{ijkl}(T) \) in Eq.(4.18), and \( c_{ik}(T, \nu_u, \nu_r) \) in Eq. (4.21). This effective cross section \( \sigma_{kl}(\nu_r, T, \nu_u) \) will be dependent on both pump and probe frequencies until sub-level populations are thermally equilibrated. Treatment of sub-level coherence (e.g. wavepackets) via \( \sigma_{kl}(\nu_r, T, \nu_u) \) is approximate, but non-equilibrium sub-level kinetics (e.g., vibrational population relaxation) can be incorporated exactly in this way.\(^{28,29}\) After coherence decay and sub-level relaxation, \( \sigma_{kl}(\nu_r, T, \nu_u) = \sigma_{kl}(\nu_r) \), the steady state cross section.

The transverse spatial profile and attenuation of the pump with depth affect the transverse spatial profile of the propagating probe intensity in Eq. (4.14). Eq. (4.14) has the same form as Beer’s law when all three of the following conditions hold: 1) the anisotropy is zero; 2) complete relaxation wipes out all correlation so that \( \sigma_{kl}(\nu_r, T, \nu_u) = \sigma_{kl}(\nu_r) \); and 3) the molecules excited by the pump are spatially uniform over the probe spatial profile.

The transmitted probe photon spectrum is

\[
\rho_r(\nu_r, f, T) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} p_r(R, \nu_r, f, T) |_{z=l} \, dX dY,
\]

so the spectrally resolved pump-probe signal is \( S_{SRPP}(\nu_r, f, T) = \rho_{r0}^{on}(\nu_r, f, T) - \rho_{r0}^{off}(\nu_r) \). The
spectrally resolved pump-probe signal is explicitly related to \( \Delta \alpha(\mathbf{R}, \nu, f, T) \) using Eq. (4.15) and Eq. (4.16):

\[
S_{SRPP}(\nu, f, T) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} p_r^{\text{eff}}(\mathbf{R}, \nu) \left|_{z=1} \right. \exp \left[ -\int_{0}^{i} \Delta \alpha(\mathbf{R}, \nu, f, T) dZ \right] - 1 \right. dXdY
\]

(4.22)

The pump-probe signal, \( S_{PP}(f, T) \) is the change in the total transmitted probe photon number caused by the pump:

\[
S_{PP}(f, T) = \int_{0}^{\infty} S_{SRPP}(\nu, f, T) d\nu_r
\]

\[
= \Delta P = P_r^{\text{on}}(f, T) - P_r^{\text{off}}
\]

(4.23)

where \( P_r^{\text{on}}(f, T) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \left[ \int_{0}^{\infty} p_r^{\text{on}}(\mathbf{R}, \nu, f, T) \left|_{z=1} \right. d\nu_r \right] dXdY \) is the total transmitted probe photon number with the pump on and \( P_r^{\text{off}}(f, T) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \left[ \int_{0}^{\infty} p_r^{\text{off}}(\mathbf{R}, \nu) \left|_{z=1} \right. d\nu_r \right] dXdY \) is the total transmitted probe photon number with the pump off.

In contrast to approaches based on the third order nonlinear susceptibility, coherent processes are formally excluded. However, correlated relaxation kinetics can be treated exactly so that some coherent processes can be incorporated approximately (e.g., the semiclassical or “doorway-window” descriptions of vibrational wavepackets in the integrated pump-probe signal). For comparison to experiment, the treatment here is primarily concerned with developing expressions that depend on the smallest set of independently measurable parameters. Furthermore, it automatically incorporates, to all orders, the “scheme S1/scheme S2” cascades (later renamed “parallel cascades”) necessary to describe propagation in concentrated samples when the cumulative effect of the pump over the sample length is not weak.
In the weak pump excitation regime, when the cumulative effect of the pump is also small, the exponential in Eq. (4.22) can be linearized to yield a simple expression for the spectrally resolved pump-probe signal:

$$S_{SRPP}(v_r, f, T) \approx \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} p_{sr}^{off}(R, v_r) \bigg|_{z=t} \cdot -\int_0^t \Delta \alpha(R, v_r, f, T) dZ \bigg] dX dY. \ (4.24)$$

This linearization requires that the integral of $\Delta \alpha$ over the propagation depth $Z$ (proportional to an effective change in absorbance) be very much less than one. In this limit, the spectrally resolved pump-probe signal becomes proportional to a probe weighted spatial average of the transverse coordinate dependent change in absorbance or optical density. This average is often reported as $\Delta A(v_r)$ or $\Delta OD(v_r)$.

Within approximation Eq. (4.24), if the population changes are linearly proportional to the pump pulse energy, then the signal is linearly proportional to the pump pulse energy. *However, population changes proportional to the pump pulse energy are not sufficient to guarantee that the measured pump-probe signal is linearly proportional to the pump pulse energy.* Thicker samples or higher sample concentrations require even lower excitation probabilities to suppress the cumulative higher order terms in the exponential. The quadratic term in the expansion of the exponential in Eq. (4.22) corresponds to a parallel cascade\textsuperscript{31,32} in which the third order pump-probe signal field radiated by one molecule propagates in the same direction as the probe and then acts as the probe field in generating a pump-probe signal from a second molecule excited by the pump (hence the quadratic dependence on $\Delta n$). The $m$th order term involves $m$ molecules, all $m$ excited by the pump, but only the first interacting with the probe, in an $m$th order parallel cascade.
Note that the transmitted probe photon number has an implicit dependence, through $\Delta n_{ij}$ in Eq.(4.19) on the pump intensity. Equations (4.14)-(4.16) and (4.22)-(4.23) are also valid with probe intensity ($I_i$) and probe energy ($U_i$) substituted for photon fluence ($p_r$) and total photon number ($P_r$), respectively.

We now apply the above equations to the relaxation kinetics of fluorescein, simplifying to two electronic levels. The initial conditions relevant to both levels are given by Eq. (4.14a) and (4.14b). For the excited level $j$,

$$\Delta n_{ij} (R, \nu_u, T) = \Delta n_{ij} (R, \nu_u, T = 0) c_{ji} (T) + \Delta n_{ij} (R, \nu_u, T = 0) c_{ij} (T) \quad \text{[Eq. (4.21) with } k = j], with

$$c_{ji} (T) = L_j (T),$$

where $L_j (T)$ is the lifetime function of $j$ [for a Bloch model, $L_j (T) = \exp (-T/T_1)$], where $T_1$ is the population lifetime]. If the levels are separated by much more than the thermal energy, the probability of a spontaneous thermal transition from $i$ to $j$ is zero, $c_{ij} (T) = 0$, so that

$$\Delta n_{ij} (R, \nu_u, T) = \Delta n_{ij} (R, \nu_u) L_j (T).$$

For the ground state $i$, $\Delta n_{ji} (R, \nu_u, T) = \Delta n_{ji} (R, \nu_u, T = 0) c_{ji} (T) + \Delta n_{ji} (R, \nu_u, T = 0) c_{ij} (T)$.

Because no other state is thermally populated, $c_{ji} (T) = 1$. The conditional probability $c_{ji} (T)$ is the population of level $i$ found by solving the relaxation equations of the system for unit population of level $j$ at $T = 0$; it is an increasing function of $T$, with $c_{ji} (0) = 0$ and $c_{ji} (T = \infty) = 1$.

The sum rule $\sum_i c_{ji} (T) = 1$ implies that $c_{ji} (T) \leq (1 - c_{ij} (T)) = (1 - L_j (T))$; the ground state recovers population no faster than the excited state loses population. Since $c_{ji} (T) = 1 - \sum_{k \neq i} c_{jk} (T)$, setting $L_i (T) = \sum_{k \neq i} c_{jk} (T) = L_j (T) + \sum_{k \neq i, j} c_{jk} (T)$ (note the hidden dependence on $j$) gives

$$c_{ji} (T) = (1 - L_i (T)),$$ so that
If only one excited level \( j \) is initially populated by the pump, then \( L_i(T) \) (which depends on the initially excited state \( j \)) is the experimentally measurable ground state population recovery lifetime function. When population from the initially excited level \( j \) relaxes directly to \( i \), and subsequent thermal equilibration within \( i \) is much faster than the population relaxation from \( j \) to \( i \), \( L_i(T) \approx L_j(T) \).

The expression for the 2D spectrum obtained by leaving out the integral over \( \nu_u \) in Eq. (4.17) and following the above derivation through to approximation (4.24) agrees with previously tested expressions [Eq. (17) and (18) in ref. 34] for the shape of the relaxed real 2D spectrum for an electronic two level system [in the \( S_{2D}^- \) representation (Eq. (21) of ref. 35)] appropriate for a reference that passes through the sample, as in the pump – probe geometry] when the proportionalities between Einstein \( B \) coefficient lineshape \( g(\nu) \) and cross section \([ g(\nu) \propto \sigma(\nu)/\nu \) ] and between intensity and fluence \([ I(\nu) \propto \nu p(\nu) \) ] are incorporated.

With a simplifying assumption that both the pump and probe pulses have no “spatial chirp”, the incident photon fluence in Eq. (4.12) may be written as a product of a spatial and a spectral distribution

\[
p_x(R,\nu_x)_{|z=0} = h_x(X,Y)\rho_x(\nu_x)_{|z=0},
\]

where \( x = u \) for pump and \( r \) for probe. With \( P = \int_0^\infty \rho(\nu)_{|z=0} d\nu \), the transverse spatial distribution must be normalized so that \( \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} h(X,Y)dXdY = 1 \). Substituting Eq. (4.27) for \( p_u \), Eq. (4.19) becomes

\[
\Delta n_{ji}(R,\nu_u, T) = -\Delta n_{ji}(R,\nu_u)L_i(T).
\]
\[ \Delta n_g(R, \nu_u) = h_g(X, Y) \rho_g(\nu_u) \mid_{Z=0} \cdot n^0_0 \mid \sigma_g(\nu_u) \mid \exp[-\alpha^0(\nu_u)Z] \]  

(4.28)

where \( h_g(X, Y) \) is the spatial profile (dimensions: 1/area) and \( \rho_g(\nu) \mid_{Z=0} \) is the frequency dependent photon number distribution (dimensions: (photons/pulse)/frequency) of the pump at the front of the sample.

Using labels \( g \) for ground state and \( e \) for excited state in a two-level system, the initial state is \( i = g \), the pump populates \( j = e \), and the probe connects level \( k = e \) to \( l = g \) for ESE, in addition to level \( k = g \) to \( l = e \) for GSB. For fluorescein at room temperature, the excited electronic state is not thermally populated and \( n^0_0 = 0 \). Using these labels and inserting Eq. (4.25) and Eq. (4.26) into Eq. (4.18), Eq. (4.17) becomes

\[
\Delta \alpha(R, \nu_r, f, T) = \int_0^T \left[ +\Delta n_g(R, \nu_u) L_g(T) \right. \\
\left. \cdot [1 + f'_{geg}(T)] \sigma_{eg}(\nu_r) \right. \\
- \Delta n_{ge}(R, \nu_u) L_g(T) \\
\left. \cdot [1 + f'_{geg}(T)] \sigma_{ge}(\nu_r) \right] d\nu_u.
\]

(4.29)

Substituting Eq. (4.28) into Eq. (4.29), Eq. (4.29) into Eq. (4.24), evaluating the integral over \( Z \), using \( p^\text{off}_r(R, \nu_r) = h_r(X, Y) \rho_r(\nu_r) \mid_{Z=0} \exp[-\alpha^0(\nu_r)Z] \) (which results from inserting Eq. (4.27) into Eq. (4.16)), and integrating over \( X \) and \( Y \) yields

\[
S_{SRPP}(\nu_r, f, T) \approx H [P_{u_0} \mid_{Z=0} - P_{u} \mid_{Z=0}] \\
\cdot \rho_r(\nu_r) \mid_{Z=0} \exp[-\alpha^0(\nu_r)] \\
\left[ +L_g(T)[1 + f'_{geg}(T)] \sigma_{eg}(\nu_r) \right] \\
- L_g(T)[1 + f'_{geg}(T)] \sigma_{ge}(\nu_r) \right],
\]

(4.30)

where

\[
H \equiv \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} h_r(X, Y) h_u(X, Y) dXdY,
\]

(4.31)
is the pump-probe transverse spatial overlap, and

$$\left[ P_u \mid_{Z=0} - P_u \mid_{Z=al} \right] \equiv \int_0^\infty \rho_u(v_u) \mid_{Z=0} [n_0^0 \sigma_{ge}(v_u) / \alpha^0(v_u)] [1 - \exp(-\alpha^0(v_u)/l)] dv_u,$$

(4.32)

is the number of pump photons absorbed by the molecules being probed. The frequency dependent fraction of photons absorbed by the molecules being probed is $[n_0^0 \sigma_{ge}(v_u) / \alpha^0(v_u)]$.

For a single solute, this fraction is equal to one if the solvent does not appreciably absorb or scatter light within the pump spectrum; in this circumstance, which applies to fluorescein in methanol near 500 nm, Eq. (4.32) simply gives the total number of pump photons absorbed.

