Topics in multidimensional optical spectroscopy

I. Theoretical studies of finite pulse effects II. Applications to plant light-harvesting complex and nanomaterials

Do, Thanh Nhut

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Topics in Multidimensional Optical Spectroscopy

I. Theoretical Studies of Finite Pulse Effects

**II. Applications to Plant Light-Harvesting Complex and
Nanomaterials**

Do Thanh Nhut

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

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Nanomaterials

Do Thanh Nhut

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

A thesis submitted to the Nanyang Technological
University in partial fulfilment of the requirement for the
degree of Doctor of Philosophy

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research done by me except where otherwise stated in this thesis. The thesis work has not been submitted for a degree or professional qualification to any other university or institution. I declare that this thesis is written by myself and is free of plagiarism and of sufficient grammatical clarity to be examined. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

13/01/2020

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DO THANH NHUT

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I have reviewed the content and presentation style of this thesis and declare it of sufficient grammatical clarity to be examined. To the best of my knowledge, the thesis is free of plagiarism and the research and writing are those of the candidate's except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

13/01/2020

Date

Assoc. Prof. TAN HOWE-SIANG

Authorship Attribution Statement

This thesis contains material from five papers published in the following peer-reviewed journals / from papers accepted at conferences in which I am listed as an author.

Chapter 2 is published as T. N. Do, M. F. Gelin, and H.-S. Tan. Simplified expressions that incorporate finite pulse effects into coherent two-dimensional optical spectra. *The Journal of Chemical Physics* **147**, 144103 (2017). DOI: 10.1063/1.4985888

The contributions of the co-authors are as follows:

- Assoc. Prof. Howe-Siang Tan initiated the project direction and revised the manuscript draft.
- I prepared the manuscript draft and the draft was revised by Dr. Maxim F. Gelin and Assoc. Prof. Howe-Siang Tan.
- I performed the theoretical derivation and numerical simulations.
- Dr. Maxim F. Gelin assisted in the interpretations and derivation for the case of arbitrary response function measured by Gaussian pulses.

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- Dr. Maxim F. Gelin and I performed the theoretical derivation and interpreted the results.
- I performed the numerical simulations.
- Dr. Lipeng Chen and Dr. Andrey K. Belyaev provided valuable comments for data interpretation.

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The contributions of the co-authors are as follows:

- Dr. Petar H. Lambrev and Assoc. Prof. Howe-Siang Tan initiated the project direction and revised the manuscript draft.
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- Dr. Adriana Huerta-Viga and I conducted the preparation and testing for the cryostat system used in temperature dependent 2DES measurements.
- Dr. Adriana Huerta-Viga and I conducted the experimental tests to determine the minimal amount of glycerol used to cool down the LHCII sample to 77 K.
- Dr. Paweł Nowakowski, Dr. Adriana Huerta-Viga, Dr. Muhammad Faisal Khyasudeen and I arranged the optical setup for 2DES experiments.
- Dr. Parveen Akhtar conducted the extraction and purification of LHCII samples.
- Dr. Parveen Akhtar, Dr. Petar H. Lambrev, Dr. Paweł Nowakowski and I jointly performed the 2DES measurements.
- Dr. Parveen Akhtar and Dr. Petar H. Lambrev interpreted the results.

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- Dr. Paweł Nowakowski, Dr. Adriana Huerta-Viga, Dr. Muhammad Faisal Khyasudeen and I arranged the optical setup for 2DES experiments.
- Dr. Parveen Akhtar conducted the extraction and purification of LHCII samples.
- Dr. Parveen Akhtar, Dr. Petar H. Lambrev, Dr. Paweł Nowakowski and I jointly performed the 2DES measurements.

- Dr. Adriana Huerta-Viga, Mr. Hoang Long Nguyen, and I outlined the initial modelling formalism and performed the fitting.
- Mr. Hoang Long Nguyen and I refined the model and reiterated the fitting.
- Mr. Hoang Long Nguyen, Dr. Petar H. Lambrev and I interpreted the fit results.

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The contributions of the co-authors are as follows:

- Assoc. Prof. Howe-Siang Tan initiated the project direction and edited the manuscript draft.
- Assoc. Prof. Howe-Siang Tan and I prepared the manuscript draft and the draft was revised by Assoc. Prof. Yinthai Chan.
- Dr. Xuanwei Ong conducted the synthesis of colloidal CdSe quantum dots sample.
- Dr. Jie Lian conducted the synthesis of CdSe nanoplatelets sample.
- Dr. Cheng Zhang and I performed the 2DES experiments on the CdSe quantum dots sample. We also interpreted the data.
- I performed the 2DES experiments on the CdSe nanoplatelets sample. I also interpreted the data.
- Assoc. Prof. Yinthai Chan assisted and provided guidance for interpreting the data of nanomaterials.

13/01/2020

Date

DO THANH NHUT

Abstract

In Nature, there are numerous important processes happening at the picosecond, femtosecond and attosecond timescales. With the development of the ultrafast laser and techniques such as ultrafast multidimensional optical spectroscopy, scientists are able to study many of such ultrafast dynamic processes. In this thesis, we present both theoretical and experimental studies on multidimensional optical spectroscopy (MDOS) and the applications of ultrafast two-dimensional electronic spectroscopy (2DES) to the study of photosynthetic pigment-protein complexes and nanomaterials.

MDOS spectra are affected by the use of finite bandwidth excitation pulses due to the convolution properties of the multi-pulse interactions. Disentangling the effects of finite bandwidth is not trivial. In Chapters 2 and 3 of this thesis, we present theoretical studies on how the finite bandwidth and the duration of excitation pulses alter the MDOS spectra. We derive an approximate and simplified expression that can be used to calculate the MDOS spectra that includes finite bandwidth effects. We found that for third-order two-dimensional optical spectra, in certain conditions, the obtained expressions are exact and contain only simple multiplications of the pulse spectra and the system response functions. We also obtained expressions for fifth-order optical spectra, such as two-quantum two-dimensional and three-dimensional optical spectra. In these cases, due to the higher complexity caused by a higher number of pulse interactions, the obtained formulae are only approximate. However, by comparing the simulations using the exact formalism and our approximated expressions, the approximated formula can reproduce most of the main spectral features very well. These results will be beneficial to the MDOS community by providing simpler and more resource-efficient ways of incorporating the finite bandwidth effects of excitation pulses into the simulation of MDOS spectra. It will also facilitate the extraction from experimental MDOS spectra, of relevant information contained in the systems' response functions.

Chapters 4 and 5 of this thesis present the application of 2DES on studying the ultrafast excitation energy transfer (EET) processes in Light-harvesting complex II (LHCII) the most abundant light-harvesting antenna on Earth. LHCII plays an important role in photosynthesis due to its main functions of harvesting sunlight energy and funnelling it to the reaction center to regulate photosynthesis. As the first pigment-protein complex in eukaryotic plants having its crystal structure solved, LHCII has been intensively studied during the past decades. Many experimental and theoretical studies have been proposed to try to solve the EET scheme. However, due to the highly congested spectral features and ultrafast dynamics in the femtosecond timescale, there is no consensus on the model for LHCII EET dynamics. In our studies, we implement 2DES with the capability of correlating the excitation and emission frequencies to track the ultrafast EET dynamics of LHCII. 2DES measurements of LHCII at various temper-

atures elucidate the temperature-dependent excitation energy transfer dynamics of the Chl *a* manifold of LHCII. Global analysis results reveal three main timescales for the energy equilibration dynamics of LHCII. A clear indication of the temperature dependence of uphill/downhill energy transfer processes was also discerned, which follows the detailed-balance condition. In Chapter 5, we take a closer look at the cryogenic 77 K 2DES measurement. We construct a phenomenological model to fit our 2DES data and extract the spectral information of the excitonic states, as well as the kinetic parameters. Due to the restriction of the model and complexity of the system, the EET network cannot be constructed uniquely. However, based on the agreement of our fitting results with the previously proposed structure-based calculations, we have proposed two EET schemes with tentative pigment assignments. In addition, the existence of a ‘bottleneck’ state in the intermediate wavelength region (660-670 nm) is supported by our results. Our model also determines three terminal excitons having the lowest energy levels, and the excitation can always be equilibrated among these three states without any significant EET directly connecting them. These equilibration processes happen via the uphill energy flow to the higher excitonic levels bridging the three lowest termini and this equilibration mechanism accelerates the EET at higher temperatures and results in the temperature dependence of EET processes in LHCII.

Chapter 6 presents the application of 2DES on studying the photophysics of nanomaterials. Due to intrinsic limitations in synthesis procedure, the nanomaterials, like quantum dots (QDs) and nanoplatelets (NPLs) always exhibit heterogeneity in size, shape, and spectroscopic properties. We perform 2DES measurements on zero-dimensional CdSe QDs and two-dimensional CdSe NPLs. Employing the nodal line slope and central line slope analysis, we can extract information about the systems’ homogeneity, inhomogeneity and spectral diffusion dynamics from our measured 2DES data. The results show that no spectral diffusion dynamics occur for the CdSe QDs. On the other hand, spectral diffusion was observed in the 5 mono-layers CdSe NPLs’ heavy-hole transition. The normalized frequency fluctuation correlation function of the CdSe NPLs’ heavy-hole transition was measured to have a major fast decay component at 80 fs, followed by a slower diffusion component of 2.5 ps.

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When I start writing this section, my four-year journey of PhD is being wrapped-up. This section takes me the longest writing time as there are many things I have experienced, many people I have met, many places I have arrived and many lessons I have learned during this long and tough journey. Writing this section is like watching a rewinding movie of my own memories contributed from many people that had come to my life and all those shape my current self.

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Abbreviations list

2D DAS	Two-dimensional decay-associated spectrum
2DES	Two-dimensional electronic spectroscopy
2DIR	Two-dimensional infrared
2DOS	Two-dimensional optical spectroscopy
2Q-2DOS	Two-quantum two-dimensional optical spectroscopy
3DOS	Three-dimensional optical spectroscopy
Chl(s)	Chlorophyll(s)
EADS	Evolution-associated decay spectrum
EET(s)	Excitation energy transfer(s)
ESA	Excited-state absorption
FFCF	Frequency fluctuation correlation function
FT	Fourier transform
GSB	Ground-state bleach
HEOM	Hierarchical equations of motion
HH	Heavy-hole
LH	Light-hole
NPLs	Nanoplatelets
LHCII	Light-harvesting complex II
MDOS	Multidimensional optical spectroscopy
PSII	Photosystem II
QDs	Quantum dots
SE	Stimulated emission
TA	Transient absorption

Chapter 1

Introduction

1.1 Ultrafast nonlinear optical spectroscopy

Optical spectroscopy is the science of using light to investigate the properties of materials based on light-matter interactions. In the nonlinear regime, where the signal is created by multiple interactions between the matter and light, we need a very strong excitation light source to make the nonlinear signal measurable. With the invention of the mode-locked laser [1–3] and pulse compression techniques [4–8], the temporal duration of a laser pulse can reach the femtosecond to attosecond regime and we can observe nonlinear physical, chemical and biological processes at this timescale. The importance of ultrafast nonlinear optical spectroscopy was recognized by the Nobel Prize in Chemistry in 1999 being awarded to Ahmed Zewail for his pioneering work on femtochemistry [9] and the Nobel Prize in Physics in 2018 to Gérard Mourou and Donna Strickland for the invention of the chirped pulse amplification technique [4].

1.1.1 Transient absorption spectroscopy

Figure 1.1. The typical pump-probe beam geometry used in a transient absorption measurement.

One of the most common nonlinear ultrafast optical experiment is the transient absorption (TA) spectroscopy, i.e., pump-probe experiment. The pump-probe beam geometry, which is commonly used in a transient absorption measurement is depicted in Fig. 1.1. The sample is excited by a femtosecond laser pulse, i.e., the pump pulse. This pump

pulse interacts twice with the sample to generate a transiently excited population state. After a delay time T , a broad-band probe pulse probes the excited population. The emitted third-order signal is then dispersed together with the probe pulse on a spectrograph to obtain the heterodyne detected intensity spectrum $I^{(3)}(T, \omega_t)$. In this case, the probe pulse can be viewed as a local oscillator. A chopper or a pulse shaper can be employed to chop the pump and the transient change of the absorption can be calculated as

$$\Delta A(T, \omega_t) = -\log \frac{I_{pump-on}^{(3)}(T, \omega_t)}{I_{pump-off}^{(3)}(T, \omega_t)}, \quad (1.1)$$

where $I_{pump-on}^{(3)}(T, \omega_t)$ and $I_{pump-off}^{(3)}(T, \omega_t)$ are the intensity spectra detected by the spectrometer when the pump pulse is on and off, respectively. Therefore, the $\Delta A(T, \omega_t)$ quantifies how much the sample absorption changes after a delay time T from the excitation of the pump. This technique is widely used to monitor dynamics in various systems [10–12]. However, due to the one-dimensionality of the spectra, TA spectroscopy can only provide the frequency resolution along the probe/detection dimension. By implementing the pulse train excitation with the controllable interpulse delay time, we can expand the frequency resolution along the pump/excitation dimension and this is the principle of the two-dimensional optical spectroscopy.

1.1.2 Multidimensional optical spectroscopy

Two-dimensional optical spectroscopy (2DOS) can be viewed as an improvement of the TA technique [13, 14]. In this thesis, we mainly discuss 2DOS measurements using the pulse shaper assisted pump-probe geometry setup as depicted in Fig. 1.2.

Figure 1.2. The pulse shaper assisted pump-probe beam geometry used in 2DOS measurements.

In the geometry shown in Fig. 1.2, a femtosecond laser pulse is shaped by a pulse shaper into a phase-locked double-pulse train having controllable relative delay time τ . Each pulse in the pulse train interacts once with the sample and after a delay time T , a pulse arrives to probe the excited population. The data are collected in a similar manner to the TA measurement described in Section 1.1.1. However, by scanning the relative delay time τ between two excitation pulses, we resolve the coherence occurring between two excitations and obtain a series of τ -dependent transient absorption spectra $\Delta A(\omega_t, T, \tau)$. A discrete Fourier transformation along τ dimension is performed and 2DOS spectra $S_{2D}(\omega_t, T, \omega_\tau)$ are retrieved. With one more dimension, the 2DOS spectrum is usually easier to visualize and can provide more information compared to a TA spectrum. We shall use a simple example to illustrate the extra information provided by 2DOS. Let's consider a system comprised of two chromophores absorbing at 670 nm and 680 nm. We further assume that there is an energy equilibration between them happening with a time-constant of 1 ps. The simulated 2DOS and TA spectra are presented in Fig. 1.3.

In principle, the 2DOS spectrum $S_{2D}(\omega_t, T, \omega_\tau)$ is a two-dimensional frequency correlation map showing the spectral correlation between excitation and emission frequencies. A 2D signal appearing at (ω_τ, ω_t) corresponds to the absorption at frequency ω_τ (or wavelength λ_τ) and the emission at frequency ω_t (or wavelength λ_t), after a delay time T . As shown in Fig. 1.3, at $T = 0$ ps, the 2DOS spectrum only shows two diagonal peaks corresponding to two absorption states at 670 nm and 680 nm. At later delay time T , we observe a decaying diagonal peak at $(\lambda_\tau, \lambda_t) = (670, 670)$ nm and a rising cross-peak at $(\lambda_\tau, \lambda_t) = (670, 680)$ nm. This spectral evolution is the downhill energy transfer from the higher energy state (670 nm) to the lower energy state (680 nm). In addition, due to the Boltzmann distribution, if the chromophores are in thermal equilibrium, the energy is expected to also flow uphill and this is reflected clearly by a rising cross-peak at $(\lambda_\tau, \lambda_t) = (680, 670)$ nm. Meanwhile, the corresponding TA spectra with the same set of population times T shown on the right column of Fig. 1.3 can only detect the decay of the 670-nm state and the rise of the 680-nm state. This is expected as due to the projection slice theorem, the TA spectrum is the integral of the corresponding 2DOS spectrum along the excitation frequency axis [15, 16]. Hence, in the TA spectra, the uphill energy flow is totally hidden by the overwhelming downhill flow. The ability of directly resolving downhill and uphill energy flows of the 2DOS technique was reported in literatures [17, 18]. In addition, the 2DOS is proven to be sufficient in determining all kinetic parameters in the case of spectrally well-separated multichromophore systems [19]. This makes 2DOS a widely applicable tool to monitor various processes such as excitation energy transfer in photosynthetic complexes [20–22], spectral dynamics of molecules and proteins [23, 24] or photo-induced dynamics in semiconductor nanomaterials [25–27]. Therefore, it is crucial to develop the

Figure 1.3. The comparison between 2DOS (left column) and TA (right column) spectra of a two-chromophore system absorbing at 670 nm and 680 nm. There is an energy equilibration process between two chromophores happening at 1 ps timescale. The corresponding population time T is written at the lower-right corner of the spectrum.

theoretical tools to get insights about 2DOS and more general multidimensional optical spectroscopy (MDOS).

The theoretical formalism for MDOS that is based on time-dependent perturbation theory is well-documented. The interactions between nonlinear response functions of the system and the electric fields can be fully derived and the corresponding MDOS spectra can be simulated [28, 29]. However, the MDOS spectra simulation procedure is still challenging as it involves the calculation of an n -layer integral, where n is the order of the nonlinear process. Due to the high computational cost of simulation, the matter-field interactions are usually treated as impulsive ones, i.e., the temporal durations of pulses are approximated as Dirac δ functions, and the finite bandwidths of the interacting pulses are neglected. Although the impulsive approximation simplifies the simulation process, the simulated spectra are not representative of the experimental data usually obtained using the finite duration pulses.

In this thesis, we derive the formalism incorporating the finite bandwidths of the interacting pulses into MDOS spectra. As the MDOS technique has a huge diversity and the scope of our study is limited, we only discuss the finite pulse effects on the most commonly used MDOS techniques including third-order 2DOS, fifth-order two-quantum two-dimensional optical spectroscopy (2Q-2DOS) and fifth-order three-dimensional optical spectroscopy (3DOS). In Chapter 2, using the time-domain approach we derive the simplified expressions that incorporate finite pulse envelope effects into 2DOS spectra. These expressions are simpler and less computationally intensive than the conventional triple integral calculations needed to simulate 2DOS spectra. The time-domain approach exactly replicates how the experimental 2DOS spectra are usually measured [30]. In Chapter 3, using the frequency-domain approach, we investigate how the finite bandwidths affect the fifth-order 2Q-2DOS and 3DOS spectra. The frequency-domain approach is also proven to be equivalent to the time-domain approach and the mathematical derivations in the frequency domain are more simplified [31]. Under certain conditions, the approximated expressions that we derived can significantly reduce the computational cost for the simulation process. The insights obtained from these studies provide new ways to remove the undesired effects from the finite bandwidth to recover the ‘pure’ nonlinear response functions, which is supposed to be measured in the impulsive limit. The obtained results are then applied in the following chapters to simulate the 2DOS spectra to get insights about the excitation energy transfer (EET) process of the photosynthetic membrane protein complex.

1.2 Excitation energy transfer in photosynthesis

In plant photosynthesis, the sunlight energy is captured by chlorophyll-type pigments, followed by various redox chemical reactions, H_2O and CO_2 are converted into organic

compounds and O_2 is released. With annual biomass production of more than 100 Pg of Carbon per year ($1 \text{ Pg} = 10^{15} \text{ g}$) [32], photosynthesis plays an important role in controlling CO_2 and O_2 levels of the Earth's atmosphere. In Nature, photosynthesis is mostly performed by bacteria, green algae and green plants. Although the detailed mechanisms differ among species, all photosynthesis processes begin with light-capturing by pigment-protein complexes. The excitation energy is then transferred into the corresponding reaction center to perform the charge separation and electron transfer chain [33].

