Two-dimensional Electronic Spectroscopy and Photosynthesis: Fundamentals and Applications to Photosynthetic Light-Harvesting

Gabriela S. Schlau-Cohen, Akihito Ishizaki, Graham R. Fleming

PII: S0301-0104(11)00144-3
DOI: 10.1016/j.chemphys.2011.04.025
Reference: CHEMPH 8200

To appear in: Chemical Physics

Received Date: 4 March 2011
Revised Date: 21 April 2011
Accepted Date: 25 April 2011


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Two-dimensional Electronic Spectroscopy and Photosynthesis: Fundamentals and Applications to Photosynthetic Light-Harvesting

Gabriela S. Schlau-Cohen, Akhiho Ishizaki, and Graham R. Fleming*
Department of Chemistry, University of California, Berkeley, CA 94720, USA and Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

In natural light harvesting systems, pigment-protein complexes are able to harvest sunlight with near unity quantum efficiency. These complexes exhibit emergent properties that cannot be simply extrapolated from knowledge of their component parts. In this Perspective, we focus on how two-dimensional electronic spectroscopy (2DES) can provide an incisive tool to probe the electronic, energetic, and spatial landscapes that must be understood to describe photosynthetic light-harvesting. We review the theoretical and experimental principles of 2DES, and demonstrate its application to the study of the Photosystem II supercomplex of green plants. We illustrate several capabilities of 2DES, including monitoring energy transfer pathways, observing excitonic coherence, determining excitonic geometry, and informing on the atomic structure.

I. INTRODUCTION

The development of a unified theoretical description of ultrafast nonlinear spectroscopic measurements by Mukamel [1] spurred a large and continuing burst of experimental development focused on dynamics of condensed phase systems. In turn, the results of these experiments have stimulated new theoretical approaches, particularly to condensed phase quantum dynamics [2–10]. Nowhere has this synergy been more evident than in studies of photosynthetic light harvesting, where increasingly sophisticated ultrafast spectroscopies have been employed. These advances have culminated in the use of two-dimensional electronic spectroscopy (2DES) [11–16], following the impressive developments in 2D infrared spectroscopy pioneered by Hochstrasser and coworkers [17]. In this perspective, we describe the theory underlying 2DES and its analysis and illustrate the application of these ideas to understanding complicated condensed phase systems. We use the Photosystem II (PSII) supercomplex of green plants [18, 19], as an example to describe how 2DES can, by probing structure-function relationships, provide an incisive probe of the overall design of this natural device. We close with some thoughts on directions for future developments in this area.

The PSII supercomplex is a particularly impressive natural apparatus which functions with remarkable near unity quantum efficiency at low light levels [22]. Components varied in size and form combine to harvest sunlight and then use every single photoexcitation to power chemical reactions [22, 23]. As shown in the structural model in Fig. 1a, the PSII supercomplex consists of several associated pigment protein complexes (PPCs), which consist of chromophores held in a protein matrix [18, 19]. Initial absorption events primarily occur on the periphery of the supercomplex in arrays of the major light-harvesting complex of PSII (LHCII). The excitation then translocates to the reaction center where it drives photochemistry. During its migration from the antenna to the reaction center, the excitation travels through so-called minor complexes, such as chlorophyll-binding protein 29 (CP29). Experimental evidence suggests that CP29, as well as participating in energy transfer processes, plays a role in non-photochemical quenching (NPQ) [24, 25],

*Electronic address: grfleming@lbl.gov

FIG. 1: Structural models of the light-harvesting pigment protein complexes from green plants where (a) shows a model of the Photosystem II supercomplex, (b) a structural model from x-ray crystallography [20] of LHCII, and (c) of the minor complex, CP29 [21]. (d) The linear absorption spectra of LHCII and CP29 at 77 K.
which is the process by which plants and algae dissipate excess energy. The structural models of LHC1 and CP29 are shown in Fig. 1b and c, respectively. These complexes, which complete efficient energy transfer, exhibit processes, such as relaxation of the phonon bath and structural fluctuations, which occur on timescales from femtosecond to hours. This combination of dynamical timescales and molecular rearrangements mean that it is difficult to identify the molecular origin of any given process.

PPCs are systems with emergent properties very different from those of their individual molecules. Their electronic absorption spectra are congested, generally without structure, and result from interactions of the chromophores (generally chlorophylls (Chl)) with this protein environment and with neighboring chromophores. The resultant states (often called excitons) are not localized on individual chromophores, interact weakly with their environment, exhibit ultrafast electronic relaxation within the exciton manifold, and exhibit long-lived electronic quantum coherence [16, 22, 26]. These properties appear to be essential for achieving the near unity quantum efficiency that photosynthetic light harvesting achieves at low light levels [22]. At light levels in excess of that usable for photosynthetic energy conversion, green plants and algae have complex mechanisms for reducing the quantum efficiency of energy transfer through the antenna to the reaction center [25]. The precise molecular mechanisms of this regulatory process is still under investigation, but undoubtedly involves, among other factors, the dynamic properties listed above.

These dynamic properties often render conventional spectroscopic techniques such as steady state and time resolved absorption and emission spectroscopy insufficiently incisive to reveal the microscopic mechanisms, and connections between the spatial, electronic, and dynamic landscapes which underlie function. 2DES offers a significantly more detailed window into the microscopic basis of light harvesting efficiency for the following reasons: (1) by spreading out the time-evolution of the spectrum onto two dimensions spectral resolution is enhanced and pathways of relaxation processes are directly revealed, (2) because the spectrum is recorded at the amplitude, rather than the intensity, level 2DES is directly sensitive to the quantum phase of the system thereby providing direct evidence of quantum coherence, (3) the polarizations of the four light pulses involved can be independently set providing the ability to connect the atomic structure (determined by x-ray crystallography, for example) with the electronic structure through the angles between the transition dipole moments. In addition in favorable cases, insight into the unknown atomic structure can be obtained and the uncoupled site energies of individual chlorophyll molecules can be estimated.

II. ELECTRONIC ENERGY TRANSFER IN PHOTOSYNTHETIC PIGMENT-PROTEIN COMPLEXES

To describe photosynthetic excitation energy transfer (EET), we consider a PPC consisting of \( N \) pigments. In this Perspective, we restrict the electronic spectrum of the \( m \)-th pigment to the ground state \( | \varphi_{m\text{g}} \rangle \) and the first excited state \( | \varphi_{m\text{e}} \rangle \) which corresponds to the Qy transition in a chlorophyll molecule. These states are obtained by the electronic Schr"{o}dinger equation \( H_{m\text{g}}(X)|\varphi_{m\text{g}}\rangle = E_{m\text{g}}(X)|\varphi_{m\text{g}}\rangle \), where \( H_{m\text{g}}(X) \) and \( E_{m\text{g}}(X) \) are the electronic Hamiltonian and energy of the \( m \)-th pigment in the absence of the inter-pigment Coulomb interaction, and depend parametrically on the set of the relevant nuclear coordinates including protein degrees of freedom, \( X \). Hence, the nuclear dynamics associated with an electronic state \( | \varphi_{ma} \rangle \) are described by \( H_{ma}(X) = E_{ma}(X) + \text{(kinetic energy)} \). The normal mode treatment is usually assumed for the PPC nuclear dynamics, because anharmonic motion with large amplitudes and long timescales produces inhomogeneous broadening on timescales irrelevant to photosynthetic EET. Such slow motions are, however, often significant for protein functionality. Further, it may be assumed that nuclear configurations for the electronic excited states of pigments are not greatly different from those for the ground states owing to the absence of large permanent dipoles on the pigments. Thus, the nuclear Hamiltonians associated with the electronic states \( | \varphi_{mg} \rangle \) and \( | \varphi_{ma} \rangle \) can be modeled as a set of displaced harmonic oscillators [16, 27],

\[
H_{mg}(X) = E_{mg}(X_{mg}) + \sum_{\xi} \frac{\hbar \omega_{m\xi}}{2} (p_{m\xi}^2 + q_{m\xi}^2), \tag{2.1}
\]

\[
H_{me}(X) = H_{mg}(X) + \hbar \Omega_m - \sum_{\xi} \hbar \omega_{m\xi} q_{m\xi} d_{m\xi} q_{m\xi}, \tag{2.2}
\]

where \( \{ q_{m\xi} \} \) are the dimensionless normal mode coordinates introduced around the equilibrium nuclear configuration associated with the \( m \)-th pigment \( X_{mg}^0 \), and \( \{ \omega_{m\xi} \} \) and \( \{ p_{m\xi} \} \) are the accompanying frequencies and momenta. In what follows we set \( E_{mg}(X_{mg}) = 0 \) to simplify our expressions. \( \hbar \Omega_m \) is the Franck-Condon transition energy, which is also termed the site energy.

In order to characterize the possible states of the whole complex of excitonic units, product states are introduced as \( \prod_{m} | \varphi_{ma} \rangle \). This Hartree-like ansatz is reasonable only if different state vectors \( | \varphi_{ma} \rangle \) do not overlap and the exchange interaction is negligible. For later convenience, we order the product state with respect to the number of elementary excitations. The overall ground state with zero excitation reads \( | 0 \rangle \equiv \prod_{m} | \varphi_{mg} \rangle \), whereas the presence of a single excitation at the \( m \)-th pigment is described by \( | m \rangle \equiv | \varphi_{me} \rangle \prod_{(\ell \neq m)} | \varphi_{\ell g} \rangle \). Description of EET on the basis of \( \{ | m \rangle \} \) is termed the site representation. The corresponding expansion of the complete PPC
Hamiltonian yields
\[ H_{\text{PPC}} = H^{(0)}_{\text{PPC}} + H^{(1)}_{\text{PPC}} + \cdots , \]  
where \( H^{(n)}_{\text{PPC}} \) (\( n = 0, 1, \ldots \)) describes \( n \)-excitation manifold comprising \( n \) elementary excitations. The Hamiltonian of the zero-excitation manifold reads
\[ H^{(0)}_{\text{PPC}} = \sum_{m} H_{mg}(X)|0\rangle\langle 0|, \]
whereas the Hamiltonian of the single-excitation manifold takes the form,
\[ H^{(1)}_{\text{PPC}} = \sum_{mn} H_{mn}(X)|m\rangle\langle m| + \sum_{mn} hJ_{mn}|m\rangle\langle n| \]
with \( H_{mn}(X) \equiv H_{mne}(X) + \sum_{k(\neq m)} H_{kg}(X) \). The electronic coupling \( hJ_{mn} \) has to be deduced from the inter pigment Coulomb interaction [28-32]. The electronic coupling may be also modulated by nuclear motions [33]. In the following, however, we assume that nuclear dependence of \( hJ_{mn} \) is vanishingly small and employ the Condon-like approximation as usual. Since the intensity of sunlight is weak, the single-excitation manifold is of primary importance under physiological conditions. However, nonlinear spectroscopic techniques such as 2D electronic photon echo spectroscopy can populate higher exciton manifolds, e.g. the double-excitation manifold comprising \( |mn\rangle \equiv (1 - \delta_{mn})|\varphi_{mne}\rangle|\varphi_{nne}\rangle \prod_{k(\neq m,n)}|\varphi_{kg}\rangle \). The Hamiltonian for the double-excitation manifolds is expressed as
\[ H^{(2)}_{\text{PPC}} = \sum_{mn} H_{mn}(X)|mn\rangle\langle mn| + \sum_{mnk} \left( hJ_{mk}|mn\rangle\langle kn| + hJ_{mk}|mn\rangle\langle mk| \right) \]
with \( H_{mn}(X) \equiv H_{mne}(X) + H_{mne}(X) + \sum_{k(\neq m,n)} H_{kg}(X) \).

From the dynamical point of view, Eqs. (2.1) and (2.2) demonstrate that the electronic energies of the pigments experience modulation by the protein motion. Due to the large number of protein degrees of freedom, such dynamical modulation can be modeled as random fluctuations. In order to describe fluctuations in electronic states, we introduce the collective energy gap coordinate \( \beta \),
\[ u_{m} = H_{mne}(X) - H_{mg}(X) - h\Omega_{m} = -\sum_{\zeta} h\omega_{\zeta} d_{m\xi} q_{m\xi}. \]
Because \( \{q_{m\xi}\} \) are normal mode coordinates or phonon modes, the dynamics of \( \tilde{u}_{m}(t) \equiv e^{iH_{mg}t/h}u_{m}e^{-iH_{mg}t/h} \) can be described as a Gaussian process [34]. Therefore, all environment-induced relaxation processes can be quantified by two-body correlation functions of \( \tilde{u}_{m}(t) \). Fluctuations in the electronic energy of the \( m \)th pigment are described by the symmetrized correlation function of \( \tilde{u}_{m}(t) \),
\[ S_{m}(t) = \frac{1}{2} \langle \tilde{u}_{m}(t)\tilde{u}_{m}(0) + \tilde{u}_{m}(0)\tilde{u}_{m}(t) \rangle_{mg}. \]
where \( \langle \cdots \rangle_{mg} \) denotes averaging over \( \rho_{mg} \equiv e^{-\beta H_{mg}}/\text{Tr}[e^{-\beta H_{mg}}] \) with \( \beta \) being inverse temperature. After electronic excitation in accordance with the vertical Franck-Condon transition, reorganization takes place from the excited nuclear configuration with respect to the electronic ground state \( |\varphi_{mne}\rangle \) to the actual equilibrium configuration in the excited state \( |\varphi_{mne}\rangle \) with dissipation of the reorganization energy \( h\lambda_{m} \). This reorganization proceeds on a characteristic timescale \( \tau_{m} \).

![FIG. 2: Schematic illustration of the electronic ground and excited states of the \( m \)th pigment, \( |\varphi_{mne}\rangle \) and \( |\varphi_{mne}\rangle \), affected by nuclear motion of the protein environment. After electronic excitation in accordance with the vertical Franck-Condon transition, reorganization takes place from the equilibrium nuclear configuration with respect to the electronic ground state \( |\varphi_{mne}\rangle \) to the actual equilibrium configuration in the excited state \( |\varphi_{mne}\rangle \) with dissipation of the reorganization energy \( h\lambda_{m} \). This reorganization proceeds on a characteristic timescale \( \tau_{m} \).](image)

The reorganization process, in principle, can be measured by the time-dependent fluorescence Stokes shift experiment [16, 35], where the direct observable quantity is the relaxation function defined by
\[ \Gamma_{m}(t) = \int_{0}^{\infty} ds \chi_{m}(s). \]

The reorganization energy \( h\lambda_{m} \), which can be measured via the Stokes shift magnitude, is given as \( \Gamma_{m}(0) = 2h\lambda_{m} \), and the characteristic timescale of the reorganization dynamics is given as \( \tau_{m} = \int_{0}^{\infty} dt \Gamma_{m}(t)/\Gamma_{m}(0) \). When the environmental degrees of freedom can be described classically, the symmetrized correlation, response, and relaxation functions satisfy the classical fluctuation-dissipation relation as [34]
\[ S_{m}(t) = \frac{1}{\beta} \Gamma_{m}(t), \quad \chi_{m}(t) = -\frac{d}{dt}\Gamma_{m}(t). \]
We note that the quantum fluctuation-dissipation relation [34] needs to be employed for low-temperature systems where the classical fluctuation-dissipation relation breaks down. These two-point correlation functions play a significant role in analyses of condensed phase laser spectroscopic signals such as 2D spectra, as will be discussed later.