Integrating over $\nu_r$, yields the pump-probe signal in the following form,

$$S_{pp}(T, f) = H\left[ P_u \mid_{Z=0} - P_u \mid_{Z=al} \right]$$

$$\left[ + G_{ge} L_g(T)[1 + f_{gege}(T)] \right]$$

$$\left[ - G_{eg} L_e(T)[1 + f_{gege}(T)] \right].$$

(4.33)

where

$$G_{ge} \equiv \int_0^\infty \rho_r(v_r) \mid_{Z=0} \exp[-\alpha^0(v_r)/l]\sigma_{ge}(v_r) dv_r,$$

(4.34)

quantifies probe spectral overlap with the ground state bleach and

$$G_{eg} \equiv \int_0^\infty \rho_r(v_r) \mid_{Z=0} \exp[-\alpha^0(v_r)/l]\sigma_{eg}(v_r) dv_r,$$

(4.35)

quantifies probe spectral overlap with the excited state emission. (The negative sign in front of $G_{eg}$ is the result of choosing a negative stimulated emission cross section).

For a two-level electronic system, the molecular parameters needed are the decadic molar extinction coefficient $\epsilon(\lambda)$ □ □ fluorescence spectrum $F(\lambda)$, the excited state lifetime function
$L_e(T)$, and the ground state bleach recovery function $L_g(T)$. Laser pulse parameters needed are
the pulse spatial profile $h(X,Y)$, and pulse spectra $\rho(\nu)|_{\nu=0}$. Methods for determining the
required parameters are outlined in the Experimental Section.

4.3 Experimental

The goal of the experiment is two-fold: 1) to measure the molecular and laser pulse
parameters required for the calculation, 2) to measure the absolute pump-probe signal strength
for comparison against the calculation. Accurate determination of the molecular parameters
required low sample concentrations and minimizing air, light and water exposure of the organic
dye solution. Measurements of concentration and sample thickness determined the decadic
molar extinction coefficient $\varepsilon(\lambda)$ to ~1% precision (with accuracy limited by mass purity).
Small error bars on the pulse parameters were needed to be reasonably confident of an agreement
between the measured and calculated absolute pump-probe signal strength; measurements of
pump and probe spectra and spatial profiles to within 5% error were therefore necessary. The
absolute pump-probe signal measurements required controlled pump-probe polarization, low
pulse energies, and near-maximum spatial pump probe beam overlap; high sample flow rates
were needed to avoid pump scatter, sample photodegradation, signal saturation, and excitation of
unrelaxed molecules.

4.3.1. Sample preparation, handling and integrity

Fluorescein (>97% purity, Invitrogen) was used without further purification. All
fluorescein samples were prepared in 0.01 N KOH in methanol (>99.8% purity, <0.1% water)
and were therefore in dianion form ($pK_a = 6.8$ for acid dissociation from monoanion to
dianion$^{36}$). The critical fluorescein concentration appropriate for optical experiments was
established by Förster\textsuperscript{37}; this critical concentration corresponds to the molecular separation below which energy transfer to neighboring molecules occurs within the excited state lifetime, and therefore can affect pump-probe signal strength. Förster calculated the critical separation for fluorescein in 0.01 N KOH water to be \(\sim 50\,\text{Å}\), corresponding to a critical concentration of \(\sim 3\,\text{mM}\) (for fluorescein in 0.01 N KOH methanol solution, we calculated the critical separation to be \(\sim 51\,\text{Å}\)). The highest concentration used in the experiment was \(\sim 0.5\,\text{mM}\), \(\sim 6\times\) below the critical concentration. The decadic molar extinction coefficient, \(\varepsilon(\lambda)\), was optically determined for concentrations ranging from 0.1 mM to 0.5 mM for two reasons: to check for aggregation which would be concentration dependent and to establish an accurate \(\varepsilon(\lambda)\). The optical determination was done by measuring the absorbance of independently known concentrations; known masses of the dye were dissolved into known volumes of solvent (both were known to \(<1\%\) error). Absorbance of the sample was measured with an absorption spectrometer (Varian Cary 500) in a 0.199±0.001 mm cuvette; the manufacturer-specified pathlength was independently confirmed by measuring the absorbance of the same solution in a 1 cm cuvette whose pathlength was determined to 0.005 mm accuracy using a Starrett small hole gauge and Mitutoyo digital calipers. When the absorbance spectra were divided by \(cl\), where \(c\) is the measured concentration and \(l\) is the sample thickness, \(\varepsilon(\lambda)\)’s agreed within 1\% error, indicating no concentration effects on \(\varepsilon(\lambda)\). We obtained \(\varepsilon_{\text{max}} = 92,400\pm90\,\text{M}^{-1}\cdot\text{cm}\) at 497 nm for fluorescein in methanol/0.01 N KOH, which agreed within the presumed error of the previously reported literature value of \(92,300\,\text{M}^{-1}\cdot\text{cm}\) for fluorescein in ethanol/0.01 N KOH\textsuperscript{38} (this is a lower bound on \(\varepsilon_{\text{max}}\) because of possible sample impurity; \(\varepsilon_{\text{max}}\) could be as high as \(95,000\,\text{M}^{-1}\cdot\text{cm}\)).
Preserving the sample integrity was important, as casual handling caused degradation manifested by a reduction in absorption peak height after the measurements. Light and air exposure during handling were minimized by wrapping the sample with aluminum foil and parafilm. The sample was typically used for measurements within two hours of preparation. During data collection, which typically lasted ~3 – 4 hours, blocking the laser beams in between measurements significantly reduced degradation. The samples were prone to photo-degradation caused by repeated excitation of unrelaxed sample if not refreshed after every laser shot; the sample was refreshed by rapidly flowing the sample using a Micropump (GA series) at a discharge of 2.5 ml/s through a sample cell with an 8 mm x 0.2 mm cross section. This flow rate ensured that the average velocity for the slowest 10% of the molecules (determined by a laminar flow calculation\(^{39}\) based on ref.\(^{13,40}\)) was sufficiently fast that they traveled completely across the laser beam before excitation by the next pulse arriving 100 µs later (10 kHz laser repetition rate). To ensure no substantial photodegradation had occurred, absorption spectra before and after the experiments were compared. The differences were less than 4% after 3 – 4 hour measurements when degradation was minimized as described above. In contrast, for an exposed and aged sample, up to 10% change was not uncommon.

4.3.2. Determination of absorption and emission cross sections

The calculation ultimately demands the absorption and emission cross sections as molecular parameters. From the decadic molar extinction coefficients, the absorption cross section (dimension: \(\text{cm}^2\)) can be derived,

\[
\sigma_{\text{abs}}(\nu) = \frac{1000(\text{cm}^3 / L) \cdot \ln(10)}{N_A} \varepsilon(\nu)
\]  

\((4.36)\)
where $\varepsilon(\nu) = \varepsilon(\lambda = c / \nu)$ is the decadic molar extinction coefficient in 1/M·cm, and $N_A$ is Avogadro’s number in 1/mol. Here, determination of the absolute stimulated emission cross section was based on measurement of the relative fluorescence spectrum, the absorption cross section, the near unit fluorescence quantum yield, and the agreement between the measured fluorescence lifetime and the radiative lifetime calculated from the absorption spectrum using the Strickler – Berg relation.\textsuperscript{41} The accuracy of the Strickler – Berg relation establishes the Einstein $A$ coefficient and lineshape for spontaneous emission, and hence the Einstein $B$ coefficient and lineshape for stimulated emission (needed to calculate the absolute stimulated emission cross section). As the Strickler – Berg relation depends on the Condon approximation, this integrated agreement between the calculated and measured fluorescence lifetimes supports the average validity of the Condon approximation for this electronic transition in fluorescein. The relative fluorescence spectrum [$F(\lambda)$ in photon counts/(s·nm)] was recorded using 470 nm excitation on a Fluorog (Horiba Jobin-Yvon) fluorimeter calibrated for vacuum wavelength. The area normalized lineshape function for the stimulated emission Einstein $B$ coefficient was derived from the fluorescence lineshape using the relation

$$g_{em}(\nu) = \left[F(\lambda = c / \nu) / \nu^5 \right] / \int_0^\infty \left[F(\lambda = c / \nu) / \nu^5 \right] d\nu$$

where $1/\nu^5 = (1/\nu^2) \times (1/\nu^3)$, with $1/\nu^2$ proportional to the Jacobian in going from even wavelength intervals to even frequency intervals ($\delta \lambda \rightarrow \delta \nu$) and $1/\nu^3$ proportional to the factor relating Einstein $A$ (spontaneous emission) and $B$ (stimulated emission) coefficients\textsuperscript{42}. The absolute stimulated emission cross section was obtained from the normalized emission lineshape function $g_{em}(\nu)$ by using the relation,
\[ |\sigma_{e\ell}(\nu)| = \frac{\hbar \nu}{c} B_{e\ell} g_{em}(\nu) = \frac{\hbar \nu}{c} B_{ge} S_{em}(\nu) \]

where \( \sigma_{ge}(\nu') \) is the frequency dependent absorption cross section and the prime denotes a dummy frequency variable. Division and multiplication by \( \nu \) inside and outside the integral on the last line arises from the relationship between \( B \) and \( \sigma \) on the first line, which also holds for the absorption cross section, \( \sigma_{ge}(\nu) = (\hbar \nu / c) B_{ge} g_{abs}(\nu) \).

The approximation in Eq. (4.38) arises because the change in equilibrium geometry between ground and excited state may allow \( B_{ge} \neq B_{eg} \) for relaxed spectra; it is exact if the Condon approximation holds over the full range of both ground and excited state equilibrium coordinate distributions. The agreement between the experimental literature lifetime (4.28 ns) and that calculated from Strickler – Berg relation (3.97 ns) was within 10% error, suggesting the Condon approximation is valid on average.

Figure 4.1 shows the \( S_0 \rightarrow S_1 \) absorption and emission cross sections as a function of frequency. The emission cross section roughly mirrors the absorption cross section. The obvious difference in the maximum cross section reflects both the wider normalized emission lineshape (~20% wider than the absorption lineshape) and the proportionality of cross section to frequency. In Figure 4.1, the laser pulse spectrum is overlaid to give an idea of how it overlaps with the cross sections; the pump-probe signal calculation essentially involved the overlap of the pulse spectra and cross section.

4.3.3. Pump-probe experiment setup

The schematics of the femtosecond laser setup employed for the pump-probe experiment are shown in Figure 4.2. The femtosecond pulses were generated from a home-built noncollinear
Figure 4.1. Optically determined $S_0$–$S_1$ cross-sections for absorption, $\sigma_{\text{gs}}(\tilde{\nu})$ (thick solid line) and emission, $|\sigma_{\text{eg}}(\tilde{\nu})|$ (thin solid line) of fluorescein in methanol/KOH (~0.01N). The absolute pump pulse laser spectrum $\rho(\tilde{\nu})$ is overlaid (vertical axis at right). The laser pulse parameters are: pulse energy, $U = 13.5 \pm 0.80$ nJ; maximum pulse intensity at $\tilde{\nu}_{\text{max}} = 20023$ cm$^{-1}$ (499.4 nm $\lambda_{\text{max}}$); width of the pulse spectrum $\Delta\tilde{\nu}_{\text{FWHM}} = 430 \pm 26$ cm$^{-1}$ (10.7 nm $\Delta\lambda_{\text{FWHM}}$). The inset shows the molecular structure of fluorescein dianion.
Figure 4.2. Femtosecond pump-probe experiment. WP1, WP2, WP3, zero order half waveplates; BS1, BS2, 50:50 fused silica femtosecond laser beamsplitter for $p$-polarized light; CP1, CP2, anti-reflection coated fused silica compensating plates; TRF, trihedral retroreflector; P1, P2, calcite cube polarizers; L1, L2, $f=10$ cm plano-convex focusing lenses; S, sample flowcell; I, iris; PD, Si photodiode detector; VND, variable neutral density filter; D, translation stage for pump-probe time delay.
optical parametric amplifier (NOPA)\(^{44,45}\), pumped by an 80 fs, 800 nm, 7 µJ regenerative amplifier running at 10 kHz repetition rate (Coherent RegA 9050). The NOPA delivered horizontally polarized pulses having up to 200 nJ energy, tunable from 480 nm to 750 nm. NOPA pulses typically had \(~400\) cm\(^{-1}\) full width half maximum (FWHM), easily compressible below 40 fs using a pair of fused-silica prisms. The spectrum was shaped to nearly Gaussian by means of razor blades placed after the second compressor prism.

The polarization of the beam before splitting it to a strong pump and weak probe was rotated to vertical, using an achromatic zero-order quartz-MgF\(_2\) \(\lambda/2\) wave plate [0.52 retardance at 500 nm (Newport 10RP52-1)]. After the beamsplitter (FABS-550-45P-PW-1006-UV – this 50:50 beamsplitter designed for \(p\)-polarized femtosecond pulses at 550 nm wavelength generates unequal pulse energies for \(s\)-polarized pulses at 500 nm wavelength), the pump and probe beams travelled separate beam paths; the probe beam path length was variable via a computer controlled (Newport MM3000) mechanical translation stage (Newport MTM200PP.1) for up to 20 cm, allowing a pump – probe delay up to \(~1\) ns. The dispersions in both beams were matched using identical uncoated air-spaced glan prism calcite polarizers (Karl Lambrecht, MGTYE8, \(5 \times 10^{-6}\) extinction), metallic variable neutral density filters (Edmund Optics), and zero-order \(\lambda/2\) waveplates (Newport 10RP52-1). The energies of the pump and probe beams were independently varied using the variable neutral density filters. For polarization dependent pump-probe measurements, the pump polarization was varied using the pump zero-order \(\lambda/2\) waveplate; the waveplate was placed after the last mirror before the sample to preserve the pump polarization, which can be scrambled if reflection off a mirror is neither \(s\) nor \(p\). The probe polarizer was placed after the last mirror before the sample to make sure the probe polarization was vertical. The pump and probe polarizations were checked by using an analyzer polarizer.
placed behind the sample after the collimating lens to measure the extinction ratios. The extinction ratio was 121:1 when setting the pump or probe polarization to either parallel or perpendicular and rotating the analyzer to give either maximum or minimum transmission.