1.2.1 Theoretical background

The existence of EET was proposed by Emerson and Arnold with the determination that under the saturated light intensity, only one O_2 molecule is produced per 2400 chlorophylls (Chls) [34]. The result showed that only a small portion of pigment molecules perform the photoredox water-splitting reaction. Most of the pigments serve as the light-harvesting antenna, capturing photons and funnelling excitation energy into the corresponding reaction center to conduct chemical reactions. With this arrangement, the excitation energy flow can be controlled and utilized with many quenching and protection mechanisms to regulate photosynthesis under different light conditions. In addition, different antennas can absorb at various spectral ranges to maximize the usage of natural sunlight for photosynthesis [35, 36]. There are many theories proposed to describe the EET process. If the chromophores are in close contact (up to several Ångströms), the EET can happen between the donor and the acceptor by exchanging the electron via intermolecular orbital overlap. This process is known as Dexter exchange [37, 38]. For longer distances, the EET is mainly governed by the Coulombic interactions between the chromophores. Based on the strength of the coupling between the interacting pigments, there are two main cases which are Förster theory [39] applied for the weakly coupled chromophores and Redfield theory [40], used to describe the strong coupling regime.

In the Förster picture, two chromophores are weakly coupled and all intramolecular conversions, such as vibrational relaxation, internal conversion, etc., are faster than the EET process. The exciton wavefunctions are localized within each chromophore and the transfer happens as the incoherent hopping of the excitons. The Förster transfer rate constant depends on the square of the dipole-dipole interaction and can be expressed as

$$k_{EET} = \frac{9\kappa^2 c^4}{8\pi\tau_D n^4 R^6} \int F_D(\omega) \varepsilon_A(\omega) \frac{d\omega}{\omega^4}, \quad (1.2)$$

where $F_D(\omega)$ and $\varepsilon_A(\omega)$ are the corrected fluorescence spectrum of the donor and the absorption spectrum of the acceptor, respectively. c is the speed of light, n is the

medium refractive index, R is the donor-acceptor distance and τ_D is the decay lifetime of the donor in the absence of the acceptor. The mutual orientation factor comes from the dipole-dipole interaction $\kappa = (\vec{\mu}_D \cdot \vec{\mu}_A) - 3 \left(\vec{R} \cdot \vec{\mu}_A \right) \left(\vec{R} \cdot \vec{\mu}_D \right)$. The Förster transfer rate decreases drastically with the sixth power of the donor-acceptor distance and is directly proportional to the spectral overlap between the donor fluorescence and the acceptor absorption.

In the Redfield picture, the EET rates are not explicitly calculated but the whole system density matrix $\rho(t)$ is propagated in time with the proper interaction operators. From the evolution of the system density matrix $\rho(t)$, the populations of the excitons can be obtained and the EET network is resolved. The Redfield equation in exciton basis normally takes the form as [41]

$$\frac{\partial}{\partial t} \rho_{\mu\nu}^{exc}(t) = -i\omega_{\mu\nu} \rho_{\mu\nu}^{exc}(t) + \sum_{\mu'\nu'} \mathcal{R}_{\mu\nu\mu'\nu'} \rho_{\mu'\nu'}^{exc}(t), \quad (1.3)$$

where, on the right hand side, the first term corresponds to the coherent evolution governed by the system Hamiltonian where $\omega_{\mu\nu}$ is the energy gap between the μ^{th} and the ν^{th} exciton. The second term describes the phonon induced dynamics governed by the Redfield tensor with $\mathcal{R}_{\mu\nu\mu'\nu'}$ as the transfer rate from $\rho_{\mu'\nu'}^{exc}$ to $\rho_{\mu\nu}^{exc}$. $\rho^{exc}(t)$ is the system density matrix written in exciton basis and μ, ν, μ' , and ν' are the exciton basis indexes. The Redfield tensor can be expressed as

$$\mathcal{R}_{\mu\nu\mu'\nu'} = \Gamma_{\nu'\nu\mu\mu'} + \Gamma_{\mu'\mu\nu\nu'}^* - \delta_{\nu'\nu} \sum_{\kappa} \Gamma_{\mu\kappa\kappa\mu'} - \delta_{\mu'\mu} \sum_{\kappa} \Gamma_{\nu\kappa\kappa\nu'}^*. \quad (1.4)$$

The Γ term is called damping matrix and can be obtained from the bath correlation function $\langle B_m(\tau) B_n(0) \rangle$ and system eigenvectors in site basis $(S_n)_{\mu\nu} = \langle \mu | n \rangle \langle n | \nu \rangle$ as

$$\Gamma_{\mu\nu\mu'\nu'} = \frac{1}{\hbar^2} \sum_{m,n} \int_0^{+\infty} d\tau \langle B_m(\tau) B_n(0) \rangle e^{i\omega_{\mu'\nu'}\tau} (S_m)_{\mu\nu} (S_n)_{\mu'\nu'}. \quad (1.5)$$

The bath correlation function $\langle B_m(\tau) B_n(0) \rangle$ is normally described by the spectral density function of the electron-phonon coupling strengths, where m and n are site basis indexes [41]. In Redfield theory, the EET rate is mainly determined by the overlap between the exciton wavefunctions under the perturbation of electron-phonon couplings as shown in Eqn. (1.5). The coupling between excitons is strong enough for the transfer to occur faster than any internal relaxation. Therefore, the transfer happens as the coherent wave-like motion of the excitons and the exciton wavefunctions are delocalized.

In reality, especially in the photosynthetic protein-pigment complexes, the excitonic coupling between chromophores and the exciton-phonon couplings between pigments and protein are of the same magnitude, i.e, the intermediate regime. This is the main

obstacle for describing the EET processes in biological systems. In this regime, the excitonic coupling between chromophores are strong enough to delocalize the exciton wavefunctions, but the vibration of the protein can partially destroy that and the exciton wavefunctions become semi-localized. This leads to the EET rates having the same timescale as the intramolecular conversion rates. To resolve this challenging problem, a method called hierarchical equations of motion (HEOM) was developed by Tanimura [42–45]. The HEOM method is used to propagate the density matrix of the quantum system non-perturbatively. System-bath interactions and thermal fluctuations are also considered. However, due to the high cost needed for computation, the application of HEOM is limited to small systems [41, 46]. To be able to apply to biological complexes, the HEOM approach needs simplifications and strong computational resources [47–50].

There is another approach to describe the EET in the intermediate regime called the combined Förster-Redfield theory. Although this method is only an approximation, it is widely applied in various systems due to the relatively lower computation cost compared to HEOM [51–55]. In this method, the crystal structure of the investigated complex is the prerequisite to determine the coupling strengths between all chromophores. The pigment molecules are then clustered into sub-domains in a way that within each sub-domain, all the chromophores are strongly coupled to each other and the Redfield transfer is applicable. The interdomain transfers are then treated with the Förster formalism. However, this approach can lead to some different EET schemes for the same system as the coupling strengths between the chromophores can be determined in different ways. More details will be discussed in the next section about the EET network of Light-harvesting complex II.

1.2.2 Light-harvesting complex II

Light-harvesting complex II (LHCII) is the peripheral antenna complex of green plant Photosystem II (PSII). LHCII binds half of all Chl pigments on the thylakoid membrane and it is the most abundant pigment-protein membrane complex in the biosphere [56]. LHCII mostly exists in nature in trimeric form. Figure 1.4 depicts the crystal structure of trimeric LHCII resolved by X-ray crystallography [57, 58].

From the crystal structure, each monomeric unit is determined to comprise a polypeptide chain of 232 amino acids and 18 pigment molecules (eight Chls *a*, six Chls *b* and four carotenoids) non-covalently bound to the protein. The polypeptide folds into three transmembrane α helices, forming a relatively rigid environment to keep all the pigments in an optimal spatial arrangement for functioning. The Chl pigments are clustered into two layers near two sides of the thylakoid membrane. The layer near the stromal side of the membrane consists of five Chls *a* and three Chls *b*: the Chls *a*610-611-612, Chls *a*602-603 and Chls *b*608-609 forming three strongly coupled domains,

Figure 1.4. The crystal structure of the monomeric and trimeric forms of LHCII isolated from pea *Pisum sativum* resolved by X-ray diffraction [57]. (Left) The sideview of monomeric unit with the upper/lower part is the stromal/luminal side of the thylakoid membrane. (Right) The topview of trimeric LHCII from the stromal side of the thylakoid membrane. The Chl *a*, Chl *b* and carotenoid pigments are coloured in green, cyan and yellow, respectively. The Chl pigments nomenclature shown in monomer structure and throughout this thesis are adopted from Liu et al. [57].

which are excitonically connected with the Chl *b*601. On the luminal side, there is a strongly coupled dimer of Chls *a*613-614 and four more Chls *a*604, *b*605, *b*606, and *b*607 located near to each other.

Four carotenoids in each monomeric unit act like the anchors to help the polypeptide chain folding and stabilize the protein 3D structure. The central part of the protein is stabilized by two all-*trans*-luteins crossing each other and holding two central α helices. The third carotenoid is the 9'-*cis*-neoxanthin protruding from the peripheral of the trimer and the bending head is hooked near the Chls *a/b* cluster in the luminal side. The fourth carotenoid is violaxanthin or its de-epoxidized form, zeaxanthin, under low- or high-light condition, respectively. This pigment is located at the interface between monomeric units and the de-epoxidation plays a role in the protection mechanism, which is known as non-photochemical quenching of the excess excitation energy under high-light condition [59].

LHCII is a complicated antenna apparatus with the ability to harvest sunlight energy as indicated from the name. It also functions as an energy regulator to ensure sufficient energy flows into the reaction center under limited-light and dissipate excess energy to avoid photodamage of the reaction center under harsh-light condition. These functions are dynamically tuned with many interactions with different components of the thylakoid membrane. In addition, being one of the first eukaryotic membrane proteins

to have its crystal structure solved [60, 61], LHCII has been studied intensively for the past decades.

The EET network in LHCII has been studied by various time-resolved techniques in both cryogenic and physiological temperatures. The main obtained result is that in LHCII, the Chl *b* $\rightarrow$ Chl *a* EET happens at the sub-picosecond timescale (0.15-0.6 ps at room temperature and 0.3-0.6 ps at low temperature) followed by a slower transfer of a few picoseconds from the intermediate absorption region Chls (660-670 nm) [62–69]. The energy equilibration process in the Chl *a* manifold has not been reported with consistent EET pathways. Various timescales for the equilibration process have been reported from few hundreds of femtoseconds to tens of picoseconds [18, 63, 65]. Due to the huge spectral overlap between the eight Chl *a* states, the experimental spectra are highly congested and the mechanistic details of the energy equilibration within the Chl *a* manifold remain elusive.

Since the publication of the crystal structure, many structure-based theoretical studies attempting to solve the EET network of LHCII have been proposed. Novoderezhkin et al. used modified Redfield theory [70] and later the combined Redfield-Förster picture [51, 52, 71] to determine the EET pathways in LHCII. Based on the crystal structure, the coupling strength between all Chl pigments are calculated using point-dipole approximation. The site energy of the pigments, transition dipole moments and spectral lineshapes are free parameters and they are optimized by fitting the transient absorption and steady-state spectra. The general results show that the fastest EETs take place between the strongly coupled pigments at the timescale of tens of femtosecond. The strongly coupled clusters are determined to be the dimers Chl *b*606-607, Chl *b*608-609, Chl *a*602-603, Chl *a*613-614 and the peripheral trimer Chl *a*610-611-612. The later domain is also determined to be the terminal energy acceptor which is the lowest excitonic energy level. The slower EET processes with the timescales ranging from a few to tens of picosecond are assigned to the energy equilibration between those domains and three Chls *a*604, *b*601 and *b*605.

Renger and Müh propose an ab initio quantum chemical electrostatic approach to calculate the site energy and the coupling strengths between all the Chl pigments [53–55, 72]. The excitonic states determined by this method are similar to Novoderezhkin’s model and even the lowest energy pool is also consistently determined as the Chl *a*610-611-612 trimer. However, there are still some disagreements between the two models. In Novoderezhkin’s scheme, the excitonic couplings between the strongly coupled pigments are strong enough to delocalize the exciton wavefunctions. However, Renger predicts that the protein vibrations can partially destroy the delocalization effects and significantly reduce the wavefunction overlap between different domains. This results in slower inter-domain transfer rates in Renger’s model compared to the ones in Novoderezhkin’s model. The assignment for the ‘bottleneck’ state is also

different between the two models. According to the modified Redfield calculation of Novoderezhkin, the Chls *b605* and *a604* are two ‘bottleneck’ states as they are located relatively far from the remaining pigments. Meanwhile, the electrostatic quantum calculation from Renger proposes that the Chl *a604* is closely connected with Chls *b606* and *b605*. This leads to an assignment of a much lower excitonic level for Chl *a604* and makes the downstream EET from Chl *a604* faster.

In summary, despite the wealth of literatures and knowledge, there is still no consensus in the model for the EET network in LHCII. The studies in this thesis mainly aim to solve or partially resolve the EET dynamics with detailed pathways connecting 14 Chl pigments in LHCII. The second part of this thesis is structured as follows. In Chapter 4, we present the experimental study using 2DES to investigate the EET dynamics in LHCII at different temperatures. Using global analysis, we can determine three main timescales of EET in LHCII and all dynamics become faster with the increase of temperature due to the broadening of spectral linewidths and the faster uphill energy flow dictated by the Boltzmann distribution. In Chapter 5, we propose a phenomenological model to describe the EET network in LHCII. Directly from the results of the fit, the six lowest excitonic energy levels of LHCII are determined as 666-668, 670-671, 673, 675, 677-678 and 680-681 nm with high confidence. Additionally, the homogeneous and inhomogeneous linewidths of these excitons are consistently obtained among the best 120 fits as 20-35 cm^{-1} and 30-55 cm^{-1} , respectively. Although the unique transfer scheme cannot be constructed, the model has successfully determined three distinct energy sinks where the excitation energy is eventually localized, regardless of the initial excitation condition. Additionally, the existence of the ‘bottleneck’ state in the intermediate region is also positively supported by our model.

Chapter 6 presents the application of 2DES in studying the spectral diffusion of nanomaterials. With the ability to correlate the excitation and emission frequencies, 2DES is an excellent tool to study heterogeneous ensembles such as CdSe quantum dots (QDs) and CdSe nanoplatelets (NPLs). By characterizing the dynamics of nodal lines in 2DES spectra of CdSe QDs and the central lines in the 2DES spectra of CdSe NPLs, the difference in spectral diffusion dynamics can be studied. This provides valuable insights about the photophysics of these nanomaterials.

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Chapter 2

Finite bandwidth effects in third-order 2DOS spectra

2.1 Introduction

Two-dimensional coherent optical spectroscopy (2DOS) has been employed as a powerful tool to investigate various physical, chemical, and biological systems [1–3]. 2DOS has been applied over a broad electromagnetic spectrum extending from IR, visible to UV regime. This includes experiments designed to probe the vibrational levels of chemical compounds or solvation dynamics using 2D IR spectroscopy [4–7], 2D and 3D electronic spectroscopy to study electronic excitation and energy transfer in photosynthesis apparatus [8–11], and UV 2D spectroscopy to observe electron transfer processes in proteins [12].

The third order non-linear optical response function formalism based on time dependent perturbation theory in the system-field interactions is usually used to form the framework to describe 2DOS spectra [13, 14]. In this formalism, the third order response functions $R^{(3)}(t_3, t_2, t_1)$ corresponding to the stimulated photon echo and anti-echo (rephasing and non-rephasing) signals of the material of interest are probed by multiple optical pulses. These response functions correlate single quantum coherences over t_1 with single quantum coherences over t_3 with population time (or waiting time) t_2 as a parameter. A typical 2DOS experiment seeks to measure the 2D spectral response function $R^{(3)}(\omega_3, t_2, \omega_1)$. This function is the Fourier transforms over t_1 and t_3 of the time-domain response function $R^{(3)}(t_3, t_2, t_1)$. However, due to the finite durations of the interacting pulses, the measured 2DOS spectrum is a non-trivial convolution of the 2D spectral response function $R^{(3)}(\omega_3, t_2, \omega_1)$ with the interacting finite pulses. Nonetheless, it is still a common practice to simulate the 2DOS spectra in the semi-impulsive limit, where the pulses are assumed to be infinitely short. In this limit, the 2DOS spectrum is assumed to be the 2D spectral response function $R^{(3)}(\omega_3, t_2, \omega_1)$. This approximation simplifies derivations and reduces the computational costs for simulation. This approximation is valid if the system dynamics time scales are much longer than the pulse durations. However, for many recent applications of 2DOS, this is not always the case. For example, the energy relaxation pathways in Fenna-Matthews-Olson (FMO) complex of green sulfur bacteria *C. tepidum* were resolved with lifetimes from 500 fs to 1000 fs using 50 fs duration laser pulses [15]. The coherent quantum beat in the above system was also observed by using 40 fs duration pulses revealing the beating frequency 250 cm^{-1} corresponding to 133 fs period [16]. In other studies on nanomaterials, the electronic coupling in GaAs double quantum wells was investigated. The observed dynamics was faster than the 150 fs pulse duration and could not be resolved [17]. In those studies, the pulse durations cannot be considered impulsive when

compared to the recovered dynamics.

There have been studies with calculations, both numerically [18–22] and analytically [23–25], that take into consideration the finite duration of the laser pulses on the 2DOS spectra. These studies provide better understanding of the bandwidth [18, 19] and pulse chirp [20] effects on the 2DOS spectra. The so-called non-perturbative methods of the calculation of 2DOS spectra take into account specific pulse shapes and finite pulse durations automatically [26–28]. The standard approach to incorporate the finite pulse effect is to perform the convolution of the interacting pulses and the response functions [18–20]. However, due to the need to numerically evaluate a triple integral, these approaches are computationally intensive. Another approach is to obtain expressions in the frequency domain which converts the convolutions into simple multiplications and thereby cutting down on the computational costs [23, 29, 30]. More recently, there have been analytical expressions developed for specific types of response functions interacting with pulses of certain analytical forms: Perlík et al. and Smallwood et al. developed analytical expressions for 2DOS spectra simulated for Bloch model third-order response functions using Lorentzian [24] and Gaussian excitation pulses [25], respectively.

In this chapter, using time domain approach to calculate third-order polarization, we derive general expressions for 2DOS spectra that take into account finite bandwidth effects for arbitrary pulse envelope functions. These general expressions aim to reduce the amount of numerical integration necessary to calculate the 2DOS spectra. It can then be shown that for certain common types of response functions, these expressions are exact or near-exact. This result is important for many 2DOS experiments that measure response functions that are represented in this study. A more detailed description of this motivation is provided in Section 2.2. The theory Section 2.3 provides the derivation of the expressions. Section 2.4 explore some applications with simulations and discussions.

2.2 Motivation

In the non-linear optical response function formalism based on time dependent perturbation theory, the observed signal derives from the polarization $P^{(n)}(t)$ which is generated by the interaction between the excitation pulses and the material response function [13, 14]. It can be expressed as

$$P^{(n)}(t) \propto \int_{-\infty}^t d\tau_n \int_{-\infty}^{\tau_n} d\tau_{n-1} \dots \int_{-\infty}^{\tau_2} d\tau_1 \times R^{(n)}(t - \tau_n, \tau_n - \tau_{n-1}, \dots, \tau_2 - \tau_1) \\ \times E(\tau_n)E(\tau_{n-1})\dots E(\tau_1), \quad (2.1)$$

where $R^{(n)}(t_n, t_{n-1}, \dots, t_1)$ is the n th-order response function of the material of interest. $E(\tau_i)$ are the electric fields interacting with the material at specific time moment τ_i to generate the polarization. Typically, in a 2DOS experiment, there may be some different processes with different corresponding properties. Hence, the $R^{(n)}(t_n, t_{n-1}, \dots, t_1)$ term can be a sum of various n th-order response functions.