The electronic coupling $h \lambda_m$, the reorganization energy $h \lambda_m$, and the characteristic timescale of the reorganization dynamics $\tau_m^{\text{ren}}$ are three fundamental quantities determining the nature of EET in photosynthetic PPCs. Ordinarily, photosynthetic EET is discussed only in terms of the mutual relation between magnitudes of the electronic coupling and the reorganization energy. If the electronic coupling is small in comparison with the reorganization energy, then the electronic coupling can be treated perturbatively. This treatment yields Förster theory [36]. In the opposite limit, it is possible to describe the interaction between electronic excitation and the environment, which is characterized by the reorganization energy, in a perturbative manner to derive quantum master equations such as Redfield theory [37]. However, we should not overlook that the nature of EET is also dependent on the mutual relation between two timescales, $\tau_m^{\text{ren}}$ and $J_m^{-1}$ [16, 33]. The inverse of the electronic coupling, $J_m^{-1}$, defines the time an electronic excitation needs to move from one pigment to another. As was emphasized in Refs. [6, 16, 38], the relation between $J_m^{-1}$ and $\tau_m^{\text{ren}}$ is more fundamental for the nature of EET than that between $h \lambda_m$ and $h \lambda_m$. In the case of $\tau_m^{\text{ren}} \ll J_m^{-1}$, it is difficult to construct a wave function straddling multiple pigments. The environmental reorganization introduces fast equilibration of the environmental configurations, and hence EET occurs after the environmental equilibration associated with the excited pigment. In this situation, EET is described as a diffusive motion similar to classical random walk; it follows classical rate laws where the transition rate is given by Förster theory. In the opposite case of $\tau_m^{\text{ren}} \gg J_m^{-1}$, the excitation can travel almost freely from one pigment to others through a photosynthetic complex as a quantum mechanical wave packet keeping its phase coherence. Thus, this EET process is termed coherent energy transfer. Obviously, there exists a regime of EET where the two coupling magnitudes and/or the two timescales compete against each other, i.e. $\lambda_m \sim \tau_m^{\text{ren}}$ and/or $\lambda_m \sim J_m^{-1}$. This intermediate regime is typical for photosynthetic EET [11, 16, 31]. In this situation, a distinct characteristic is not straightforward because characteristics of both coherent energy transfer and diffusive motion coexist. In order to address the issue, two of the authors developed a formally exact solution from the second-order cumulant time-nonlocal quantum master equation which reduces to the conventional Förster and Redfield theories in their respective limit of validity [6]. In the regime of coherent wavelike motion the equation predicts several times longer lifetime of quantum coherence between electronic excited states of the pigments than does the conventional Redfield equation. Reference [6] demonstrated that quantum coherent motion can be observed even when reorganization energy is large in comparison to intersite electronic coupling, although this situation has often been regarded as the regime of incoherent hopping diffusive motion. This will be discussed in more detail in Sec. V.C.

One of the viable approaches to observe EET dynamics in photosynthetic PPCs is optical spectroscopy. As molecular systems become increasingly complex, conventional optical spectroscopic techniques such as time- or frequency-resolved absorption spectroscopy or spontaneous emission spectroscopy become of limited use for extraction of direct information on the molecular properties such as site energies, $h \Omega_m$, electronic couplings, $h J_m$, environmental reorganization energies, $h \lambda_m$, timescales of environmental reorganization dynamics, $\tau_m^{\text{ren}}$, and the EET dynamics induced by these quantities in photosynthetic PPCs. This is because conventional one-dimensional (1D) spectroscopies, which project all the information onto a single time- or frequency-axis, are not capable of separating the variety of structural and dynamic contributions present on the 1D axis. In order to disentangle the 1D signals, one needs to explore multidimensional observables, which project the structural and dynamic information onto multiple axes. In this regard, 2DES, based on the heterodyne-detected four-wave mixing technique, can provide us with far more detailed information on photosynthetic PPCs.

III. FOUNDATIONS OF TWO-DIMENSIONAL ELECTRONIC SPECTROSCOPY

A. Nonlinear optical responses

In third-order four-wave mixing techniques, the incoming electric field can be written in the form [39, 40],

$$E(t) = \sum_{i=1}^{3} \tilde{E}_i A_i (t - \tau_i) e^{-i \omega_i (t - \tau_i)} \hat{e}_i + \text{c.c.},$$

(3.1)

where the laser pulse centered at time $\tau_i$ ($\tau_1 \leq \tau_2 \leq \tau_3$) is linearly polarized along the direction characterized by the unit vector $\hat{e}_i$. In Eq. (3.1), $\omega_i$, $\hat{e}_i$, $A_i (t)$ and $\phi_i$ represent the carrier frequency, wavevector, temporal envelop, and phase of the $i$th field, respectively. The third-order polarization per unit volume, $P^{(3)}(t)$, can be expressed as [1]

$$P^{(3)}(t) = n_{\text{PPC}} \int_0^\infty dt_3 \int_0^\infty dt_2 \int_0^\infty dt_1 \Phi(t_3, t_2, t_1) \times E(t - t_3) E(t - t_3 - t_2) E(t - t_3 - t_2 - t_1),$$

(3.2)

with $n_{\text{PPC}}$ being the number density of PPCs. $\Phi(t_3, t_2, t_1)$ is a forth-rank tensor indicating the third-
order response function expressed as

\[ \Phi(t_3,t_2,t_1) = \left( \frac{i}{\hbar} \right)^3 \times \left( \mu(t_1 + t_2 + t_3), \mu(t_1 + t_2), \mu(t_1) \right) \rho_{\text{PPC}}^{\text{eq}}, \]

(3.3)

where \( \mu(t) \equiv \delta_{\text{HERC}/t} / \mu \) has been introduced. In what follows we ignore \( \hbar^3 \) in the denominator and \( n_{\text{PPC}} \) for simplicity of the expression. Due to the three-times commutations, the third-order response function contains \( 2^3 = 8 \) contributions (\( 2^3 = 8 \) different Liouville space pathways),

\[ \Phi(t_3,t_2,t_1) = i^3 \sum_{\alpha=1}^4 \left[ R_{\alpha}(t_3,t_2,t_1) - R_{\alpha}^*(t_3,t_2,t_1) \right] \]

(3.4)

where we have defined the four-point correlation function of \( \mu(t) \) as

\[ F(t_3,t_2,t_1) = \langle \mu(t_3) \mu(t_2) \mu(t_1) \rangle \rho_{\text{PPC}}^{\text{eq}}. \]

(3.5)

The macroscopic polarization in Eq. (3.2) generates electric fields in the phase-matching directions of \( k_s = \pm k_1 \pm k_2 \pm k_3 \),

\[ E_{\text{sig}}(t) \propto i P^{(3)}(t). \]

(3.6)

In the heterodyne detection scheme, an additional electric field called the local oscillator is applied, \( E_{\text{LO}}(t) e^{i \phi_{\text{LO}}} \) with \( \phi_{\text{LO}} \) being a controllable phase parameter, and then the detected signal is given as \[ S = \int_0^{\infty} dt E_{\text{LO}}^*(t) \cdot E_{\text{sig}}(t). \]

(3.7)

Here we consider the local oscillator to be an impulsive field \( E_{\text{LO}}(t) \equiv \delta(t - t_m) \), where the pulse centered at time \( t = t_m \) is linearly polarized along the direction characterized by the unit vector \( \hat{E}_{\text{LO}} \). Thus, the detected signal can be a function of a time variable as

\[ S(t_m) = \hat{E}_{\text{LO}} \cdot i P^{(3)}(t_m). \]

(3.8)

B. 2D electronic photon echo spectroscopy

Photon echo (PE) spectroscopy is an optical counterpart of the spin echo in nuclear magnetic resonance (NMR) spectroscopy [35, 41-47]. Originally based on pump-probe, i.e. dynamic hole-burning spectroscopy [17], this is one of the most widely used 2D infrared/electronic spectroscopic techniques. Using the above third-order response function theory, one can formulate the photon echo polarization and signal electric field. Typically photon echo spectroscopy measures the echo signal field emitted in the direction of \( k_1 = -k_1 + k_2 + k_3 \) for the incident laser pulses in Eq. (3.1). In what follows time zero is set at the center of the third pulse, i.e. \( \tau_3 = 0 \). It is customary to define the coherence period \( \tau \) as the separation between the centers of the first two pulses, \( \tau \equiv \tau_2 - \tau_1 \geq 0 \), and the waiting period \( T \) as the difference between the second and third pulses, \( T \equiv -\tau_2 \geq 0 \) to ensure pulse ordering.

The expression for the incident laser pulses in Eq. (3.1) is fairly general for an arbitrary nondegenerate photon echo spectroscopy. Two different frequencies \( \omega_1 = \omega_3 \neq \omega_2 \) were employed for a two-color electronic coherence photon echo experiment [14], while \( \omega_1 = \omega_2 \neq \omega_3 \) was adopted for the two-color three pulse echo peak shift experiment [48-52]. In addition, two-color 2D infrared/electronic photon echo spectroscopies were implemented [53, 54]. However, most photon echo experiments have been performed by using pulses with the same center frequency \( \omega_1 = \omega_2 = \omega_3 \equiv \omega_0 \) (the degenerate limit). The degenerate photon echo polarization \( P_{\text{PE}} \) is dependent on the controllable parameters \( (T, \tau) \) in addition to \( t \), and then is given as

\[ P_{\text{PE}}(t, T, \tau) = e^{-i \omega_0 t + i \omega_0 \tau} \int_0^{\infty} dt_3 \int_0^{\infty} dt_2 \int_0^{\infty} dt_1 \times \phi(t_3, t_2, t_1) E_{\text{LO}} A_3(t - t_3) E_{\text{LO}} A_2(t + T - t_3 - t_2) \times E_1 A_1^*(t + T + \tau - t_3 - t_2 - t_1) e^{i \omega_0 (t_3 - t_2 - t_1)}, \]

(3.10)

where the wavevector part, \( \exp[i(\mathbf{k}_1 + \mathbf{k}_2 - \mathbf{k}_3) \cdot \mathbf{r}] \), and the phase part, \( \exp[i(\phi_3 + \phi_2 - \phi_1)] \), have been ignored for simplicity of the expression. In Sec. IV, the phase part will be discussed in more detail.

The third-order response function consists of eight different contributions as shown in Eqs. (3.4) and (3.5), each of which has a different coherent oscillating pattern in time. If the laser frequency \( \omega_0 \) approximately matches an electronic transition frequency in the PPC of interest, some of the phase factors originating from the response function may cancel with those originating from the laser field. For instance, the coherence oscillation term of \( \phi(t_3, t_2, t_1) \) is \( \exp(-i \Omega_{\text{eg}} t_3 + i \Omega_{\text{eg}} t_1) \) with \( \Omega_{\text{eg}} \) being a characteristic optical transition frequency of the PPC. Therefore, this contribution cancels with the electric field component oscillating as \( \exp(i \omega_0 t_3 - i \omega_0 t_1) \) when \( \omega \equiv \Omega_{\text{eg}} \). Thus, under the integration in Eq. (3.10) we would have slowly varying terms where oscillatory factors canceled and fast oscillating terms where phase factors added. After the integration, oscillatory terms result in a much smaller contribution than slowly varying ones, and we can neglect them. This is usually referred to as rotating wave approximation (RWA). In Eq. (3.10) under the RWA, the surviving components among the eight different response function contributions are only \( \phi(t_3, t_2, t_1), \phi(t_3, t_2, t_1) \) and \( \phi^*(t_3, t_2, t_1) \), which are classified to be the rephasing pathways. Thus, the
rephasing echo signal \( S_R(t, T, \tau) \equiv \hat{E}_{\text{LO}} \cdot i \hat{P}_{\text{PE}}(t, T, \tau > 0) \) can be expressed as
\[
S_R(t, T, \tau) = [R_2(t, t_2, t_1) + R_3(t_2, t_2, t_1)]
- R^*_1(t, t_2, t_1)] \hat{E}_{\text{LO}} \cdot \hat{E}_3 \cdot \hat{E}_2 \cdot \hat{E}_1,
\]
where we have employed the impulsive limit of \( A_i(t - \tau) \simeq \delta(t - \tau) \). In the above, the rephasing echo spectroscopy was discussed, where the echo signal field propagating in the direction of \( \mathbf{k}_1 = - \mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3 \) was measured. One can also selectively measure the terms \( R_1, R_4 \), and \( R^*_2 \) with coherence oscillation \( \exp(-i \Omega_{\text{PE}} \tau_3 - i \Omega_{\text{PE}} \tau_1) \) by focusing on the emitted signal in the direction of \( \mathbf{k}_1 = \mathbf{k}_1 - \mathbf{k}_2 + \mathbf{k}_3 \) instead. This has been called the nonrephasing photon echo (NR-PE) measurement. The degenerate nonrephasing photon echo signal \( S_{\text{NR}}(t, T, \tau) \equiv \hat{E}_{\text{LO}} \cdot i \hat{P}_{\text{NR-PE}}(t, T, \tau > 0) \) in the impulsive limit is then given as
\[
S_{\text{NR}}(t, T, \tau) = [R_1(t, T, \tau) + R_4(t, T, \tau)]
- R^*_1(t, T, \tau)] \hat{E}_{\text{LO}} \cdot \hat{E}_3 \cdot \hat{E}_2 \cdot \hat{E}_1.
\]
Experimentally, one does not detect the polarizations of Eqs. (3.11) and (3.12) directly as a function of \( t \) but instead with a spectral interferogram as a function of the conjugate frequency \( \omega_i \), i.e. \( S\bar{h}(\omega_i, T, \tau) \) and \( S_{\text{NR}}(\omega_i, T, \tau) \). Mathematically, however, there are three possibilities of double Fourier-Laplace transform of the echo field by choosing a pair of time variables from \( \tau \), \( T \), and \( t \). The most popular choice has been to take \( \tau \) and \( t \) variables for the transform, and thus 2D rephasing spectrum and 2D nonrephasing spectrum are given as
\[
S_R(\omega_i, T, \omega_T) = \int_0^\infty dt \int_0^\infty d\tau e^{i \omega_T \tau} S_R(t, T, \tau),
\]
(3.13)
\[
S_{\text{NR}}(\omega_i, T, \omega_T) = \int_0^\infty dt \int_0^\infty d\tau e^{i \omega_T \tau} S_{\text{NR}}(t, T, \tau),
\]
(3.14)
which allow us to explore electronically excited states in photosynthetic PPC and their dynamics during the waiting time \( T \).