The pump and probe beams were focused into the sample by a 10 cm focal length BK7 glass lens, coated to prevent reflective loss in the visible range. The pump and probe beams were overlapped at the focus by maximizing their transmission through a 50 µm pinhole. The pump and probe beams were separated by 1.2 cm on the 10 cm focal length lens. At 500 nm wavelength, the beam crossing angle becomes 5° in the methanol sample, so the optical pathlength is a negligible 0.1% larger than the sample thickness. The interferometer alignment was checked by monitoring probe transmission through the pinhole over a 1 ns time delay; the change in transmitted probe energy varied less than 2%, indicating a good alignment over the entire probe delay range.

Once a good spatial overlap was found, the zero pump-probe delay was found by autocorrelation with 100 µm thick KDP cut at 40 °, which also allowed pulse duration measurements. After further optimization of dispersion compensation by changing the tip to tip prism distance and amount of prism insertion into the beam, ~38 fs pulse duration was obtained for a beam centered at 500 nm with ~12 nm FWHM (34 fs transform limited pulse duration). After crossing the pump and probe beams in the sample, the transmitted probe energy was measured by a silicon PIN photodiode with an active area of 4.6 mm diameter and a risetime of 30 ns (Electro-Optics Technology ET2040) (the pump beam was blocked by an iris as soon as it exited the sample). To reduce the scattered beams reaching the probe photodiode, several irises were placed along the probe path from sample to photodiode, the photodiode was placed in a blackened box, and stray lights were blocked at their sources.
For pump-probe transients (the transmitted probe photon number as a function of pump-probe delay) lock-in detection was used. The 500 Hz modulated probe signal from the photodiode was sent to a digital lock-in amplifier (Stanford Research SR830) referenced at the pump chopping frequency. The pump-induced change in transmitted probe photon number was detected by the lock-in amplifier and sent to the A/D converter (Stanford Research SR245). The lock-in time constant was set at 10 ms which was sufficient for smoothing fast fluctuating noise, mainly coming from particle scatter in the sample (no liquid filters were used in the sample flow system because they can collect molecules, changing the concentration). The sample cell was translated along the focus to maximize the pump – probe signal for 0.2 mM fluorescein, and fixed there for all measurements. Over the 0.2 mm sample pathlength, beams overlapped in the middle of the sample cell walk off by 9 µm at each end. For the 43 µm beam diameter, (see below) this reduces the pump – probe spatial overlap integral $H$, averaged over the sample length, by 2%, justifying neglect of spatial walk-off.

For fixed delay absolute signal strength measurements, the absolute voltages generated by the probe photodiode with the pump on ($V_{on}$) and off ($V_{off}$) were measured using a gated integrator (boxcar). Multiple sequences of pump–on and –off voltages were recorded by chopping the pump beam at 4 Hz. The output of the probe diode was amplified $5 \times$ by a Stanford Research SR445A preamplifier and sent to a gated integrator and boxcar (Stanford Research SR245). The boxcar was triggered by the laser amplifier signal from the RegA electronics. The boxcar integrated the photodiode output for each pulse over a 100 ns gate and the “last sample” output was read by a 13-bit resolution analog to digital converter through a GPIB interface. Measurement data files generated using the boxcar measurements were analyzed by a software algorithm to extract average $V_{on}$ and $V_{off}$. The difference in voltages $\Delta V = V_{on} - V_{off}$, divided by
the voltage with the pump off \( (V_{\text{off}}) \), was equal to \( \Delta P_r / P_{\text{off}} \) where \( P_{\text{off}} \) was the transmitted photon number with the pump off and \( \Delta P_r \) is the change in transmitted probe photon number caused by the pump [the pump – probe signal we are interested in, see Eq. (4.23)]. For both pump and probe, incident and transmitted photon numbers were determined with a power meter [Coherent FieldMaster GS display (1% accuracy) and LM-2Vis sensor (5% accuracy)] to within 6% error; thus \( \Delta P_r \) was determined with better than 6% error from the voltage measurements.

### 4.3.4. Determination of the laser spatial profile and spectra

The femtosecond pulses were characterized to determine their spatial and spectral profiles. The spatial profile was determined by imaging the focused beam on a CMOS based color web camera with its lens removed (ZoomCam 1598, 640×480 resolution; we were unable to obtain a pixel size specification). By imaging a 1.00±0.025 mm grid pattern (National Brand, engineering form graph paper # 12-188) attached to the surface of the CMOS censor, the average pixel spacing was determined in both dimensions to be 7.35 µm/pixel. The beam images were corrected for saturation using the measured detector saturation function vs. beam intensity. For the beam focused with 20 cm lens, the spatial profile fitted a 2-D Gaussian of the form

\[
h(X,Y) = A \exp[-\{(X^2 / w_X^2) + (Y^2 / w_Y^2)\}]\]

where \( A \) is a constant, \( X \) and \( Y \) are the Cartesian coordinate distances from beam center and \( w_{x,y} \) the 1/e half widths along two orthogonal axes (not necessarily aligned with the CMOS array axes). The 2D profile was nearly circular with \( w_x : w_y \) ratio ~0.9. The resolution of the camera was inadequate to image the beam focused by the 10 cm lens. For the beam focused with the 10 cm lens, the beam diameter (defined as that of the circular aperture giving 50% transmission) was determined by energy transmission through 25, 50, and 75 µm diameter pinholes. The diameters estimated with different pinhole sizes all
agreed within 6% of 43 µm. This diameter was within 4% of the diffraction limited focal spot size for a Gaussian beam. The Rayleigh range, over which focused Gaussian beams increase by $2^{1/2}$ in size, is 8 mm, justifying the approximation of collimated Gaussian beams for the 0.2 mm sample pathlength.

The pulse spectra as a function of wavelength were determined using a grating spectrometer with a linear silicon CCD array detector (Ocean Optics, USB4000-UV-VIS). The beam was diffused by passing through a few layers of tissue and guided into the spectrometer via multimode optical fiber (Ocean Optics P300-1-SR). The spectrometer measured photons per unit wavelength. The calibrated pulse spectra corresponding to photons per unit frequency [\(\rho(\nu)\)], were obtained by: 1) multiplying the corresponding frequency scale spectra by \(1/\nu^2\), the Jacobian in going from wavelength to frequency intervals; 2) area normalizing the frequency spectra from step 1; and 3) multiplying the area normalized frequency spectrum by the total number of photons in the pulse \(U/\hbar\nu_{500nm}\) [where \(U\) is the pulse energy determined with the silicon photodiode power meter (Coherent FieldMaster LM-2Vis), set on the energy calibration for 500 nm, by measuring average power and dividing by the laser repetition rate]. The pump and probe spectra were identical within <0.1% error and were fitted to a Gaussian of the form \(\rho'(\tilde{\nu}) = \rho_{\max} \exp[-(\tilde{\nu} - \tilde{\nu}_0)^2 / \Delta^2]\), where \(\rho_{\max}\) is the maximum number of photons per unit frequency interval, \(\tilde{\nu}_0\) is the frequency maximum in cm\(^{-1}\), and \(\Delta\) is half the 1/e width (=FWHM/2\(\sqrt{\ln(2)}\)) in cm\(^{-1}\) (the prime serves to denote \(\rho'\) as a fit function that differs from the actual spectrum). Area normalizing the Gaussian, \(\rho_{\max} = U / (\hbar\nu_{500nm}\sqrt{\Delta^2\pi})\). From the fit, \(\tilde{\nu}_0 = 19991 \pm 2\) cm\(^{-1}\) and \(\Delta = 257 \pm 0.5\) cm\(^{-1}\). [Although determined from the same spectrum, \(\tilde{\nu}_0 \neq \tilde{\nu}_{\max}\) and \(\Delta \neq 2\sqrt{\ln(2)}\Delta\tilde{\nu}_{FWHM}\) in Fig. 4.1 because \(\tilde{\nu}_{\max}\) and \(\Delta\tilde{\nu}_{FWHM}\) are determined directly from \(\rho(\tilde{\nu})\);
similarly, $\lambda_{\text{max}}$ and $\Delta\lambda_{\text{FWHM}}$ are determined from $\rho(\lambda)$, so $\tilde{\nu}_{\text{max}} \neq 1/\lambda_{\text{max}}$ because the wavelength interval contained in a constant frequency interval depends on wavelength (as reflected in the Jacobian).] The frequency dependent photon distribution function in terms of the pulse energy, center frequency and the width is

$$\rho'(\tilde{\nu}) = U / (h\nu_{500\text{nm}}\sqrt{\Delta^2\pi}) \exp[-(\tilde{\nu} - \tilde{\nu}_0)^2 / \Delta^2].$$

Stable operation of the femtosecond laser (typically, pulse to pulse energy fluctuation was ~1%) was critical to maintaining uniform spatial and spectral pulse profiles over the course of measurements and was, in turn, necessary to achieve a good signal to noise ratio.

4.3.5. Beer’s law check

Beer’s law requires linear pulse propagation that is explicitly assumed in the calculation. Energetic femtosecond pulses that generate very high peak powers with low average powers can cause a breakdown of Beer’s law.\textsuperscript{20} Sufficiently weak femtosecond pulses may propagate linearly, with attenuation governed by Beer’s law (and phase shift governed by the refractive index).\textsuperscript{46} To check that Beer’s law was obeyed for the femtosecond laser pulses used in the experiments, we measured the transmitted pulse energy as a function of incident pulse energy and compared the measurements to calculations using the laser pulse spectrum and absorption spectrum. The measured fraction of photons absorbed, $0.94\pm0.02$, matched well with the calculated fraction of $0.93\pm0.01$ using the following independently measured experimental parameters: 0.81 mM fluorescein in a 0.200 mm thick flow cell (peak absorbance of 1.28 at 496 nm); 0.3 nJ to 13.5 nJ incident laser pulse energy (0.2% to 8.8% excitation probability when averaged over the 43 µm diameter Gaussian beam and the 0.2 mm sample pathlength); $\tilde{\nu}_{\text{max}} = 20023 \text{ cm}^{-1}$; $\Delta\tilde{\nu}_{\text{FWHM}} = 430 \text{ cm}^{-1}$; 38 fs pulse duration. Reflective losses from the front and back
surfaces (~ 8% in total) were accounted for by measuring transmitted power through the flow cell with a methanol blank solution.

4.3.6. The pump-probe signal linearity check

The linearity of the pump-probe signal with respect to the pump pulse energy was checked by power dependence measurements at two fixed pump-probe delays; one at \( T = 100 \, \text{fs} \) when the pulse overlap was over and one at \( T = 100 \, \text{ps} \) when all vibrational coherence had decayed. The experimental parameters were: 0.298 ± 0.004 mM fluorescein in a 0.20 mm thick flow cell; \( \bar{v}_{\text{max}} = 20023 \, \text{cm}^{-1} \); \( \Delta \nu_{\text{FWHM}} = 430 \, \text{cm}^{-1} \); 38 fs pulse duration. The pump pulse energy was varied from 1 nJ to 8 nJ in 0.5 nJ steps; the weighted average of the excitation probability ranged from 1% to 8.9%. After carefully establishing the zero signal level, the pump-probe signal vs. pulse energy fitted a linear function \( S = c U_u + d \), where \( c \) is a proportionality constant, \( U_u \) is the pump pulse energy and \( d \) an arbitrary constant, with reduced \( \chi^2 = 1.16 \). The fit parameters were: \( c = 0.0510 \pm 0.0004 \) (at 100 fs) and 0.0384 ± 0.0002 (at 100 ps) and \( d = -0.02 \pm 0.02 \) (at 100 fs) and \( -0.014 \pm 0.015 \) (at 100 ps) for signals \( S \) ranging from 0 to 0.2. Since intercepts were poorly determined (errors are bigger than the intercepts), the fit constrained them to zero, which did not significantly increase the reduced \( \chi^2 \). A quadratic coefficient also did not improve the fit and was zero within error. The slopes are determined to within 1% error, indicating near perfect linearity for pump pulse energies varying nearly one order of magnitude.

4.4 Results

First, the pump-probe measurements as a function of pump-probe delay at parallel, perpendicular and magic angle pump-probe polarizations are presented. The time dependent kinetics is described and compared to the literature. Next, we show the calculation compared to
measurements. Two calculations are discussed; one for the time dependence of the pump-probe signal with magic angle polarization and another for the concentration dependence of the pump-probe signals at a fixed pump-probe delay (long after vibrational and rotational coherences have decayed).