In linear spectroscopy where $n = 1$, the first-order polarization $P^{(1)}(t)$ is a convolution between the first order response function and the optical field,

$$P^{(1)}(t) \propto \int_{-\infty}^t d\tau_1 R^{(1)}(t - \tau_1) E(\tau_1). \quad (2.2)$$

Upon Fourier transform along t , the polarization expression $P^{(1)}(t)$ gives the frequency expression

$$S(\omega_t) \equiv \int_{-\infty}^{+\infty} dt P^{(1)}(t) e^{i\omega_t t} = \tilde{E}(\omega_t) R^{(1)}(\omega_t). \quad (2.3)$$

In this convenient form, the absorption spectrum is simply a multiplication of the frequency domain linear response and the optical pulse spectrum. The convolution in Eqn. (2.2) can be avoided. Equation (2.3) is a statement of the convolution theorem where the Fourier transform of the convolution of two functions is equal to the multiplication of the individual Fourier transform of the two functions.

For the third-order response function used in 2DOS where $n = 3$, Eqn. (2.1) becomes

$$P^{(3)}(t, T, \tau) \propto \int_{-\infty}^t d\tau_3 \int_{-\infty}^{\tau_3} d\tau_2 \int_{-\infty}^{\tau_2} d\tau_1 R^{(3)}(t - \tau_3, \tau_3 - \tau_2, \tau_2 - \tau_1) E(\tau_3) E(\tau_2) E(\tau_1). \quad (2.4)$$

In a typical 2DOS measurement, the sample is usually excited by a three-pulse train as depicted in Fig. 2.1. The optical field used for such experiments typically consists of three pulses expressed as

$$\begin{aligned} E(t) &= E_1(t + T + \tau) + E_2(t + T) + E_3(t) \\ &= \mathcal{E}_1(t + T + \tau) e^{-i\omega_L(t+T+\tau)} + \mathcal{E}_2(t + T) e^{-i\omega_L(t+T)} + \mathcal{E}_3(t) e^{-i\omega_L t}, \end{aligned} \quad (2.5)$$

where τ and T are the time delays from the first to the second and from the second to the third pulse. $\mathcal{E}_i(t)$ are the envelopes of the i th pulses and ω_L is the laser frequency. In this thesis, we use $\mathcal{E}_i(t)$ to denote the pulse envelope and $E_i(t)$ to denote the optical pulse field that includes the pulse envelope and carrier wave. $\tilde{\mathcal{E}}_i(\omega)$ and $\tilde{E}_i(\omega)$ are the Fourier transform of $\mathcal{E}_i(t)$ and $E_i(t)$, respectively.

Typically, in order to obtain the third order polarization, the triple integral in Eqn.

Figure 2.1. The three-pulse train illustration with the average of response function centered at T and weighted by the pulse envelopes.

(2.4) needs to be calculated. However, with Eqn. (2.3) as an example, it is therefore attractive to seek analogous expressions for higher orders that consist of multiplications of the response function in the frequency domain and the interacting pulse spectra. In the case of third order, it is possible to derive and show that the triple Fourier transform of the third order polarization signal yields [23, 25, 29, 30]

$$\begin{aligned}
 S(\omega_t, \omega_T, \omega_\tau) &\equiv \iiint_{-\infty}^{+\infty} dt dT d\tau P^{(3)}(t, T, \tau) e^{i\omega_t t} e^{i\omega_T T} e^{i\omega_\tau \tau} \\
 &\simeq \tilde{E}_1(\omega_\tau) \tilde{E}_2(\omega_T - \omega_\tau) \tilde{E}_3(\omega_t - \omega_T) R^{(3)}(\omega_t, \omega_T, \omega_\tau), \quad (2.6)
 \end{aligned}$$

where $R^{(3)}(\omega_3, \omega_2, \omega_1)$ is the triple Fourier transform of the third-order response function $R^{(3)}(t_3, t_2, t_1)$ along all three time domains. Analogous to Eqn. (2.3), Equation (2.6) can be viewed as a statement of the convolution theorem. This kind of expression is very useful as the finite pulse effect can be taken into consideration from a direct multiplication of the interacting pulse spectra and the frequency domain expression of the response function. Equations like Eqn. (2.6) have been derived by Belabas and Jonas for applications in analyzing pulse propagation effects in 2DOS [29, 30]. Schweigert and Mukamel derived general expressions which extended applications to higher dimensional optical spectroscopies [23] of which Eqn. (2.6) can be considered a special case.

However, 2D photon echo experiments are usually presented as 2D spectra of frequency axes ω_t and ω_τ with the population time T as a parameter. The standard approach to acquire a 2DOS spectrum with finite pulse effect is to obtain the third-order polarization by performing the triple integral in Eqn. (2.4) followed by Fourier transformations over τ and t . We shall henceforth label this as the standard triple integration (STI) approach. As the STI approach is computationally intensive, and with Eqn. (2.6)

as a motivation, it is natural to seek such expressions for the 2DOS signal function,

$$S(\omega_t, T, \omega_\tau) \equiv \int_{-\infty}^{+\infty} dt d\tau P(t, T, \tau) e^{i\omega_t t} e^{i\omega_\tau \tau} \approx \tilde{E}_1(\omega_\tau) \tilde{E}_2(\omega_\tau) \tilde{E}_3(\omega_t) R^{(3)}(\omega_t, T, \omega_\tau), \quad (2.7)$$

where analogous to Eqn. (2.6), we have the spectra of the three interacting optical fields multiplied by the 2D spectral response function $R^{(3)}(\omega_3, \omega_2, \omega_1)$ which is the Fourier transform of the third-order response function along t_3 and t_1 domains. It is known that for the case where there is no longer any correlation between the excitation and detection frequencies, the 2D spectral response function is simply the product of the linear absorption spectra along both frequency dimensions, and Eqn. (2.7) is valid [19]. This typically is the case for long population time t_2 , for systems undergoing spectral diffusion. One major purpose of this article is to determine the general conditions under which the expression of Eqn. (2.7) is valid.

In Section 2.3, we present the derivation of expressions that will allow us to answer our questions better. We will then show that for the cases where 2D spectral response functions are not t_2 -dependent, the expression is exact for waiting times significantly longer than the pulse width. For t_2 -dependent 2D response functions, generally, we can only approximate the expressions. However, for two cases as shown below, the t_2 -oscillating and t_2 -exponentially decaying 2D response functions, simplified expressions related to Eqn. (2.7) are exact.

2.3 Theory

In this section, we present the derivation of the simplified expressions for the 2DOS signal function $S(\omega_t, T, \omega_\tau)$. Equation (2.4) is evaluated with the pulse train expressed in Eqn. (2.5). The first interaction comes from the first pulse presented in Eqn. (2.4) as the first integral along τ_1 . Similarly, the second and the third integrals denote the second and the third interactions between the sample and the second and the third pulses, respectively.

The kind of pulse trains in Eqn. (2.5) describes a train consisting of replica pulses that can be created by translational delay stages and are representative of pulses used in the phase matching non-collinear beam geometry, sometimes known as the Boxcar geometry. Substituting the electric field in Eqn. (2.1) and assigning $E_i(\tau_i)$ to be the i th

interaction, we have

$$\begin{aligned}
P^{(3)}(t, T, \tau) &\propto \int_{-\infty}^t d\tau_3 \mathcal{E}_3^{(\eta_3)}(\tau_3) e^{-i\eta_3 \omega_L \tau_3} \int_{-\infty}^{\tau_3} d\tau_2 \mathcal{E}_2^{(\eta_2)}(\tau_2 + T) e^{-i\eta_2 \omega_L (\tau_2 + T)} \\
&\times \int_{-\infty}^{\tau_2} d\tau_1 \mathcal{E}_1^{(\eta_1)}(\tau_1 + T + \tau) e^{-i\eta_1 \omega_L (\tau_1 + T + \tau)} R^{(3)}(t - \tau_3, \tau_3 - \tau_2, \tau_2 - \tau_1).
\end{aligned} \tag{2.8}$$

Here, the response function is either of the rephasing $R_R^{(3)}$ or non-rephasing $R_{NR}^{(3)}$ processes which is associated with the different sets of interaction indices η_i , respectively,

$$\begin{aligned}
R_R^{(3)} &\text{ with } [\eta_1, \eta_2, \eta_3] = [-1, +1, +1], \\
R_{NR}^{(3)} &\text{ with } [\eta_1, \eta_2, \eta_3] = [+1, -1, +1].
\end{aligned} \tag{2.9}$$

We use the notation of Smallwood et al. for the interaction indices η_i dictating whether an electric field ($\eta_i = 1$) interaction or a complex conjugated field ($\eta_i = -1$) interaction is being considered [25],

$$\mathcal{E}_i^{(\eta_i)}(\tau_i) e^{-i\eta_i \omega_L \tau_i} = \begin{cases} \mathcal{E}_i(\tau_i) e^{-i\omega_L \tau_i} & \text{if } \eta_i = +1, \\ \mathcal{E}_i^*(\tau_i) e^{+i\omega_L \tau_i} & \text{if } \eta_i = -1. \end{cases}$$

We can substitute the upper bound of the integrals with infinity by adding the Heaviside step functions $\Theta(t)$ to satisfy the time order of the pulse sequence,

$$\begin{aligned}
P^{(3)}(t, T, \tau) &\propto \int_{-\infty}^{+\infty} d\tau_3 \mathcal{E}_3^{(\eta_3)}(\tau_3) e^{-i\eta_3 \omega_L \tau_3} \Theta(t - \tau_3) \\
&\times \int_{-\infty}^{+\infty} d\tau_2 \mathcal{E}_2^{(\eta_2)}(\tau_2 + T) e^{-i\eta_2 \omega_L (\tau_2 + T)} \Theta(\tau_3 - \tau_2) \\
&\times \int_{-\infty}^{+\infty} d\tau_1 \mathcal{E}_1^{(\eta_1)}(\tau_1 + T + \tau) e^{-i\eta_1 \omega_L (\tau_1 + T + \tau)} \Theta(\tau_2 - \tau_1) \\
&\times R^{(3)}(t - \tau_3, \tau_3 - \tau_2, \tau_2 - \tau_1).
\end{aligned} \tag{2.10}$$

The emitted signal that is proportional to the polarization is then resolved in a spectrometer which effectively carries out a Fourier transform along t . The first coherence time τ is scanned and Fourier transformed to produce the 2DOS signal function. As indicated in Eqn. (2.7), we shall define the 2DOS signal function $S(\omega_t, T, \omega_\tau)$ as the

Fourier transform result over t and τ domains of the polarization $P^{(3)}(t, T, \tau)$,

$$S(\omega_t, T, \omega_\tau) = \int_{-\infty}^{+\infty} d\tau e^{i\omega_\tau \tau} \int_{-\infty}^{+\infty} dt e^{i\omega_t t} P^{(3)}(t, T, \tau). \quad (2.11)$$

We notice that in Eqn. (2.10), τ containing terms only appear in the first integral. We assume that all considered functions are well-behaved and satisfy the conditions for integration interchangeability [31, 32]. We can interchange the integration sequence and Eqn. (2.11) becomes

$$\begin{aligned} S(\omega_t, T, \omega_\tau) &\propto \int_{-\infty}^{+\infty} dt e^{i\omega_t t} \int_{-\infty}^{+\infty} d\tau_3 \mathcal{E}_3^{(\eta_3)}(\tau_3) e^{-i\eta_3 \omega_L \tau_3} \Theta(t - \tau_3) \\ &\quad \times \int_{-\infty}^{+\infty} d\tau_2 \mathcal{E}_2^{(\eta_2)}(\tau_2 + T) e^{-i\eta_2 \omega_L (\tau_2 + T)} \Theta(\tau_3 - \tau_2) \\ &\quad \times \int_{-\infty}^{+\infty} d\tau_1 R^{(3)}(t - \tau_3, \tau_3 - \tau_2, \tau_2 - \tau_1) \Theta(\tau_2 - \tau_1) \\ &\quad \times \int_{-\infty}^{+\infty} d\tau e^{i\omega_\tau \tau} \mathcal{E}_1^{(\eta_1)}(\tau_1 + T + \tau) e^{-i\eta_1 \omega_L (\tau_1 + T + \tau)}. \end{aligned} \quad (2.12)$$

After the operation of the two Fourier transformations, the obtained 2DOS signal function becomes

$$\begin{aligned} S(\omega_t, T, \omega_\tau) &\propto \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_\tau - \omega_L) \int_{-\infty}^{+\infty} d\tau_3 \mathcal{E}_3^{(\eta_3)}(\tau_3) e^{-i\eta_3 \omega_L \tau_3} e^{i\omega_t \tau_3} \\ &\quad \times \int_{-\infty}^{+\infty} d\tau_2 \mathcal{E}_2^{(\eta_2)}(\tau_2 + T) e^{-i\eta_2 \omega_L (\tau_2 + T)} e^{-i\omega_\tau (\tau_2 + T)} \Theta(\tau_3 - \tau_2) \\ &\quad \times R(\omega_t, \tau_3 - \tau_2, \omega_\tau). \end{aligned} \quad (2.13)$$

We note that Eqn. (2.13) is still equivalent to Eqn. (2.11). The first thing to notice is that in this case, the first interaction pulse is now recast as a pulse spectrum. The triple integral has been reduced to a double integral multiplied by a pulse spectrum. Mathematically, the Heaviside function $\Theta(\tau_3 - \tau_2)$ in Eqn. (2.13) ensures time-ordered interactions, i.e., the third pulse will always interact after the second pulse. The Heaviside function is important in cases where the second and the third pulses overlap in time. However, for cases in which the second and the third pulses do not overlap, i.e., in the case that the waiting time delay T is significantly larger than the pulse durations

of the two last pulses, the time ordering is ensured even without the Heaviside function. In most experimental conditions, 2D spectra are presented only for T larger than the pulse widths. This is due to the fact that when pulses two and three overlap, there are inadvertently coherent artifacts that distort the 2D spectra and are usually not taken into consideration [8, 33–35]. Therefore, for such cases, we can drop the Heaviside function to obtain

$$\begin{aligned}
S(\omega_t, T, \omega_\tau) &\propto \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1\omega_\tau - \omega_L) \int_{-\infty}^{+\infty} d\tau_3 \mathcal{E}_3^{(\eta_3)}(\tau_3) e^{-i\eta_3\omega_L\tau_3} e^{i\omega_t\tau_3} \\
&\times \int_{-\infty}^{+\infty} d\tau_2 \mathcal{E}_2^{(\eta_2)}(\tau_2 + T) e^{-i\eta_2\omega_L(\tau_2+T)} e^{-i\omega_\tau(\tau_2+T)} R(\omega_t, \tau_3 - \tau_2, \omega_\tau).
\end{aligned} \tag{2.14}$$

From Eqn. (2.14), a clear physical interpretation can be discerned for the remaining two integrals. It is the average of the 2D spectral response function centered at T , weighted by the pulse envelopes of the second and the third pulses as shown in Fig. 2.1. In Sections 2.3.1 – 2.3.3, we consider further simplification of Eqn. (2.14) under certain conditions.

2.3.1 t_2 -independent response functions and slowly varying response functions

We consider here for the case that the 2D spectral response function of the investigated system is t_2 -independent. In this case, the 2D spectral response function $R(\omega_t, t_2, \omega_\tau) = R(\omega_t, \omega_\tau)$ and can be taken out of the integrals in Eqn. (2.14) to give

$$\begin{aligned}
S(\omega_t, T, \omega_\tau) &= R(\omega_t, \omega_\tau) \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1\omega_\tau - \omega_L) \int_{-\infty}^{+\infty} d\tau_3 \mathcal{E}_3^{(\eta_3)}(\tau_3) e^{-i\eta_3\omega_L\tau_3} e^{i\omega_t\tau_3} \\
&\times \int_{-\infty}^{+\infty} d\tau_2 \mathcal{E}_2^{(\eta_2)}(\tau_2 + T) e^{-i\eta_2\omega_L(\tau_2+T)} e^{-i\omega_\tau(\tau_2+T)}.
\end{aligned} \tag{2.15}$$

The two remaining integrals can be easily evaluated as

$$\int_{-\infty}^{+\infty} d\tau_2 \mathcal{E}_2^{(\eta_2)}(\tau_2 + T) e^{-i\eta_2\omega_L(\tau_2+T)} = \tilde{\mathcal{E}}_2^{(\eta_2)}(-\eta_2\omega_\tau - \omega_L), \tag{2.16}$$

$$\int_{-\infty}^{+\infty} d\tau_3 \mathcal{E}_3^{(\eta_3)}(\tau_3) e^{-i\eta_3\omega_L\tau_3} e^{i\omega_t\tau_3} = \tilde{\mathcal{E}}_3^{(\eta_3)}(\eta_3\omega_t - \omega_L). \tag{2.17}$$

Finally, the 2DOS signal function can be expressed as

$$S(\omega_t, \omega_\tau) = R(\omega_t, \omega_\tau) \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^{(\eta_2)}(-\eta_2 \omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^{(\eta_2)}(-\eta_2 \omega_\tau - \omega_L). \quad (2.18)$$

For cases that the 2D spectral response function varies with a time scale that is slow compared to the pulse width, we can make the approximation that the response function does not vary so much over the last two pulse widths between the two integration time points $\tau_3 - \tau_2$ (see Fig. 2.1) and assume that the response function bears the value at T , i.e., $R^{(3)}(\omega_t, \tau_3 - \tau_2, \omega_\tau) \approx R^{(3)}(\omega_t, T, \omega_\tau)$ in Eqn. (2.14), the resultant 2D spectra can be approximated as

$$S(\omega_t, T, \omega_\tau) \approx R(\omega_t, T, \omega_\tau) \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^{(\eta_2)}(-\eta_2 \omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^{(\eta_2)}(-\eta_2 \omega_\tau - \omega_L). \quad (2.19)$$

The result presented here physically means that the 2D spectrum obtained using the pulse train expressed in Eqn. (2.5) can be approximated as the 2D spectral response function of the system in the spectral domain multiplied by the spectra of three excitation laser pulses. An expression similar to Eqn. (2.19) has been stated previously [36]. The 2DOS of transitions that undergo spectral diffusion can also be described by Eqn. (2.19) at sufficiently large waiting time, T , when the transitions are no longer correlated between the excitation frequencies ω_τ and detection frequencies ω_t [19].