In practice, the rephasing and nonrephasing echo signals can be detected in the same direction \(- \mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3\) by exchanging the time-ordering of the first two incident pulses, \( \mathbf{k}_1 \) and \( \mathbf{k}_2 \), in Eq. (3.1). By redefining \( T \equiv \min(-\tau_2, -\tau_1) \geq 0 \) and \( \tau \equiv \tau_2 - \tau_1 \), \( P_{\text{PE}}(t, T, \tau > 0) \) is the rephasing photon echo polarization whereas \( P_{\text{PE}}(t, T, \tau < 0) \) corresponds to the nonrephasing photon echo polarization. Therefore, a 2D photon echo spectrum can be obtained as [39, 40, 55]
\[
S_{\text{PE}}(\omega_i, T, \omega_T) = \int_0^\infty dt e^{i \omega_i t} \int_{-\infty}^\infty d\tau e^{-i \omega_T \tau} \hat{E}_{\text{LO}} \cdot \hat{P}_{\text{PE}}(t, T, \tau)
\]
(3.15)
for positive \( \omega_i \) and \( \omega_T \). It should be noted that the lower-limit of the \( \tau \)-integration is \(-\infty \) instead of \( 0 \). This 2D photon echo spectrum is identical to 2D correlation spectrum defined as the sum of the 2D rephasing and nonrephasing spectra [56, 57],
\[
S_{\text{PE}}(\omega_i, T, \omega_T) = S_R(\omega_i, T, \omega_T) + S_{\text{NR}}(\omega_i, T, \omega_T).
\]
(3.16)

\section{2D lineshape of a monomer}

In order to clarify the information contained in 2D rephasing, nonrephasing, and photon echo spectra, it is advisable to begin by examining the 2D lineshapes of a monomer. Because of the Gaussian process of the collective energy gap coordinate in Eq. (2.7), the four-body correlation function given in Eq. (3.6) for the \( m \)th pigment can be written as [1]
\[
F(\tau_4, \tau_3, \tau_2, \tau_1) \hat{E}_{\text{LO}} \cdot \hat{E}_3 \cdot \hat{E}_2 \cdot \hat{E}_1 = e^{-i H_m (\tau_4 - \tau_3 + \tau_2 - \tau_1) + f_m (\tau_4, \tau_3, \tau_2, \tau_1)},
\]
(3.17)
where we have ignored the constant prefactor, and the auxiliary function \( f_m \) has been introduced as
\[
f_m (\tau_4, \tau_3, \tau_2, \tau_1) = -g_m (\tau_4 - \tau_3) + g_m (\tau_4 - \tau_2) - g_m (\tau_4 - \tau_1)
- g_m (\tau_3 - \tau_2) + g_m (\tau_3 - \tau_1) - g_m (\tau_2 - \tau_1).
\]
(3.18)
in terms of the linebroadening function,
\[
g_m (t) = \frac{1}{h^2} \int_0^t ds_1 \int_0^{s_1} ds_2 \left[ S_m (s_2) - \frac{1}{2} \chi_m (s_2) \right].
\]
(3.19)
To calculate the linebroadening function, we employ the overdamped Brownian oscillator model \( \Gamma_m (t) = 2 h \lambda_m e^{-t^2/\tau_m^{\text{exc}}} \), which has been successfully applied for theoretical analyses of experimental results [2, 7, 12, 58, 59]. From the classical fluctuation-dissipation relation in Eq. (2.11), the symmetrized correlation and response functions are evaluated as \( S_m (t) = h^2 \Delta_m^2 e^{-t^2/\tau_m^{\text{exc}}} \) and \( \chi_m (t) = (2 h \lambda_m/\tau_m^{\text{exc}}) e^{-t^2/\tau_m^{\text{exc}}} \), where \( h\Delta_m = h/2 \lambda_m/\hbar \beta \) stands for the root-mean-square amplitude of the electronic energy fluctuations of the \( m \)th pigment. Consequently, the linebroadening function can be expressed in the form,
\[
g_m (t) = \left[ (\Delta_m \tau_m^{\text{exc}})^2 - i \lambda_m \tau_m^{\text{exc}} \right] \left( e^{-t^2/\tau_m^{\text{exc}}} + t/\tau_m^{\text{exc}} - 1 \right).
\]
(3.20)
For simplicity, we focus on a situation where \( \tau_m^{\text{exc}} \) is much faster than the timescale of interest. In this Markov limit, Eq. (3.20) is approximated as a linear function of time variable,
\[
g_m (t) \simeq (\Delta_m^2 \tau_m^{\text{exc}} - i \lambda_m) t,
\]
(3.21)
and thus the rephasing echo signal in the impulsive limit are expressed as

\[ S_R(t, T, \tau) = 2e^{-i\Omega_m t - g_m(t)}e^{i\Omega_m \tau - g_m(\tau)}, \]  

(3.22)

where the \( R \) Liouville space pathway does not contribute for the case of a two-level transition. It should be noted that \( t, T, \) and \( \tau \) dependences are separable only when the linebroadening function is expressed as a linear function of the time variable. This time-domain signal is converted into a complex 2D spectrum by a double Fourier-Laplace transform with respect to \( t \) and \( \tau \). By separating the absorptive and dispersive contributions, the 2D spectrum is expressed as

\[ S_R(\omega_t, T, \omega_\tau) = 2[a(\omega_t)a(\omega_\tau) + d(\omega_t)d(\omega_\tau)] - i2[d(\omega_t)a(\omega_\tau) - a(\omega_t)d(\omega_\tau)] \]  

(3.23)

in terms of the absorption and dispersive components,

\[ a(\omega) = \frac{\Delta^2_{m, \text{reph}}}{(\omega - \Omega^0_m)^2 + (\Delta^2_{m, \text{reph}})^2}, \]  

(3.24)

\[ d(\omega) = \frac{-\omega + \Omega^0_m}{(\omega - \Omega^0_m)^2 + (\Delta^2_{m, \text{reph}})^2}, \]  

(3.25)

respectively, where \( \Omega^0_m = \Omega_m - \lambda_m \) has been introduced. See Fig. 2. The real part of \( S_R(\omega_t, T, \omega_\tau) \) has a ‘mixed phase’ [41] because it consists of a superposition of a pure 2D absorptive peak and a pure 2D dispersive peak. This 2D lineshape is referred to as phase-twist in the literature of 2D NMR [60]. It should be noticed that the decay of the dispersive components is much slower than that of the absorptive components, and thus it is ambiguous to extract accurate information from the phase-twisted lineshapes of 2D rephasing spectra for complex systems such as photosynthetic PPCs. It is difficult to distinguish absorptive features from dispersive contributions because the phase-twisted lines sometimes look like absorptive off-diagonal peaks in multi-component 2D spectra, as shown in Fig. 3. Suppression of the dispersive component is therefore desirable for improved resolution. On the other hand, the nonrephasing echo signal in the impulsive limit, Eq. (3.12), is expressed as

\[ S_{NR}(t, T, \tau) = 2e^{-i\Omega_m t - g_m(t)}e^{-i\Omega_m \tau - g_m(\tau)}, \]  

(3.26)

which delivers the 2D nonrephasing spectrum as

\[ S_{NR}(\omega_t, T, \omega_\tau) = 2[a(\omega_t)a(\omega_\tau) - d(\omega_t)d(\omega_\tau)] - i2[d(\omega_t)a(\omega_\tau) + a(\omega_t)d(\omega_\tau)]. \]  

(3.27)

The real part of the 2D nonrephasing spectrum is also contaminated by the phase-twisted lineshape due to the dispersive component. Comparing the rephasing and nonrephasing spectra in Eqs. (3.23) and (3.27), however, we can observe that the dispersive components in the real parts of the two spectra exactly cancel out, and thus we arrive at

\[ S_{FE}(\omega_t, T, \omega_\tau) \propto a(\omega_t)a(\omega_\tau) - i d(\omega_t)a(\omega_\tau). \]  

(3.28)

In summary, the 2D photon echo spectrum or 2D correlation spectrum provides us with a pure absorptive peak [56, 57], in contrast to the 2D rephasing and nonrephasing spectra.

IV. EXPERIMENTAL BACKGROUND

In order to describe experimental implementations of 2DES, we begin by summarizing the experiment using the two apparatuses in our laboratory as an example system. We then discuss which experimental considerations and requirements are more stringent than in conventional ultrafast techniques.

A. Overview of 2D Spectroscopic Measurement

As noted above, 2DES is a four-wave-mixing technique, where three interactions between the incident laser fields with the sample induce emission of a signal field. The experiment can be performed with a variety of pulse geometries, methods of delay control, and pulse orderings. Schematics of two apparatuses in our laboratory are shown in Fig. 4.

The temporal sequence of the incident pulses is shown in Fig. 5a. In order to produce a 2D spectrum, four pulses impinge on the sample. Pulses 1, 2 and 3 generate the signal and pulse 4, called the local oscillator (LO), is used for heterodyne detection. As was described in Sec. III, the delay between pulses 1 and 2, \( \tau \), is known as the coherence time, between pulses 2 and 3, \( T \), is known as the waiting time, and between pulses 3 and the signal emission, \( t \), is known as the detection time. A Fourier-transform across \( \tau \) and \( t \) for a given value of \( T \) produces a 2D spectrum, which is a correlation map of excitation and emission energies as a function of the time, \( T \), between excitation and emission events.
The excitation frequency is measured indirectly by scanning in time. Pulses 1 and 2 prepare, or excite, the system. When pulse 1 impinges on the sample, the oscillation of the electric field under the envelope of the laser pulse induces an oscillation of the transition dipoles within the ensemble of molecules in the sample, or a coherence |g⟩⟨e|. The frequency of the oscillation of each member of the ensemble is proportional to the excited state energy, $\hbar \omega_a$, because every element contributing to the ensemble propagates with its phase factor, $e^{i\theta_a \tau}$. The collective oscillation of the phases of the transition dipoles is shown in Fig. 5a, bottom. Scanning the coherence time samples this collective oscillation because it imprints phase onto the signal with increasing $\tau$. Interaction of the sample with the second laser pulse stops the oscillation between the ground and excited states. Phase can accumulate during the waiting time, but only from coherence pathways, which will be discussed in Sec. V.C. After excitation, or interaction with pulses 1 and 2, the system evolves until the arrival of pulse 3. The waiting time is the period over which the photophysics of the system are monitored. The arrival of the third pulse again produces a coherence in the ensemble, $|e⟩⟨g|$. The resulting oscillatory macroscopic polarization within the sample drives the emission of an electric field, the signal field. Methods of isolating the signal field, which depends on interactions with all three incident beams, from other emitted electric fields will be discussed in Sec. IV.C.

In the experimental implementation of 2DES, the data is collected in the time domain along the $\tau$ axis, which is Fourier-transformed to produce the excitation frequencies. In most apparatuses, the Fourier-transform of the $t$ axis into the frequency domain, however, is performed experimentally by frequency-dispersing the collinear signal and local oscillator and detecting by spectral interferometry. The detected interference, which oscillates in amplitude along $\tau$ and exhibits spectral fringes along $\omega_t$, is depicted in Fig. 5b. In Fig. 5c, an example of raw 2D data is presented in which the oscillations along both axes are visible in the intensity of the detected signal. A Fourier-transform across the $\tau$ axis generates the 2D spectrum as shown in Sec. III.

The example implementations in Fig. 4 use a fully non-collinear geometry [61]. Figure 4a shows an approach with mechanical delays and pairwise phase stability from a diffractive-optic based approach [61, 62]. The laser beam is first split by a beam splitter, after which the two pulses are delayed relative to each other by a retroreflector on a delay stage. The two beams are focused onto a diffractive optic optimized for ±1 orders, which generates
two pulse pairs. Glass wedges inserted into beams 1 and 2 introduce delays with attosecond precision. The signal emerges collinear with the local oscillator and the two beams are spectrally dispersed and detected on a charge coupled device in the frequency domain. Figure 4b illustrates a pulse-shaper based implementation based on Ref. [63]. Delays are introduced by phase applied with a reflective 2D spatial light modulator and the four pulses are selected from an expanded laser beam with a spatial mask. The signal is detected as in Fig. 4a.

B. Phase stability

1. Phase in 2D spectroscopy

2D spectroscopy is a measurement technique that recovers a complex-valued signal electric field by detecting at the amplitude, not intensity, level [39]. Determining the phase of the electric field separates the real (absorptive) and imaginary (dispersive) contributions to 2D spectra. In order to prevent mixing of the real and imaginary components, the two beams used to scan a Fourier-transformed axis must be phase stable, i.e. there must be pairwise phase stability. The measured signal is proportional to the complex-valued, third order polarization, which depends on the phase of the incident electric fields in the following way,

\[ D(\omega_1, \omega_2, \omega_3) = e^{-i\phi_1} e^{i\phi_2} e^{i\phi_3} \]  

where \( \phi_i \) is the phase of the ith field as defined in Eq. (3.1). Acquired phase or phase errors, \( \delta \phi \), in the laser fields which generate the signal become mapped onto the measured polarization. As this is a heterodyne experiment, the relationship between the interferometrically detected phase and the phase \( \phi_{\text{sig}} \) has the following form [62],

\[ \Delta \phi_{\text{detected}} = \Delta \phi + \phi_{\text{LO}} + \phi_{\text{sig}} \]

which illustrates why pairwise phase stability is sufficient to remove phase errors. Therefore, any phase difference between pulse pairs introduced by interferometer imperfections in the setup imprints onto the signal, and in this way mixes the real and imaginary parts of the generated 2D spectrum [55].

There are both active and passive approaches to generate phase stable pulse pairs. Active phase stabilization methods involve running HeNe tracer beams across each arm of the 2D spectrometer and continuously correcting for path length errors [64, 65]. This requires interferometers between each arm. Passive phase stabilization methods include diffractive-optic approaches [61, 62], approaches with conventional optics where phase errors cancel [66], and fully or partly collinear geometries where phase cycling isolates the signal [67, 68]. For adaptive-optic based apparatuses, using partially or fully collinear geometries can impede the introduction of phase errors [68]. 2DES apparatuses generally have phase stability in the range of \( \lambda/60 \) to \( \lambda/95 \) over many hours [61, 62, 66].

2. Detection of complex signals

**Heterodyne Detection.** Heterodyne, or interferometric, detection has been described extensively in several references and is a method for measuring a complex field [1, 69]. In most 2D apparatuses, measurement occurs by spectrally dispersing the collinear signal and local oscillator fields and recording in the frequency domain. The frequency domain, heterodyne-detected signal is the square modulus of the sum of the two fields,

\[ I(\tau_0, T_0, \omega_1) = |E_{\text{LO}}(\omega_1)e^{i\omega_1\Delta t} + E_{\text{sig}}(\tau_0, T_0, \omega_1)|^2 \]

\[ = |E_{\text{LO}}(\omega_1)|^2 + E_{\text{LO}}^*(\tau_0, T_0, \omega_1)E_{\text{LO}}(\omega_1)e^{i\omega_1\Delta t} \]

\[ + E_{\text{LO}}(\tau_0, T_0, \omega_1)E_{\text{LO}}(\omega_1)e^{-i\omega_1\Delta t} \]

\[ + |E_{\text{LO}}(\tau_0, T_0, \omega_1)|^2 \]  

(4.3)

One of the non-interferometric components, \( |E_{\text{LO}}|^2 \), can be measured separately and subtracted. The final term, which scales quadratically with the low amplitude signal field, becomes vanishingly small and so can be ignored [40]. The interference fringes are illustrated along the \( \omega_1 \) axis of Figs. 5b and c. They can be written as 2Re[\( E_{\text{sig}}(\tau_0, T_0, \omega_1)E_{\text{LO}}(\omega_1) \cos (\Delta \phi(\omega_1)) \)], and so the interference term oscillates with the phase difference between the two fields. From this information, the total characterization of a field distribution, or the separation of the absorptive (real) and dispersive (imaginary) components, can be completed using a known reference field [1]. That is, the frequency domain fringes in the cross term contain information about the phase of the signal with respect to the local oscillator [69]. The frequency of the fringes \( \Delta \phi \) is the sum of acquired phase from the time delay, \( \omega_1 \Delta t \), and from the phase difference, \( \phi_{\text{LO}} - \phi_{\text{sig}} \), between the signal and the local oscillator. Because the temporal offset can be independently measured, the phase of the signal can be recovered.