4.4.1 Pump-probe transients

Figure 4.3 shows the time dependent pump-probe signals measured at parallel, perpendicular and magic angle polarization. Overlaid is the magic angle signal reconstructed from the parallel and perpendicular signals and fits. The parallel, perpendicular and magic angle pump–probe signals were globally fitted to a model function based on Eq.(4.39),

\[ S_{pp}(f, T) = S_{iso}(T)[1 + fr(T)] \] (4.39)

where \( S_{iso}(T) \) described the isotropic dynamics (ideally, the magic angle signal), \( r(T) \) is the time dependent anisotropy, and the index \( f \) quantifies the relative angle between pump and probe polarization [\( f \) is defined between Eq.(4.12) and (4.13); \( f = 2 \) for parallel, 0 for magic angle, and -1 for perpendicular]. The isotropic dynamics is modeled by a sum of three exponentials [Eq.(4.40)],

\[ S_{iso}(T) = A_1 \exp(-T / \tau_1) + A_2 \exp(-T / \tau_2) + A_3 \exp(-T / \tau_3) \] (4.40)

with two exponentials describing dynamics faster than 20 ps and one exponential describing nanosecond dynamics. The anisotropy is modeled by a sum of a Gaussian and an exponential [Eq.(4.41)],

\[ r(T) = A_G \exp[-(T / \tau_G)^2] + A_{aniso} \exp(-T / \tau_{aniso}) \] (4.41)
Figure 4.3. Measured absolute fluorescein pump-probe signal as a function of pump-probe delay for parallel (black), perpendicular (red) and magic angle (blue) pump-probe polarization. The vertical axis is the change in transmitted probe photon number. The magic angle signal constructed from the parallel and perpendicular signal $MA = (PA + 2PE)/3$ is overlaid (cyan). Inset: Same plot for the first 5 ps. Fluorescein concentration in 0.01N KOH, 0.298 ± 0.004 mM; pump pulse energy $U_u = 3.12 ± 0.19$ nJ; probe pulse energy $U_r = 0.506 ± 0.03$ nJ; beam diameter 43.5 ± 2.6 µm (50% transmission through a 43.5 µm diameter pinhole); maximum pulse intensity at $\tilde{\nu}_{max} = 20023$ cm$^{-1}$; width of pulse spectrum $\Delta\tilde{\nu}_{FWHM} = 430 ± 26$ cm$^{-1}$; 38 fs pulse duration. The probe transmission with the pump off was 0.33 ± 0.02. The total incident probe photon number was $(1.27 ± 0.08) \times 10^9$. 
Table 4.1. Global fit parameters for fluorescein dianion in basic methanol recovered from parallel, perpendicular and magic angle pump–probe experiments. The fit function was 
\[ A_1 \exp(-t / \tau_1) + A_2 \exp(-t / \tau_2) + A_3 \exp(-t / \tau_3) \cdot [1 + f \cdot A_G \exp(-(t / \tau_G)^2) + A_{aniso} \exp(-(t / \tau_{aniso})^2)] \]

\( f \) was set at 2 for parallel and -1 for perpendicular but was allowed to float for magic angle (the best fit value was \( f = 0.04 \) when ideally \( f = 0 \); the best fit \( f \) corresponds to a polarizer set at 53.9°, a 0.8° deviation from the 54.7° magic angle). The nominal 1σ error bars correspond to a unit increase in \( \chi^2 \). *The error bars for \( A_3 \) and \( \tau_3 \) increase to ±0.33 ns, respectively, when an overall signal offset is allowed to float with the ±0.003 uncertainty of the zero baseline established for signal at negative \( T \).
with the Gaussian describing inertial rotational dynamics\textsuperscript{47} and the single exponential describing rotational diffusion.\textsuperscript{5} The results of the global fit are summarized in Table 4.1. The 331±22 fs component, which accounts for ~17% of the total amplitude, coincides reasonably with methanol polar solvation; literature timescales are 280 fs\textsuperscript{48} and 340 fs.\textsuperscript{42} The 9.54±0.33 ps component with ~13% of the total amplitude is roughly intermediate between the two components at 3.20 and 15.3 ps that Horng et al.\textsuperscript{48} report in studies of methanol solvation dynamics using a coumarin dye (fluorescein contains much of the coumarin structure); the two components have amplitudes approximately equal to each other in their study. It is possible that the fitting routine used here assigned a single “averaged” component that might separate into two with higher signal to noise. Vibrational relaxation may also occur on 10 – 100 ps timescales. We attribute the 3.58±0.33 ns component, which accounts for ~70% of the total amplitude, to the fluorescein fluorescence lifetime. This time constant is ~17% smaller than the literature value (4.28±0.07 ns)\textsuperscript{43}, but the measurement here had a 1 ns maximum pump-probe delay and so is unlikely to accurately capture a ~ 4 ns lifetime. A shorter lifetime could also arise from fluorescence quenching (for example, by dissolved oxygen\textsuperscript{49}) in the measurements reported here. The 137±1.5 ps anisotropy decay component is in good agreement with the 140 ps literature value.\textsuperscript{50} The experimental initial anisotropy, \(r(0) = 0.396±0.003\) is close to the expected value of 2/5; this agreement suggests that saturation (which reduces the initial anisotropy in pump-probe experiments\textsuperscript{13}) is negligible (the statistical error bar does not account for systematic error from the polarization extinction ratio of 121:1). The Gaussian inertial component was necessary to accurately capture the first 2 ps data; without it, there was a systematic under-estimation in the fit. The 2.7±0.5 ps inertial component, which accounts for ~4\% of the total anisotropy decay amplitude, is longer than that reported in the ultrafast dichroism study of anthracene (~400 fs).\textsuperscript{47} Although the
Figure 4.4. Magic angle change in transmitted probe photon number as a function of pump-probe delay. Inset: The same plot for the first 20 ps. The experimental measurement (red) is compared to a calculation without any adjustable parameters (black). The error in the calculation is indicated assuming ±6% variation in the beam diameter, ±6% variation in the pump and probe pulse energies. Fluorescein concentration in 0.01N KOH, 0.298±0.004 mM; pump pulse energy $U_u = 3.12\pm0.19$ nJ; probe pulse energy $U_r = 0.506\pm0.03$ nJ; beam diameter 43.5±2.6 µm (50% transmission through a 43.5 µm diameter pinhole); maximum pulse intensity at $v_{max} = 20023$ cm$^{-1}$; width of the pulse spectrum $\Delta v_{FWHM} = 430\pm26$ cm$^{-1}$; pulse duration 38 fs. The probe transmission with the pump off was 0.33±0.02. The total incident probe photon number was $(1.27\pm0.08)\times10^9$. 
Gaussian component alleviated the misfit in the first 2 ps of the anisotropy, the signal to noise ratio may not be sufficient to pin down the timescale.

**4.4.2 Comparison to calculation** Figure 4.4 shows the calculated [using Eq.(4.33)] and measured time dependent pump-probe signal at magic angle pump-probe polarization. The calculation agreed with experiment within 10% error. The spatial profiles were modeled by a circular 2D Gaussian; 

$$h'(X,Y) = 1/(\pi w^2) \exp[-(X^2 + Y^2)/w^2]$$ (the prime is to denote it’s a fit function) where \((X^2 + Y^2)^{1/2}\) is the radius and \(w\) is the 1/e half width (the 50% transmission beam diameter is \(2w(\ln(2))^{1/2}\)). Assuming the pump and probe beam to be identical and have perfect spatial overlap (these assumptions were justified by the measurements), 

$$H = 1/(2w^2\pi).$$

In general, \(H\) can be evaluated numerically without any assumption using the measured \(h(X,Y)\).

The pump pulse spectra \(\rho_u(\nu_u)\) and probe pulse spectra \(\rho_r(\nu_r)\) at the sample spot were obtained by the method detailed in the Experimental Section. Raw spectra (not the Gaussian fit) were used in the calculation. However, the previously defined fit functions \(h'(X,Y)\) and \(\rho'(\nu)\) were useful because the relationship between the pulse parameters and the strength of the pump-probe signal was explicitly revealed; assuming spatially and spectrally identical pump and probe pulses, the pump-probe signal is proportional to the factors, \((1/2w^2\pi) \cdot (1/\Delta^2\pi)\) where \(w\) is half the 1/e beam diameter and \(\Delta\) is half the 1/e spectral width (the first factor arises from the normalization constant for the spatial Gaussian fit function and the second factor is the square of the normalization constant for the spectral Gaussian fit function). The polarization index \(f\), was set to 0 for magic angle pump-probe polarization. The parallel and perpendicular signals were calculated using the literature \(^{50}\) anisotropy function \(r(T)\) which was assumed to be the same for the excited and ground state. The calculation agreed with measurements within \(\sim 10\%\) error (see
Figure 4.4). The time dependent dynamics was solely dictated by the lifetime function for which the lifetime constant was set to the literature result, $4.28 \pm 0.07$ ns$^{43}$. The inset in Figure 4.4 shows the first 20 ps of the same plot. The calculation deviated from the measurement by more than 10% for pump-probe delay below 20 ps. This discrepancy was expected from the fit results of the experimental data that showed a few hundreds femtoseconds to picoseconds dynamics; the calculations did not include solvation or vibrational wavepacket dynamics [which can be incorporated through correlation in the cross section $\sigma(v_r, T, v_e)$]. This comparison established that, for delays of less than ~20 ps, the approach taken to calculate the pump-probe signal is inadequate and a treatment based on the third order nonlinear susceptibility or response function is needed.

Figure 4.5 shows calculated and measured concentration dependent pump-probe signal with the parallel pump-probe polarization at 600 ps pump-probe delay. The rotational relaxation was over by 600 ps so the signals were independent of pump-probe polarization; the signals from all three polarizations converged to the same value within 3% error for 0.20 and 0.56 mM concentrations. The measured signals fell within ~10% of the calculation for the range of concentrations measured (from 0.03 to 0.56 mM). This concentration range contains both the rise and fall of the signal around its maximum.

4.5 Discussion

The good agreement between the calculations and measurements demonstrates that the required set of theoretical assumptions and experimental conditions are sufficiently met for fluorescein. All calculated population changes in the ground and excited states are accounted for by the measurement within error. Experimental requirements for this agreement are uniform
pump–probe spatial overlap throughout the sample, determination of the beam diameter and spectrum width to within 5% and keeping the laser noise to less than 1%, in order to keep the

**Figure 4.5.** Measured (solid circle) and calculated (× and line) pump–probe signal as a function of concentration. Discrete calculations (× and error bars) used the experimental parameters of the individual measured signals. The thick continuous line is a calculation using the average pump and probe pulse energies for the entire series of measurements. Thin grey lines indicate calculated upper and lower bounds on the calculated signals using the average pulse energies for the series. Vertical bars just to the left of data points indicate lower bounds on concentration. Parallel polarization; pump-probe delay $T = 600$ ps; pump pulse energy $U_p$ ranged from 4.55 to 4.61 nJ with ~6% systematic error; probe pulse energy $U_r$ ranged from 0.464 to 0.480 nJ with ~6% systematic error; beam diameter 43.5 ± 2.6 µm (50% transmission through a 43.5 µm diameter pinhole); maximum pulse intensity at $\nu_{max}$ = 20023 cm$^{-1}$, width of the pulse spectrum $\Delta \nu_{FWHM} = 430 \pm 26$ cm$^{-1}$; 38 fs pulse duration.
error in the calculation less than \( \sim 10\% \); the good agreement allows for quantitative physical interpretation (see below). \( \varepsilon(\lambda) \) and \( F(\lambda) \) can be determined with better than 1\% precision but are prone to systematic errors with too high concentration or poorly determined sample path length; we have observed distortion of the absorption shape at \( \text{OD} \sim 1.5 \) and the peak of Figure 4.5 shift by 10\% for 7.5\% error in the sample length. Checks for linear pulse propagation and linear pump pulse energy dependence of the pump-probe signal are important to establish that data are free of high order effects that are not included in the theoretical treatment; in our experiments, measurements confirmed the pulse propagation and signal linearity. In femtosecond measurements, low excitation probabilities are necessary. However, for thick samples or higher concentrations, even lower excitation probabilities may be required to ensure linearity against cascades. The calculation of excitation probabilities as a function of the sample depth for the pulses used in the experiments shows that the local excitation probability is a steeply varying function of depth; for \( C = 0.298 \text{ mM}, U_u = 3.12 \text{ nJ}, \) and \( w = 26.1 \mu\text{m} \) (50\% transmission through a 43.5 \( \mu\text{m} \) diameter pinhole), the local excitation probabilities in the center of Gaussian beam where the energy is highest were \( \sim 12\% \) at the front and \( \sim 4\% \) at the back of the sample, although the average excitation probability over the sample depth and transverse profile is \( \sim 6\% \). With a polarized beam, for molecules aligned parallel to the laser polarization, the excitation probability is three times higher (36\% in the “worst case scenario” at sample front and beam center). Thicker samples or higher sample concentrations run the risk of cumulative effects that manifest as a parallel cascade; this effect corresponds to the quadratic and higher order terms in the expansion of the outer exponential of Eq. (4.22), which are neglected in Eq. (4.24).
Experimentally significant cumulative effects are not detected in this data; the calculations suggest that quadratic terms contribute less than 3% to the total signal.