We now take a closer look at Eqn. (2.18) for the two cases of non-rephasing and rephasing processes with the corresponding η_i value sets [Eqn. (2.9)]. The non-rephasing (anti-echo) and rephasing (echo) 2DOS signal functions, from Eqn. (2.18), are, respectively,

$$\begin{aligned} S_{NR}(\omega_t, \omega_\tau) &= R_{NR}(\omega_t, \omega_\tau) \tilde{\mathcal{E}}_1(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_3(\omega_t - \omega_L), \\ S_R(\omega_t, \omega_\tau) &= R_R(\omega_t, \omega_\tau) \tilde{\mathcal{E}}_1^*(-\omega_\tau - \omega_L) \tilde{\mathcal{E}}_2(-\omega_\tau - \omega_L) \tilde{\mathcal{E}}_3(\omega_t - \omega_L). \end{aligned} \quad (2.20)$$

In a typical 2DOS experiment, after three interactions with the excitation laser pulses, the generated third order polarization results in an electric field emitting along the phase matching direction,

$$S(t, T, \tau) \propto -iP^{(3)}(t, T, \tau). \quad (2.21)$$

To retrieve the phase and time dependence of this emitted field $S(t, T, \tau)$, we need to perform a heterodyne detection using a local oscillator which is either an extra pulse in a BOXCAR geometry or the probe pulse in a pump-probe geometry. The local oscillator and emitted signal are detected in the frequency domain in a spectrometer using a

square-law detector. Hence, the detected signal at the spectrometer is expressed as

$$\begin{aligned} & \left| \int_{-\infty}^{+\infty} dt e^{i\omega_t t} [S(t, T, \tau) + E_{LO}(t)] \right|^2 \\ &= \left| \tilde{E}_{LO}(\omega_t) \right|^2 + 2\Re \left[S(\omega_t, T, \tau) \tilde{E}_{LO}^*(\omega_t) \right] + |S(\omega_t, T, \tau)|^2. \end{aligned} \quad (2.22)$$

The last term of Eqn. (2.22) $|S(\omega_t, T, \tau)|^2$ is the intensity spectrum of the emitted field which is negligible compared to the other terms and is typically dropped. The first term $\left| \tilde{E}_{LO}(\omega_t) \right|^2$ is the intensity spectrum of the local oscillator. The heterodyne detected spectrum $\mathbb{S}(\omega_t, T, \tau)$ is the second term of Eqn. (2.22),

$$\mathbb{S}(\omega_t, T, \tau) = 2\Re \left[S(\omega_t, T, \tau) \tilde{E}_{LO}^*(\omega_t) \right] = S(\omega_t, T, \tau) \tilde{E}_{LO}^*(\omega_t) + S^*(\omega_t, T, \tau) \tilde{E}_{LO}(\omega_t). \quad (2.23)$$

To obtain the 2DOS spectrum $\mathbb{S}(\omega_t, T, \omega_\tau)$, discrete Fourier transformation is performed along τ ,

$$\begin{aligned} \mathbb{S}(\omega_t, T, \omega_\tau) &= \int_{-\infty}^{+\infty} d\tau e^{i\omega_\tau \tau} \left[S(\omega_t, T, \tau) \tilde{E}_{LO}^*(\omega_t) + S^*(\omega_t, T, \tau) \tilde{E}_{LO}(\omega_t) \right] \\ &= S(\omega_t, T, \omega_\tau) \tilde{E}_{LO}^*(\omega_t) + S^*(\omega_t, T, -\omega_\tau) \tilde{E}_{LO}(\omega_t). \end{aligned} \quad (2.24)$$

According to Eqn. (2.24), the heterodyne detected 2DOS spectrum consists of two terms. The first term $S(\omega_t, T, \omega_\tau) \tilde{E}_{LO}^*(\omega_t)$ is the conventional term with rephasing (echo) signal appearing at the $(\omega_t, \omega_\tau) = (+, -)$ quadrant and non-rephasing (anti-echo) signal at the $(\omega_t, \omega_\tau) = (+, +)$ quadrant. The second term $S^*(\omega_t, T, -\omega_\tau) \tilde{E}_{LO}(\omega_t)$ is the complex conjugation and mirror image of the first term over the $\omega_\tau = 0$ axis.

With the heterodyne detection taken into consideration, the final measured 2DOS spectrum $\mathbb{S}(\omega_t, T, \omega_\tau)$ becomes

$$\mathbb{S}(\omega_t, T, \omega_\tau) = S(\omega_t, T, \omega_\tau) \tilde{E}_{LO}^*(\omega_t) + S^*(\omega_t, T, -\omega_\tau) \tilde{E}_{LO}(\omega_t). \quad (2.25)$$

For 2DOS performed in the phase matching non-collinear beam geometry, where the non-rephasing and rephasing signals are acquired separately, the measured 2DOS spectra for the non-rephasing and rephasing processes are, respectively,

$$\begin{aligned} \mathbb{S}_{NR}(\omega_t, \omega_\tau) &= R_{NR}(\omega_t, \omega_\tau) \tilde{\mathcal{E}}_1(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_3(\omega_t - \omega_L) \tilde{E}_{LO}^*(\omega_t), \\ \mathbb{S}_R(\omega_t, \omega_\tau) &= R_R^*(\omega_t, -\omega_\tau) \tilde{\mathcal{E}}_1(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_3^*(\omega_t - \omega_L) \tilde{E}_{LO}(\omega_t). \end{aligned} \quad (2.26)$$

We have only included the measured 2DOS spectra in the positive quadrant $(\omega_t, \omega_\tau) = (+, +)$, as is usually presented.

For 2DOS that is performed in the pump-probe geometry, the local oscillator is the probe pulse that is the same pulse as the third interacting pulse, i.e., $\tilde{E}_{LO}(\omega_t) = \tilde{\mathcal{E}}_3(\omega_t - \omega_L)$. The signal measured in the pump-probe geometry is in purely absorptive form, as both rephasing and non-rephasing signals are measured simultaneously [37–40]. The measured 2D spectrum will be

$$\begin{aligned} \mathbb{S}_{abs}(\omega_t, T, \omega_\tau) = & R_{NR}(\omega_t, \omega_\tau) \tilde{\mathcal{E}}_1(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_3(\omega_t - \omega_L) \tilde{\mathcal{E}}_3^*(\omega_t - \omega_L) \\ & + R_R^*(\omega_t, -\omega_\tau) \tilde{\mathcal{E}}_1(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_3^*(\omega_t - \omega_L) \tilde{\mathcal{E}}_3(\omega_t - \omega_L). \end{aligned} \quad (2.27)$$

In most cases, only the real part of Eqn. (2.27) is presented.

2.3.2 Response functions with t_2 -dependent oscillation or exponential decay

As presented in Eqn. (2.19), if the 2D spectral response function is t_2 -dependent, the multiplication expression is an approximation. However, for some cases of t_2 -dependent 2D spectral response functions, we can also derive exact expressions. Let us assume that the 2D spectral response function takes the form of

$$R(\omega_t, t_2, \omega_1) \equiv R(\omega_3, \omega_1) \times e^{-i\Xi t_2}, \quad (2.28)$$

where $\Xi = \Omega - i\gamma$ is a complex number with two real valued coefficients Ω and γ . The above expression can describe the oscillation in the peaks amplitude and shape, which is one aspect of the 2DOS spectra for vibrational wave packet motion [36] and quantum coherent beatings [41, 42] ($\Xi = \Omega$ being the corresponding frequency) or the influence of vibrational relaxation during the population time ($\Xi = -i\gamma$, γ being the corresponding rate) [43] and linear combination of these expressions describes fairly general response function in the sum-over-state representation. The expressions of Eqn. (2.28) can be substituted into the response function in Eqn. (2.14). After simplification, the expression for 2DOS signal becomes

$$\begin{aligned} S(\omega_t, T, \omega_\tau) = & R(\omega_t, \omega_\tau) e^{-i\Xi T} \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_\tau - \omega_L) \int_{-\infty}^{+\infty} d\tau_3 \mathcal{E}_3^{(\eta_3)}(\tau_3) e^{-i\eta_3 \omega_L \tau_3} e^{i\omega_t \tau_3} e^{-i\Xi \tau_3} \\ & \times \int_{-\infty}^{+\infty} d\tau_2 \mathcal{E}_2^{(\eta_2)}(\tau_2 + T) e^{-i\eta_2 \omega_L (\tau_2 + T)} e^{-i\omega_\tau (\tau_2 + T)} e^{i\Xi (\tau_2 + T)}. \end{aligned} \quad (2.29)$$

The subsequent integration then gives

$$S(\omega_t, T, \omega_\tau) = R(\omega_t, \omega_\tau) e^{-i\Xi T} \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_\tau - \omega_L) \\ \times \tilde{\mathcal{E}}_2^{(\eta_2)}(-\eta_2 \omega_\tau - \omega_L + \eta_2 \Xi) \tilde{\mathcal{E}}_3^{(\eta_3)}(\eta_3 \omega_t - \omega_L - \eta_3 \Xi). \quad (2.30)$$

This expression is similar to Eqn. (2.18) except for the fact that the second and the third pulse spectra are shifted from its original spectrum by Ξ .

Let us first consider the case where the Ξ term is purely real, i.e., $\Xi = \Omega$. This represents a 2D spectral response function whose amplitude oscillates with t_2 . From Eqn. (2.30), the resultant expressions for the 2DOS signal become

$$S(\omega_t, T, \omega_\tau) = R(\omega_t, \omega_\tau) e^{-i\Omega T} \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_\tau - \omega_L) \\ \times \tilde{\mathcal{E}}_2^{(\eta_2)}(-\eta_2 \omega_\tau - \omega_L + \eta_2 \Omega) \tilde{\mathcal{E}}_3^{(\eta_3)}(\eta_3 \omega_t - \omega_L - \eta_3 \Omega). \quad (2.31)$$

The expression in Eqn. (2.31) shows that the 2D spectra consist of the 2D spectral response function oscillating during T multiplied with the first pulse spectrum. The second and the third pulse spectra that are multiplied onto the 2D spectral response function are shifted by oscillating frequency Ω . More detailed analysis will be conducted in Section 2.4.

We now consider the case where the Ξ term contains only an imaginary value, i.e., $\Xi = -i\gamma$. In this case, the t_2 -dependence is described as an exponential decay representing, for example, population transfer dynamics. Substituting $\Xi = -i\gamma$ into Eqn. (2.30), we have the expression for the 2DOS signal function as

$$S(\omega_t, T, \omega_\tau) = R(\omega_t, \omega_\tau) e^{-\gamma T} \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_\tau - \omega_L) \\ \times \tilde{\mathcal{E}}_2^{(\eta_2)}(-\eta_2 \omega_\tau - \omega_L - \eta_2 i\gamma) \tilde{\mathcal{E}}_3^{(\eta_3)}(\eta_3 \omega_t - \omega_L + \eta_3 i\gamma). \quad (2.32)$$

Similar to the previous case, the expression for the 2DOS signal function is the product of the 2D spectral response function and the three excitation pulses. However, the second and the third pulse spectra contain an imaginary frequency $i\gamma$. More detailed interpretation will be presented in Section 2.4.

2.3.3 Cases with arbitrary 2D spectral response function and analytical pulse spectra

In some cases, it may be sufficient to use an analytical function to represent the pulse spectra. For example, Gaussian functions are regularly used to represent ultrashort laser pulses. The 2D spectra of an arbitrary t_2 -dependent response function can be generated taking into account the finite pulse effect. We can reduce the two integrals needed in Eqn. (2.14) to one integral. Equation (2.14) can be expressed after changing the time

variables $\tau_3 - \tau_2 = t_2$ to give

$$S(\omega_t, T, \omega_\tau) \propto \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1\omega_\tau - \omega_L) \int_0^{+\infty} dt_2 \mathcal{E}_3^{(\eta_3)}(\tau_2 + t_2) e^{-i\eta_3\omega_L(\tau_2+t_2)} e^{i\omega_t(\tau_2+t_2)} \\ \times \int_{-\infty}^{+\infty} d\tau_2 \mathcal{E}_2^{(\eta_2)}(\tau_2 + T) e^{-i\eta_2\omega_L(\tau_2+T)} e^{-i\omega_\tau(\tau_2+T)} R(\omega_t, t_2, \omega_\tau), \quad (2.33)$$

where the integral over τ_2 no longer involves the response function. By making the substitution $\tau_2 + t_2 = x - \xi$ and $\xi = (T - t_2)/2$, we then obtain

$$S(\omega_t, T, \omega_\tau) \propto \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1\omega_\tau - \omega_L) \int_0^{+\infty} dt_2 \Phi(\Omega_+, \xi) e^{-i\xi\Omega_-} R(\omega_t, t_2, \omega_\tau), \quad (2.34)$$

where

$$\Omega_+ = \eta_3\omega_L - \omega_t + \eta_2\omega_L + \omega_\tau, \\ \Omega_- = -\eta_3\omega_L + \omega_t + \eta_2\omega_L + \omega_\tau.$$

The quantity

$$\Phi(\Omega_+, \xi) = \int_{-\infty}^{+\infty} dx \mathcal{E}_2^{(\eta_2)}(x + \xi) \mathcal{E}_3^{(\eta_3)}(x - \xi) e^{-ix\Omega_+} \quad (2.35)$$

is known as the Wigner spectrogram [44, 45]. It can be easily evaluated analytically for certain functions. For example, for pulses represented by a Gaussian function, $\mathcal{E}_i^{(\eta_i)}(t) = e^{\Gamma^2 t^2}$, one obtains

$$\Phi(\Omega_+, \xi) \propto e^{-2\Gamma^2 \xi^2} e^{-\Omega_+^2/(8\Gamma^2)}. \quad (2.36)$$

Equation (2.36) can then be substituted into Eqn. (2.34), where only one integration needs to be performed. In the event if the pulses are short on the time scale of the population dynamics, $\Phi(\Omega_+, \xi)$ is sharply peaked around $\xi = 0$, one can write

$$S(\omega_t, T, \omega_\tau) \approx R(\omega_t, T, \omega_\tau) \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1\omega_\tau - \omega_L) \times 2 \int_{-\infty}^{+\infty} d\xi \Phi(\Omega_+, \xi) e^{-i\xi\Omega_-}. \quad (2.37)$$

Equation (2.37) is equivalent to Eqn. (2.19). The approximation made in the deduction of Eqn. (2.37) from Eqn. (2.34) is similar to the so-called doorway-window approximation, which is frequently used for the description of femtosecond signals induced by

temporally well-separated pulses [46].

2.3.4 Double-sided Feynman diagram

In nonlinear optical spectroscopy, double-sided Feynman diagram (DSFD) is a convenient tool to enumerate the nonlinear processes perturbatively. As the DSFD has been well-documented in literatures [13, 14], this section only discusses some properties of the DSFD related to our study. A typical DSFD of a third-order process for a two-level system $|0\rangle$ and $|1\rangle$ is depicted in Fig. 2.2.

Figure 2.2. A typical DSFD for a third-order process.

As shown in Fig. 2.2, a typical DSFD always has two vertical lines. The left vertical line denotes the time-evolution of the ket of the system's density matrix and the right line shows the evolution of the bra. Each arrow represents the matter-field interaction between the system and the laser pulse. The last arrow indicates the nonlinear polarization emission denoted by a different type of arrow. As mentioned before, each DSFD represents a nonlinear optical process coming from the time-dependent perturbation expansion of the interaction between the system's response function and the electric field. It is important to understand the meaning of each DSFD to be able to enumerate all possible DSFDs accurately to simulate the comprehensive nonlinear optical spectra. In each DSFD:

- The time flows from the bottom to the top. The system is usually assumed to be at the ground state $|0\rangle\langle 0|$ at zero time.
- The last interaction, representing the nonlinear polarization emission, must yield a population state, i.e., the same state between the ket and the bra, as it is the trace of the density matrix after the last operation of the transition dipole moment operator $P^{(n)}(t) = \text{Tr}[\hat{\mu}\rho(t)]$.
- Between two interaction, the density matrix element oscillates with the frequency $\omega = \omega_{ket} - \omega_{bra}$, which is the different between the ket and bra frequencies.

- The arrow pointing to the right represent the electric field having interaction index $\eta_i = +1$, i.e., $E_i(t) = \mathcal{E}_i(t)e^{-i\omega_L t}$. On the other hand, the arrow pointing to the left showing the interaction with the conjugated field, $\eta_i = -1$, i.e., $E_i(t) = \mathcal{E}_i^*(t)e^{+i\omega_L t}$. The last arrow is the emission of the nonlinear polarization having the frequency and wavevector is the sum of the input frequencies and wavevectors. This is the result of the momentum and energy conservation.

2.4 Applications and Simulations

2.4.1 t_2 -independent response functions

A common example of a t_2 -independent 2D spectral response function is a system with linear spectrum that contains homogeneous and inhomogeneous linewidths. This is typically termed a Voigt's profile which is a convolution between a Lorentzian profile for the homogeneous broadening and a Gaussian profile for the inhomogeneous distribution. In such cases, the response functions for rephasing and non-rephasing processes can be expressed as

$$\begin{aligned} R_{NR}(t_3, t_2, t_1) &= - \left(\frac{i}{\hbar} \right)^3 \mu^4 \Theta(t_3) \Theta(t_2) \Theta(t_1) e^{-i\omega_0(t_3+t_1)} e^{-\Gamma(t_3+t_1)} e^{-\Delta_0^2(t_3-t_1)^2}, \\ R_R(t_3, t_2, t_1) &= - \left(\frac{i}{\hbar} \right)^3 \mu^4 \Theta(t_3) \Theta(t_2) \Theta(t_1) e^{-i\omega_0(t_3-t_1)} e^{-\Gamma(t_3+t_1)} e^{-\Delta_0^2(t_3-t_1)^2}. \end{aligned} \quad (2.38)$$

To affirm that Eqn. (2.18) is indeed the analytical equivalent of Eqn. (2.11) for t_2 -independent 2D spectral response functions, we perform calculations of the 2DOS spectra that are generated using the STI approach and using Eqn. (2.26). For simplicity, in the subsequent simulations, the center frequency of the response function is set as $\omega_0 = 0$. We investigate an ensemble of two-level systems having a homogeneous linewidth Γ of 0.2 eV and an inhomogeneous linewidth Δ_0 of 0.2 eV. Although the pulse spectra can be of arbitrary functions, for simplicity, we assume all three excitation pulses used are transform-limited Gaussian pulses with a full-width at half-maximum (FWHM) duration of 20 fs. We calculate the spectrum at population time $T = 100$ fs. The local oscillator in this simulation is the same as the last excitation laser pulse.

In Fig. 2.3(a), the spectrum depicts the real part of the non-rephasing 2DOS spectrum measured by impulsive pulses (δ pulses). It is therefore simply the 2D spectral response function which is obtained by the Fourier transform over t_3 and t_1 of the $R_{NR}(t_3, t_2, t_1)$ shown in Eqn. (2.38). Figure 2.3(b) is obtained using the simplified expression in Eqn. (2.26). The multiplication scheme is illustrated in the figure. The spectrum of the first pulse $\tilde{\mathcal{E}}_1(\omega_\tau - \omega_L)$ and the complex conjugated spectrum of the second pulse $\tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L)$ are multiplied along the ω_τ domain. Both of them are cen-

Figure 2.3. Simulation results for the 2D spectra of an ensemble of two-level systems. Figure 2.3(a) is the non-rephasing (anti-echo) response function, i.e., 2D spectrum in the impulsive regime. Figure 2.3(b) is the 2D spectrum obtained from the multiplication of Fig. 2.3(a) with suitable pulse spectra as presented. Figure 2.3(c) is the 2D spectrum obtained by calculating the triple integral in Eqn. (2.8). All spectra are normalized to the maxima.

tered at ω_L . The last pulse spectrum $\tilde{\mathcal{E}}_3(\omega_t - \omega_L)$ and the local oscillator spectrum $\tilde{E}_{LO}^*(\omega_t) = \tilde{\mathcal{E}}_3(\omega_t - \omega_L)$ are multiplied along the ω_t dimension. Figure 2.3(c) presents the real part of 2DOS spectrum calculated using the STI approach. It can be seen that Figs. 2.3(b) and 2.3(c) are virtually identical. The only difference is the numerical background noise that comes from finite numerical integrations of the triple integral in Fig. 2.3(c).