In a 2D experiment, heterodyne detection offers two primary advantages over homodyne, or intensity, detection. The first advantage is the separation of the real and imaginary components of the emitted field. The second is that simultaneous detection of two co-propagating fields aids in measuring a low amplitude signal. The magnitude of the cross terms scale linearly with the signal and with the local oscillator. Because the amplitude of the local oscillator can be set to an easily detectable level, the mixed term essentially enhances a low amplitude signal.

**Retrieval of absolute phase.** In the case of a perfectly functioning apparatus, with no phase offset between beams, precise time delays, and no errors in the arms of the interferometer, the heterodyne detected signal has the correct absolute phase [58], meaning no mixing of the real and imaginary components.
This accuracy and precision is very challenging to achieve experimentally. Both phase jitter underneath the pulse envelope and small temporal distortions manifest as phase errors in the detected signal. As can be seen from the form of the electric field given in Eq. (3.1), a timing error $\Delta t$ has a similar effect to a phase error $\Delta \phi$, because it imprints phase $\omega \Delta t$. Thus, subwavelength temporal accuracy is required to maintain phase stability [70]. For example, with the 660 nm pulses, as used in the experiments described in Sec. V, an optical cycle has a duration of 2.2 fs. Therefore, a 1 fs error produces a phase error of almost $\pi$. This then requires the following temporal condition for phase stability,

$$-\Delta t_1 + \Delta t_2 + \Delta t_3 - \Delta t_{LO} = 0$$

Experimental apparatuses often contain differences in lengths of the arms of the interferometer, jitter in the arms of the interferometer, small errors in time steps, and independent fluctuations of the four beam paths which destroy phase stability [40]. There are experimental and analytical methods to correct for constant phase errors, which will be discussed in Sec. IV B 4.

3. Retrieval of Excitation Energies

During the coherence time, the system evolves in a coherency, so for a given $\tau_0$, the system acquires a phase $e^{i\omega_\tau \cdot \tau_0}$ as illustrated in Fig. 5a, bottom. Because the experiment is performed on an inhomogeneously broadened ensemble, the slightly different excited state energy of each member produces an oscillation at a slightly different frequency, which causes the depopulation in the collective oscillation shown in Fig. 5a, bottom. Thus, for a given value $\omega_\tau$, the interference fringes exhibit an oscillation in amplitude along $\tau$ as shown by the dashed line in Fig. 5b, where for each additional increment $\Delta \tau = 2\pi / \omega_\tau$, the signal undergoes a full phase rotation. This resultant oscillation in signal amplitude can also be seen along the $\tau$ axis in the raw data shown in Fig. 5c. A Fourier-transform across the $\tau$ axis retrieves frequencies $\omega_\tau$ and generates the 2D spectrum. Because the excitation energy is retrieved by controlling the time that the dipole within the sample undergoes a collective oscillation, the measurement requires highly accurate time delays and phase stable pulses. The phase stability is required because an additional phase offset will imprint phase onto the signal similarly to an additional time increment.

**Time delays** In this experiment, the time delay between beams 1 and 2 must be set with higher accuracy and precision than in other techniques. Because scanning this time delay constructs a temporal axis which is then Fourier-transformed to generate frequencies, errors in temporal sampling produce errors in the frequency domain information. Sub-wavelength accuracy is required to retrieve excitation frequencies without the introduction of Fourier artifacts.

The method by which temporal delays are introduced also varies among 2D apparatuses. In Fig. 1, the introduction of controllable amount of glass using wedges on stepper motors allows < 50 attosecond precision, which is more precise than with conventional delay methods. In Fig. 1b, the pulses are delayed by the application of linear phase using the SLM, which provides 10-50 as temporal resolution [63].

Because of the short period of an optical cycle, reconstructing the frequency information through sampling at the Nyquist frequency requires a very large number of data points. While the precision requirement remains, the number of data points needed can be decreased by two approaches, undersampling or sampling in the rotating frame. For the apparatus in Fig. 1a, the $\tau$ delay is often sampled at approximately an odd multiple of the Nyquist frequency of the excitation frequency. The frequency components are limited to those within the bandwidth of the laser, so the detected signal can be unfolded to retrieve the frequency domain information. This method is described in detail in several references [41, 71]. In this way, temporal step size can be increased by undersampling the time axis and extracting the frequency through aliasing methods [41, 71].

The second approach is to detect within the rotating frame and is the method applied in the apparatus in Fig. 4b. We now describe two ways of thinking about applying delays using the rotating wave approximation. First, in the frequency domain, a time delay manifests as a phase factor, $e^{i\omega \cdot \tau}$. The additional phase accrued, therefore, is linear in frequency. Because an SLM directly controls the phase of the beams, an SLM can be used to essentially offset the additional phase along the frequency axis, which results in terms $e^{i(\omega - \omega_\tau) \cdot \tau}$. The phase offset along the frequency axis is the rotating frame frequency, $\omega_\text{RF}$. Therefore, as additional time delays are applied, the oscillation frequency along the $\tau$ axis decreases.

In the second picture, delays introduced in the rotating frame can be understood as arising from the fact that the phase control introduced by the SLM allows both phase and time delays to be applied. When the temporal delay is introduced, a phase shift is added as well. The additional phase at each $\tau$ is chosen to maintain a constant phase at a selected carrier frequency, and all other frequencies components are delayed relative to the reference frequency. Instead of introducing only delay with $e^{i\omega \cdot \tau}$, both a time and phase component are mapped onto the pulse, $e^{i(\omega_\tau - \omega_\tau) \cdot \tau}$. While delays introduced using conventional techniques introduce delay $e^{i\omega \cdot \tau}$, in rotating wave detection, at each time step, the addition of a phase $\omega \Delta \tau - \Delta \phi$ means that scanning an optical cycle in $\tau$ does not necessarily correspond to a full phase rotation. In the apparatus in Fig. 4b, the oscillatory cycle is often set to 20 fs, which oversamples the oscillations for higher accuracy.
4. Methods to generate correctly phased spectra

2D electronic spectroscopy almost always requires a phase correction factor to account for experimental phase offsets, which arise from problems such as unequal lengths between the two arms, e.g. $\phi_0 = \phi_1 - \phi_2$. In order to maintain phase stability, however, any phase jitter, $\delta\phi$, must occur on both beams, or during the course of data collection $\delta\phi_1 = \delta\phi_2$ and $\delta\phi_3 = \delta\phi_{LO}$. While there are no methods to account for any unequal phase jitter terms, there are several experimental and analytical methods to correct for a constant phase difference. An algorithm-based method has been developed, although it has yet to be widely implemented [72]. The analytical method does, however, offer the potential for significant experimental simplification.

In the passively-stabilized, diffractive optic based setup in Fig. 4a, phase offset in 2D data is corrected by comparing to separately collected pump-probe data [40]. The projection-slice theorem of Fourier transforms states that the projection in one domain is equivalent to a slice in another domain. This equivalency gives rise to a relationship between the pump-probe data and the 2D data [41, 71]. The projection along the $\omega_2$ axis is related to the slice at $\gamma = 0$, which is a pump-probe measurement. The projection slice theorem [39, 41] relates the real component of a projection of the 2D data to spectrally resolved pump-probe data in the following way,

$$S_{\text{pump-probe}}(\omega_1, T_0) = \text{Re} \left[ \omega_1 E_{\text{probe}}(\omega_1) \int_{-\infty}^{\infty} \frac{d\omega_2}{2\pi} S_{\text{FE}}(\omega_1, T_0, \omega_2) \right]$$

(4.5)

where $E_{\text{probe}}(\omega_1)$ is the electric field of the probe pulse. Transient absorption experiments are able to recover the correct phase because pump-probe experiments involve only two pulses, where the first two interactions are both from pulse 1 and the third interaction and the local oscillator are from pulse 2. Therefore, it is an inherently phased measurement. By fitting the projection of a 2D spectrum to the spectrally resolved pump-probe data, the absolute phase of the measured data can be retrieved. A zeroth and first order phase correction term, $e^{i\phi_0 + \omega_1 \Delta t}$, is generally sufficient to match the 2D spectra to the pump-probe data. This approach, however, only works when the spectrum ($\omega_1 = \omega_2, \omega_3 = \omega_{LO}$) and the polarization ($\hat{E}_1 = \hat{E}_2, \hat{E}_3 = \hat{E}_{LO}$) are the same for each pulse pair.

C. Beam geometry

A 2D spectrum is a sum of the selected energy and momentum conserving pathways, $k_s = -k_1 + k_2 + k_3$. The experiment can be performed in fully, partially, or non-collinear beam geometries. In the case of non-collinear geometries, the condition of momentum conservation limits the spectrum from the signal detected in the spatially phase-matched direction to a subset of third order signals, or a subset of molecular processes [1]. The selected third-order signal can be extracted through spatially windowing and detecting using a box geometry. In this geometry, there is background free detection of the generated signal. In the case of fully or partially collinear geometries, phase cycling is used to isolate the signal [68]. In phase cycling, the signal is isolated by a linear combination formed from selectively multiplying each beam or combinations of beams by a phase factor, $e^{i\phi}$. For this process, because the signal has phase dependence on all three excitation pulses, multiplying any odd number of pulses by the phase factor $e^{i\phi}$ flips the phase of the signal. Multiplying an even number of pulses by the phase factor does not flip the phase of the signal. Subtracting the sum of the odd permutations from the even permutations separates the third order signal, which depends on all three incident pulses. A phase cycling sequence can also aid in background removal for fully non-collinear geometries because scattered light does not share the third order phase dependence.

D. Artifacts and spectral phase

In ultrafast experiments, transform-limited compression is often difficult to achieve, particularly for the case of broad bandwidths. Using laser pulses with different orders of spectral phase can lead to various artifacts within a 2D spectrum. Figure 6a-c are experimental, absolute value, correlation 2D spectra of the non-resonant response of sapphire. Three different spectral phases were applied to the four laser pulses using the SLM in the experimental apparatus from Fig. 4b. Figure 6a displays the spectrum in the case of almost transform-limited pulses. In this case, basically the spectral profile of the laser pulse is recovered. For the spectrum in Fig. 6b, a parabolic phase was applied to the laser pulse. It is important to note here that the peak shape generated by the chirped pulse can easily be confused with correlated excited states, or an inhomogeneously broadened peak. For a non-resonant response, however, a correlation is physically impossible. Cubic spectral phase, as shown in Fig. 6c, is characterized by an x-shaped artifact.

For experiments such as pump-probe spectroscopies, after characterizing dispersion in the laser pulses, algorithms have been used to remove the effects of spectral phase [73–75]. In 2D spectroscopy, however, algorithms to remove these artifacts from spectral phase are much more complicated [76]. Each peak within the generated spectrum is a function of four light-matter interactions. The two frequency axes of the spectrum, however, only provide information about the frequency of two of the four interactions. The two unknown frequencies complicate determination of the effect of sub-optimal compression, even for a well-characterized spectral phase.

The spectral phase can change the time delays between the four interactions. Sample Liouville pathways, cor-
II (PSII) supercomplex of higher plants. PSII is a sophisticated natural nano-device, with several associated PPCs, each containing multiple pigments, with dynamics governed by interactions within and between these PPCs. Understanding the molecular machinery and relating structure to function is particularly challenging because of the nonequivalent geometries of the chlorophyll within the PPCs and the resultant rugged energy landscape of plant systems.

A. 2D electronic spectrum of pigment-protein complexes

2D electronic spectroscopy provides an incisive tool to study electronic couplings and electronic energy transfer among pigments. In general, optical spectroscopy provides us with the information projected onto energy eigenstates, and therefore it is common to employ energy eigenstates of electronic excitations, which are termed excitons in the literature. For this purpose, we rewrite the PPC Hamiltonian by substituting Eqs. (2.1)-(2.7) into Eqs. (2.5) and (2.6) as

\[
H_{\text{PPC}}^{(n)} = H_{\text{site}}^{(n)} + H_{\text{site-env}}^{(n)} + H_{\text{env}}^{(n)}.
\]

(5.1)

The first term on the right hand side is the electronic excitation Hamiltonian comprised of the Franck-Condon energies and electronic couplings,

\[
H_{\text{site}}^{(1)} = \hbar \sum_m \Omega_m |m\rangle \langle m| + \hbar \sum_{m,n} J_{mn} |m\rangle \langle n|,
\]

(5.2)

\[
H_{\text{site}}^{(2)} = \hbar \sum_{m,n} (\Omega_m + \Omega_n) |mn\rangle \langle mn| + \hbar \sum_{m,n,k} \left( J_{mk} |mn\rangle \langle kn| + J_{mk} |mn\rangle \langle kn| \right).
\]

(5.3)

The second part in Eq. (5.1) corresponds to the coupling of environmental degrees of freedom to the electronic excitations,

\[
H_{\text{site-env}}^{(1)} = \sum_m u_m |m\rangle \langle m|,
\]

(5.4)

\[
H_{\text{site-env}}^{(2)} = \sum_{m,n} (u_m + u_n) |mn\rangle \langle mn|.
\]

(5.5)

The last term in Eq. (5.1) is a set of normal mode Hamiltonians, i.e. the phonon Hamiltonian expressed as

\[
H_{\text{env}} = \sum \hbar \omega_q (p_q^2 + q_q^2)/2.
\]

To obtain the one- and two-excitation energies and states, the Hamiltonians in Eqs. (5.2) and (5.3) are diagonalized with orthogonal transformation operators \( V \) and \( W \) as

\[
H_{\text{ex}}^{(1)} = V^{-1} H_{\text{site}}^{(1)} V,
\]

(5.6)

\[
H_{\text{ex}}^{(2)} = W^{-1} H_{\text{site}}^{(2)} W.
\]

(5.7)

where the diagonal elements of \( H_{\text{ex}}^{(1)} \) and \( H_{\text{ex}}^{(2)} \) are the eigenenergies in the single- and double-excitation manifolds. The one- and two-excitation states belonging to