The calculation and measurement are not simple, and their agreement for fluorescein suggests that both are correct within the limits stated. In experiments carried out before the fluorescein measurements, measurement and calculation did not agree for three structurally related tricarbocyanine dyes (IR144, IR125, and HDITCP), even though they are well known to behave as electronic two-level systems near 800 nm\(^1\) and the dynamics on femtosecond and picosecond timescales have been attributed to solvation and vibrational relaxation in several studies.\(^{42,52-54}\) The preliminary measurements of the absolute pump-probe signal on these tricarbocyanines are consistent with relaxation channels that drain up to half of the excited state population within a few hundred femtoseconds. Mechanisms for fast population relaxation have been described for smaller symmetrical cyanines.\(^{55-57}\) The presence of fast population decay channels may partially explain why the reported 2% fluorescence quantum yield for IR 144 in methanol\(^58\) is significantly lower than the \(~8\)% quantum yield that would be deduced from the slowest exponential decay of 445 ps (previously interpreted as the lifetime)\(^{42,58}\) and the radiative lifetime of \(~5.8\) ns that we calculate from the Strickler–Berg relation.\(^{41}\) Evidently, some of the femtosecond and picosecond signal decay previously attributed to solvation of these tricarbocyanines arises from vibrational or solvent coordinate dependent ultrafast excited state population decay, and their non-exponential lifetime function \(L_e(T)\) must be determined by comparing calculated and measured absolute pump-probe signal strengths. To enable a test without unknown parameters, fluorescein was chosen for subsequent experiments because the fluorescence quantum yield is almost one,\(^{38,43}\) the emission lifetime obeys the Strickler-Berg relation,\(^{41}\) and the absorption spectrum of the first excited singlet state\(^{59}\) does not overlap the
pulse spectra. The pump-probe method demonstrated here may also determine the excited state absorption cross-section if the lifetime function is known.

While the agreement in fluorescein is good for pump-probe delays greater than 20 ps, matching the measured and calculated signals at earlier times requires either the extension to time dependent cross sections [2D spectroscopy seems to naturally measure the products $\sigma_{ji}(\nu_a)c_{ik}(T,\nu_a,\nu_e)\sigma_{ik}(\nu_e,T,\nu_a)$] or, more generally, use of the third order nonlinear susceptibilities. The approach presented here can anchor the absolute signal for these more general approaches at large pump–probe delays. Meanwhile, the current approach can be improved in at least 3 ways: 1) an absolute radiative rate can be used to determine the emission cross section\(^{60}\) removing the need for the Condon approximation\(^{61}\); 2) the measured pump-probe signal can be spectrally dispersed to obtain transient spectra that are calibrated in absolute photon number (combined with global analysis, this may be sufficient for many multilevel systems); 3) the two beam pump-probe experiment for measuring the spectrally resolved pump-probe transients can be converted to a “HARD 2D”\(^{62}\) experiment to obtain 2D spectra with amplitude calibrated in absolute photon number (this should enable determination of excitation energy dependent lifetimes).

### 4.6 Conclusions

The measured and calculated absolute pump-probe signal strengths of fluorescein dianion in basic methanol match within 10% error. The calculations use the decadic molar extinction coefficient, the relative fluorescence spectrum, the pulse spectrum and the pulse transverse profile from measurements; the expression assumes a vibrationally relaxed electronic two-level system, unity fluorescence quantum yield, and the Condon approximation and can be applied to
calculate signal strength under linear pulse propagation and weak pump conditions. The formula can be applied to polarized experiments because the effect of rotational anisotropy is explicitly included. The absolute measurement of the nonlinear signal presented in this paper provides a way to determine the excited state absorption cross section or excited state quantum yields (and can determine both if a broadband probe is spectrally resolved).

4.7 Appendix 4A

When the pump and probe can be regarded as collinear, the populations excited by the pump have an exponential depth dependence for every transverse coordinate, and Eq.(4.8) is readily solved analytically. If \( p_u(R, \nu_u) \big|_{z=0} \) is the density of incident pump photons per unit area per unit frequency, then

\[
\Delta p_u(R, \nu_u) = p_u(R, \nu_u) \big|_{z=0} \left[ \exp[-\alpha^0(\nu_u)Z] - 1 \right]
\]

(4A.42)

is the accumulated (integrated from 0 to \( Z \)) change in pump photon fluence (a negative number), which is equal in magnitude and opposite in sign to the accumulated density of excited molecules left in the wake of the pump at \( T = 0 \). The partial derivative of \(-\Delta p_u\) with respect to \( Z \) is the number of molecules excited by the pump per unit volume per unit pump frequency.

\[
\Delta n(R, \nu_u) = -\left( \frac{\partial \Delta p_u}{\partial z} \right) = p_u(R, \nu_u) \big|_{z=0} \alpha^0(\nu_u) \exp[-\alpha^0(\nu_u)Z]
\]

(4A.43)

The change in number density in each excited sub-level \( j \) may be calculated by recognizing that each absorption channel is independently absorbing photons at a rate proportional to \( nB \), where \( n \) is number density and \( B \) is the Einstein \( B \) coefficient. For stimulated emission, the photon absorption rate is negative. The net sum of absorption and stimulated
emission gives the net depletion of photons (as in the attenuation coefficient of Eq.(4.2)) and the corresponding net excitation of molecules. Thus the fraction transferred to level $j$ from level $i$ through either absorption or stimulated emission is

$$ n_i^0 |\sigma_{ij}(\nu)| \sum_{k,l} n_k^0 \sigma_{kl}(\nu) = n_i^0 |\sigma_{ij}(\nu)| / \alpha^0(\nu) \quad (4A.44) $$

The absolute value of the cross section arises because, regardless of whether photons are absorbed (positive cross section) or emitted (negative cross section), molecules are transferred to the final state of the transition. For electronic transitions, the initial excited state molecular number density $n_j^0$ is usually zero so that the pump does not stimulate emission. Multiplying Eq. (4A.43) by Eq.(4A.44), the change in number density in level $j \neq i$, caused by transitions from $i$ to $j$ through pump fluence $p_u(R,\nu_u)$ per unit frequency is

$$ \Delta n_j(R,\nu_u) = p_u(R,\nu_u)|_{\nu=0} n_i^0 |\sigma_{ij}(\nu_u)| \
\cdot \exp[-\alpha^0(\nu_u)Z] \quad (4A.45) $$

By conservation of the molecular number density, the change in the number of molecules in the initial level $i$ caused by transitions from $i$ to $j$ is $-\Delta n_j(R,\nu_u)$. If there is thermal population in an upper level excited by the pump, both that upper level and the lower level of that transition will experience partially opposing changes in number density.

The treatment is now extended to incorporate the angular and spatial distribution of the change in population on level $j$, which will be specified by, $\Delta n_j(R,\Theta,\nu_u)$, where $R$ specifies the molecular spatial coordinates in the laboratory frame, $\Theta$ specifies the Euler angles for the molecular axes in the laboratory frame, and $\nu_u$ specifies the pump frequency ($u$ is the first letter
of “pump” that does not also appear in “probe”). Integration over the angular distribution recovers the total number density deduced from pump pulse photon number depletion:

$$\Delta n_{ij}(\mathbf{R}, \nu_u) = \int \Delta n_{ij}(\mathbf{R}, \Theta, \nu_u) d\Theta$$

(4A.46)

$\Delta n_{ij}(\mathbf{R}, \Theta, \nu_u)$ is integrated over the sub-levels of $j$ excited at $\nu_u$. The angular distribution of excited molecules is initially aligned to the pump laser polarization. As a result, the probe propagates in a transiently dichroic and birefringent sample. For a linearly polarized pump and a dipolar pump transition, the transient electric susceptibility is like that of a uniaxial crystal\textsuperscript{64}, with one principal axis parallel to the pump polarization and two equivalent principal axes perpendicular. Therefore, probe pulses polarized parallel and perpendicular to the pump propagate without change of polarization. Pulses polarized at all other angles should be decomposed into fields along two principal axes, which are propagated separately in amplitude and phase. In such circumstances a linearly polarized probe can become elliptically polarized.

To evaluate the change in absorption parallel and perpendicular to the pump polarization axis, the angular coordinates can be integrated over both the pump and probe interactions, leaving a polarization index $f$ and an anisotropy that depends on the initial ($i$), intermediate ($j, k$) and final ($l$) states. The intermediate states $k$ include the initial state of the pump transition, $i$ (which is depleted), the initially excited state, $j$ (which is populated), and all states connected to either (or both) of these states by relaxation during the time delay $T$ between pump and probe (which may be either populated or depleted). Defining $\Delta n_{ijk}$ as the change in number density of state $k$ caused by the pump transition from $i$ to $j$, $\Delta n_{ijk}$ may be calculated from $\Delta n_{ij}$ if the relaxation kinetics are known. For linearly polarized pulses and dipole transitions, the effective change
(caused by the pump transition from $i$ to $j$) in the number density of absorbers for the transition from intermediate state $k$ to final state $l$ is

$$
\Delta n_{ijkl}(R, f, \nu_u, T) = \Delta n_{ijk}(R, \nu_u, T)[1 + f r_{ijkl}(T)] ,
$$

(4A.47)

where $r_{ijkl}(T)$ is the anisotropy for excitation from level $i$ to level $j$ (pump excitation) and transition between the levels $k$ to $l$ (probe transition), $f$ is determined by the relative pump and probe polarization ($f = 2$ for parallel and $f = -1$ for perpendicular), and $T$ specifies the pump-probe delay. The total change in attenuation coefficient is given by the sum

$$
\Delta \alpha(R, \nu_r, f, T) = \sum_{i,j,k,l} \left[ \int_0^\infty \Delta n_{ijkl}(R, f, T, \nu_u) \sigma_{kl}(\nu_r, \nu_u) d\nu_u \right],
$$

(4A.48)

where the indices run over the levels.

The treatment here assumes that probe transitions from $k$ can be described by absorption and stimulated emission cross sections $\sigma_{kl}(T, \nu_u, \nu_r)$ that depend on the final state $l$, the pump-probe delay, the pump frequency, and the probe frequency ($\nu_r$). This allows inclusion of non-equilibrium correlation between the pumped and probed sub-level populations. In the bi-linear pulse propagation limit considered here, the signals from more than one starting state are additive.

**References**


(39) Assuming uniform flow, this corresponds to a linear velocity of 1.56 m/s in the 0.200 mm x 5 mm rectangular cell. Approximating the rectangular cross-section with an ellipse, laminar flow calculations yield an average flow velocity of 2.67 m/s. The slowest
10% molecules (near the boundary of the flow cell) yield an average velocity of 0.40 m/s (40 microns in 100 microsecond).


CHAPTER 5

SIMULTANEOUS ALL – OPTICAL DETERMINATION OF MOLECULAR CONCENTRATION AND EXTINCTION COEFFICIENT

The contents of this chapter are adapted from the paper titled “Simultaneous all – optical determination of molecular concentration and extinction coefficient” published in May 2013 in the Journal of Analytical Chemistry. This chapter extends the theoretical and experimental framework developed in Chapter 4 to simultaneously determine absolute molecular number concentration and extinction coefficient using linear and nonlinear spectroscopic measurements. This method is based on measurements of absolute femtosecond pump probe signals. Accounting for pulse propagation, a closed form expression is presented for molecular number concentration in terms of absorbance, fluorescence, absolute pump probe signal, and laser pulse parameters (pulse energy, spectrum, and spatial intensity profile); all quantities are measured optically. As in gravimetric and coulometric determinations of concentration, no standard samples are needed for calibration. The extinction coefficient can then be determined from the absorbance spectrum and the concentration. For fluorescein in basic methanol, the optically determined molar concentrations and extinction coefficients match gravimetric determinations to within 10% for concentrations from 0.032 to 0.540 mM, corresponding to absorbance from 0.06 to 1. In principle, this photonumeric method is extensible to transient chemical species for which other methods are not available.

5.1 Introduction

Absolute quantification of molecular concentration is critical in many chemical and biochemical analyses\textsuperscript{1-4}. Often, Beer’s law is used; absorbance determines molar concentration if molar extinction coefficient and the sample length are known (spectrophotometry).\textsuperscript{5-8} This
optical method is convenient because measurements are done *in situ*, but the method requires a known molar extinction coefficient. Measurements of molecular molar extinction coefficients rely upon a known concentration; most ultimately depend on measurements of mass, volume, and molecular mass (gravimetric and electrogravimetric methods), charge and volume (coulometric methods), or gas pressure and temperature for some sample of known composition. Indirect measurements of the extinction coefficient for photochemical intermediates rely upon known molar extinction coefficients for reactants (the method of ground state depletion and its multi-component extensions), known concentrations for reactants (the method of total depletion), actinometry (the measurement of photon numbers) with known quantum yields (ultimately resting upon a known molar extinction coefficient), or stoichiometric comparison to a co-product with a known molar extinction coefficient. In some cases, quantum yields are inferred from a kinetic model (e.g. ref. and used to estimate extinction coefficients with accuracy dependent upon that of the model. An optically based method that avoids isolation and physical handling of the chemical species for absolute quantification of concentration would be a powerful mechanistic and analytical tool.

For isolated atoms in the gas-phase, Einstein’s relations between absorption and spontaneous emission have been used to determine absorption cross-sections from radiative lifetimes since the 1920s. Even for atoms, the approach is only valid when the upper level emits to only one lower level. However, once such a case is found, it can be used to determine concentration, and thus other absorption cross-sections. For molecules, which usually change geometry upon electronic excitation, Einstein’s relation between absorption and spontaneous emission holds instantaneously, before the geometry change, but may not hold between steady state absorption and steady state emission; this severely limits the applicability of Einstein’s
absorption-spontaneous emission relationship to molecules. Time-resolved emission spectroscopy is sometimes considered a nonlinear optical measurement, suggesting other, fundamentally related, approaches may become practical as nonlinear optical measurements find applications in analytical chemistry.