Comparing Figs. 2.3(b) and 2.3(c) with Fig. 2.3(a), we clearly see that the main difference is that the finite bandwidth of laser pulses serves as a window and limits the accessible spectral range. This effect narrows the measured 2DOS spectra compared to the ‘ideal’ 2D spectral response function. As a further test, we also consider cases when the pump pulse spectra are asymmetric. We employ the first pulse as $E_1(t) = \mathcal{E}_1(t) [e^{-i\omega_L t} + 2e^{-i(\omega_L + \zeta)t}]$ and the second pulse as $E_2(t) = \mathcal{E}_2(t) [e^{-i\omega_L t} + 2e^{-i(\omega_L + \zeta)t}]$. This translates into pulse spectrum which is a linear combination of two Gaussian functions (centered at ω_L and $\omega_L + \zeta$) of different amplitudes [see pulse spectrum on the left of Fig. 2.4(a)], thus having asymmetric spectrum. The third interacting pulse is a transform limited Gaussian pulse and the local oscillator is taken to be the same as the third interacting pulse. All excitation pulses have the temporal width of 25 fs and the simulation results presented with $\zeta = 0.1$ eV. Population time T is set as 100 fs.

In Fig. 2.4(a), we depict the 2D spectral response function which is obtained by the Fourier transform over t_3 and t_1 of the $R_{NR}(t_3, t_2, t_1)$ shown in Eqn. (2.38). Figure 2.4(b) is obtained using the simplified expression in Eqn. (2.26). The spectrum of the first pulse $\tilde{\mathcal{E}}_1(\omega_\tau - \omega_L)$ and the complex conjugated spectrum of the second pulse $\tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L)$ are asymmetric. The last pulse spectrum $\tilde{\mathcal{E}}_3(\omega_t - \omega_L)$ and the local oscillator spectrum $\tilde{E}_{LO}^*(\omega_t) = \tilde{\mathcal{E}}_3^*(\omega_t - \omega_L)$ are symmetric. Figure 2.4(c) presents the real part of 2DOS spectrum calculated using the STI approach. It can be seen that Figs. 2.4(b) and 2.4(c) are virtually identical. This further shows that the expressions derived also work for pulses with asymmetric spectra and other arbitrary shapes.

We note that although we perform the simulation to verify the validity of our expressions on this relatively simple system, our expressions in Eqns. (2.26) and (2.27) are applicable for arbitrary 2D spectral response functions that are t_2 -independent. Examples of systems with t_2 -independent 2D spectral response functions include the Fano model which is typically used to describe photoionization of atoms and photodissociation of molecules [47].

Figure 2.4. Simulation results for non-rephasing t_2 - independent response function using asymmetric spectral pulses. All the notations are the same as Fig. 2.3.

2.4.2 Quantum beat response functions

We now take a look at 2DOS spectra with response functions whose amplitude oscillates with t_2 . We have shown that the 2DOS signal function behaves according to Eqn. (2.31) if we include finite pulse effects. This can come in the form of quantum beats generated by the coherence between different excited states during the population time. One typical system with such quantum beats is a system of atomic electronic levels with one ground state connected with many excited states. If the excitation laser spectrum is broad enough to cover two or more excited states, the quantum beat coherence can be created and the signal exhibits beatings during population time. One example of such a system is a three-level system having two excited states with slightly different resonant frequencies connected through a shared ground state as shown in Fig. 2.5. Quantum beats have also featured prominently in the discussions on efficient energy transfer dynamics in photosynthetic light harvesting systems [16]. Cheng and Fleming proposed a model to study the interplay between rephasing and non-rephasing contributions into the quantum beat signal in 2D spectra [41]. Ishizaki and Fleming examined the decay of quantum beat with two models and determined the observed decay is the ensemble dephasing [42]. The beating peaks are different for rephasing and non-rephasing signals. The beating signals only appear in non-rephasing diagonal peaks and rephasing cross-peaks as presented by Khalil et al. [48]. For a comprehensive simulation of 2DOS spectrum of this three-level system, all accessible double-sided Feynman diagrams should be enumerated and treated separately, i.e., the beating and non-beating processes have different finite pulse effects. The resultant 2DOS spectrum is the sum of all individually treated processes. In the scope of this paper, we present simulation of one specific non-rephasing diagonal beating peak and interpret the finite pulse effects. The corresponding double-sided Feynman diagram of the mentioned non-rephasing process is shown in Fig. 2.5.

Figure 2.5. (Left) Double-sided Feynman diagram of the interested non-rephasing beating process. (Right) Energy level diagram of the investigated three-level system.

For a non-rephasing process, the expression for the heterodyne detected 2DOS spec-

trum in Eqn. (2.31) becomes

$$\begin{aligned} \mathbb{S}_{NR}(\omega_t, T, \omega_\tau) &= R_{NR}(\omega_t, \omega_\tau) e^{-i\Omega T} \tilde{\mathcal{E}}_1(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L - \Omega) \\ &\times \tilde{\mathcal{E}}_3(\omega_t - \omega_L - \Omega) \tilde{E}_{LO}^*(\omega_t). \end{aligned} \quad (2.39)$$

When a 2DOS spectrum is presented, only the real part of Eqn. (2.39) is usually taken. This gives rise to the oscillation of the 2DOS spectrum's peak amplitude and shape. In this simulation, we consider the same parameters as in Section 2.4.1 with both homogeneous and inhomogeneous linewidths of 0.2 eV. Three transform-limited Gaussian laser pulses have the same FWHM duration of 20 fs and are resonant with the transition $|0\rangle \rightarrow |1\rangle$. We let the local oscillator be the same as the third laser pulse, i.e., $\tilde{E}_{LO}^*(\omega_t) = \tilde{\mathcal{E}}_3^*(\omega_t - \omega_L)$. The energy difference between the $|1\rangle$ and $|1'\rangle$ states is set to 0.1 eV and yields the beating frequency $\Omega = 0.1$ eV which corresponds to a beating period of around 41.36 fs. This peak lies on the diagonal and its amplitude oscillates with population time.

Figure 2.6 is presented in a similar fashion as Fig. 2.3. It shows the simulated 2DOS spectra for the investigated non-rephasing process depicted in Fig. 2.5. Figure 2.6(a) is the real part of the 2DOS spectrum obtained with impulsive pulses (real valued 2D spectral response function). In this example, we have calculated the 2DOS spectra when the population time $T = 82.72$ fs was chosen to be twice as the beating period to keep the 2D spectral response function real valued (for ease of portrayal in the figure). The multiplication scheme according to Eqn. (2.39) between Fig. 2.6(a) and the four pulse spectra leads to Fig. 2.6(b). Figure 2.6(c) shows the 2DOS spectrum calculated using the STI approach. The similarity between the two spectra of Figs. 2.6(b) and 2.6(c) shows the validity of our derivation. For comparison, Fig. 2.6(d) depicts the 2DOS spectrum calculated via Eqn. (2.26). It is clear that the simple expressions of Eqn. (2.26) are not adequate for this case. From Eqn. (2.39) and Fig. 2.6, we can see that the first two pulse spectra $\tilde{\mathcal{E}}_1(\omega_\tau - \omega_L)$ and $\tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L - \Omega)$ are not centered at the same frequency. The second pulse spectrum has an apparent shift by Ω along the ω_τ dimension relative to the first pulse spectrum. In the ω_t dimension, the third pulse spectrum $\tilde{\mathcal{E}}_3(\omega_t - \omega_L - \Omega)$ also has an apparent shift of Ω from the local oscillator $\tilde{\mathcal{E}}_3^*(\omega_t - \omega_L)$. These frequency shifts cause an asymmetric distortion of the 2D peakshape. The 2D peak is more elongated towards the higher frequency. We perform further simulations for different population times T . They similarly show the validity of our expression [Eqn. (2.39)]. They also show the trend of the asymmetric distortion of the 2D peakshapes compared with the spectra generated with the simple expressions of Eqn. (2.26) (see below).

The shift in the frequency for the second and third pulses in Eqn. (2.39) may seem puzzling initially, as all the excitation pulses have the same central frequencies. A more

Figure 2.6. Simulation results for 2D spectra of non-rephasing beating peak. Population time $T = 82.72$ fs was chosen to be twice as the beating period to keep the 2D spectral response function real valued (for ease of presentation in the figure). Figure 2.6(a) is the non-rephasing response function in the spectral domain. Figure 2.6(b) is the result of multiplication of Fig. 2.6(a) with the appropriate pulse spectra as depicted. Figure 2.6(c) is the calculated 2DOS spectrum using the STI approach. Figure 2.6(d) is obtained by a simple expression of Eqn. (2.26) and presented for comparison.

physical interpretation of this shift can be made if we view it as a shift in the resonance frequency rather than a shift in the pulse center frequency. This can be seen from the double sided Feynman diagram (Fig. 2.5), where the second and third interactions are the $|0\rangle$ to $|1'\rangle$ transitions which are shifted from the $|0\rangle$ to $|1\rangle$ transition (which is set as 0 frequency) by Ω . This interpretation is also in line with the expectation that with a bigger separation between states $|1\rangle$ and $|1'\rangle$, the intensity of resultant peaks will decrease with the separation of the first and the second pulse spectra, and one of the third and local oscillator pulse spectra. As Ω becomes large compared with the pulse spectral widths, the pulse spectra will cease to cover simultaneously both the $|0\rangle \rightarrow |1\rangle$ and $|0\rangle \rightarrow |1'\rangle$ transitions.

In Fig. 2.6, for simplicity, we only present the simulation results corresponding to population time T equal to twice the beating period. However, the real part of Eqn. (2.39) is shown as usually the case of presenting 2DOS spectra. The term $e^{-i\Omega T}$ then gives rise to the oscillation of the 2DOS spectrum's peak amplitude and shape. In the following Figs. 2.7-2.10, we present a series of results analogous to Fig. 2.6 – (a) Real part of 2D spectral response function, (b) Calculations using Eqn. (2.39), (c) Calculation using the STI approach, and (d) Calculation using Eqn. (2.26), at various waiting time T to provide a clearer picture of the 2DOS peak oscillation. They similarly show the validity of our expression in Eqn. (2.39). They also show the trend of the asymmetric distortion of the 2D peakshapes compared with the spectra generated with the simple expression of Eqn. (2.26).

Figure 2.7. Simulation results for quantum beat response function with waiting time $T = 2$ oscillation periods. This figure is presented as the same fashion as Fig. 2.6 in Section 2.4.2. The multiplication scheme was omitted for clarity.

Figure 2.8. Simulation results for quantum beat response function with waiting time $T = 2.25$ oscillation periods. This figure is presented as the same fashion as Fig. 2.7.

Figure 2.9. Simulation results for quantum beat response function with waiting time $T = 2.5$ oscillation periods. This figure is presented as the same fashion as Fig. 2.7.

Figure 2.10. Simulation results for quantum beat response function with waiting time $T = 2.75$ oscillation periods. This figure is presented as the same fashion as Fig. 2.7.

2.4.3 Exponential decay response functions

We now discuss the expressions that describe 2DOS peaks whose amplitudes decay exponentially with t_2 . Examples of such features are commonly observed in 2DOS spectra. 2DOS is routinely deployed to measure the excitonic energy transfer (EET) dynamics of light-harvesting complexes [8, 9, 11]. In such experiments, the amplitudes of the various peaks in the 2DOS spectrum reveal the strength of coupling and transfer rates between different coupled excitonic states. The change of the peak amplitude is typically modeled with exponential decays, and the response functions of the systems will include forms resembling Eqn. (2.28), with imaginary $\Xi = -i\gamma$, where γ is the decay rate constant. The resultant 2D spectral function is described in Eqn. (2.32). Here we simulate the measured purely absorptive 2DOS spectrum that is typically used in studies of EET processes,

$$\begin{aligned} \mathbb{S}_{abs}(\omega_t, T, \omega_\tau) = & \\ & R_{NR}(\omega_t, \omega_\tau) e^{-\gamma T} \tilde{\mathcal{E}}_1(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L + i\gamma) \tilde{\mathcal{E}}_3(\omega_t - \omega_L + i\gamma) \tilde{E}_{LO}^*(\omega_t) \\ & + R_R^*(\omega_t, \omega_\tau) e^{-\gamma T} \tilde{\mathcal{E}}_1(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L - i\gamma) \tilde{\mathcal{E}}_3^*(\omega_t - \omega_L + i\gamma) \tilde{E}_{LO}(\omega_t). \end{aligned} \quad (2.40)$$

In this simulation, we consider the same parameters as in Section 2.4.1 with both homogeneous and inhomogeneous linewidths of 0.2 eV. Three laser pulses having a duration of 20 fs are used to excite the system resonantly. The population time T of 100 fs is significantly larger than the pulse durations. We consider two cases here. In the first case, the decay time is of the similar order as the pulse temporal width and the second case for the decay time scale is much longer than the pulse duration. In the first case, we assume a decay rate $\gamma = 0.1$ eV (corresponding to a lifetime of ~ 40 fs) in the 2D spectral response function. Figure 2.11 illustrates the resultant purely absorptive 2DOS spectra.

Figure 2.11 is presented in a similar fashion to Fig. 2.3. Figure 2.11(a) presents the real part of the purely absorptive 2DOS spectrum obtained with impulsive pulses. Figure 2.11(b) presents the real part of the purely absorptive 2DOS spectrum as calculated from Eqn. (2.40), while Fig. 2.11(c) presents the real part of the 2DOS spectrum as calculated using the STI approach. It is clear that apart from the numerical noise, the spectra are identical. Figure 2.11(d) presents the real part of the 2DOS calculated from applying the simple expression of Eqn. (2.27). The obvious difference is that the simple expression of Eqn. (2.27) results in a narrower peakshape than the actual peakshape. This can be understood by looking at the expression of Eqn. (2.29). The two integrals that give rise to the pulse spectra with imaginary frequency shift can be viewed as Fourier transforms over the pulse envelope multiplied by the exponential decay $e^{-\gamma T}$. The resultant convolution introduces complex valued pulse spectra which upon the ap-

Figure 2.11. Simulation results of purely absorptive 2D spectra for the population transfer response function. Figure 2.11(a) is the purely absorptive 2D spectrum obtained using semi-impulsive pulses. Figure 2.11(b) is obtained using Eqn. (2.40) with the multiplication scheme presented. Figure 2.11(c) is calculated using the STI approach. Figure 2.11(d) is the purely absorptive 2DOS spectrum without population decay calculated using Eqn. (2.27) and shown for comparison purpose. All 2D spectra are normalized to the maxima.

plication of Eqn. (2.40) mixes the dispersive part of the 2D spectral response function with the purely absorptive portions to give a broadened 2D peakshape [Fig. 2.11(b)] compared to Fig. 2.11(d). In the case above, the decaying lifetime is in a similar order as the pulse durations, and the effects of the finite pulse width are apparent. We now consider the case where the population transfer lifetime is long compared to the pulse widths. In this case, we let the lifetime be 400 fs ($\gamma = 1/400 \text{ fs}^{-1}$) and the results are shown in Fig. 2.12.

The scheme of Fig. 2.12 follows that of Fig. 2.11. Figure 2.12(a) presents the real part of the purely absorptive 2DOS spectrum obtained with impulsive pulses. Figure 2.12(b) presents the real part of the 2DOS spectrum as calculated from Eqn. (2.40), while Fig. 2.12(c) presents the real part of the 2DOS spectrum as calculated using the STI approach for comparison. Figure 2.12(d) presents the real part of the 2DOS spectrum calculated from applying Eqn. (2.27). In this case, Fig. 2.12(d) is similar to the spectra in Figs. 2.12(b) and 2.12(c).

Figure 2.12. Simulation results for slow decaying response function. All notations are the same as Fig. 2.11.

Therefore, by comparing the simulations in Figs. 2.11 and 2.12, we can conclude that the finite pulse effect is significant and apparent in the regime where the population decay time scale is of the same order as the pulse durations. If the decay rate is slow compared to the excitation pulse duration, the approximation of Eqn. (2.19) applies.

2.5 Conclusion

In this chapter, using the time domain approach, we derive simplified expressions for third-order 2DOS spectra that incorporate effects of finite pulse durations with arbitrary excitation pulse spectral profile. Compared to the standard triple integration method, the simplified expressions are computationally less intensive. The expressions can be reduced to one integral for arbitrary t_2 -dependent 2D spectral response functions with analytical Gaussian shaped excitation pulses. For certain cases, the expressions can be reduced to the simple product of the 2D spectral response function $R(\omega_3, T, \omega_1)$ with arbitrary excitation pulse spectra. These cases include t_2 -independent 2D spectral response functions, quantum beat systems, and population evolving dynamics. For 2D spectral response functions that vary slowly with t_2 , the expression of Eqn. (2.19) is a good approximation. We performed simulations to verify that the simplified expressions

are indeed equivalent to the standard triple integration method. We note that although in our simulations we used Gaussian pulse spectra, the expressions are applicable for arbitrary pulse spectra, as is the case for a realistic experiment. There are recent studies that consider analytical expressions for 2D spectra that incorporate pulse shapes of known functional forms [24, 25]. In the expressions we develop here, the pulse spectra can be arbitrary functions or simply the experimental pulse spectra that can be easily measured in an experiment. Our current work therefore is complementary to and a generalization of these previous studies.

The results of the presented work should be useful for the 2DOS community. With efficient procedures to simulate the 2DOS spectra and the resulting knowledge of how the finite pulse duration can alter 2DOS spectra, this opens up the possibility of recovering the original 2D spectral response functions of the material of interest by the deconvolution of the experimental 2DOS spectra. Trial 2D spectral response functions can be multiplied with the measured spectra of the pulses used, according to the appropriate expressions derived in this chapter, to generate numerical 2DOS spectra which can then be compared with the experimentally measured 2DOS spectra. An optimal search loop can then be formulated to arrive at a best fit 2D spectral response function.

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Chapter 3

Finite bandwidth effects in fifth-order 2Q-2DOS and 3DOS spectra

3.1 Introduction

As discussed in the previous chapter, 2DOS has been shown to be a powerful third-order four-wave-mixing spectroscopic technique [1–4]. 2DOS can be implemented in several different ways [5] and possesses the highest information content among all third-order nonlinear optical spectroscopies. It is capable of monitoring the dynamical changes of material systems during three sequential time intervals determined by the time delays between the three interacting laser pulses typically being used in a 2DOS experiment. If the durations of the pulses are short enough for their time envelopes to be approximated by Dirac-delta functions (the so-called impulsive limit), then one obtains the 2D spectra that represent the complete third order 2D spectral response function of the material under study. Otherwise, the measured 2DOS spectra will in general depend on the carrier frequencies and the time envelopes of the pulses. Although this creates a possibility to use the laser pulse properties to control the system responses [6], this pulse effects on the spectra may be undesirable. As has been recently demonstrated [7–9], careful account of pulse-envelope effects may become crucial for adequate interpretation of the measured 2DOS spectra.

In principle, the available theoretical methods are capable of the simulation of 2DOS spectra induced by any arbitrary shaped pulses [10, 11], and several theoretical studies were focused on how pulse-envelope effects modify 2DOS spectra of material systems of different complexity [12–20]. Yet the rigorous analysis of these effects is system-dependent and requires, in general, extensive simulations. To overcome this difficulty, analytical expressions for 2DOS spectra for simple model systems measured using exponential [21] and Gaussian [22] pulses have been developed. A more general and computationally convenient approach to take into account pulse-envelope effects [23] have also been presented in the previous chapter. Although under some circumstances, only approximated expressions can be derived.