V. APPLICATIONS TO PHOTOSYNTHETIC COMPLEXES

As an example of the utility of 2D electronic spectroscopy, we discuss how 2D spectroscopy has been applied to disentangling the function of the Photosystem
the eigenenergies are expressed as
\[ |e_\alpha\rangle \equiv \sum_m V^{-1}_{\alpha m}|m\rangle; \quad H^{(1)}_{ex}(e_\alpha) = \hbar \Omega_\alpha |e_\alpha\rangle, \]  \hspace{1cm} (5.8)
\[ |f_\gamma\rangle \equiv \sum_m W^{-1}_{\gamma mn}|mn\rangle; \quad H^{(2)}_{ex}(f_\gamma) = \hbar \Omega_\gamma |f_\gamma\rangle. \]  \hspace{1cm} (5.9)

We note that Roman letters are employed for the site representation while Greek letters are used for the exciton representation. Once the eigensystems are determined, the exciton transition dipoles can be expressed as linear combinations of pigments’ transition dipoles,
\[ \mu_{\alpha 0} = \langle e_\alpha | \mu | 0 \rangle = \sum_m V^{-1}_{\alpha m}\mu_{m 0}, \]  \hspace{1cm} (5.10)
\[ \mu_{\gamma 0} = \langle f_\gamma | \mu | e_\alpha \rangle = \sum_m W^{-1}_{\gamma(mn)}V^{-1}_{\alpha m}\mu_{m 0}. \]  \hspace{1cm} (5.11)

The excitation-environment coupling Hamiltonian in Eqs. (5.4) and (5.5) is also transformed as
\[ H^{(1)}_{ex-env} \equiv V^{-1}H^{(1)}_{site-env}V, \]  \hspace{1cm} (5.12)
\[ H^{(2)}_{ex-env} \equiv W^{-1}H^{(2)}_{site-env}W. \]  \hspace{1cm} (5.13)

In this exciton representation, the diagonal matrix elements of the excitation-environment coupling Hamiltonians are interpreted as fluctuations in the exciton energies, whereas the off-diagonal elements contribute to transition or relaxation processes among excitons. For example, the matrix element
\[ \langle e_\alpha | H^{(1)}_{ex-env} | e_\beta \rangle = \sum_m u_m V_{\alpha m}\nu_{\beta m}, \]  \hspace{1cm} (5.14)
causes exciton \( \alpha \) to relax to relaxation \( \beta \), \( \langle e_\alpha | e_\alpha \rangle \rightarrow \langle e_\beta | e_\beta \rangle \), in the single-exciton manifold. Here, one should not overlook that these eigenstates and eigenenergies are obtained via diagonalization of the Hamiltonian comprised of the Franck-Condon transition energies. However, energies of the actual excitations will deviate from these values as the environmental reorganization (Stokes shift) takes place [3, 4, 6, 77]. Also, the concept of the exciton relaxation is useful and intuitive in analyzing ensemble averaged behaviors of PPCs which are observed by means of condensed phase spectroscopy. Nevertheless, one should be cautious in discussing microscopic dynamics in individual PPCs [78]. Although optical spectra provide us with the information projected onto energy eigenstates, this does not necessarily mean that PPCs are always in their energy eigenstates.

### B. Monitoring Pathways of Energy Transfer

#### 1. Electronic couplings between pigments

2D electronic spectroscopy is the optical counterpart of 2D NMR spectroscopic methods such as COSY and

![Rephasing Diagram](Image1.png)

**FIG. 7:** Double-sided Feynman diagrams which represent the Liouville space pathways contributing cross peaks \((\omega_1, \omega_2) = (\Omega_\beta, \Omega_\alpha)\) and \((\Omega_\alpha - \Omega_\beta, \Omega_\alpha)\) in 2D electronic spectra. The corresponding energy-level diagrams are also shown, where the solid arrows represent interactions of electric fields with ket-states while the dashed arrows represent interactions of electric fields with bra-states.

NOESY, which are powerful tools for organic structural analysis and structural biology [41]. Electronic couplings among different pigments can be identified visually from off-diagonal peaks termed cross peaks. Figure 7 presents the double-sided Feynman diagrams producing cross peaks. The Liouville space pathways \(R_1\) and \(R_4\) contribute to the ground state bleaching (GSB) in the language of pump-probe spectroscopy, and produce a cross peak at \((\omega_1, \omega_2) = (\Omega_\beta, \Omega_\alpha)\) whose intensity is proportional to \(\langle (\mu_{\alpha 0} \cdot \mathbf{E}_L) | (\mu_{\beta 0} \cdot \mathbf{E}_R) | (\mu_{\beta 0} \cdot \mathbf{E}_L) | (\mu_{\alpha 0} \cdot \mathbf{E}_R) \rangle\) or \(\langle \mathbf{E}_L \mathbf{E}_R \rangle \Omega_\alpha\). On the other hand, the pathways \(R_1^*\) and \(R_4^*\) describe the excited state absorption (ESA) and give a cross peak at \((\omega_1, \omega_2) = (\Omega_\alpha - \Omega_\beta, \Omega_\alpha)\) whose intensity is \(\propto \langle (\mu_{\alpha 0} \cdot \mathbf{E}_L^\dagger) | (\mu_{\beta 0} \cdot \mathbf{E}_L^\dagger) | (\mu_{\alpha 0} \cdot \mathbf{E}_L^\dagger) \rangle\) or \(\langle \mathbf{E}_L^\dagger \mathbf{E}_L^\dagger \rangle \Omega_\alpha\). The ESA is observed as negative-going peaks in the conventional 2D electronic spectra with use of all-parallel polarization, \(\mathbf{E}_L = \mathbf{E}_R = \mathbf{E}_L^\dagger = \mathbf{E}_R^\dagger\). We note that the
Spatial transport of electronic excitation energy in photosynthetic PPCs is observed as stepping down a ladder of excitonic energies as a function of the waiting period $T$, where the electronic excitation is transferred from a higher energy exciton to a lower energy one because of the matrix element given in Eq. (5.14). The signature of such relaxation appears in 2D electronic spectra as the emergence of a cross peak $(\omega_1, \omega_u) = (\Omega_\beta, \Omega_u)$ between a lower energy exciton $|e_\beta\rangle$ during the detection period $t$ and a higher energy exciton $|e_u\rangle$ during the coherence period $\tau$, as demonstrated with the double-sided Feynman diagrams $R_2$ and $R_1$ in Figure 8. Simultaneously, this relaxation process reduces the magnitude of peaks at $(\omega_1, \omega_u) = (\Omega_\alpha - \Omega_\beta, \Omega_u)$ located around the diagonal line, as shown in the diagram for $R_1^*$ and $R_2^*$. Monitoring electronic energy migration in photosynthetic PPCs with use of 2D electronic spectra has been extensively discussed [11, 12, 58, 79–83].

3. Relaxation Pathways in LHCII

While off-diagonal peaks at $T = 0$ indicate coupled excited states, the appearance of off-diagonal peaks with increasing waiting time indicates energy transfer. The appearance and evolution of these peaks can be simultaneously observed for all states within the spectrum of the laser pulse. Although there are other ultrafast techniques that also probe femtosecond dynamics, they require either integrating over excitation energy or using spectrally narrow pulses, which mean a loss of temporal resolution. As described above, in 2D spectroscopy, spectral resolution is maintained across excitation and emission frequencies, with no need to lose temporal resolution between excitation and emission events. In the case of closely spaced or overlapping excited states, resolution across these three dimensions becomes necessary to elucidate dynamics.

Examination of the major light-harvesting complex of Photosystem II (LHCII) by 2D spectroscopy has proved particularly informative. LHCII is the most abundant pigment–protein complex and binds over 50% of the world’s chlorophyll, the primary light-harvesting molecule. Arrays of LHCII complete the majority of light absorption in green plants. The crystal structure model is shown in Fig. 1a [20]. Each monomer of trimeric LHCII contains fourteen chlorophyll found in two spectral and structural variants, 8 Chl-a and 6 Chl-b. LHCII contains a set of discrete excited states, or excitons, that exhibit relaxation on multiple timescales. Because the fourteen chlorophyll per monomer give rise to fourteen closely spaced excited states, the congestion has often hindered measurements of the dynamics of individual donor and acceptor states. While other techniques had provided much insight by previously identifying the existence of various relaxation timescales within many spec-
tral regions [84–88], 2D experiments improved the temporal resolution of observed dynamics, allowed identification and isolation of intermediates in relaxation pathways, and surveyed the dynamics throughout the entire Qβ region [81].

Previously unobserved sub-100 fs dynamics were detected within all spectral regions. As seen in Fig. 9a, by 30 fs (\(T = 30\) fs) multiple energy transfer processes are evidenced by the appearance of cross-peaks where indicated by the white arrows. These peaks grow in for waiting times greater than zero, thus showing energy transfer. In Fig. 9b, there is increased relative amplitude in the low energy Chl-a band. The change in relative amplitude of these peaks at 70 fs indicates that there are multiple processes occurring within the first 100 fs. Rapid relaxation within the Chl-b band is also visible in the asymmetry of the Chl-b peak in the relaxation spectrum [81]. That is, there are several faster energy transfer steps within LHII than found using other experimental techniques or predicted by theory.

FIG. 9: Real, 2D spectra of LHII for selected waiting times \(T = 30, 70, 200, 300, 1.3\) fs with relaxation spectra (left) and non-rephasing spectra (right). Dynamics are highlighted by arrows. This figure was reproduced with permission from [81].

By simultaneously recording all dynamics within the Qβ region, the 2D spectra map out the relaxation pathways within LHII. Real (absorptive) 2D spectra for selected time points are shown in Fig. 9, left, and real, non-rephasing spectra are in Fig. 9, right. The spectra are divided into three energy regions, indicated by tickmarks: 1) the Chl-a region (14,700 – 15,000 cm\(^{-1}\)), which contains a low and mid energy band; 2) an intermediate region (15,000 – 15,200 cm\(^{-1}\)), which contains high energy Chl-a and low energy Chl-b; and 3) the Chl-b region (15,200 – 15,500 cm\(^{-1}\)). The appearance and evolution of five cross-peaks show energy transfer between these states. Arrows within the 2D spectra highlight these off-diagonal peaks in the non-rephasing spectra, because off-diagonal peaks in non-rephasing spectra arise almost entirely from energy transfer. It is important to note that the phase-twisted lineshape, or mixed absorptive and dispersive contributions to peaks in the non-rephasing spectra, can be interpreted as additional features. Comparison to the relaxation spectra is, therefore, important when using the non-rephasing spectra to elucidate the dynamics.

After the initial population and subsequent depopulation of mid-energy Chl-a band, these states again serve as intermediates. In Fig. 9c, in the 200 fs spectrum, there is still increased relative amplitude in the low energy Chl-a region: By 300 fs, cross-peaks 3 and 5 again increase in relative amplitude, as seen in Fig. 9d. The change in which states are populated between 200 fs and 300 fs indicates that energy transfers into the mid-energy Chl-a band from both the Chl-b and intermediate regions on a few hundred femtosecond timescale.

All picosecond spectra display very similar features, and Fig. 9e is shown as a representative timepoint (\(T = 13\) ps). From 1 ps to 20 ps, when the signal amplitude decreases to a level below the noise floor, the spectra contain two notable features. First, the horizontal band at the energy of the low energy Chl-a indicates most of the population across all spectral regions has relaxed into the low energy Chl-a band. Thus, initial excitations into Chl-b, intermediate, and mid-energy Chl-a regions transfer into the low energy Chl-a states. Second, the appearance of a new cross-peak shows energy transfer from the low energy Chl-b into a long-lived intermediate state in the mid-energy Chl-a region. Excitation remains in the intermediate state, labeled as cross-peak 6, until the signal dies by 20 ps [81]. Therefore, energy transfers out of the intermediate state much more slowly than in the case of other energy transfer steps within LHII.

After identifying each energy transfer step, understanding how these processes arise requires elucidating how the molecular components give rise to the observed relaxation pathways. The information provided by 2D spectroscopy can greatly aid correlating the individual chlorophyll within the x-ray crystallography structure with each of the observed relaxation steps. Within PPCs, this detailed modeling of relaxation pathways is often difficult. Despite the structural information, as discussed in Sec. III, there is little direct information about the de-
tails of the pigment Hamilton and pigment-protein interaction. In combination with theoretical methods, however, the dynamics of each energy transfer step were assigned to individual chlorophyll, as described in detail in Ref. [81]. The interpretation and theoretical results also provided a general picture of how excitation energy flows through the complex. The chlorophyll within LHCII are grouped into strongly coupled (20–100 cm⁻¹) clusters of 2-3 molecules. Because of the strong coupling, and large spatial overlap of the excited state wavefunctions, rapid (sub-100 fs) relaxation occurs within these molecules. Energy transfer between these clusters, or between excited states mostly localized on more weakly coupled molecules, occurs in several hundred femtoseconds. Within the structural model of LHCII, there are two chlorophyll well separated from, and thus weakly coupled to, lower energy chlorophyll. These chlorophyll serve as the sites of long-lived intermediate states.

From this picture of excitation energy flow within LHCII several fundamental principles behind nature’s design for efficient light-harvesting are observed. First, excitation energy eventually relaxes to the low energy Chl-a band, most likely into states on the external edge of LHCII. The emergent horizontal band which emits from the low energy Chl-a region indicates a pooling of energy into these states. The model of delocalized excited states creates multiple excited states on an exit site, proximal to nearby complexes, thereby aiding migration of the excitation out of LHCII and towards the reaction center. Second, there is a variety of excitation energy transfer timescales due to a range of couplings. Along each relaxation pathway, the combination of timescales may aid in unidirectional energy transfer by using coupling to overcome energy gaps larger than kBT and by using small energy gaps to overcome long-lived quantum beating. Long-lived quantum beating must be overcome because perpetual oscillatory motion means there is no permanent spatial translocation of excitation energy. In these systems, the excitation must end up localized on the reaction center. Coherent interaction, however, plays an important role in how the excitation migrates.

C. Quantum Coherent Electronic Energy Transfer

1. Quantum Coherence among Excitons

Coherent electronic energy transfer indicates that electronic excitations travel through PPCs as a quantum mechanical wave packet keeping their phase coherence, as was discussed in Sec. II. In the exciton representation, this process can be observed as time-evolution of superposition or coherence between different excitons, e.g.,

$$e^{-iH^{(1)}_{ab}T/\hbar}|\psi_{ab}\rangle = e^{iH^{(1)}_{ab}T/\hbar}|\psi_{ab}\rangle \approx e^{-i(\Omega_a - \Omega_b)T}|\psi_{ab}\rangle$$.

(5.15)

This quantum coherence manifests itself as oscillations in the peak amplitudes of 2D photon echo spectra as a function of the waiting time T with beat frequencies equal to the energy differences between the contributing excitons, $\Omega_{ab} - \Omega_{ab}$. In congested spectra where excitons are closely spaced energetically, cross peaks will appear near their diagonal counterparts. As a result, oscillations in the amplitude of cross peaks interfere with those of the peaks directly on the diagonal peaks, making it extremely difficult to isolate individual coherence contributions in conventional 2D photon echo spectra. As shown in Fig. 10, however, the coherence induces oscillatory behavior of a diagonal peak at $(\omega_r, \omega_r) = (\Omega_a, \Omega_a)$ and a cross peak located near the diagonal line $(\Omega_{ab} = \Omega_a, \Omega_b)$ in 2D nonrephasing spectra [89], and thus coherence pathways associated with diagonal peaks can be isolated through analysis of 2D nonrephasing spectra.