Germann and Rakestraw showed that by combining linear and nonlinear optical measurements, one could, in principle, determine the absolute concentration of transient gaseous HCl in a combustion reaction. The method is based on the different functional dependencies of linear and nonlinear optical signals on concentration and transition dipole moment (the extinction coefficient depends on transition dipole moment and lineshape). Thus, in principle, the two independent measurements allow simultaneous determination of the two unknowns. In practice, however, difficulties have always necessitated introduction of an in situ calibrant with a known concentration and transition dipole moment (gaseous NO in ref. ), and the absolute nonlinear optical signal has not been measured. The internal calibrant approach has recently been exploited to determine concentration and extinction coefficient using two-dimensional infrared spectroscopy as the nonlinear measurement.

In this paper, we present a method which is also based on the idea of combining the linear and nonlinear optical techniques, but differs from and builds upon prior work in the following critical aspects: 1) no internal calibrant is needed; 2) weak pump and probe pulses are used so that each input field acts and propagates linearly in generating the nonlinear signal; 3) attenuation of pulses as they propagate through the sample is accounted for; 4) polarization effects are explicitly incorporated through measurement of the anisotropy ; 5) the effect of changes in molecular geometry and environment upon electronic excitation are included through the steady state fluorescence spectrum, ; 6) the stimulated emission cross-section is
determined from an Einstein relation; and 7) the absolute nonlinear signal is measured along with
the beam parameters it depends on. Specifically, we utilize the absolutely calibrated femtosecond
pump probe signal, $S_{pp}(f, T)$ (as nonlinear optical signal) and steady state absorbance spectrum
$A(\nu)$ (as linear optical signal).

5.2 Theory

The pump – probe signal is defined here as the change in the transmitted probe photon
number caused by the pump excitation. After the pump pulse and subsequent vibrational
relaxation, Einstein’s analysis of the kinetics of absorption and stimulated emission (Einstein $B$
coefficients) is valid for weak pulses (low excitation probability). The linear propagation of the
pump through the equilibrium sample causes changes in level populations that depend on the
transverse beam profile and decrease exponentially with depth. When these altered level
populations are included in calculating the linear propagation of the probe, the pump – probe
signal may be expressed in terms of cross sections in the following way\textsuperscript{44} [assuming no excited
state absorption and no “spatial chirp” (i.e., the input pulse spectra do not depend on the
transverse spatial coordinates $X$ and $Y$)]:

$$S_{pp}(f, T) = H\Delta P_u\left[G_gL_g(T) + G_eL_e(T)\right][1 + fr(T)].$$

In Eq.(5.49), $H = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} h_t(X, Y)h_u(X, Y)dXdY$ is the spatial overlap of the pump and probe
(throughout the paper the subscript $u$ is for pump and $r$ is for probe) [dimension: 1/area];

$$\Delta P_u = \int_0^{\infty} \rho_u(\nu_u)|_{Z=0} [1 - \exp(-\alpha^0(\nu_u)l)]d\nu_u$$

is the total absorbed pump photon number [dimension: photons] evaluated at the back of the sample $Z = l$ [dimension: length], $\rho_u(\nu_u)|_{Z=0}$ is the pump spectrum [dimension: photons/frequency] at the front of the sample (so the total
number of incident pump photons, integrated over all frequency, is \( P_u^0 = \int_0^\infty \rho_u(v_u) \big|_{Z=0} dv_u \),

\( \alpha^0(\nu) \) is the equilibrium frequency dependent exponential absorption coefficient in

\[
I(\nu) = I^0(\nu)e^{-\alpha^0(\nu)l} \quad [\text{dimension: } 1/\text{length}],
\]

\( G_{ge}(\nu) \equiv \int_0^\infty \rho_r(\nu) \big|_{Z=0} \exp[-\alpha^0(\nu)l] |\sigma_{ge(eg)}(\nu_r) | dv_r \)

is the overlap of the frequency dependent absorption (stimulated emission) cross section \( \sigma_{ge(eg)} \)

[dimension: area] with the attenuated probe spectrum evaluated at the back of the sample \( Z = l \)

(the total number of incident probe photons, integrated over all frequency, is

\( P_r^0 = \int_0^\infty \rho_r(\nu_r) \big|_{Z=0} dv_r \)). The subscript \( g \) stands for ground state and \( e \) for excited state. For a single absorbing species in the ground state, the exponential absorption coefficient is

\( \alpha^0(\nu) = N_g^0 \sigma_{ge}(\nu) \) where \( N_g^0 \) is the equilibrium molecular number density [dimension: 1/volume].

\( f = 3 \cos^2 \phi - 1 \) quantifies the relative polarization of linearly polarized pulses where \( \phi \) is the angle between pump and probe optical electric fields (\( f = 0 \) for magic angle, \( f = 2 \) for parallel, \( f = -1 \) for perpendicular); \( r(T) \) is the time dependent anisotropy (which accounts for the non-equilibrium orientational distribution excited by polarized light); \( L_{ge}(T) \) quantifies the time dependent loss (recovery) of population for state \( g(e) \) [\( L(T = 0) = 1 \) and \( L(T = \infty) = 0 \)].

Eq. (5.1) says that the pump-probe signal is proportional to the number of molecules excited by the pump \( (\Delta P_u) \), the overlap of the transmitted probe photon spectrum with the molecular absorption and emission cross-sections \( (G) \), the spatial overlap of the pump and probe \( (H) \), and the fraction of excited molecules that survive \( (L) \) in the correct alignment \( (r) \) to interact with the polarized probe. For fixed pump and probe spectra, the pump-probe signal in Eq. (5.1) is proportional to the total number of incident pump photons, \( P_u^0 \), and to the total number of incident probe photons, \( P_r^0 \). In Eq. (5.1), molecular number density and sample pathlength
always appear together as their product, $N_{ge}^0 I$. For a nearly transparent sample ($N_{ge}^0 N_l \ll 1$) with a long lifetime that is pumped and probed by parallel polarized pulses with a narrow spectrum (width $\delta$) that does not overlap the emission spectrum, Eq. (5.1) reduces to

$$S_{pp} = H \cdot [(\rho_u \delta) N_{ge}^0 \sigma_{ge} l][\rho_r \delta] \sigma_{ge} [9 / 5],$$

(5.50)

where the first factor in brackets arises from $\Delta P_u$, the second from $G$, and the third from $r$. Aside from measurable pulse properties in $H \cdot (\rho_u \delta)(\rho_r \delta)$, $S_{pp}$ is proportional to the absorbance $[A = (N_{ge}^0 \sigma_{ge} l) / \ln(10)]$ and the absorption cross section, enabling determination of the absorption cross section from $S_{pp}$, the pulse properties, and the absorbance. Eq. (5.50) is analogous to Eq. 2 of ref. 41, but explicitly includes the pulse properties needed for an absolute measurement. Eq. (5.50) is only valid for a nearly transparent sample; Eq. (5.49) includes pulse attenuation in an absorbing sample, which is necessary for an optimal measurement. We have tested Eq.(5.49) on fluorescein dissolved in basic methanol; the calculated pump probe signal matched to within 10% error with the measured absolute probe photon numbers. 44

With this quantitative agreement, the molar concentration can now be absolutely determined by explicitly connecting $S_{pp}(f,T)$ to molar concentration. This new analysis of the absolute pump probe signals from ref. 44 begins by recasting the absorption and emission cross sections in Eq.(5.49) in terms of the experimentally measured absorbance spectrum, $A(\nu)$, and the relative fluorescence spectrum $F(\nu)$. In the following, we express the absorption and emission cross sections in term of $A(\nu)$ and $F(\nu)$. First, the absorption cross section is written as [Eq. (5.51)],

$$\sigma_{ge}(\nu) = \frac{\kappa}{C} A(\nu)$$

(5.51)
where \( \kappa = 1000 \text{cm}^3 / L \cdot \text{ln}(10) / N_A l \), \( N_A \) is Avogadro’s number, \( l \) is the sample length (dimension: \( \text{cm} \)), \( C \) is the unknown molar concentration (dimension: \( M \)) and \( A(\nu) \) is the absorbance spectrum (dimensionless)\(^{25,45}\). Using absorbance, the exponential terms in \( \Delta P_g \) and \( G_{g(e)} \) are conveniently rewritten as \( \exp[-\alpha^0(\nu \lambda) l] = 10^{-A(\nu)} \).

Next, we express the frequency dependent stimulated emission cross section using \( F(\lambda = c / \nu) \), the steady state relative fluorescence spectrum as a function of wavelength. There are two ways to do this; one using the absorption spectrum and making the Condon approximation and one using only the radiative rate. Both approaches use the relative fluorescence spectrum \( F(\lambda = c / \nu) \) to calculate the normalized stimulated emission lineshape \( g_{eg}^{\text{stim}}(\nu) = \int_{Band} [F(\lambda = c / \nu) / \nu^5] d\nu \) and normalized spontaneous emission lineshape \( g_{eg}^{\text{spon}}(\nu) = F(\lambda = c / \nu) / \nu^2 \). The first route to the absolute stimulated emission cross section uses the Condon approximation,\(^{32,47,48}\) which assumes the transition dipole moment is independent of vibrational coordinates and thus the same for absorption and emission (Eq. 30 of ref. \(^{25}\)), giving:

\[
|\sigma_{eg}(\nu)| = \frac{\kappa}{C} E_{CA}(\nu) \tag{5.52}
\]

where \( E_{CA}(\nu) = \nu g_{eg}^{\text{stim}}(\nu) \int_{Band} A(\nu') / \nu' d\nu' \),\(^{32,47,48}\) the prime denotes a dummy frequency variable [dimension of \( g_{eg} \): \( 1 / \text{frequency} \), \( E_{CA} \) is dimensionless], and subscript \( CA \) indicates the Condon approximation.

Substituting Eqs.(5.51) and (5.52) into Eq.(5.49), and rearranging to isolate concentration yields
\[
C = \kappa \frac{[1 + f(T)]}{S_{pp}(f,T)} H \Delta P_u \Gamma(T)
\]

(5.53)

where

\[
\Delta P_u = \int_0^\infty \rho_u(nu) |_{\nu=0} [1 - 10^{-4\nu}] d\nu_u,
\]

(5.54)

and

\[
\Gamma(T) = \int_0^\infty \rho_r(nv_r) |_{\nu=0} 10^{-4\nu} \left[ L_g(T) A(nv_r) + L_e(T) E_{ca}(nv_r) \right] d\nu_r.
\]

(5.55)

The measured pump-probe signals, \(S_{pp}\), changes in pump-photon number upon transmission through the sample, \(\Delta P_u\), and probe/absorption-emission overlap, \(\Gamma(T = 600\,\text{ps})\), calculated from Eq. (5.55) are tabulated in supporting information along with the gravimetric concentrations and pulse energies used for each measurement.

The second approach is based on exploiting Einstein’s relation between spontaneous and stimulated emission (Einstein \(A_{21}\) and \(B_{21}\) coefficients), preferably using a directly measured total radiative rate, \(k_{rad}\) [dimension: photons/(time \(\times\) molecules)] to determine the emission cross section.

\[
|\sigma_{eg}(\nu)| = \frac{c^2}{8\pi n^2} k_{rad} v \left[ g_{eg}^{spon}(\nu) / \nu^3 \right]
\]

(5.56)

where \(c\) is the speed of light in vacuum, and \(n\) is the refractive index (the origin of the factor 8\(\pi\) is traced to the electromagnetic energy density in a black body\(^{24}\)). If there is a single excited state with a single exponential lifetime function, then \(k_{rad} = \phi/\tau_f\) where \(\tau_f\) is the measured lifetime of fluorescence and \(\phi\) is the fluorescence quantum yield,\(^{49}\) which can be difficult to measure accurately.\(^{50}\) The Einstein relation connecting the stimulated emission cross section to \(k_{rad}\) and \(g_{eg}^{spon}(\nu)\) was tested for molecules in solution during the early days of the dye laser,\(^{51}\) and is used
for dopants in solids\textsuperscript{52,53} (where the radiative rate equals the fluorescence lifetime for the lowest excited level in the absence of collisions) and molecules in solution.\textsuperscript{13,32-34} Substituting Eqs. (5.51) and (5.56) into Eq.(5.49) and rearranging for concentration,

$$ C = \kappa \left[ \frac{1 + fr(T)}{S_{pp}^{GSB}(f,T)} \right] H \Delta P_o \Gamma_g(T) \tag{5.57} $$

where $S_{pp}^{GSB}(f,T) = S_{pp}(f,T) - H \Delta P_o G_e L_e(T)[1 + fr(T)]$ and

$$ \Gamma_g(T) = \int_0^\infty \rho_r(v_r)|_{\nu=0} 10^{-A(v_r)} L_g(T) A(v_r) dv_r. \quad S_{pp}^{GSB}(f,T) \text{ is the ground state bleach contribution to total pump – probe signal; it is the total pump – probe signal, } S_{pp}(f,T) \text{ minus the excited state emission contribution (} = H \Delta P_o G_e L_e(T)[1 + fr(T)]\text{). Eq.(5.57) differs from Eq.(5.53) in that the emission cross section is absolutely determined from the spontaneous emission rate without making the Condon approximation; only the ground state absorbance spectrum is needed, as indicated by the dependence of } \Gamma_g(T) \text{ on the overlap integral between the attenuated probe spectrum and the steady state absorption spectrum. Note that this route does not use the Condon approximation to relate the absorption and emission cross sections, but determines them independently.}

All parameters on the right hand side of Eqs.(5.53) and (5.57) are optically determined: $A(\nu)$ and $F(\nu)$ are obtained from steady state spectra; $S_{pp}(f,T)$, $r(T)$ and $L(T)$ are obtained by pump probe experiments; $h_{r,u}(X,Y)$ and $\rho_{r,u}(\nu_{r,u})$ are obtained from laser pulse characterization.