As the third-order 2DOS has been developed into a versatile and mature diagnostic tool, the forefront of contemporary research is shifting toward even more sophisticated fifth-order two-quantum 2D optical spectroscopy (2Q-2DOS) and three-dimensional optical spectroscopy (3DOS), in infrared [4, 24–26], tetra-hertz (THz) [27], and visible regimes [28–32] (see also recent reviews [33, 34]). Analogous to the third-order 2DOS technique, in a typical 2Q-2DOS experiment, a two-pulse train is used to excite the sample before interacting and probed with a probe pulse. Each of the excitation

pulse interacts twice and the probe pulse interacts once with the sample to generate a fifth-order polarization which is then heterodyne detected by the same probe pulse. Due to the double interaction from the first pulse, this 2Q-2DOS technique can reveal the coherence between the ground state and the double-excited state of the investigated system. For 3DOS experiment, a four-pulse train comprising four pulses with controllable delays is typically used to excite the sample and followed by a probe pulse. This type of pulse sequence creates three coherence and two population time intervals. With the three coherence periods that are Fourier transformed to give the three frequency dimensions, this 3DOS technique can resolve the three-point frequency correlation or multi-step population transfer of the system. These novel techniques have enormous potential, opening the world of multi-stage photophysical processes and photochemical reactions to detailed spectroscopic scrutiny. However, just like the third-order 2DOS situation, unless the experiments are performed in the impulsive limit, where the measured 2Q-2DOS and 3DOS spectra will fully represent the 2Q-2D and 3D spectral response functions, respectively, there will be non-trivial finite pulse effects that need to be considered. Therefore, a careful analysis of pulse-envelope effects is of great importance.

In this chapter, we extend our analysis of the finite bandwidth effects in third-order 2DOS spectra [23] in the previous chapter to consider the fifth-order 2Q-2DOS and fifth-order 3DOS spectra. We introduce the gate functions which relate the 3D or 2Q-2D spectral response function and the 3DOS or 2Q-2DOS spectra measured under typical experimental conditions with finite pulses. We show that these can be performed in a computationally efficient way. By making the appropriate approximations, we derive simple analytical formulae, which express the measured 2Q-2DOS or 3DOS spectra through the 2Q-2D or 3D spectral response function multiplied by the appropriate pulse-shape factors.

The chapter is structured as follows. Section 3.2 introduces the starting equations and definitions. Section 3.3 applies the frequency domain approach to investigate the finite bandwidth effects in third-order 2DOS spectra to show the equivalent between time and frequency domain approaches. Section 3.4 and 3.5 present, respectively, the explicit expressions for the fifth-order 2Q-2DOS and 3DOS spectra. Section 3.6 illustrates the results of Sections 3.4 and 3.5 by the explicit comparisons of the spectral response functions, the approximate, and the exact fifth-order 2Q-2DOS and 3DOS spectra calculated for a representative model system. The results are summarized in Section 3.7.

3.2 Preliminaries and definitions

According to time dependent perturbation theory, any n th-order optical signal is determined by the nonlinear polarization $P^{(n)}(t)$ which is generated by n interactions of the

material system with external fields [10],

$$P^{(n)}(t) \propto \int_0^{+\infty} dt_n \int_0^{+\infty} dt_{n-1} \dots \int_0^{+\infty} dt_1 R^{(n)}(t_n, t_{n-1}, \dots, t_1) \\ \times E_n(t - t_n) E_{n-1}(t - t_n - t_{n-1}) \dots E_1(t - t_n - t_{n-1} - \dots - t_1). \quad (3.1)$$

Here $R^{(n)}(t_n, t_{n-1}, \dots, t_1)$ is the n th-order response function of the material system of interest and $E(t)$ are the optical fields of the laser pulses involved. This response function is causal in time to ensure the time ordering of interactions. For higher-order (fifth, seventh, etc.) spectroscopies, $R^{(n)}$ can be calculated analytically for a system of (slightly) anharmonic displaced oscillators [35] or $P^{(n)}(t)$ can be evaluated non-perturbatively for any $E_n(t)$ [36]. Equation (3.1) can equivalently be written in the frequency domain [10] as

$$P^{(n)}(t) \propto \int_{-\infty}^{+\infty} d\omega_n \int_{-\infty}^{+\infty} d\omega_{n-1} \dots \int_{-\infty}^{+\infty} d\omega_1 R^{(n)}(\omega_1 + \dots + \omega_n, \omega_1 + \dots + \omega_{n-1}, \dots, \omega_1) \\ \times e^{-i(\omega_1 + \omega_2 + \dots + \omega_n)t} \tilde{E}_n(\omega_n) \tilde{E}_{n-1}(\omega_{n-1}) \dots \tilde{E}_1(\omega_1). \quad (3.2)$$

Here

$$R^{(n)}(\Omega_n, \Omega_{n-1}, \dots, \Omega_1) = \int_0^{+\infty} dt_n \int_0^{+\infty} dt_{n-1} \dots \int_0^{+\infty} dt_1 e^{i(\Omega_n t_n + \Omega_{n-1} t_{n-1} + \dots + \Omega_1 t_1)} \\ \times R^{(n)}(t_n, t_{n-1}, \dots, t_1)$$

is the (one-sided) Fourier transform (FT) of the response function and

$$\tilde{E}_n(\omega) = \int_{-\infty}^{+\infty} dt e^{i\omega t} E_n(t)$$

is the FT of the external field. We also introduce mixed time-frequency representation of the response functions. For example, $R^{(n)}(\Omega_n, t_{n-1}, \dots, \Omega_1)$ means that the FT has been made with respect to all time variables but t_{n-1} . Hereafter, we adopt the following notation: ω_n and Ω_n in the expressions for response functions and pulse envelopes will always refer to the FTs, while t_n and τ_n will always refer to the time domain. The electric fields can be explicitly written as

$$E_n^{(\eta_n)}(t) = e^{-i\eta_n \Omega_n^p(t - \tau_n)} \mathcal{E}_n^{(\eta_n)}(t - \tau_n) \quad (3.3)$$

where τ_n is the central time of the pulse, Ω_n^p is its carrier frequency, and $\mathcal{E}_n^{(\eta_n)}(t)$ is its envelope. The quantities $\eta_n = \pm 1$ determine the sign of the phase factor and will be referred to as interaction indexes as described in the previous chapter [23]. Therefore,

$$\tilde{E}_n^{(\eta_n)}(\omega) = e^{i\omega\tau_n} \tilde{\mathcal{E}}_n^{(\eta_n)}(\eta_n\omega - \Omega_n^p), \quad (3.4)$$

where $\tilde{\mathcal{E}}_n^{(\eta_n)}(\omega)$ is the FT of the pulse envelope. We also introduce the pulse durations, which can be defined, for example, as

$$\tau_n^p = \int_{-\infty}^{+\infty} dt \frac{\mathcal{E}_n^{(\eta_n)}(t)}{\mathcal{E}_n^{(\eta_n)}(t=0)} = \frac{\tilde{\mathcal{E}}_n^{(\eta_n)}(\omega=0)}{\mathcal{E}_n^{(\eta_n)}(t=0)}.$$

3.3 Third-order 2DOS spectra

In Chapter 2, we discuss about the finite bandwidth effects in the third-order 2DOS spectra using the time domain approach. In time domain approach, the nonlinear polarization is calculated and then followed by the proper Fourier transformations to result the corresponding 2DOS spectra. This approach can replicate exactly how the nonlinear optical experiment is usually conducted. However, the direct calculation of nonlinear polarization is not always analytically possible, especially when for the higher order signal. In this chapter, we are investigating the fifth-order process and the complexity of the formula makes the time domain approach is not very feasible. In this section, we present the derivation for third-order 2DOS spectra using frequency domain approach and we show that the new approach is totally equivalent and it can significantly simplify the derivation for the fifth-order cases. In this chapter, for simplicity, we always assume the local oscillator spectrum is a white-light continuum, i.e., $\tilde{E}_{LO}(\omega) = \text{const.}$ Hence, the heterodyne detected 2D(3D) spectra are directly the 2D(3D) Fourier transform of nonlinear polarizations.

The third-order polarization $P^{(3)}(t, T, \tau)$ emitting in the third-order 2DOS experiment can be expressed as

$$\begin{aligned} P^{(3)}(t, T, \tau) \propto & \int_0^{+\infty} dt_3 \int_0^{+\infty} dt_2 \int_0^{+\infty} dt_1 R^{(3)}(t_3, t_2, t_1) \\ & \times E_3(t - t_3) E_2(t - t_3 - t_2) E_1(t - t_3 - t_2 - t_1) \end{aligned} \quad (3.5)$$

where the pulses defined in Eqn. (3.3) have the time delays $\tau_1 = -\tau - T$, $\tau_2 = -T$ and

$\tau_3 = 0$. The measured 2DOS spectrum is defined as

$$\mathbb{S}^{(3)}(\omega_t, T, \omega_\tau) = \int_{-\infty}^{+\infty} d\tau e^{i\omega_\tau \tau} \int_{-\infty}^{+\infty} dt e^{i\omega_t t} P^{(3)}(t, T, \tau). \quad (3.6)$$

Now we change to frequency domain representation of Eqn. (3.2), the third-order polarization expression becomes

$$\begin{aligned} P^{(3)}(t, T, \tau) &\propto \int_{-\infty}^{+\infty} d\omega_3 \int_{-\infty}^{+\infty} d\omega_2 \int_{-\infty}^{+\infty} d\omega_1 R^{(3)}(\omega_3 + \omega_2 + \omega_1, \omega_2 + \omega_1, \omega_1) \\ &\times e^{-i(\omega_1 + \omega_2 + \omega_3)t} e^{-i(\omega_1 + \omega_2)T} e^{-i\omega_1 \tau} \\ &\times \tilde{\mathcal{E}}_3^{(\eta_3)}(\eta_3 \omega_3 - \Omega_3^p) \tilde{\mathcal{E}}_2^{(\eta_2)}(\eta_2 \omega_2 - \Omega_2^p) \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_1 - \Omega_1^p). \end{aligned} \quad (3.7)$$

Inserting the expression in Eqn. (3.7) into the Eqn. (3.6), two integrals along t and τ dimensions can be easily performed and produce the product of two δ -functions, $\delta(\omega_1 + \omega_2 + \omega_3 - \omega_t) \delta(\omega_1 - \omega_\tau)$. As a result,

$$\begin{aligned} \mathbb{S}^{(3)}(\omega_t, T, \omega_\tau) &\propto \int_{-\infty}^{+\infty} d\omega_2 R^{(3)}(\omega_t, \omega_\tau + \omega_2, \omega_\tau) e^{-i(\omega_\tau + \omega_2)T} \\ &\times \tilde{\mathcal{E}}_3^{(\eta_3)}[\eta_3(\omega_t - \omega_2 \omega_\tau) - \Omega_3^p] \tilde{\mathcal{E}}_2^{(\eta_2)}(\eta_2 \omega_2 - \Omega_2^p) \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_\tau - \Omega_1^p). \end{aligned} \quad (3.8)$$

We note that Eqn. (3.8) is exact. Introducing the mixed time-frequency response function $R^{(3)}(\omega_t, t_2, \omega_\tau)$, Eqn. (3.8) can be rewritten as

$$\mathbb{S}^{(3)}(\omega_t, T, \omega_\tau) \propto \int_0^{+\infty} dt_2 R^{(3)}(\omega_t, t_2, \omega_\tau) \Phi^{(3)}(t_2 - T), \quad (3.9)$$

where

$$\Phi^{(3)}(t_2) = \int_{-\infty}^{+\infty} d\omega_2 e^{i(\omega_\tau + \omega_2)t_2} \tilde{\mathcal{E}}_3^{(\eta_3)}[\eta_3(\omega_t - \omega_2 \omega_\tau) - \Omega_3^p] \tilde{\mathcal{E}}_2^{(\eta_2)}(\eta_2 \omega_2 - \Omega_2^p) \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_\tau - \Omega_1^p). \quad (3.10)$$

Here $\Phi^{(3)}$ is the gate function (cf. refs. [37–40]). It depends on the pulse envelopes and can be expressed in terms of the Wigner spectrograms of the pulses [23]. $\Phi^{(3)}$ (and all other gate functions of the present work) can be analytically evaluated for Gaussian or exponential pulse envelopes and can be calculated by a numerical integration for any arbitrary pulse envelopes.

Equation (3.9) is still exact. Now, assuming that the pulses are short on the time scale of the vibrational dynamics of the system, we can replace $R^{(3)}(\omega_t, t_2, \omega_\tau)$ by $R^{(3)}(\omega_t, T, \omega_\tau)$ in Eqn. (3.9). The replacement is also correct if the response function are independent or weakly dependent on the population time t_2 . (This is equivalent with the case considered in Section 2.3.1). One thus arrives at the approximate expression

$$\mathbb{S}^{(3)}(\omega_t, T, \omega_\tau) \propto \mathcal{A}^{(3)}(\omega_t, \omega_\tau) R^{(3)}(\omega_t, T, \omega_\tau), \quad (3.11)$$

where

$$\mathcal{A}^{(3)}(\omega_t, \omega_\tau) = \int_{-T}^{+\infty} dt_2 \Phi^{(3)}(t_2).$$

After the replacement of T by ∞ (this is correct if $T > \tau_2^p + \tau_3^p$), the integration over t_2 yields $\delta(\omega_2 + \omega_\tau)$. Hence, Eqn. (3.10) simplifies to

$$\mathcal{A}^{(3)}(\omega_t, \omega_\tau) = \tilde{\mathcal{E}}_3^{(\eta_3)}(\eta_3 \omega_t - \Omega_3^p) \tilde{\mathcal{E}}_2^{(\eta_2)}(-\eta_2 \omega_\tau - \Omega_2^p) \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_\tau - \Omega_1^p). \quad (3.12)$$

The expression in Eqn. (3.12) is also obtained in Chapter 2 using time domain approach, i.e., directly calculate the polarization in Eqn. (3.5). Therefore, the time and frequency domain approaches are totally equivalent.

3.4 Fifth-order 2Q-2DOS spectra

The fifth-order resonant 2Q-2D optical spectroscopy (2Q-2DOS) has been proposed in ref. [41]. The pulse delays are defined in Eqn. (3.13) and the pulse train for 2Q-2DOS measurement is depicted in Fig. 3.1.

$$\tau_1 = \tau_2 = -\tau - T, \quad \tau_3 = \tau_4 = -T, \quad \tau_5 = 0. \quad (3.13)$$

The corresponding fifth-order polarization is denoted as $P_{2D}^{(5)}(t, T, \tau)$ and the 2Q-2DOS spectrum is defined as

$$\mathbb{S}_{2D}^{(5)}(\omega_t, T, \omega_\tau) = \int_{-\infty}^{+\infty} d\tau e^{i\omega_\tau \tau} \int_{-\infty}^{+\infty} dt e^{i\omega_t t} P_{2D}^{(5)}(t, T, \tau), \quad (3.14)$$

Figure 3.1. Scheme of the pulse train of 2Q-2DOS measurement. The pulse envelopes are depicted by the blue lines. The oscillatory (straight) orange lines indicate the time intervals during which the system evolves in electronic coherence (population).

where $P_{2D}^{(5)}(t, T, \tau)$ can be evaluated by Eqn. (3.1) with $n = 5$. For the pulse delays defined per Eqn. (3.13), one obtains

$$\begin{aligned}
 P_{2D}^{(5)}(t, T, \tau) &\propto \int_0^{+\infty} dt_5 \int_0^{+\infty} dt_4 \int_0^{+\infty} dt_3 \int_0^{+\infty} dt_2 \int_0^{+\infty} dt_1 R_{2D}^{(5)}(t_5, t_4, t_3, t_2, t_1) \\
 &\times e^{-i\eta_5 \Omega_5^p(t-t_5)} \mathcal{E}_5^{(\eta_5)}(t-t_5) e^{-i\eta_4 \Omega_4^p(t-t_5-t_4+T)} \mathcal{E}_4^{(\eta_4)}(t-t_5-t_4+T) \\
 &\times e^{-i\eta_3 \Omega_3^p(t-t_5-t_4-t_3+T)} \mathcal{E}_3^{(\eta_3)}(t-t_5-t_4-t_3+T) \\
 &\times e^{-i\eta_2 \Omega_2^p(t-t_5-t_4-t_3-t_2+T+\tau)} \mathcal{E}_2^{(\eta_2)}(t-t_5-t_4-t_3-t_2+T+\tau) \\
 &\times e^{-i\eta_1 \Omega_1^p(t-t_5-t_4-t_3-t_2-t_1+T+\tau)} \mathcal{E}_1^{(\eta_1)}(t-t_5-t_4-t_3-t_2-t_1+T+\tau).
 \end{aligned} \tag{3.15}$$

Switching into frequency domain representation, the Eqn. (3.15) becomes

$$\begin{aligned}
 P_{2D}^{(5)}(t, T, \tau) &\propto \int_{-\infty}^{+\infty} d\omega_5 \int_{-\infty}^{+\infty} d\omega_4 \int_{-\infty}^{+\infty} d\omega_3 \int_{-\infty}^{+\infty} d\omega_2 \int_{-\infty}^{+\infty} d\omega_1 \\
 &\times R_{2D}^{(5)}(\omega_1 + \omega_2 + \omega_3 + \omega_4 + \omega_5, \omega_1 + \omega_2 + \omega_3 + \omega_4, \omega_1 + \omega_2 + \omega_3, \omega_1 + \omega_2, \omega_1) \\
 &\times e^{-i(\omega_1 + \omega_2 + \omega_3 + \omega_4 + \omega_5)t} e^{-i(\omega_1 + \omega_2 + \omega_3 + \omega_4)T} e^{-i(\omega_1 + \omega_2)\tau} \tilde{\mathcal{E}}_5^{(\eta_5)}(\eta_5 \omega_5 - \Omega_5^p) \\
 &\times \tilde{\mathcal{E}}_4^{(\eta_4)}(\eta_4 \omega_4 - \Omega_4^p) \tilde{\mathcal{E}}_3^{(\eta_3)}(\eta_3 \omega_3 - \Omega_3^p) \tilde{\mathcal{E}}_2^{(\eta_2)}(\eta_2 \omega_2 - \Omega_2^p) \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1 \omega_1 - \Omega_1^p).
 \end{aligned} \tag{3.16}$$

Substituting the expression in Eqn. (3.16) into the Fourier transform in Eqn. (3.14), one can perform two integrations along t and τ to yield the product of two δ -functions as

$\delta(\omega_1 + \omega_2 + \omega_3 + \omega_4 + \omega_5 - \omega_t)\delta(\omega_1 + \omega_2 - \omega_\tau)$. As a result,

$$\mathbb{S}_{2D}^{(5)}(\omega_t, T, \omega_\tau) \propto \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} d\omega_1 d\omega_3 d\omega_4 R_{2D}^{(5)}(\omega_t, \omega_\tau + \omega_3 + \omega_4, \omega_\tau + \omega_3, \omega_\tau, \omega_1) \Phi_{2D}^{(5)}(\omega_4, \omega_3, \omega_1), \quad (3.17)$$

in which

$$\begin{aligned} \Phi_{2D}^{(5)}(\omega_4, \omega_3, \omega_1) = & e^{-i(\omega_\tau + \omega_3 + \omega_4)T} \tilde{\mathcal{E}}_5^{(\eta_5)} [\eta_5(\omega_t - \omega_\tau - \omega_3 - \omega_4) - \Omega_5^p] \tilde{\mathcal{E}}_4^{(\eta_4)} (\eta_4\omega_4 - \Omega_4^p) \\ & \times \tilde{\mathcal{E}}_3^{(\eta_3)} (\eta_3\omega_3 - \Omega_3^p) \tilde{\mathcal{E}}_2^{(\eta_2)} [\eta_2(\omega_\tau - \omega_1) - \Omega_2^p] \tilde{\mathcal{E}}_1^{(\eta_1)} (\eta_1\omega_1 - \Omega_1^p). \end{aligned} \quad (3.18)$$

Equation (3.17) is exact and fully accounts for finite pulse durations. It involves triple convolution of the response function in the frequency domain with the gate function $\Phi_{2D}^{(5)}(\omega_4, \omega_3, \omega_1)$ defined through the pulse envelopes per Eqn. (3.18). The method of gate functions was used previously to describe time- and frequency-resolved spontaneous emission [37, 38, 42] and four-wave-mixing responses [39]. Equation (3.17) is more efficient computationally than a straightforward evaluation of $P_{2D}^{(5)}(t, T, \tau)$ by Eqn. (3.15) (which involves 5-fold integration) following the double FT according to Eqn. (3.14).