FIG. 10: Double-sided Feynman diagrams which represent the electronic quantum coherence $|\psi_{ab}\rangle/|\psi_{ab}\rangle$ in the waiting period. The corresponding energy-level diagrams are also shown. This process is observed as the beating of cross peaks $(\omega_r, \omega_r) = (\Omega_a, \Omega_b)$ and $(\Omega_r - \Omega_a, \Omega_a)$ in 2D rephasing spectrum, and as the beating of diagonal peaks $(\omega_r, \omega_r) = (\Omega_a, \Omega_a)$ and $(\Omega_r - \Omega_b, \Omega_a)$ in 2D nonrephasing spectrum.
2. Quantum Coherent Energy Transfer in LHCII

In recent years Fleming and coworkers have investigated the Fenna-Matthews-Olson (FMO) pigment-protein complex isolated from a green sulfur bacterium by means of 2D electronic photon echo spectroscopy [58, 59, 79, 80]. One of their experiments succeeded in observing long-lasting quantum beating providing direct evidence for long-lived electronic coherence [80]. The observed coherence lasts for time scales similar to the EET timescales, implying that electronic excitations move coherently through the FMO protein rather than by incoherent hopping motion, although it had been generally assumed that electronic coherence decay so rapidly that it does not affect the EET [22]. Observation of long-lasting quantum coherence is not unique to the FMO complex. Coherent EET dynamics in the reaction center of a purple bacterium were also revealed by applying the two-color electronic coherent photon echo technique [14]. Although the electronic quantum coherence in the PPCs was originally observed outside the physiological range of temperatures, the presence of quantum coherence lasting up to ~300 fs was detected in a conjugated polymers [90], the antenna complexes in marine cryptophyte algae [82] and the FMO complex [91] at physiological temperatures. Interestingly, this timescale was consistent with a previous theoretical prediction [7]. However, the significance of quantum coherence to electronic energy transfer in photosynthetic light harvesting cannot be argued without it first being established as a universal phenomenon. If coherence is essential for efficient energy transfer, it should be present in the large antenna complexes whose sole responsibility is to absorb solar energy and funnel it to the reaction centers. Despite having energy transfer as its primary function, the FMO complex is not a key photosynthetic complex with its analog largely absent in higher plants. This begs the question as to whether electronic coherence was important enough to survive evolution. Recent 2D electronic spectroscopic experiments have revealed evidence of electronic coherence in LHCII at cryogenic temperature, 77 K [92].

Figure 11(a) presents the diagonal cut of 2D non-rephasing spectra shown in Fig. 9 as a function of $T$. The strong peaks assigned to Chl-a ($\omega \sim 14,500 - 15,000 \text{ cm}^{-1}$) as well as the weaker peaks from Chl-b ($\omega \sim 15,500 \text{ cm}^{-1}$) demonstrate clear beating throughout the entire 500 fs duration of the experiment. Because the excitons responsible for these beating peaks originate from individual Chl molecules, the coherent dynamics can be mapped back onto the site basis. As seen in other photosynthetic complexes [14, 80, 82, 91], the coherence is long-lived in LHCII, with the quantum beating lasting beyond 500 fs scan of the experiment and beyond the lifetimes of many excitons in the system, suggesting that this is a general phenomenon in photosynthetic light harvesting systems. The observations of long-lived quantum coherence have led to speculation that coherence may contribute to optimizing photosynthetic energy transfer efficiency. A potential role of quantum coherence is to overcome local energetic traps and aid smooth and robust energy flow toward targets such as the reaction centers by taking advantage of quantum coherence and the energy landscape of pigments tuned by the protein scaffold [7].

While these studies provide insight into the role of quantum coherence in photosynthetic EET, questions remain regarding the underlying mechanisms preserving the long-lived quantum coherence. In order to elucidate the origin of the long-lived quantum coherence and its interplay with the protein environment, Ishizaki and Fleming presented the second-order cumulant time-nonlocal quantum master equation which reduces to the conventional Förster and Redfield theories in their respective limit of validity [6]. In the regime of coherent wave-like motion, the equation predicts several times longer lifetime of quantum coherence between electronic excited states of pigments than the Redfield equation does. Reference [6] also demonstrated that quantum coherent motion can be observed even when reorganization energy is large in comparison to intersite electronic coupling, although this situation is usually regarded as the Förster regime. The reason for the long-lived quantum coherence is as follows: In a region of small reorganization energy, the slow fluctuation sustains longer-lived coherent oscillation, whereas the Markov approximation in the Redfield framework causes infinitely fast fluctuations which then collapse the quantum coherence. In the region of large reorganization energy, on the other hand, the sluggish reorganization dynamics allows the excitation to stay above an energy barrier separating two local minima, which correspond to the two sites in the adiabatic potential surface, for a prolonged time. The dynamic behavior of the inter-
mediate regime, which is typical in photosynthetic EET, can be understood as the combined influence of the slow fluctuation effect in the regime of small reorganization energy and the slow dissipation effect in the large reorganization energy. In order to visualize this statement, it is helpful to consider the following minimal model instead of the PPC Hamiltonian in Eqs. (2.1)-(2.5) [6, 16]

\[ H_{\text{PPC}} = \sum_{m=0}^{2} \epsilon_m(q)|m\rangle\langle m| + hJ_{12}(|1\rangle\langle 2| + |2\rangle\langle 1|) \]  

(5.16)

where

\[ \epsilon_1(q) = \epsilon_0(q) + h\Omega_1 - h\omega_{\text{ph}} dq_{1}, \]  

(5.17a)

\[ \epsilon_2(q) = \epsilon_0(q) + h\Omega_2 - h\omega_{\text{ph}} dq_{2}, \]  

(5.17b)

with

\[ \epsilon_0(q) = \frac{h\omega_{\text{ph}}}{2} q_1^2 + \frac{h\omega_{\text{ph}}}{2} q_2^2. \]  

(5.18)

The reorganization energy is expressed as \( h\lambda_m = h\omega_{\text{ph}}d^2/2 \). The Hamiltonian Eq. (5.16) can be easily diagonalized, and then adiabatic excitonic potential surfaces in the single-exciton manifold can be obtained as

\[ E^\pm(q) = \frac{\epsilon_1(q) + \epsilon_2(q)}{2} \pm \sqrt{(hJ_{12})^2 + \left[\frac{\epsilon_1(q) - \epsilon_2(q)}{2}\right]^2} \]  

(5.19)

In Fig. 12, we draw the adiabatic potential surfaces for the lower energy, \( E^-(q) \), as a function of two phonon coordinates, \( q_1 \) and \( q_2 \). The parameters in this model are chosen to be \( \Omega_1 - \Omega_2 = 100 \text{ cm}^{-1}, J_{12} = 100 \text{ cm}^{-1}, \omega_{\text{ph}} = 53 \text{ cm}^{-1}, \) and \( \lambda_1 = \lambda_2 = 500 \text{ cm}^{-1} \). Since the reorganization energy is large compared with the electronic coupling, we can observe two local minima which represent the two sites, \( |1\rangle = |\varphi_{1e}\rangle|\varphi_{2g}\rangle \) and \( |2\rangle = |\varphi_{2e}\rangle|\varphi_{1g}\rangle \). Incoherent hopping EET describes the transition between the local minima. Attention will now be given to the point of origin, which corresponds to the Franck-Condon state. The energy of the point is higher than the barrier between the minima; therefore, we find that the electronic excited state is delocalized just after the excitation despite being in the incoherent hopping regime, \( \lambda > J_{12} \). As time increases, the dissipation of reorganization energy proceeds and the excitation will fall off into one of the minima and become localized.

Another possible reason for the long-lived quantum coherence is strong correlation between fluctuations in electronic energies of different pigments, as supported by the experimental results of Lee, Cheng and Fleming [14]. If different pigments share the dynamic effects of their local environmental, fluctuations in their electronic states would be correlated. If fluctuations in electronic energies of different pigments are correlated or almost synchronized, the EET process among the pigments would not experience any noise or dynamic disturbance. In this situation, the phase coherence of the quantum mechanical wave packet involved in the EET process would be preserved. In order to obtain further insight into this effect, it is also useful to consider the minimal model of Eq. (5.16). Here we consider the following instead of Eq. (5.17) [16, 93]:

\[ \epsilon_1(q) = \epsilon_0(q) + h\Omega_1 - h\omega_{\text{ph}} dq_{1} - h\omega_{\text{ph}}\zeta_1 dq_{2}, \]  

(5.20a)

\[ \epsilon_2(q) = \epsilon_0(q) + h\Omega_2 - h\omega_{\text{ph}} dq_{2} - h\omega_{\text{ph}}\zeta_2 dq_{1}, \]  

(5.20b)

where the reorganization energy is expressed as \( h\lambda_m = (1 + \zeta_m^2)h\omega_{\text{ph}}d^2/2 \), and the collective energy gap coordinates can be expressed as \( u_m = -h\omega_{\text{ph}} dq_{m} - h\omega_{\text{ph}}\zeta_m dq_{n} \) for \( m \neq n \). As a result, the cross-correlation function of \( u_m(t) \) and \( u_n(t) \) is

\[ \langle u_m(t)u_n(0) \rangle = (\zeta_m + \zeta_n)C_{qq}(t), \]  

(5.21)

with \( C_{qq}(t) \equiv \hbar^2\omega_{\text{ph}}d^2\langle q_m(t)q_m(0) \rangle \). We note that this cross-correlation function can be positive or negative, as discussed in the context of 2D-IR spectroscopy [57, 94]: the situations of \( \zeta_1 + \zeta_2 > 0 \) and \( \zeta_1 + \zeta_2 < 0 \) correspond to the positive and negative correlations, respectively. Figure 13 illustrates how the dimer system discussed in Fig. 12 varies for different extremes of environmental correlation. Similar to Fig. 12, the adiabatic exciton potential surfaces are drawn as a function of two phonon coordinates with minima representing two electronic states. In the case of positive correlation, Fig. 13a, the minima move into the same quadrant reducing their spacing and hence the energy barrier between them. A Franck-Condon transition to the origin lies above the energy barrier resulting in a delocalized excited state promoting coherence, and hence the EET is robust against environment-induced fluctuations [93]. However, for the negative correlation case illustrated in Fig. 13b, the minima exist in opposite quadrants. The large energy barrier
between them results in a localized state upon excitation to the origin thus eliminating coherence, and hence the EET becomes slower or more inefficient because the EET must overcome a higher energy barrier between the two states [93].

In addition to providing insights into the electronic energy transfer in photosynthetic light harvesting, the observed quantum beating is useful for identifying the PPC energy landscape or the exciton energies [92]. Fourier transforming the diagonal cut of the 2D nonrephasing spectra in Fig. 11(a) with respect to the waiting time T produces a power spectrum. In Fig. 11(b), a portion of the power spectrum from the Chl-a region is shown. Peaks appearing at smaller beat frequencies indicate coherence between excitons that arise from the same chlorophyll variant. In Fig. 11(b), these low energy beat frequencies exhibit strong coherence amongst Chl-a excitons. Meanwhile coherences between Chl-a and Chl-b excitons are evident due to the presence of beat frequencies  > 500 cm\(^{-1}\). In addition, as each exciton in LHCCI produces a beating diagonal peak with unique frequency components, the power spectrum allows the electronic energy levels to be deconvolved. Beat frequency peaks align vertically at positions corresponding to individual exciton energies. Analysis of the full power spectrum reveals a relatively evenly-spaced energy landscape in LHCCI with a high energy Chl-a exciton (\(\omega \sim 15, 130 \text{ cm}^{-1}\)) located intermediate to the Chl-a and Chl-b bands. The resulting small energy gap between the Chl-a and Chl-b excited states and the coherences observed between these bands facilitate ultrafast relaxation within LHCCI [92].

D. Polarization dependence of 2D spectra

1. Orientational Prefactor

As was shown in the above, the 2D rephasing, non-rephasing, and photon echo spectra involve averaging over molecular orientations. In order to take into consideration the orientational average, we assume that the angular part of the PPC Hamiltonian, Eq. (2.3) are statistically separable from the electronic transition and excitation dynamics implicit in the ensemble average. Therefore, individual Liouville space pathways contributing to 2D spectra, Eqs. (3.11)-(3.12), can be expressed as

\[
R_{\alpha}(t, T, \tau) \tilde{E}_{\alpha,LO} \tilde{E}_{\beta,LO} \tilde{E}_{1} = Y_{\text{ori}} R_{\alpha}(t, T, \tau) \quad (5.22)
\]

The prefactor of the right hand side, \(Y_{\text{ori}}\), is the so-called orientational prefactor expressed as

\[
Y_{\text{ori}} = \langle(\mu^{4} \cdot \tilde{E}_{\alpha,LO})(\mu^{3} \cdot \tilde{E}_{\beta})(\mu^{1} \cdot \tilde{E}_{1})\rangle\text{ori}.
\]

with \(\mu^{i}\) being the unit vector characterizing the transition dipole orientation conjugated to the \(i\)th laser pulse. \(R_{\alpha}\) is a scalar part of \(R_{\alpha}\), which describes the electronic transition and excitation dynamics. Interestingly, Eqs. (5.22) and (5.23) indicate that controlling the polarization direction of one of the excitation laser pulses and the local oscillator enables us to manipulate individual Liouville space pathways and deconvolute 2D spectra by selecting out certain Liouville space pathways [58, 59, 95–101].

For evaluation of the orientational factor in Eq. (5.23), we let \(\{e_{x}, e_{y}, e_{z}\}\) and \(\{e_{x}, e_{y}, e_{z}\}\) be the basis vectors of the laboratory-fixed and molecule-fixed coordinate systems, respectively. The molecular-fixed vectors \(\{e_{x}, e_{y}, e_{z}\}\) can be expressed with the laboratory-fixed basis \(\{e_{x}, e_{y}, e_{z}\}\) with the help of the Euler angles, and thus the following formula is obtained [102]:

\[
\langle(e_{x}, e_{y}, e_{z}) | (e_{x}, e_{y}, e_{z}) | (e_{x}, e_{y}, e_{z}) \rangle\text{ori} = \frac{1}{30} \begin{bmatrix} \delta_{x y} \delta_{y z} \delta_{z x} \\ \delta_{x y} \delta_{y z} \delta_{z x} \\ \delta_{x y} \delta_{y z} \delta_{z x} \end{bmatrix} \begin{bmatrix} 4 & -1 & -1 \\ -1 & 4 & -1 \\ -1 & -1 & 4 \end{bmatrix} \begin{bmatrix} \delta_{x y} \delta_{y z} \delta_{z x} \\ \delta_{x y} \delta_{y z} \delta_{z x} \\ \delta_{x y} \delta_{y z} \delta_{z x} \end{bmatrix} = \begin{bmatrix} \delta_{x y} \delta_{y z} \delta_{z x} \\ \delta_{x y} \delta_{y z} \delta_{z x} \\ \delta_{x y} \delta_{y z} \delta_{z x} \end{bmatrix}
\]

for \(\lambda_{i} \in \{x, y, z\}\) and \(I_{i} \in \{X, Y, Z\}\). Because of \(\mu^{i} = \sum_{X} \lambda_{i}(\mu^{i} \cdot e_{X}) e_{X} = \sum_{X} (\tilde{E}_{X} \cdot e_{X}) e_{X}\), Eq. (5.23) can be recast into

\[
Y_{\text{ori}} = \frac{1}{30} \left( \cos \theta_{43} \cos \theta_{21} \right) + \left( \cos \theta_{42} \cos \theta_{31} \right) + \left( \cos \theta_{41} \cos \theta_{32} \right)
\]

for \(\Sigma = \cos \theta_{43} \cos \theta_{21} + \cos \theta_{42} \cos \theta_{31} + \cos \theta_{41} \cos \theta_{32}\).