A proof of principle demonstration of the method described above is now presented. To simultaneously quantify the molar concentration and extinction coefficient of a dye in solution, a set of linear and nonlinear spectroscopic measurements were performed on fluorescein solutions for which concentrations are known by independent measurements of mass of the dye and
volume of the solvent (the extinction coefficient is, therefore, known from absorbance measurements if the sample path length is known). The all-optical determinations are then compared to the gravimetrically (mass and volume) determined concentrations and extinction coefficient for concentrations ranging over more than an order of magnitude.

5.3 Experimental

Fluorescein (C_{20}H_{12}O_{5}, MW 332.31 g/mol) was purchased from Invitrogen (specified mass purity > 97%) and used without further purification. The fluorescein solutions were made in alkaline methanol (0.01 N KOH), forming fluorescein dianion. 44.8 mg of dry powdered fluorescein was dissolved in 250.0 ml of alkaline methanol to make up a concentrated stock solution. From successive dilutions of the stock solution, 538 (522), 314 (305), 200 (194), 147 (143), 94 (91), 63 (61), and 32 (31) µM solutions were prepared (the numbers in the parentheses indicate the lower bound on concentration for 97% pure fluorescein; errors from uncertainties in dye mass and solvent volumes are less than <1%).

The absorbance spectra \(A(\nu)\) were measured in a 200 µm pathlength cuvette with a scanning grating spectrophotometer (Varian Cary 500 Scan). After base-lining against air, a wavelength range spanning 350 – 600 nm was scanned, covering the \(S_0 – S_1\) band in 1 nm scan steps with 100 ms integration time. A spectrum of a basic methanol solvent blank was taken under the identical conditions and subtracted from fluorescein solution spectra. Overlaid single scan extinction coefficient spectra show a maximum difference of less than 2% (not shown), suggesting negligible concentration dependent changes in the samples. Absorbance is determined with minimum signal to noise ratio \(\sim 333:1\). The sample length (0.199±0.001 mm) was determined by independently measuring the absorbance of the same solutions in a 1cm
cuvette whose path length was determined to 0.005 mm accuracy using a Starrett hole gauge and Mitutoyo digital calipers.

The normalized fluorescence lineshape \( g_{eg}^{\text{norm}}(\nu) \) was determined from a fluorescence spectrum taken with Horiba Jobin Yvon Fluorog fluorimeter. The excitation wavelength was 470 nm. For a 6.4 µM solution (prepared from the 32 µM solution by 5× dilution) in a 1 cm cuvette (peak absorbance ~0.01), fluorescence emitted at 90° to the excitation beam was collected for a 50 ms integration time for emission wavelengths ranging from 400 – 800 nm in 1 nm steps. The fluorescence spectrum was taken with ~ 1000:1 signal to noise ratio in a single scan. The emission lineshape function was derived from the fluorescence spectrum using procedures described in Yu et al.\(^{45}\)

Two sets of pump probe measurements were carried out: the pump probe signal was measured at a fixed pump probe polarization and delay to determine \( S_{pp}(f, T) \); pump probe signals were measured as a function of the pump probe delay and polarization to determine the lifetime function, \( L(T) \) and anisotropy, \( r(T) \). The pump – probe measurements were made using apparatus described previously.\(^{54}\) Briefly, the light source was derived from a noncollinear optical parametric amplifier (NOPA)\(^{55,56}\) pumped by a Ti:sapphire regenerative amplifier (Coherent RegA 9050, 7 µJ/pulse, 800 nm center wavelength, 80 fs pulse width, 10 kHz) seeded by a home-built Ti:sapphire oscillator (4 nJ/pulse, 800 nm center wavelength, 30 fs pulse width, 76 MHz repetition rate). The NOPA produced horizontally polarized pulses tunable between 480 – 750 nm, having up to 200 nJ/pulse energy and ~430 cm\(^{-1}\) full width half maximum bandwidth (~34 fs transform limited pulse width). After pulse compression with a pair of fused silica prisms that pre-compensated for material dispersion further along the beam path, the NOPA beam was split into a strong pump and weak probe by a femtosecond broadband
dielectric beam splitter (CVI Melles-Griot, W2-PW-1006-UV-550-45P). To vary the probe time delay with respect to the fixed pump delay, the probe optical path length was adjusted using a trihedral retro-reflector mounted on a computer controlled mechanical delay stage. The intensities of the pump and probe beams were varied independently with metallic variable neutral density filters (Edmund Optics). The polarizations of the pump and probe beams were controlled with a combination of calcite cube polarizers (Karl Lambrecht, MGTYE8, $5 \times 10^{-6}$ extinction) and zero order half-wave waveplates (Newport 10RP52-1); the extinction ratio was 121:1. Parallel pump and probe beams were focused to cross in the sample with a 10 cm singlet fused silica lens. The pulse duration was determined by second harmonic generation autocorrelation using 100 µm thick KDP cut at 40°. Assuming a Gaussian temporal envelope, the FWHM pulse duration was ~38 fs. The sample flowed through a 0.199 mm pathlength × 5 mm width flowcell with a discharge of 2.5 ml/s; assuming laminar flow (which implies zero fluid velocity at the windows), more than >90% of the sample moves out of the laser beam diameter between laser shots at 10 kHz repetition rate.

For absolute $S_{pp}(f,T)$ determination, relative probe photon numbers were measured with a biased silicon PIN photodiode (4.5 mm diameter active area, 30 ns risetime), which was amplified 5x, fed into a gated integrator (boxcar), and digitized. Using a 100 ns gate, boxcar output voltages generated by the probe beam with ($V_{on}$) and without ($V_{off}$) the pump excitation were measured and used to calculate a difference voltage ($\Delta V = V_{on} - V_{off}$). The boxcar output voltages were calibrated into absolute photon numbers by measuring the corresponding pulse energies; for example, the absolute probe photon number change caused by the pump is $\Delta P_r = \Delta V P_{r_{off}} / V_{off}$, where $P_{r_{off}}$ is the transmitted probe photon number with the pump off. For both pump and probe, incident and transmitted photon numbers were determined to 6% accuracy.
by measuring, $U_x$, the pulse energies (where $x = u$ for pump and $r$ for probe) with a power meter [Coherent FieldMaster GS display (1% accuracy) and LM-2Vis sensor (5% accuracy)] and dividing by the photon energy. ~1% change in absolute voltage caused by the pump was detected with a signal-to-noise ratio of ~20:1 in a single measurement. Multiple sequences of pump on and off voltages were measured by blocking and unblocking the pump beam with a mechanical chopper at 4 Hz. A software program extracted the average $\Delta V$ and $V_{off}$ from a set of at least 40 pump on and off cycles. $f$ was set to 0 ($\phi = 54.7^\circ$) for magic angle pump probe polarization57 at $T = 600$ ps.

For $L(T)$ and $r(T)$ determination, the voltage changes brought about by the probe beam intensity change as a result of pump excitation were measured using lock-in detection. Modulated voltage changes referenced at 500 Hz mechanical pump beam chopping were detected by a lock-in amplifier. The time varying pump probe signal traces from -5 ps to 1 ns pump probe delay were measured at parallel, perpendicular and magic angle polarization and were used to extract $L(T)$ and $r(T)$.

The transverse laser intensity profiles needed to determine $h_u,r(X,Y)$ were determined by beam imaging at the sample position. The beam images taken from a CMOS based sensor (ZoomCam 1598, 640×480 resolution) revealed a nearly Gaussian spatial intensity profile in two dimensions, with aspect ratio >0.9. Images were corrected for detector saturation and pixel spacing [calibrated by an 1.00±0.025 mm grid pattern63 (National Brand, engineering form graph paper #12-188)], fits gave 43.5 µm full width half maximum (FWHM) diameter. The FWHM beam diameters were further checked by measuring beam transmission through a set of pinholes; using 25, 50, and 75 µm diameter pinholes, all power transmission were consistent with a 43 µm diameter to within 6% error.
The laser pulse spectra $\rho_{u,r}(\nu_{u,r})$ were obtained from relative wavelength spectra recorded using a silicon CCD array spectrograph. The pulse energies were measured using a silicon sensor power meter (Coherent FieldMaster GS display and LM-2Vis). The spectra were calibrated in photons per unit frequency using the procedure outlined in Yu et al.\textsuperscript{45} The frequency integral of the product of the absolute pulse spectrum with the photon energy $[\rho(\nu)h\nu]$ equals the pulse energy and is used to determine absolute pulse spectra from the relative spectra and pulse energies. The spectra had a Gaussian profile; fit to a model Gaussian function yielded FWHM width of 430 cm\textsuperscript{-1}.

5.4 Results and Discussion

Fig. 5.1 compares concentration using Eqs.(5.53) and (5.57) to the concentration determined from mass and volume measurements for fluorescein dianion in basic methanol. From 0.032 mM to 0.54 mM (absorbance = 0.06 to 1), the optically determined concentrations agree with the mass and volume determined concentrations to within 10% error. The errors in the concentration (along the abscissa) are dominated by uncertainty in purity of the fluorescein, which could be as low as 97% (the stated lower limit purity from supplier) and the lower bounds are shown as the vertical bars. The calculated error bars for the optically determined concentrations (along the ordinate) are found by propagating errors in the independently measured parameters through Eq. (5.53) and Eq. (5.57), respectively. They stem primarily from a combination of uncertainties in
**Figure 5.1.** Optically determined concentration vs. mass and volume concentration. Concentrations determined using the Condon approximation (cross) and the Einstein relation (open circles) are overlaid. Errors in the optically determined concentration using the Condon approximation are indicated. The diagonal line has a slope of 1. The lower bound concentration assuming 97% fluorescein purity is indicated by the vertical bars. The best fit line to data (dotted) has the slope of 0.96 ± 0.02 when the intercept is fixed at the origin (the error in the slope is larger if the intercept is floated).
beam diameter [−6%, affecting \( h_u(X, Y) \) and \( h_r(X, Y) \)], spectral width [−5%, affecting \( \rho_u(\nu_u) \) and \( \rho_r(\nu_r) \)] and systematic errors in pump and probe pulse energies due to power meter calibration [−5%, affecting \( \rho_u(\nu_u) \) and \( \rho_r(\nu_r) \)] that dominate over other measurement uncertainties. The calculated standard error in concentration increases from ±3 µM at 32 µM to ±40 µM at 540 µM; however, as concentration increases, the calculated fractional error in concentration decreases from 11% to 7% over this range. This reduction in fractional error can be traced to the functional sensitivity of concentration to pump-probe signal and absorbance (see below). It should be noted that the three largest contributions to the calculated error bars indicated above are all constant throughout the experiment, and thus determine primarily a single error in the slope of the optical vs. gravimetric concentration that is also constant throughout the experiment. For ideal measurements, the theoretical slope is equal to the mass purity of the sample (specified as greater than 0.97), so the fitted slope of 0.96±0.02 is about 2.5× more accurate than should be expected from the 5% calculated standard error in optical determination of the highest concentrations used. This indicates a fortuitous partial cancellation of the three largest sources of systematic error.