We can rewrite the Eqn. (3.17) by introducing the mixed time-frequency response function $R_{2D}^{(5)}(\omega_t, t_4, \omega_\tau + \omega_3, \omega_\tau, \omega_1)$ as

$$\mathbb{S}_{2D}^{(5)}(\omega_t, T, \omega_\tau) \propto \int_0^{+\infty} dt_4 \int_{-\infty}^{+\infty} d\omega_1 d\omega_3 R_{2D}^{(5)}(\omega_t, t_4, \omega_\tau + \omega_3, \omega_\tau, \omega_1) \Phi_{2D}^{(5)}(t_4 - T, \omega_3, \omega_1), \quad (3.19)$$

where

$$\begin{aligned} \Phi_{2D}^{(5)}(t_4, \omega_3, \omega_1) = & \int_{-\infty}^{+\infty} d\omega_4 e^{-i(\omega_\tau + \omega_3 + \omega_4)t_4} \tilde{\mathcal{E}}_5^{(\eta_5)} [\eta_5(\omega_t - \omega_\tau - \omega_3 - \omega_4) - \Omega_5^p] \\ & \times \tilde{\mathcal{E}}_4^{(\eta_4)} (\eta_4\omega_4 - \Omega_4^p) \tilde{\mathcal{E}}_3^{(\eta_3)} (\eta_3\omega_3 - \Omega_3^p) \tilde{\mathcal{E}}_2^{(\eta_2)} [\eta_2(\omega_\tau - \omega_1) - \Omega_2^p] \tilde{\mathcal{E}}_1^{(\eta_1)} (\eta_1\omega_1 - \Omega_1^p). \end{aligned} \quad (3.20)$$

Assuming that the pulses are short on the time scale of vibrational motion or the response function is weakly dependent on population time t_4 , we can replace t_4 by T and

the response function term $R_{2D}^{(5)}$ can be brought out of the integral to yield

$$\mathbb{S}_{2D}^{(5)}(\omega_t, T, \omega_\tau) \propto \int_{-\infty}^{+\infty} d\omega_1 d\omega_3 R_{2D}^{(5)}(\omega_t, T, \omega_\tau + \omega_3, \omega_\tau, \omega_1) \Phi_{2D}^{(5)}(\omega_3, \omega_1), \quad (3.21)$$

where

$$\begin{aligned} \Phi_{2D}^{(5)}(\omega_3, \omega_1) = & \int_{-\infty}^{+\infty} d\omega_4 \int_{-T}^{+\infty} dt_4 e^{-i(\omega_\tau + \omega_3 + \omega_4)t_4} \tilde{\mathcal{E}}_5^{(\eta_5)} [\eta_5(\omega_t - \omega_\tau - \omega_3 - \omega_4) - \Omega_5^p] \\ & \times \tilde{\mathcal{E}}_4^{(\eta_4)} (\eta_4\omega_4 - \Omega_4^p) \tilde{\mathcal{E}}_3^{(\eta_3)} (\eta_3\omega_3 - \Omega_3^p) \tilde{\mathcal{E}}_2^{(\eta_2)} [\eta_2(\omega_\tau - \omega_1) - \Omega_2^p] \tilde{\mathcal{E}}_1^{(\eta_1)} (\eta_1\omega_1 - \Omega_1^p). \end{aligned} \quad (3.22)$$

If, furthermore, the forth and the fifth pulses are well separated ($T > \tau_4^p + \tau_5^p$), we can replace the lower bound $-T$ by $-\infty$. The resulting integration over dt_4 in Eqn. (3.22) produces $\delta(\omega_3 + \omega_4 + \omega_\tau)$ and we obtain

$$\begin{aligned} \Phi_{2D}^{(5)}(\omega_3, \omega_1) = & \tilde{\mathcal{E}}_5^{(\eta_5)} (\eta_5\omega_t - \Omega_5^p) \tilde{\mathcal{E}}_4^{(\eta_4)} [-\eta_4(\omega_\tau + \omega_3) - \Omega_4^p] \tilde{\mathcal{E}}_3^{(\eta_3)} (\eta_3\omega_3 - \Omega_3^p) \\ & \times \tilde{\mathcal{E}}_2^{(\eta_2)} [\eta_2(\omega_\tau - \omega_1) - \Omega_2^p] \tilde{\mathcal{E}}_1^{(\eta_1)} (\eta_1\omega_1 - \Omega_1^p). \end{aligned} \quad (3.23)$$

The measured 2Q-2DOS spectrum $\mathbb{S}_{2D}^{(5)}(\omega_t, T, \omega_\tau)$ can be expressed through the response function by Eqns. (3.21) and (3.23) which involves only two integrations. To derive approximate analytical formulae for $\mathbb{S}_{2D}^{(5)}(\omega_t, T, \omega_\tau)$, it is worthwhile to consider separately two situations (hereafter, A and B) for which we can introduce 2Q-2D spectral response functions (which are the 2Q-2DOS spectra measured using Dirac- δ pulses and independent of the pulse envelopes) and relate them with 2Q-2DOS spectra measured using finite pulses.

3.4.1 Situation A: Short pulses relative to the electronic dephasing time

If the pulse durations are short (but not short as δ pulses) on the timescale of vibrational motion and electronic dephasing, the time intervals t_1 and t_3 in Eqn. (3.15) are controlled by the pulse durations not by the dephasing rates. We can therefore write, approximately

$$R_{2D}^{(5)}(\omega_t, T, t_3, \omega_\tau, t_1) \approx R_{2D}^{(5)}(\omega_t, T, 0, \omega_\tau, 0) e^{i\eta_3\nu_3 t_3} e^{-i\eta_1\nu_1 t_1}, \quad (3.24)$$

where ν_1 and ν_3 are electronic energies. We thus extracted the fastest electronic contributions from the response function and assumed that the remaining time-dependence, which is determined by the vibrational motion, can be neglected during t_1 and t_3 . Note that the sign of the electronic phase $e^{\pm i\nu_k t_k}$ in the response functions should be oppo-

site to the sign of the phase of the corresponding laser field, $e^{\mp i\Omega_k^p t_k}$. This connection between a specific pulse sequence and a specific response function guarantees that the nonlinear response depends on $\nu_k - \Omega_k^p$ but is independent of $\nu_k + \Omega_k^p$, as it should be in the rotating wave approximation [8]. Hence, the interaction indexes η_1 and η_3 appear in the response function of Eqn. (3.24).

In frequency domain, Eqn. (3.24) reads

$$R_{2D}^{(5)}(\omega_t, T, \omega_\tau + \omega_3, \omega_\tau, \omega_1) \approx R_{2D}^{(5)}(\omega_t, T, 0, \omega_\tau, 0) \frac{1}{\epsilon - i(\eta_3 \nu_3 + \omega_\tau + \omega_3)} \frac{1}{\epsilon + i(\eta_1 \nu_1 - \omega_1)}, \quad (3.25)$$

$\epsilon \rightarrow 0$. Substituting the frequency domain response function in Eqn. (3.25) into Eqn. (3.21), the measured 2Q-2DOS spectra can be expressed as

$$\mathbb{S}_{2D}^{(5A)}(\omega_t, T, \omega_\tau) \propto \mathcal{A}_{2D}^{(5A)}(\omega_t, \omega_\tau) R_{2D}^{(5)}(\omega_t, T, t_3 = 0, \omega_\tau, t_1 = 0), \quad (3.26)$$

where

$$\mathcal{A}_{2D}^{(5A)}(\omega_t, \omega_\tau) = \iint_{-\infty}^{+\infty} d\omega_1 d\omega_3 \Phi_{2D}^{(5)}(\omega_3, \omega_1) \frac{1}{\epsilon - i(\eta_3 \nu_3 + \omega_\tau + \omega_3)} \frac{1}{\epsilon + i(\eta_1 \nu_1 - \omega_1)}, \quad (3.27)$$

and $\Phi_{2D}^{(5)}(\omega_3, \omega_1)$ is defined in Eqn. (3.23). The two-layer integral in Eqn. (3.27) can be solved by changing the integration variables $\bar{\omega}_1 = \omega_1 - \eta_1 \nu_1$, $\bar{\omega}_3 = \eta_3 \nu_3 + \omega_\tau + \omega_3$ and making use of the Sokhotskii's formula [43, 44]

$$\frac{1}{\epsilon \mp i\omega} = \pi \delta(\omega) \pm iP \left(\frac{1}{\omega} \right),$$

the Eqn. (3.27) becomes

$$\begin{aligned} \mathcal{A}_{2D}^{(5A)}(\omega_t, \omega_\tau) &= \alpha_{2D}^{(5A)}(\omega_t) \alpha_{2D}^{(5A)}(\omega_\tau), \\ \alpha_{2D}^{(5A)}(\omega_t) &= \tilde{\mathcal{E}}_5^{(\eta_5)}(\eta_5 \omega_t - \Omega_5^p), \\ \alpha_{2D}^{(5A)}(\omega_\tau) &= A_{21}(\eta_2 \omega_\tau - \eta_1 \eta_2 \nu_1 - \Omega_2^p, \nu_1 - \Omega_1^p) A_{43}(\eta_3 \eta_4 \nu_3 - \Omega_4^p, -\eta_3 \omega_\tau - \nu_3 - \Omega_3^p), \end{aligned} \quad (3.28)$$

where

$$A_{mn}(x, y) = \pi \tilde{\mathcal{E}}_m^{(\eta_m)}(x) \tilde{\mathcal{E}}_n^{(\eta_n)}(y) + iB_{mn}(x, y), \quad (3.29)$$

and

$$B_{mn}(x, y) = \lim_{\epsilon \rightarrow 0} \left(\int_{-\infty}^{-\epsilon} + \int_{\epsilon}^{+\infty} \right) d\omega \frac{\tilde{\mathcal{E}}_m^{(\eta_m)}(x - \omega) \tilde{\mathcal{E}}_n^{(\eta_n)}(y + \omega)}{\omega}. \quad (3.30)$$

Unfortunately, the evaluation of $B_{mn}(x, y)$ requires the calculation of the principle value of the integral in Eqn. (3.30), which can be done analytically for exponential pulse envelopes only. For other pulse envelopes, the integration in Eqn. (3.30) should be performed numerically. Note that $B_{mn}(0, 0) = 0$, hence for small detuning numbers x and y , $B_{mn}(x, y)$ can therefore be neglected and

$$\alpha_{2D}^{(5A)}(\omega_\tau) \propto \tilde{\mathcal{E}}_3^{(\eta_3)}(\eta_3\omega_\tau - \nu_3 - \Omega_3^p) \tilde{\mathcal{E}}_2^{(\eta_2)}(\eta_2\omega_\tau - \eta_1\eta_2\nu_1 - \Omega_2^p). \quad (3.31)$$

We dropped the constant factors $\tilde{\mathcal{E}}_4^{(\eta_4)}(\eta_3\eta_4\nu_3 - \Omega_4^p)$ and $\tilde{\mathcal{E}}_1^{(\eta_1)}(\nu_1 - \Omega_1^p)$ in the above expression.

3.4.2 Situation B: Long pulses relative to the electronic dephasing time

Another situation which is quite common in femtosecond spectroscopy is that the excitation pulse durations are short on the timescale of vibrational motion, but long on the timescale of electronic dephasing (see ref. [10]). The latter assumption implies that the time intervals t_1 and t_3 in Eqn. (3.15) are controlled by fast electronic dephasing described by the response functions. In frequency domain, this means that $\tilde{\mathcal{E}}_3^{(\eta_3)}(\eta_3\omega_3 - \Omega_3^p)$ and $\tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1\omega_1 - \Omega_1^p)$ are close to $\delta(\eta_3\omega_3 - \Omega_3^p)$ and $\delta(\eta_1\omega_1 - \Omega_1^p)$ and we can replace $R_{2D}^{(5)}(\omega_t, T, \omega_\tau + \omega_3, \omega_\tau, \omega_1)$ by $R_{2D}^{(5)}(\omega_t, T, \omega_\tau + \eta_3\Omega_3^p, \omega_\tau, \eta_1\Omega_1^p)$ in Eqn. (3.21). We then arrive the approximate formula for the measured 2Q-2DOS spectra

$$\mathbb{S}_{2D}^{(5B)}(\omega_t, T, \omega_\tau) \propto \mathcal{A}_{2D}^{(5B)}(\omega_t, \omega_\tau) R_{2D}^{(5)}(\omega_t, T, \omega_\tau + \eta_3\Omega_3^p, \omega_\tau, \eta_1\Omega_1^p), \quad (3.32)$$

where

$$\mathcal{A}_{2D}^{(5B)}(\omega_t, \omega_\tau) = \int_{-\infty}^{+\infty} d\omega_1 d\omega_3 \Phi_{2D}^{(5)}(\omega_3, \omega_1), \quad (3.33)$$

and $\Phi_{2D}^{(5)}(\omega_3, \omega_1)$ is given by Eqn. (3.23). To evaluate the above integral, one notices that

$$\int_{-\infty}^{+\infty} d\omega \tilde{\mathcal{E}}_m(\omega_\tau - \omega - \Omega_m^p) \tilde{\mathcal{E}}_n(\omega - \Omega_n^p) = \tilde{\mathcal{E}}_m \otimes \tilde{\mathcal{E}}_n(\omega_\tau - \Omega_m^p - \Omega_n^p) = \tilde{\mathcal{E}}_{mn}(\omega_\tau - \Omega_m^p - \Omega_n^p).$$

This results the expression for $\mathcal{A}_{2D}^{(5B)}(\omega_t, \omega_\tau)$ as

$$\begin{aligned}\mathcal{A}_{2D}^{(5B)}(\omega_t, \omega_\tau) &= \alpha_{2D}^{(5B)}(\omega_t) \alpha_{2D}^{(5B)}(\omega_\tau), \\ \alpha_{2D}^{(5B)}(\omega_t) &= \tilde{\mathcal{E}}_5^{(\eta_5)}(\eta_5 \omega_t - \Omega_5^p), \\ \alpha_{2D}^{(5B)}(\omega_\tau) &= \tilde{\mathcal{E}}_{34}^{(\eta_{34})}(-\eta_{34} \omega_\tau - \Omega_3^p - \Omega_4^p) \tilde{\mathcal{E}}_{12}^{(\eta_{12})}(\eta_{12} \omega_\tau - \Omega_3^p - \Omega_4^p),\end{aligned}\quad (3.34)$$

and

$$\tilde{\mathcal{E}}_{mn}^{(\eta_{mn})} = \left[\tilde{\mathcal{E}}_m^{(\eta_m)} \otimes \tilde{\mathcal{E}}_n^{(\eta_n)} \right] (\omega) = \int_{-\infty}^{+\infty} dt e^{i\omega t} \mathcal{E}_m^{(\eta_m)}(t) \mathcal{E}_n^{(\eta_n)}(t) \quad (3.35)$$

is the convolution of the two pulse spectra, which can be expressed as a FT of the product of two pulse time-envelopes. If the pulse envelopes are the same, the right-hand side of Eqn. (3.35) yields the FT of the pulse intensity. Note a close similarity of Eqn. (3.32) and Eqn. (3.34) with Eqn. (3.11) and Eqn. (3.12) (in Section 3.3) derived for the third-order 2DOS spectrum. There are substantial differences, the envelopes of the pulse amplitude $\tilde{\mathcal{E}}_n^{(\eta_n)}(\omega)$ in Eqn. (3.12) are replaced by the envelopes of the pulse intensity $\tilde{\mathcal{E}}_{mn}^{(\eta_{mn})}$ in Eqn. (3.34), and ω_τ in Eqn. (3.34) is peaked at the double transition frequency, as expected.

3.5 Fifth-order 3DOS spectra

The typical 3DOS measurement has been described in literatures [4, 25–28, 31]. The pulse delays are defined in Eqn. (3.36) and the five-pulse train used in 3DOS experiment is depicted in Fig. 3.2

$$\tau_1 = -\tau - T - \tau' - T', \tau_2 = -T - \tau' - T', \tau_3 = -\tau' - T', \tau_4 = -T', \text{ and } \tau_5 = 0. \quad (3.36)$$

The fifth-order polarization is denoted as $P_{3D}^{(5)}(t, T', \tau', T, \tau)$ and the corresponding 3DOS spectrum reads

$$\mathbb{S}_{3D}^{(5)}(\omega_t, T', \omega'_\tau, T, \omega_\tau) = \int_{-\infty}^{+\infty} dt \int_{-\infty}^{+\infty} d\tau' \int_{-\infty}^{+\infty} d\tau P_{3D}^{(5)}(t, T', \tau', T, \tau). \quad (3.37)$$

In impulsive limit, $P_{3D}^{(5)}(t, T', \tau', T, \tau) \propto R_{3D}^{(5)}(\omega_t, T', \omega'_\tau, T, \omega_\tau)$ and the 3DOS spectrum reduces to the 3D spectral response function which can be expressed as

$$\mathbb{S}_{3D}^{(5)}(\omega_t, T', \omega'_\tau, T, \omega_\tau) \propto R_{3D}^{(5)}(\omega_t, T', \omega'_\tau, T, \omega_\tau). \quad (3.38)$$

Figure 3.2. Scheme of the pulse train of 3DOS measurement. The pulse envelopes are depicted by the blue lines. The oscillatory (straight) orange lines indicate the time intervals during which the system evolves in electronic coherence (population).