In Eq. (5.25), \(\theta_{ab}\) is the angle between the ath and bth laser pulse polarizations, whereas \(\theta_{ac}\) is the angle between \(\mu^{a}\) and \(\mu^{c}\). The average over the distributions of PPCs’ static structure \(\langle \cdots \rangle\) has further been introduced.

A particularly useful choice of the polarization orientation is the sequence \(\{(\pi/3 \text{ [pulse 1]}, -\pi/3 \text{ [pulse 2]}), 0 \text{ [pulse 3]}, 0 \text{ [pulse LO]}\}\), i.e.

\[
\theta_{43} = 0, \quad \theta_{42} = \pi/3, \quad \theta_{41} = \pi/3, \quad \theta_{32} = \pi/3, \quad \theta_{31} = \pi/3, \quad \theta_{21} = 2\pi/3.
\]

FIG. 13: Adiabatic excitonic potential surfaces for the cases of (a) positive and (b) negative correlated fluctuations. In eqn (5.20), we have chosen \(\zeta_{1} = \zeta_{2} = 0.5\) for (a) and \(\zeta_{1} = \zeta_{2} = -0.5\) for (b). The other parameters are the same as in Fig. 12. Figure is reprinted with permission from Ref. [16].
For this sequence, the orientational prefactors for the Liouville pathways contributing diagonal peaks vanish, enabling a closer look at the cross-peaks as the remaining [58, 99]. Therefore, this polarization sequence is termed the cross-peak-specific sequence. However, we note that diagonal peaks in 2D nonrephasing spectra presenting electronic quantum coherence, i.e. $R_1$ in Fig. 10, do not necessarily vanish because $\cos \theta^{43} = \cos \theta^{21} = \cos \theta^{42} = \cos \theta^{43} = \mu_{\theta,0} : \mu_{\theta,0}^*$ and $\cos \theta^{43} = \cos \theta^{42} = 1$.

The polarization sequence $(\pi/4$ [pulse 1], $-\pi/4$ [pulse 2], $\pi/2$ [pulse 3], 0 [pulse LO]), i.e.

\[
\begin{align*}
\theta_{43} &= \pi/2, & \theta_{42} &= \pi/4, & \theta_{41} &= \pi/4, \\
\theta_{32} &= 3\pi/4, & \theta_{31} &= \pi/4, & \theta_{21} &= \pi/2,
\end{align*}
\]

(5.27)

also eliminates the prefactors for the rephasing Liouville pathways contributing diagonal peaks [99]. However, the prefactors for the nonrephasing Liouville pathways contributing the beating diagonal peaks do not vanish, e.g. $R_1$ in Fig. 10. Interestingly, this polarization sequence eliminates all response terms except for those arising from pathways where the system is in a coherence state during the population time, regardless of whether the pathways contribute to diagonal or cross peaks [58]. Thus, this polarization sequence may be termed the coherence-specific sequence.

Equation (5.25) leads to the well-known tensor relation,

\[
(ZZZZ) = (ZZYY) + (ZYZY) + (ZYZZ),
\]

(5.28)

where we have defined $\langle I_1 I_2 I_3 I_4 \rangle \equiv \langle (\hat{\mu}^1 \cdot \hat{e}_1)(\hat{\mu}^2 \cdot \hat{e}_2)(\hat{\mu}^3 \cdot \hat{e}_3)(\hat{\mu}^4 \cdot \hat{e}_4) \rangle_{\text{ori}}$. Moreover, the orientational prefactors for the cross-peak-specific polarization and sequences in Eq. (5.26) and for the coherence-specific sequence in Eq. (5.27) can be expressed as [96, 99]

\[
\begin{align*}
3(ZZYY) &= (ZZZZ), \\
(ZYZY) &= (ZYZZ),
\end{align*}
\]

(5.29)

(5.30)

respectively. Equations (5.28) and (5.29) play a role in the polarization-phased 2D electronic spectroscopy for the minor antenna complex CP29 of photosystem II [103], as will be discussed below.

3. Isolating obscured features

The difference in scaling of individual peaks under different polarization sequences can decongest crowded 2D spectra. As seen in Sec. VB3, the diagonal and off-diagonal regions of 2D spectra often contain multiple overlapping peaks, where each peak may have contributions from multiple Liouville pathways. Because many of the peaks arise from transition dipole moments with different relative angles, their amplitudes scale differently under the two polarization sequences. The cross-peak-specific polarization sequence described in the previous section removes peaks corresponding to linear absorption that lie along the diagonal. These peaks often have much larger amplitude than other pathways and so can dominate 2D spectra by obscuring weaker, near-diagonal features.

This approach also applies to the off-diagonal regions of 2D spectra. In Fig. 14a and b, a feature that appears to be a shoulder under the all-parallel polarization has been isolated into a separate peak under the cross-peak-specific polarization. After isolating this feature, it can then be analyzed to extract information about the dynamics and structural origins of the corresponding energy transfer step. As seen in Fig. 14b, the donor state has an energy of $\sim 15,400 \text{ cm}^{-1}$ and the acceptor state has an energy of $15,176 \text{ cm}^{-1}$ (determined by fitting slices from the 2D spectra). As described in detail in Ref. [101], the 2D spectra showed that the acceptor state was quickly populated, beginning at around 100 fs, and the excitation was observed to remain in this state for the lifetime of the signal (5 ps). Due to the relatively rapid population from other Chl-b states and the very slow depopulation, the acceptor state was assigned to be localized on Chl-b 605 (where numbering is from the structural model from x-ray crystallography) because only this chlorophyll is coupled to other Chl-bs, but very weakly coupled to the Chl-a band. While modeling based on the crystal structure had suggested this chlorophyll as the site of a long-lived intermediate state, no direct spectroscopic information had characterized the energy transfer processes into and out of the excited state localized mostly on Chl-b 605. As this example illustrates, rotation of the polarization of the incident laser beams can selectively dissect crowded regions of 2D spectra.

3. Elucidating Exciton Geometry

In addition to examining the excited state dynamics and observing the phenomena behind energy transfer, 2D electronic spectroscopy can access information to better characterize the excited states. More specifically, the ability to independently control the polarization of the incident laser pulses provides a means to measure the angle between the transition dipole moments of the excited states that contribute to a given peak [59, 101].

The delocalized excited states, or the excitonic states observed spectroscopically, do not have an one-to-one correspondence with single chlorophylls. As discussed in Sec. II, the excitonic states are delocalized excited states determined by the coupling between chlorophylls and the transition energies of the uncoupled chlorophylls, which in turn depend on the surrounding protein environment. Figure 14c and d illustrate the case where each chlorophyll contains an entirely localized transition (Fig. 14c) and the case where each transition contains contributions from multiple chlorophylls (Fig. 14d). The drastic differences in position and direction between the two panels indicate how the excitonic states often have forms which are not obvious based on the structural model. Describ-
ing these states, therefore, requires spectroscopic information.

The orientational prefactor, or scaling factor, can be measured with polarized 2D spectroscopy [101]. Every Liouville pathway, or molecular process, within a 2D spectrum is scaled by an orientational prefactor, as discussed in Sec. V.D. As described previously, the magnitude of the orientational prefactor depends on the dot product of the angles between transition dipole moments and angles between the electric fields of the incident laser pulses averaged over an isotropic ensemble. The orientational prefactor for a peak in each one of a set of spectra can be found by amplitude changes in the peak due to changes in laser pulse polarization. A comparison of spectra under two known polarization sequences can determine the orientational prefactor for a peak in both spectra [101].

![Diagram](image)

**FIG. 14:** a) Absolute value non-rephasing spectrum of LHCII at $T = 400\text{fs}$ with $(0,0,0)$ polarization of the incident pulses. b) Absolute value non-rephasing spectrum of LHCII $T = 400\text{fs}$ with $(\pi/3, -\pi/3, 0, 0)$ polarization of the incident pulses. c) Transition dipole moments in the absence of coupling. d) Transition dipole moments where the excitons are changed by the presence of coupling. e) Transition dipole moments where the excitons are changed by both the presence of coupling and different site energies.

This approach was applied to the spectra in Fig. 14a and b. Figure 14 contains absolute value, non-rephasing 2D spectra of LHCII under ca) all-parallel $(0,0,0,0)$ and b) cross-peak-specific $(\pi/3, -\pi/3, 0, 0)$ polarization conditions for $T = 400\text{fs}$. The spectra are again characterized by many of the same features shown in the all-parallel spectra of Fig. 9. The spectra are again divided into the Chl-a, intermediate, and Chl-b regions, with the strongest peaks at the low energy Chl-a, the mid-energy Chl-a and the Chl-b energies. While the congestion of the spectra means many amplitude changes are indiscernible under the two polarization conditions, the peak highlighted by an arrow has much larger amplitude in Fig. 14b than in Fig. 14a. Therefore, the orientational prefactor must be a large value under the cross-peak-specific polarization sequence and a small one under the all-parallel one, because only where there is a large difference in scaling would the spectra show a visible change in amplitude.

A measured orientational prefactor, for known polarization sequences, can be used to estimate the angle between the transition dipole moments of the excitons involved in a given peak. Because the scaling factor depends on angles between transition dipole moments and on angles between laser pulse polarizations, if the scaling can be determined for known polarization sequences, the angles between transition dipole moments can be extracted. In the case of the peak highlighted in Fig. 14a and b, the angle is the one between the donor and acceptor states for this energy transfer cross-peak. Figure 15d plots the orientational prefactor for the two polarization sequences shown for an energy transfer peak. Because of the increased relative amplitude in Fig. 14b, the angle between donor and acceptor states must be close to $90^\circ$. Only for a small region around $90^\circ$ would there be an visible increase in amplitude relative to the many nearby peaks [101].

4. **Determination of site energies**

Because the angles between transition dipole moments described in the previous section provide another observable for the system, the information can also access a new parameter of the system. Quantitatively relating structure to function requires a mathematical description of the molecular system. Specifically, a model requires detailed information about the pigment Hamiltonian and the pigment-protein coupling.

The diagonal elements of the pigment Hamiltonian vary because of differences in the protein pockets which surround the chlorophyll. These values are the uncoupled transition energies (site energies). The site energies depend on tuning of the local optical and electrochemical properties by the protein environment through effects such as electrostatic interactions with amino acid side chains, ligation of the central Mg of the chlorophyll, hydrogen bonding, conformation of the pigment (bowing of the porphyrin ring), and position and relative orientation of the chlorophyll to the electric field originating from the dipole of the α-helix backbone.

Neither experimental nor theoretical methods have been able to easily access the values of the site energies. As described in Sec. II, the spectroscopic results depend not only on the pigments themselves, but also on the coupling between the pigments and the protein. It is difficult to identify any one parameter of the PPC because of the multiple values which contribute to a spectrum. Also, because site energies differ based on the multiple parameters described above, and all these values have similar magnitude, the site energies are difficult to calculate theoretically. Until now, there have been no spectroscopic techniques to access the site energies, and thus provide a
benchmark for the theoretical results.

The measured angles between transition dipole moments can be used to find site energies. The directions of the transition dipole moments depend on site energies, electrodynamic coupling between the chlorophylls, and the position and direction of the transition dipole moments of the individual chlorophyll. The later two parameters can be relatively straightforwardly calculated based on the X-ray crystallography structure. Because the site energies depend on many parameters within the protein matrix, the molecular structure does not help describe the contributions of each molecular component. After having measured the angle between transition dipole moments, bounds can be placed on the remaining unknown, the site energies. The angle between donor and acceptor excitons was found to be close to $90^\circ$, and the only site energy values which produce this angle are $15,630 - 15,710 \text{ cm}^{-1}$ for Chl-b 606 and $15,680 - 15,760 \text{ cm}^{-1}$ for Chl-b 607 [101]. Figure 14d and e show pictorially why the angle between transition dipole moments is an accurate method of determining site energies. In these two panels, the parameters used for coupling and site basis transition dipole moments are the same, and only the site energies vary. The resultant drastic effect in angles between transition dipole moments emphasizes why this angular information provides a measure of the site energy values. The reason why the angle between transition dipole moments is an effective way to determine site energies is because the angle between transition dipole moments depends non-linearly on the site energies. In contrast, excited state energies have a primarily linear dependence on site energies and thus are a much less sensitive measure.

In addition to better describing the system, determination of the site energies provides insight into how the energy landscape is created. Variations in site energies lead to variations in excited state energies. Thus, the site energies are a critical component of how a single molecule, chlorophyll, is used in multiple complexes with different functions that combine for directional energy transfer. The breadth of site energies also serves to broaden the excited state absorption bands, and therefore allows chlorophyll to absorb across more of the solar spectrum.

E. Comparison to Homology Models

The applications discussed in the preceding sections involved extracting information about the functionality of the complexes from the spectroscopic results and then relating those conclusions to the molecular structure using models based on X-ray crystallography. For many complexes within the PSII supercomplex, however, a high resolution structural model has not yet been determined. Additionally, structure determination experiments, such as X-ray diffraction, are done in conditions which may differ significantly from physiological conditions. Because of the precision and breadth of information extracted from a 2D experiment, the results can be used to determine functionality, and interpret the dynamics within the context of spatial structure, without the need for previously-acquired high resolution spatial information [83, 103, 104]. In this section, we discuss how these approaches have been applied to the CP29 light-harvesting complex to determine the pathways of energy flow and to build a model of spatial positioning of excited states. Understanding CP29 has been challenging because until the recent structural model from X-ray crystallography [21], the structural model was based on partial sequence homology with LHCII [105].

CP29 is one of the three so-called minor complexes that sits between LHCII, where the majority of light-harvesting occurs, and the PSII core complexes, where charge separation takes place. Experimental evidence suggests CP29 contributes to light-harvesting and also plays a role in photoprotection [24]. According to this model, CP29 must absorb and transfer excitation energy and have mechanisms to dissipate excess energy. Based on the X-ray crystallography information, CP29 contains 13 chlorophyll, with 8 Chl-a and 4 Chl-b. As opposed to LHCII, CP29 has a mixed binding site, with a non-specific pockets that can hold either Chl-a or Chl-b [21].