Fig. 5.2 shows the optically determined extinction coefficient at 496 nm using \( \epsilon = A / Cl \) (where \( C \) is the optically determined concentration) as a function of the mass and volume determined concentration. The optically determined extinction coefficients have calculated error bars derived from the calculated standard errors in concentration using Eq. (5.51), lie within ~10% error of the mass and volume determined extinction coefficient whose upper and lower bounds are indicated by horizontal lines (the lower line represents 100% purity, and the upper line represents 97% purity). Calculated errors in molar extinction coefficient decrease with concentration from 9200/M•cm (10%) at 32 µM to 6000/M•cm (6%) at 540 µM.
Figure 5.2. All – optical determination of the extinction coefficient at 496 nm (cross) vs. gravimetric concentration. The optically determined extinction coefficients used the Condon approximation. The Beer’s law extinction coefficient determined from absorbance measurements and the gravimetric concentration is indicated by the horizontal lines; the lower line assumes 100% purity and the upper line assumes 97% purity. The inset shows the 0.31 mM fluorescein absorbance spectrum, $A$, and stimulated emission spectrum, $E_{CA}$ (from the Condon approximation) overlaid with the laser pulse spectrum, $\rho$. 
The overall error can be significantly reduced by more accurate determination of the pulse parameters. Two sets of improvements are suggested; the first requires less effort to implement but is less accurate than the second. The overall error can be reduced to \( \sim 4\% \) by implementation of commercially available technologies: 2\% accuracy in beam diameter with a beam profiler\(^{58}\); 2\% accuracy in photon flux using actinometry developed in the 1950’s;\(^ {59}\) and 1\% accuracy in spectral width using broad NOPA pulses optimized for stability are feasible.\(^ {56,60,61-62}\) The overall error might be further reduced to \( \sim 1.5\% \) by employing more advanced methods: 0.2\% accuracy in beam diameter by a precise calibration of the spatial dimension in a beam profiler using a grid pattern, 1\% accuracy in photon flux with modern actinometry\(^ {64}\), and 0.3\% accuracy in the spectral width using Ti:sapphire oscillator pulses may be attainable.\(^ {45,65,66}\)

The viability of the presented method to simultaneously determine two unknown quantities solely through optical techniques is demonstrated by the optically determined molar concentration and extinction coefficient recovering the mass and volume determined concentration and extinction coefficient to within 10\% error. Critical to the good agreement are the absolutely calibrated femtosecond pump probe signals and the calculation matching within 10\% error. Experimentally, the success in absolute calibration entails a spatially and spectrally well-behaved and reproducible femtosecond laser beam that allows use of weak pump and probe pulses so that each propagates linearly and contributes linearly to a nonlinear signal that also propagates linearly. The agreement between measurement and calculation of the femtosecond pump probe signals indicates that the lifetime functions are correct to within 10\%.

Given the many parameters involved, it is not possible to fully analyze the conditions required for optimal accuracy here. However, some guidelines are suggested by analyzing the sensitivity of the extinction coefficient and concentration to the absorbance and pump-probe
signal. Measurements of concentration from absorbance are typically most accurate for absorbance of $A \sim 0.3$ to $0.7$. For fixed pathlength and pulse parameters, the pump–probe signal given by Eq. (5.49) is maximized, as a function of concentration at an absorbance of $A \sim 0.32$. As both the pump–probe signal and absorbance accuracies are fairly flat around the optimum concentration, the overlap between the two ranges is likely near optimal if the same sample cell must be used for both measurements (which is not always the case). Conditions for optimal pump-probe measurements have been thoroughly treated on the assumption that the sample is ideal. Although larger beam diameters theoretically improve signal to noise, the beam diameter is usually limited by some combination of scattering in the sample, the need to refresh the sample between laser shots, and available pump pulse energy. Using Eq. (5.49) for the pump-probe signal and Eq. (5.53) or Eq. (5.57) for the concentration, the pump and probe photon numbers enter linearly in both numerator and denominator of the expressions for concentration, suggesting the pump photon number should be chosen for most accurate measurement of $S_{pp}$ and $\Delta P_u$. In pump-probe, the optimal probe photon number is determined by the noise characteristics of the detector and the beam diameter.

Accurate recovery of sample concentration requires that the pump-probe signal lie on a relatively flat part of the surface of concentration $C$ as a function of pump-probe signal $S_{pp}$ and absorbance $A$ shown in Figure 5.3. Figure 5.3 shows the concentration as a function of absorbance and the absolute pump–probe signal (divided by the pump and probe pulse energies), as given by Eq. (5.53). The surface is constructed for a fixed pathlength. Figure 5.3 also shows the optically determined concentrations as functions of absorbance and the absolutely measured
Figure 5.3. Surface plot of concentration as a function of absolute pump–probe signal and absorbance. The surface was calculated using Eq.(5.53); the absolute pump–probe signal was divided by the average incident pump and probe pulse photon numbers \[1.2 \times 10^{10} \text{ photons (4.62 nJ)} \times 1.2 \times 10^{9} \text{ photons (0.47 nJ)}\]. Optically determined concentrations as functions of absolutely measured pump–probe signals and absorbance are overlaid (black balls with lines connecting to the \(C-A_{\text{max}}\) and \(S_{pp}-A_{\text{max}}\) planes); the measured pump–probe signals were divided by the pump and probe pulse photon numbers used for individual measurements. The size of the spherical ball roughly corresponds to \(\pm 0.05 \text{ mM}\) in the concentration dimension (it was chosen to show intersection with the surface and does not indicate the size of the error bars). The curve in the \(S_{pp}-A_{\text{max}}\) plane is calculated using Eq. (5.1). Parameters used in the calculation are: spatial overlap \(H = 2.3 \times 10^4 \text{ cm}^{-2}\); total number of incident pump photons \(P_u^0 = 1.2 \times 10^{10} \text{ photons}\); total number of incident probe photons \(P_r^0 = 1.2 \times 10^{9} \text{ photons}\); pump – probe delay \(T = 600 \text{ ps}\); population factors \(L_c(600 \text{ ps}) = L_g(600 \text{ ps}) = 0.85\); anisotropy \(r(600 \text{ ps}) = 0\); sample pathlength \(l = 200 \mu\text{m}\); pump – probe polarization index \(f = 2\) (parallel polarization). Color changes are spaced at 0.1 mM. The linear fit through the projections of the data points onto the \(C-A_{\text{max}}\) plane is shown, revealing the Beer’s law relationship between concentration and absorbance in the data. Experimental parameters: Parallel polarization; pump-probe delay \(T = 600 \text{ ps}\); pump pulse energy \(U_u\) ranged from 4.55 to 4.61 nJ with ~6% systematic error; probe pulse energy \(U_r\) ranged from 0.464 to 0.480 nJ with ~6% systematic error; beam diameter 43.5 \(\pm 2.6 \mu\text{m}\) (50% transmission through a 43.5 \(\mu\text{m}\) diameter pinhole); maximum pulse intensity at \(\tilde{\nu}_{\text{max}} = 20023 \text{ cm}^{-1}\); width of the pulse spectrum \(\Delta \tilde{\nu}_{\text{FWHM}} = 430 \pm 26 \text{ cm}^{-1}\); 38 fs pulse duration; sample pathlength \(l = 199 \pm 1 \mu\text{m}\).
pump–probe signal (where each signal was divided by the individual pump and probe pulse energies used in that measurement); the data points, in which concentration was the only independent variable, follow a curved trajectory along the surface. In keeping with the Beer’s law relationship between concentration and absorbance, the projection of the data points onto the concentration vs. absorbance plane is linear. The calculated curve in the $S_{pp}$ vs. $A_{\text{max}}$ plane shows how the pump-probe signal at first increases with absorbance because of the increasing number of molecules contributing to the signal and then decreases with absorbance as the pulses are attenuated by absorption in the back of the sample – this makes concentration and sample pathlength important experimentally controllable parameters in maximizing the pump-probe signal. It can be seen from Figure 5.2 that the most accurate determination of the extinction coefficient occurs for $A \approx 0.4$ where the data point resides on a relatively flat part of the surface in Figure 5.3 and hence is likely to suffer less from uncertainties in $S_{pp}$ and $A$ (near this absorbance, $S_{pp}$ is also maximized, typically optimizing the signal to noise ratio). At this absorbance, the spatially and orientationally averaged excitation probability (calculated from the molecular number density of excited states given by Eq. (A2) of ref. 44) is $\sim 1/10$ – larger values accessed by increasing the pump pulse energy will give only a marginal improvement as they push up against the requirement that the pump propagate linearly in the sample for the analysis to remain valid.

Theoretical requirements may be readily satisfied in highly fluorescent samples\(^3\) enabling a direct application of Eq.(5.53) or (5.57). Caution is advised, though, when applying Eq.(5.53); the Condon approximation breaks down in molecules when the transition dipole moment varies significantly with nuclear configuration.\(^3\) Eq.(5.57) can be applied to molecules to avoid such a problem if the true radiative rate is known; however, this equation can suffer
from error when a significant proportion of excited population rapidly relaxes via a non-radiative decay channel (perhaps accessed when electronically excited molecules are vibrationally hot before cooling) so that the measured fluorescence lifetime scaled by the fluorescence quantum yield does not give the true radiative rate. Therefore, although a 93% fluorescence quantum yield for fluorescein dianion\(^32\) points to mostly radiative excited population relaxation, it does not preclude the possibility of error stemming from a rapid 7% loss of excited state population via a non-radiative channel (the initial decay component with a timescale of a few hundred femtoseconds in fluorescein pump – probe signals is ordinarily attributed to polar solvation dynamics).\(^45,69\) If this were rapid population decay of vibrationally hot, electronically excited fluorescein, the inverse of the slowest time constant in pump – probe measurement would be the true radiative rate, and require no scaling by the fluorescence quantum yield.

Equation (5.49) can be generalized to include excited state absorption, but these cross sections are often unknown; similarly, an unknown lifetime function also presents a challenge to experiments with identical pump and probe pulses. An arrangement akin to \(V\) – type double resonance\(^24\) is suggested to alleviate some of the difficulties associated with uncertainties in excited state properties; in such a scheme, the probe connects the common ground state to another excited state not directly excited by the pump. This ability to tune the probe so that the probe spectrum does not overlap with the stimulated emission spectrum leads to simplification of Eqs.(5.53) and (5.57), because the overlap integral of the probe spectrum with the emission spectrum is zero, the second term in the right hand side of the equation for \(\Gamma(T)\) [after Eq.(5.53)] involving \(E_C(\nu)\) vanishes and \(S_{pp}^{GSB}(f, T) = S_{pp}(f, T)\) in Eq.(5.57). Further, because only ground state bleach is probed, excited state absorption can be neglected, the fluorescence quantum yield is not needed and \(L_g(T)\) is the readily determined experimental ground state...
recovery time. Such two-color extensions might also make the approach suitable for different absorbance ranges than absorption spectroscopy and more amenable to analysis of mixtures with overlapping peaks in the absorption spectrum.

In this paper, we have utilized the femtosecond pump–probe and linear spectroscopic techniques as the independent optical measurements to ascertain two unknown parameters, the molar concentration and extinction coefficients; we have demonstrated that $S_{pp}(f,T), A(\nu)$, and $E_{C,1}(\nu)$ determine the molar concentration [Eq.(5.53)], with the validity of the Condon approximation; when instead $S_{pp}(f,T), A(\nu)$, and the absolutely determined stimulated emission cross-section $\sigma_{eg}(\nu)$ are used, the molar concentration is determined without making the Condon approximation [Eq.(5.57)]. This suggests another approach which involves $A(\nu)$, $\sigma_{eg}(\nu)$, and the Condon approximation; from the stimulated emission cross section $\sigma_{eg}(\nu)$, the transition strength from the ground to the excited state can be obtained by the Condon approximation, allowing determination of concentration from $A(\nu)$.

Fundamentally, the combined use of absolutely measured linear and nonlinear spectroscopic signals to determine concentration rests on measurements of the number of photons in the incident and transmitted beams to determine the number concentration. This is analogous to Coulometric methods, which fundamentally measure the number of electrons. Because it ultimately relies on counting photons, we suggest the all-optical method be called a photonumeric determination of concentration and extinction coefficient.

Although this paper demonstrates determination of unknown equilibrium ground state concentration, the method can be adapted to determine excited state concentration: a pump–pump–probe type experiment, for example, may be able to determine absolute intermediate
concentration and extinction coefficient; absolute calibration of transient spectra enables absolute quantification of time-varying concentration of transient species.

5.5 Conclusions

We have demonstrated an all-optical, photonumeric method, based on absolute calibration of femtosecond pump – probe signals, to determine absolute concentration and extinction coefficient of fluorescein dianion in basic methanol. This in situ method allows absolute and simultaneous determination of unknown concentration and extinction coefficient without the need for physical handling of sample under interrogation. This method is extensible to measure concentrations of intermediate chemical species where in situ determination is the only option.
5.6 Appendix 5A

Table 5A.1. Measured experimental parameters and calculated quantities used for the optically determined concentrations in Fig. 5.1, the optically determined extinction coefficients in Fig. 5.2, and the surface/data points in Figure 5.3. The first column is the measured gravimetric concentration (used only for comparison in Figure 5.1). The second column is the measured absolute pump-probe signal. The third column is the measured change in transmitted pump photon number. In the fourth column, $\Gamma$ is calculated from the absorption, emission, and pulse spectra (shown in the inset to Figure 5.2) using Eq. (5.7). The last two columns give the incident pump and probe pulse energies used for measurement at each concentration.

<table>
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<th>$C_{\text{gravimetric}}$ (µM)</th>
<th>$S_{PP}$ ($10^6$ photons)</th>
<th>$\Delta P_u$ ($10^9$ photons)</th>
<th>$\Gamma (600\text{ps})$ ($10^6$ photons)</th>
<th>Incident pump pulse energy (nJ)</th>
<th>Incident probe pulse energy (nJ)</th>
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<td>9.96</td>
<td>165</td>
<td>4.63</td>
<td>0.466</td>
</tr>
</tbody>
</table>

References


(66) Fransted, K. A.; Caram, J. R.; Hayes, D.; Engel, G. S.: Two-dimensional electronic spectroscopy of bacteriochlorophyll a in solution: Elucidating the coherence dynamics of


(211) Assuming uniform flow, this corresponds to a linear velocity of 1.56 m/s in the 0.200 mm x 5 mm rectangular cell. Approximating the rectangular cross-section with an
ellipse, laminar flow calculations yield an average flow velocity of 2.67 m/s. The slowest 10% molecules (near the boundary of the flow cell) yield an average velocity of 0.40 m/s (40 microns in 100 microsecond).