2DES was used to survey the relaxation dynamics for CP29. 2D spectra are presented in Fig. 15a-c. Similarly to LHCII, the spectra show excitation of Chl-b, Chl-a and the region between the two bands. However, as seen both in the 2D spectra of Fig. 15 and the linear absorption spectrum of Fig. 1, CP29 exhibits less collective oscillator strength in the Chl-b region than is the case.
for LHCII, which makes Chl-b to Chl-a energy transfer more challenging to observe. Relaxation from the Chl-b to the Chl-a band is still visible. As the CP29 spectra in Fig. 15a and b illustrate, relaxation occurs from low energy and high energy Chl-b states into the Chl-a band giving rise to two cross-peaks. Although these peaks appear as a horizontal band in the all parallel spectrum, the additional structure provided by rotation of the polarization of the laser pulses (Fig. 15c) highlights that two features with different relative amplitudes are indeed visible. These two peaks are indicated with arrows in Fig. 15c. Both of these transfer processes occur within 3.6 ps. Within CP29, according to the structural model, there are four Chl-b molecules, which are part of three Chl-b/Chl-a clusters of chlorophyll, as shown in Fig. 1c. These two relaxation processes occur within these three clusters.

Novel polarization experiments were used to generate the spectrum of CP29 shown in Fig. 15. There are only three unique polarization sequences, as described in Sec. V.D, and all other polarization sequences are a linear combination of these three, as described in Eq. (5.25). For this experiment, these four polarization sequences were recorded, the three independent polarization sequences and their linear sum. From the tensor relation of Eq. (5.25), the three unique spectra were normalized relative to each other. This allowed arithmetic-generation of arbitrary polarization combinations. The CP29 polarized spectrum in Fig. 15c, generated from a linear combination of two of the recorded spectra, is data under the same polarization sequence as that in Fig. 14b. This spectrum illustrates that this approach can be used to create spectra under all possible polarization sequences. This technique could be applied, for example, to scan through polarizations in order to selectively enhance and suppress different peaks within congested off-diagonal regions, such as shown in Fig. 14a and b. A polarization scanning approach has also been suggested using beam 3 [106, 107].

The two Chl-b to Chla peaks can provide structural information. From the two Chl-b to Chla peaks, using the same approach as described in Sec. V.D.3, the angles within the heterodimer were determined to be 138° for the low energy Chl-b to Chla peak and 53° for the high energy Chl-b to Chla peak. Thus, there must be transition dipole moments of Chl-b and Chl-a which give rise to observed angles close to these values. With further analysis, this information will aid in localizing the observed dynamics to specific pigments within the structural model from x-ray crystallography and constraining the parameters of the excited states produced by those pigments.

Because CP29 sits between LHCII and the reaction center, the complex functions as a pathway for energy flow, and when necessary, a site of energy dissipation. The strong Chl-b to Chl-a coupling allows energy transfer to the Chl-a states and thus the Chl-b molecules can broaden absorption for auxiliary light-harvesting. The Chl-a molecules, therefore, are located within the complex along a potential path from LHCII to the reaction center. This creates a chain of low energy states, or a path, for excitation energy to flow towards the site of charge separation. It is also interesting to note that neighboring this path are carotenoid molecules, which are thought to play a role in photoprotection [24, 108]. The location of the carotenoids relative to the low energy states suggests that they are well-positioned for when quenching mechanisms are triggered.

VI. CONCLUSIONS AND OUTLOOK

2DES measures the full, complex third order nonlinear response, and contained within this function is the information that reveals the ways in which molecular machinery is designed to give rise to ultrafast dynamics. This method is particularly useful in systems that have structures with multiple components, such as PPCs which contain interacting chromophores that in turn interact with a surrounding protein scaffold to produce migration of excitation energy through the light-harvesting structure. The spectral and temporal resolution provided by 2DES, as well as additional information accessed by control over polarization of the incident beams, provides a means to dissect congested spectra to elucidate the overall functionality of PPCs.

The groups of associated PPCs that form a PSII supercomplex, exhibit a remarkable ability to harvest sunlight with near unity quantum efficiency. Understanding the relationship between the molecular structure and light-harvesting functionality of PSII is critical in the context of our growing need for devices that provide renewable energy sources. 2DES provides a tool to investigate how molecular structure produces spatio-temporal dynamics in a system as sophisticated and varied as the PSII supercomplex. In addition to the studies of LHCII and CP29 described here, 2DES applied to the PSII reaction center, which is highlighted in the structural model shown in Fig. 1a, displayed both ultrafast energy transfer, and electron transfer on a variety of timescales [83]. These different spectroscopic features from the PSII reaction center, with the addition of electron transfer chains, underscore the complexity of the excited state dynamics in the photosynthetic apparatus.

As advances in 2DES experimental and theoretical tools continue, these new techniques have the potential to further disentangle the structure-function relationships that underlie ultrafast dynamics in natural and artificial systems. There are several promising new directions for the method. Variations in frequency, polarization, orderings, and number of the incident pulses can access different information about the sample. Firstly, because 2DES is sensitive to couplings, there are means to selectively investigate joint functionality of different types of molecules, which often exhibit well-spaced resonances. Non-degenerate four wave mixing experiments can exami-
the coupling between these spectrally separated states to directly probe the interaction between different types of molecules. For example, chromophore-protein interactions can be studied directly in a background free measurement with a visible-IR two-color experiment. Secondly, as discussed in Sec. VD, the ability to independently control the four incident pulses also allows polarization shaping of the fields. Besides the polarization schemes discussed in the previous sections, additional polarization control, which is more challenging to implement in the electronic regime, has been explored theoretically and experimentally in the IR region [109–111]. In addition, fifth order implementations of multidimensional spectroscopy offer the potential to directly probe higher order correlation functions, or non-gaussian effects of the surrounding protein environments. Furthermore, different ordering of the incident pulses has recently been shown to access electron correlation effects [112]. Finally, one of the most promising areas of development is constructing an approach to access a microscopic picture of how individual systems function.

Experimentally, EET dynamics in photosynthesis have been investigated by synchronizing initial electronic excitation in the entire ensemble by means of ultrashort laser pulses. Detected signals in such experiments are electric fields generated by macroscopic polarizations defined as the ensemble average of microscopic dipole moments, as expressed in Eq. (3.2). For the analyses of the spectra, commonly used theoretical approaches are statistical mechanical methods based on quantum master equations. In these approaches, the key quantity of interest is the reduced density matrix, i.e. the partial average of the total density matrix over the protein environmental degrees of freedom. It is undeniable that the combination of condensed phase laser spectroscopy and the reduced density matrix approaches have provided much useful insight into photosynthetic EET. However, in natural light harvesting, the initial event is the absorption of one sunlight photon by a single PPC, followed by EET in the PPC independently of the ensemble averaged behavior. Considering the difference between EET dynamics in individual PPCs and their ensemble average means such ensemble approaches might limit our understanding of the design principles because the resultant spectra and the reduced density matrix both involve ensemble averages which may wash out the microscopic details.

Despite the good agreement between the ensemble experiments and the ensemble averaged theories [7, 11, 31, 79, 80, 91], it is still irresistible to ask questions such as

1) Which have we observed in 2DES, true quantum mechanical decoherence or ensemble dephasing which is also called “fake decoherence” [113]?

2) How does EET proceed in a single PPC? 3) Does the initial electronic quantum coherence just decay irreversibly or can it recur? 4) Can the coherence appear in a PPC excited not by light, but by EET from another PPC? 5) How relevant are the laser-based results to photosynthesis in natural sunlight? Providing a precise answer to these questions requires experimental/theoretical approaches that look at the dynamics prior to the ensemble average. Recently, Ishizaki and Fleming [78] examined such a theoretical approach by employing a mixed quantum/classical simulation which is termed the time-dependent self-consistent field (TDSCF) approach [114–116]. In the approach, only the electronic excitation was treated quantum mechanically, while the environmental degrees of freedom were described as classical variables. Although the combination of two fundamentally different descriptions of nature provided by quantum and classical mechanics might cause serious problems of consistency in general, their simulations [78] showed encouraging agreement with results from the more accurate reduced density matrix approach [6] with respect to destruction of quantum coherent behavior when the ensemble average is considered. This agreement implies the following points as a possible interpretation of the experimentally observed long-lived electronic quantum coherence: 1) The behavior of the reduced density matrix can be interpreted as the ensemble average of the quantum dynamics in individual PPCs, i.e. ensemble dephasing or “fake decoherence” rather than true decoherence. 2) The quantum coherent motion may be robust under individual realizations of the environment-induced fluctuations contrary to intuition obtained from the reduced density matrix. 3) The existence of electronic quantum coherence does not depend on the method of preparing the electronic excitation, i.e. whether by laser pulse or natural sunlight photon. 4) Experimentally detected static delocalized states (excitons) in the ensemble-averaged behavior indicate the existence of wave-like EET dynamics in individual PPCs. Figure 16 presents EET dynamics in a four-site system calculated from the mixed quantum/classical simulation. The excitation Hamiltonian and the excitation-environment coupling parameters are given in the figure caption. Sites 1-2 and sites 3-4 make two strongly coupled dimers, while the two dimers are weakly coupled. This situation is not unusual in photosynthetic complexes. Figure 16(A) presents the ensemble averaged behavior corresponding to the reduced density matrix. In Fig. 16(A) we observe quantum coherent wave-like motion between sites 1 and 2 up to 500 fs. However, EET in the 3-4 dimer and between the dimers do not exhibit any coherent motion; they are usually regarded as describable as incoherent diffusion. On the other hand, Fig. 16(B) shows the EET dynamics influenced by a particular realization of the environment-induced fluctuations without an ensemble average. Although the ensemble averaged behavior exhibits destruction of the wave-like motion, the individual system EET does not. Comparison between Figs. 16(A) and 16(B) clearly demonstrates the quantum coherent effects are washed out in the average or dephasing mechanism. Interestingly, after the “incoherent diffusion” from the 1-2 dimer to the 3-4 dimer, wave-like motion is intensified in the 3-4 dimer contrary to the intuition obtained from the reduced density matrix.
However, these are theoretical predictions. In order to fully elucidate photosynthetic EET, particularly possible roles of electronic quantum coherence in photosynthetic light harvesting, it is important to experimentally overcome the intrinsic coarse-grained nature of the electronic quantum coherence observed for the ensemble of PPCs. For this purpose, it is intriguing to explore a single photosynthetic PPC or a dilute ensemble far from the thermodynamic limit. It is becoming possible to merge ultrafast spectroscopy and single-molecule detection [117–119], for example, Brinks et al. [119] reported the observation of vibrational coherence in individual molecules at ambient temperature by means of the phase-locked spontaneous light emission technique [120, 121]. Applications of this technique to detection of electronic quantum coherence in PPCs would provide further insights into photosynthetic EET and are currently in progress in our laboratory.

The photosynthetic apparatus of plants, algae and bacteria is a complex system with emergent properties that cannot be simply extrapolated from the properties of the component parts. This consideration also applies at the individual PPC level. An essential step on the path to a full understanding of the design principles is the experimental determination of the energetic and electronic landscapes on which the dynamics play out. Building on the dramatic increase in knowledge of the spatial landscapes of PPCs, 2DES has provided fascinating new insights into the complementary energetic and electronic landscapes. With further application of basis set polarization spectroscopy, multi-color 2DES, use of other phase matching directions to study electron correlation effects, and with development of improved methods to study the role of the protein motion, a full understanding of how these extraordinary examples of nature’s engineering may be within reach.

In a monograph entitled What is Life? [122], Schrödinger summarized the emerging molecular aspects of biology as of 1944 from his perspective. The book spearheaded the rise of molecular biology and had a tremendous impact on the development of 20th-century biology. There he stated as follows: “... incredibly small groups of atoms, much too small to display exact statistical laws, do play a dominating role in the very orderly and lawful events within a living organism.” Further he said, “A single group of atoms existing only in one copy produces orderly events, marvelously tuned in with each other and with the environment according to most subtle laws.” These statements are both suggestive and inspiring toward elucidation of the physical origins of the remarkably high quantum efficiency of photosynthetic EET. One needs to explore individual proteins embedding pigments not only as randomly fluctuating dissipative environments from the statistical point of view as usual in the literature of photosynthetic EET, but also as functional environments on the basis of atomic-level understanding. This standpoint should be generally significant to understand stochasticity and selectivity in molecular systems, in particular to elucidate directionality dominating biophysical and biochemical events.

Acknowledgments

This work was supported by the Director, Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under Contract. DE-AC02-05CH11231 and by the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences, U.S. Department of Energy under contract DE-AC03-76SF000998. The authors would like to thank Jahan M. Dawlaty and Doran Bennett for providing the data shown in Fig. 6. G.S.S.-C. is grateful for an American Fellowship from the American Association of University Women.

Gabriela Schlau-Cohen obtained her ScB in Chemical Physics from Brown University in 2003. She then joined the group of Prof. Graham Fleming as a doctoral student at the University of California, Berkeley and the Lawrence Berkeley National Lab. Her research interests focus on using non-linear spectroscopy to study photosynthetic energy transfer, in particular the relationship between the structure and the efficient light-harvesting functionality of pigment-protein complexes.

Akihito Ishizaki received his PhD in theoretical chemistry from Kyoto University, Japan in 2008. He then joined the group of Prof. Graham Fleming at the University of California, Berkeley as a JSPS Postdoctoral Fellow for Research Abroad. He is currently a postdoctoral fellow at Lawrence Berkeley National Laboratory. His research interests include open quantum systems, condensed phase chemical dynamics, modeling of optical responses, and design principles of the primary steps in photosynthesis, especially as they pertain to quantum coherence.

Graham Fleming currently serves as UC Berkeley’s Vice Chancellor for Research, a position which he assumed in April 2009. Fleming served as the Deputy Director of Lawrence Berkeley National Laboratory from 2005 through 2007. Through joint appointments as Melvin Calvin Distinguished Professor of Chemistry at UC Berkeley, and Founding Director of both the Berkeley Lab's Physical Biosciences Division and UC Berkeley’s California Institute for Quantitative Biosciences (QB3), he has re-shaped the intersection of physical and biological sciences, while maintaining his own investigations into ultrafast chemical and biological processes, in particular, the primary steps of photosynthesis. Throughout his administrative career, Fleming has remained a highly active scientific researcher. He has authored or co-authored more than 400 publications, and is widely considered to be one of the world's foremost authorities on ultrafast processes. In addition to his many other activities, Fleming has given numerous talks around the world on the inter-relation and inter-complexity of energy, climate and photosynthesis. In 2007, Fleming led the effort (with co-chair Mark Ratner) to define Grand Challenges in Basic Energy Science for DOE/BES, resulting in “Directing Matter and Energy: Five Challenges for Science and the Imagination.”
We review the theoretical principles and experimental implementation of 2D electronic spectroscopy.

2D electronic spectroscopy can be applied to monitor energy transfer dynamics, observe quantum coherence, determine excited state geometry, and compare to homology models.

2D electronic spectroscopy reveals structure-function relationships in the Photosystem II supercomplex.