The frequency domain representation of the fifth-order polarization $P_{3D}^{(5)}(t, T', \tau', T, \tau)$ can be expressed as

$$\begin{aligned}
 P_{2D}^{(5)}(t, T, \tau) &\propto \int_{-\infty}^{+\infty} d\omega_5 \int_{-\infty}^{+\infty} d\omega_4 \int_{-\infty}^{+\infty} d\omega_3 \int_{-\infty}^{+\infty} d\omega_2 \int_{-\infty}^{+\infty} d\omega_1 \\
 &\times R_{3D}^{(5)}(\omega_1 + \omega_2 + \omega_3 + \omega_4 + \omega_5, \omega_1 + \omega_2 + \omega_3 + \omega_4, \omega_1 + \omega_2 + \omega_3, \omega_1 + \omega_2, \omega_1) \\
 &\times e^{-i(\omega_1 + \omega_2 + \omega_3 + \omega_4 + \omega_5)t} e^{-i(\omega_1 + \omega_2 + \omega_3 + \omega_4)T'} e^{-i(\omega_1 + \omega_2 + \omega_3)\tau'} e^{-i(\omega_1 + \omega_2)T} e^{-i\omega_1\tau} \\
 &\times \tilde{\mathcal{E}}_5^{(\eta_5)}(\eta_5\omega_5 - \Omega_5^p) \tilde{\mathcal{E}}_4^{(\eta_4)}(\eta_4\omega_4 - \Omega_4^p) \tilde{\mathcal{E}}_3^{(\eta_3)}(\eta_3\omega_3 - \Omega_3^p) \tilde{\mathcal{E}}_2^{(\eta_2)}(\eta_2\omega_2 - \Omega_2^p) \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1\omega_1 - \Omega_1^p).
 \end{aligned} \tag{3.39}$$

Inserting Eqn. (3.39) into Eqn. (3.37), one can perform the three integrations along t , τ' and τ dimensions which produce three δ -functions as $\delta(\omega_1 + \omega_2 + \omega_3 + \omega_4 + \omega_5 - \omega_t)$, $\delta(\omega_1 + \omega_2 + \omega_3 - \omega'_\tau)$, and $\delta(\omega_1 - \omega_\tau)$. As a result, the expression for the measured 3DOS spectra in Eqn. (3.37) is reduced to

$$\mathbb{S}_{3D}^{(5)}(\omega_t, T', \omega'_\tau, T, \omega_\tau) \propto \int_{-\infty}^{+\infty} d\omega_4 d\omega_2 R_{3D}^{(5)}(\omega_t, \omega'_\tau + \omega_4, \omega'_\tau, \omega_\tau + \omega_2, \omega_\tau) \Phi_{3D}^{(5)}(\omega_4, \omega_2), \tag{3.40}$$

where, the 3D gate function $\Phi_{3D}^{(5)}(\omega_4, \omega_2)$ is defined as

$$\begin{aligned}
 \Phi_{3D}^{(5)}(\omega_4, \omega_2) &= e^{-i(\omega'_\tau + \omega_4)T'} e^{-i(\omega_\tau + \omega_2)T} \tilde{\mathcal{E}}_5^{(\eta_5)}[\eta_5(\omega_t - \omega'_\tau - \omega_4) - \Omega_5^p] \\
 &\times \tilde{\mathcal{E}}_4^{(\eta_4)}(\eta_4\omega_4 - \Omega_4^p) \tilde{\mathcal{E}}_3^{(\eta_3)}[\eta_3(\omega'_\tau - \omega_\tau - \omega_2) - \Omega_3^p] \\
 &\times \tilde{\mathcal{E}}_2^{(\eta_2)}(\eta_2\omega_2 - \Omega_2^p) \tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1\omega_\tau - \Omega_1^p).
 \end{aligned} \tag{3.41}$$

The expression in Eqn. (3.40) involves double integration and is exact. Hence only two integrations are necessary to exactly evaluate the 3DOS spectrum for any pulse envelopes. To simplify the expression into more convenient form, we define the mixed time-frequency response function $R_{3D}^{(5)}(\omega_t, t_4, \omega'_\tau, t_2, \omega_\tau)$ and rewriting the Eqn. (3.40) as

$$\mathbb{S}_{3D}^{(5)}(\omega_t, T', \omega'_\tau, T, \omega_\tau) \propto \int_{-\infty}^{+\infty} dt_4 \int_{-\infty}^{+\infty} dt_2 R_{3D}^{(5)}(\omega_t, t_4, \omega'_\tau, t_2, \omega_\tau) \Phi_{3D}^{(5)}(t_4 - T', t_2 - T), \quad (3.42)$$

where

$$\begin{aligned} \Phi_{3D}^{(5)}(t_4, t_2) = & \int_{-\infty}^{+\infty} d\omega_4 \int_{-\infty}^{+\infty} d\omega_2 e^{-i(\omega'_\tau + \omega_4)t_4} e^{-i(\omega_\tau + \omega_2)t_2} \tilde{\mathcal{E}}_5^{(\eta_5)} [\eta_5(\omega_t - \omega'_\tau - \omega_4) - \Omega_5^p] \\ & \times \tilde{\mathcal{E}}_4^{(\eta_4)} (\eta_4\omega_4 - \Omega_4^p) \tilde{\mathcal{E}}_3^{(\eta_3)} [\eta_3(\omega'_\tau - \omega_\tau - \omega_2) - \Omega_3^p] \\ & \times \tilde{\mathcal{E}}_2^{(\eta_2)} (\eta_2\omega_2 - \Omega_2^p) \tilde{\mathcal{E}}_1^{(\eta_1)} (\eta_1\omega_\tau - \Omega_1^p). \end{aligned} \quad (3.43)$$

Equation (3.42) is exact. Assuming that the pulses are short on the vibrational dynamics timescale, we can replace $R_{3D}^{(5)}(\omega_t, t_4, \omega'_\tau, t_2, \omega_\tau)$ by $R_{3D}^{(5)}(\omega_t, T', \omega'_\tau, T, \omega_\tau)$ in Eqn. (3.42) (this is also a good approximation if the response function weakly depends on t_4 and t_2). Then one arrives

$$\mathbb{S}_{3D}^{(5)}(\omega_t, T', \omega'_\tau, T, \omega_\tau) \propto \mathcal{A}_{3D}^{(5)}(\omega_t, \omega'_\tau, \omega_\tau) R_{3D}^{(5)}(\omega_t, T', \omega'_\tau, T, \omega_\tau), \quad (3.44)$$

where

$$\mathcal{A}_{3D}^{(5)}(\omega_t, \omega'_\tau, \omega_\tau) = \int_{-T'}^{+\infty} dt_4 \int_{-T}^{+\infty} dt_2 \Phi_{3D}^{(5)}(t_4, t_2) \quad (3.45)$$

The replacement of T and T' by ∞ (this is correct if $T > \tau_3^p + \tau_2^p$ and $T' > \tau_5^p + \tau_4^p$) and performing the integrations produces the product of two δ -functions, $\delta(\omega_4 + \omega'_\tau)\delta(\omega_2 + \omega_\tau)$. Hence one obtains

$$\begin{aligned} \mathcal{A}_{3D}^{(5)}(\omega_t, \omega'_\tau, \omega_\tau) &= \alpha_{3D}^{(5)}(\omega_t) \alpha_{3D}^{(5)}(\omega'_\tau) \alpha_{3D}^{(5)}(\omega_\tau), \\ \alpha_{3D}^{(5)}(\omega_t) &= \tilde{\mathcal{E}}_5^{(\eta_5)} (\eta_5\omega_t - \Omega_5^p), \\ \alpha_{3D}^{(5)}(\omega'_\tau) &= \tilde{\mathcal{E}}_4^{(\eta_4)} (-\eta_4\omega'_\tau - \Omega_4^p) \tilde{\mathcal{E}}_3^{(\eta_3)} (\eta_3\omega'_\tau - \Omega_3^p), \\ \alpha_{3D}^{(5)}(\omega_\tau) &= \tilde{\mathcal{E}}_2^{(\eta_2)} (-\eta_2\omega_\tau - \Omega_2^p) \tilde{\mathcal{E}}_1^{(\eta_1)} (\eta_1\omega_\tau - \Omega_1^p) \end{aligned} \quad (3.46)$$

3.6 Illustrative simulations

In this section, we compare the fifth-order 2Q-2DOS and 3DOS spectra evaluated by the exact numerical simulations to that calculated from the approximate formulas derived in Sections 3.4 and 3.5. Although response functions in the expressions of Sections 3.4 and 3.5 can be arbitrary, i.e., they can include, for example, spectral diffusion and population transfer, the simulations in this section are performed for a simple but nontrivial response function

$$R^{(5)}(t_5, t_4, t_3, t_2, t_1) = - \left(\frac{i}{\hbar} \right)^5 \prod_{k=1}^5 \Theta(t_k) e^{-i\nu_k t_k} e^{-\Gamma_k t_k} \quad (3.47)$$

Here, ν_k and Γ_k are the system frequencies and dephasing rates at the k th time interval. Equation (3.47) can be regarded as a typical fifth-order response function for atomic systems having well-defined electronic energy levels and homogeneously broadened lineshapes controlled by dephasing rates Γ_k . For more complex systems, the response function of Eqn. (3.47) corresponds to a specific pathway. The entire response is given then by a linear combination of similar functions, in which ν_k and Γ_k are different for each pathway (the so-called sum-over-states representation of the total response function [10]). Hence the validity of our approach for a typical response function of Eqn. (3.47) guarantees its validity for a multi-path response.

As mention in Section 3.2, the local oscillator is taken as a white-light continuum, i.e., $\tilde{E}_{LO}(\omega_t) = \text{const.}$ For brevity, we present only the real parts of the simulated spectra.

3.6.1 Fifth-order 2Q-2DOS spectra

For 2Q-2DOS spectra, the response function in Eqn. (3.47) can be constructed from the three-level Hamiltonian

$$H = \begin{pmatrix} \varepsilon_0 & 0 & 0 \\ 0 & \varepsilon_1 & 0 \\ 0 & 0 & \varepsilon_2 \end{pmatrix},$$

where ε_0 , ε_1 and ε_2 are the energies of the ground, first excited, and second excited states (see the left panel of Fig. 3.3). In the simulations, we set $\varepsilon_1 - \varepsilon_0 = 450$ THz and $\varepsilon_2 - \varepsilon_1 = 430$ THz. This yields the 2Q frequency $\varepsilon_2 - \varepsilon_0 = 880$ THz. We simulate the 2Q-2DOS spectrum in the non-rephasing (anti-echo) phase-matching direction. The double-sided Feynman diagram (DSFD) for this signal is depicted in the middle panel of Fig. 3.3. It corresponds to the interaction indexes $[\eta_1, \eta_2, \eta_3, \eta_4, \eta_5] = [+1, +1, -1, -1, +1]$. We note that this simulation is performed for only one specifi-

Figure 3.3. (Left) Energy levels of the three-level system. (Middle) DSFD for the 2Q-2DOS non-rephasing process simulated in this section. (Right) DSFD for the 3DOS non-rephasing process simulated in this section.

cally mentioned non-rephasing process. For a comprehensive simulation of 2Q-2DOS spectrum, we need to enumerate all the necessary DSFDs, treating each of them separately as they have different sets of interaction indexes. They are then summed up to obtain the final 2Q-2DOS spectrum. For the mentioned above process, the frequencies entering the response function of Eqn. (3.47) can be expressed as follows: $\nu_1 = \nu_5 = \varepsilon_1 - \varepsilon_0$, $\nu_2 = \varepsilon_2 - \varepsilon_0$, $\nu_3 = \varepsilon_2 - \varepsilon_1$, and $\nu_4 = 0$.

Situation A

In situation A, the pulses are short on the timescale of vibrational motion and electronic dephasing. In the frequency domain, this yields the pulse spectra which are broad compared to the spectral shape of the response function. In the simulation, all five interacting pulses are taken as transform-limited Gaussian pulses having the same full-width at half-maximum (FWHM) of 45 THz in the spectral domain, which corresponds to pulse durations of $\tau_n^p = 20$ fs. All dephasing times Γ_k^{-1} are fixed at 300 fs. All frequencies of the excitation pulses are set at $\Omega_k^p = \omega_L = 450$ THz and in resonance with the $|0\rangle \rightarrow |1\rangle$ transition. With the corresponding interaction indexes $[\eta_1, \eta_2, \eta_3, \eta_4, \eta_5] = [+1, +1, -1, -1, +1]$, the formula of approximate 2Q-2DOS spectrum from Eqn. (3.26) becomes

$$\begin{aligned} \mathbb{S}_{2D}^{(5A)}(\omega_t, T, \omega_\tau) &\propto R_{2D}^{(5)}(\omega_t, T, t_3 = 0, \omega_\tau, t_1 = 0) \alpha_{2D}^{(5A)}(\omega_t) \alpha_{2D}^{(5A)}(\omega_\tau) \\ \alpha_{2D}^{(5A)}(\omega_t) &= \tilde{\mathcal{E}}_5(\omega_t - \omega_L), \\ \alpha_{2D}^{(5A)}(\omega_\tau) &= A_{21}(\omega_\tau - \nu_1 - \omega_L, \nu_1 - \omega_L) A_{43}(\nu_3 - \omega_L, \omega_\tau - \nu_3 - \omega_L). \end{aligned} \quad (3.48)$$

Figure 3.4 contrasts the real parts of the 2Q-2D spectral response function (a), the approximate (b) and the exact (c) 2Q-2DOS spectra. The spectrum (a) is the 2Q-2DOS

Figure 3.4. (a) Real part of the 2Q-2D spectral response function. (b) Approximate 2Q-2DOS spectrum evaluated by Eqn. (3.48). The contributions of the factor $\alpha_{2D}^{(5A)}(\omega_\tau)$ ($\alpha_{2D}^{(5A)}(\omega_t)$) along ω_τ (ω_t) are depicted on the left (above) of panel (a). (c) Exact 2Q-2DOS spectrum obtained by the triple integration via Eqn. (3.17). The dashed white lines in the 2D spectra correspond to the 2Q diagonal line $\omega_\tau = 2\omega_t$. All 2D spectra are normalized to their maxima.

spectrum measured using δ -pulses. The approximate spectrum (b) is calculated by Eqn. (3.48) and the exact spectrum (c) is obtained from the triple integration of Eqn. (3.17). The condition of the validity of the approximate evaluation of the spectra ($\tau_n^p \ll \omega_L - \nu_3$, Γ_k^{-1}) is quite well fulfilled in the present case. That is why the approximate (b) and exact (c) 2Q-2DOS spectra are almost identical but differ significantly from the 2Q-2D spectral response function (a). The spectrum (a) is symmetric with respect to the anti-diagonal (as it should be for non-rephasing signal), while the exact and approximate spectra are significantly skewed. Note the contributions $\alpha_{2D}^{(5A)}(\omega_\tau)$ and $\alpha_{2D}^{(5A)}(\omega_t)$ [see Eqn. (3.48)], which are shown, correspondingly, on the left and top sides of panel (a). The product of $\alpha_{2D}^{(5A)}(\omega_\tau)$ and $\alpha_{2D}^{(5A)}(\omega_t)$ with the 2Q-2D spectral response function (a) results in the approximate spectrum (b) according to Eqn. (3.48). We emphasize here that $\alpha_{2D}^{(5A)}(\omega_\tau)$ is complex, since both real and imaginary parts of A_{21} and A_{34} are important, while $\alpha_{2D}^{(5A)}(\omega_t)$ is real. The complex valued $\alpha_{2D}^{(5A)}(\omega_\tau)$ deforms the spectrum along the ω_τ axis, leading to its skewness. Note also that $\alpha_{2D}^{(5A)}(\omega_t)$ is determined solely by the fifth pulse, while $\alpha_{2D}^{(5A)}(\omega_\tau)$ depends on the pulse envelopes of the first four pulses.

Situation B

In situation B, the pulses are assumed to be short on the timescale of vibrational motion, but long on electronic dephasing timescale. For the simulations, we fix the FWHM of all pulse spectra at 5 THz, which corresponds to the pulse durations of $\tau_n^p = 176$ fs. All dephasing times Γ_k^{-1} are fixed at 5 fs. The formula for the approximate 2Q-2DOS spectrum of this specific non-rephasing process becomes

$$\begin{aligned} S_{2D}^{(5B)}(\omega_t, T, \omega_\tau) &\propto R_{2D}^{(5)}(\omega_t, T, \omega_\tau - \omega_L, \omega_\tau, \omega_L) \alpha_{2D}^{(5B)}(\omega_t) \alpha_{2D}^{(5B)}(\omega_\tau) \\ \alpha_{2D}^{(5B)}(\omega_t) &= \tilde{\mathcal{E}}_5(\omega_t - \omega_L), \\ \alpha_{2D}^{(5B)}(\omega_\tau) &= \left[\tilde{\mathcal{E}}_1 \otimes \tilde{\mathcal{E}}_2 \right] (\omega_\tau - 2\omega_L) \left[\tilde{\mathcal{E}}_3^* \otimes \tilde{\mathcal{E}}_4^* \right] (\omega_\tau - 2\omega_L). \end{aligned} \quad (3.49)$$

Figure 3.5. (a) Real part of the 2Q-2D spectral response function. (b) Approximate 2Q-2DOS spectrum evaluated by Eqn. (3.49). The contributions of the factor $\alpha_{2D}^{(5B)}(\omega_\tau)$ ($\alpha_{2D}^{(5B)}(\omega_t)$) along ω_τ (ω_t) are depicted on the left (below) panel (a). (c) Exact 2Q-2DOS spectrum obtained by the triple integration via Eqn. (3.17). The dashed white lines in the 2D spectra correspond to the 2Q diagonal line $\omega_\tau = 2\omega_t$. All 2D spectra are normalized to their maxima.

Let us now consider the formation of the approximate 2Q-2DOS spectrum in Fig. 3.5(b) in more detail. According to Eqn. (3.49), this spectrum is obtained by the multiplication of the 2Q-2D spectral response function of panel (a) by the product of $\alpha_{2D}^{(5B)}(\omega_\tau)$ and $\alpha_{2D}^{(5B)}(\omega_t)$. As distinct from the situation A, both of these factors are real. $\alpha_{2D}^{(5B)}(\omega_\tau)$ is centered at the 2Q frequency $2\omega_L$, while $\alpha_{2D}^{(5B)}(\omega_t)$ is peaked at ω_L . The response function of Eqn. (3.47) does not exhibit population dynamics ($\nu_4 = 0$), and the condition of the validity of the approximate evaluation of spectra ($\tau_n^p \gg \Gamma_k^{-1}$) is fulfilled well. We thus expect that the approximate Fig. 3.5(b) and exact Fig. 3.5(c) 2Q-2DOS spectra should match each other quite well. Indeed, the shapes of the main positive central peaks in panels (b) and (c) are very similar. However, the exact spectrum (c) has two negative wings on the two sides of the main peak. Similar spectral features were observed in third-order 2Q-2DOS spectra [40]. The discrepancy between the approximate and exact spectra may be caused by the approximations made in the derivation of Eqn. (3.32). We assume that $\tilde{\mathcal{E}}_3^{(\eta_3)}(\eta_3\omega_3 - \Omega_3^p)$ and $\tilde{\mathcal{E}}_1^{(\eta_1)}(\eta_1\omega_1 - \Omega_1^p)$ are close to $\delta(\eta_3\omega_3 - \Omega_3^p)$ and $\delta(\eta_1\omega_1 - \Omega_1^p)$ and we can replace $R_{2D}^{(5)}(\omega_t, T, \omega_\tau + \omega_3, \omega_\tau, \omega_1)$ by $R_{2D}^{(5)}(\omega_t, T, \omega_\tau + \eta_3\Omega_3^p, \omega_\tau, \eta_1\Omega_1^p)$ in Eqn. (3.21). By doing so, we may lose some phase information which is responsible for the missing wings in Fig. 3.5(b).

3.6.2 Fifth-order 3DOS spectra

Fifth-order 3D optical spectroscopy is an extension of the conventional third-order 2DOS. With two population time and three frequency dimensions, the 3D spectrum is a three-point correlation map, which offers more information about the system than a conventional 2D spectrum [29–31]. In this simulation, we consider a system which is described by the two-level Hamiltonian

$$H = \begin{pmatrix} \varepsilon_0 & 0 \\ 0 & \varepsilon_1 \end{pmatrix},$$

The corresponding response function is given by Eqn. (3.47), where $\nu_1 = \nu_3 = \nu_5 = \varepsilon_1 - \varepsilon_0 = 450$ THz, $\nu_2 = \nu_4 = 0$ and all dephasing times Γ_k^{-1} are set to 50 fs. All excitation pulse spectra have FWHM of 150 THz (pulse durations of $\tau_n^p \approx 6$ fs) and are resonant with the $|0\rangle \rightarrow |1\rangle$ transition. We present here the simulation results for a specific non-rephasing process corresponding to the DSFD depicted in the right panel of Fig. 3.3 and the interaction indexes $[\eta_1, \eta_2, \eta_3, \eta_4, \eta_5] = [+1, -1, +1, -1, +1]$. The

Figure 3.6. (a) The real part of 3D spectral response function. (b) Approximate 3DOS spectrum calculated by Eqn. (3.50). (c) Exact 3DOS spectrum calculated by Eqn. (3.40). The isosurfaces correspond to -20%, -10%, 10%, 20% and 30% of the maximum. Negative and positive-valued isosurfaces are rendered in orange and blue color, respectively.

formula for approximate 3DOS spectrum of this specific non-rephasing process is

$$\begin{aligned}
 \mathcal{A}_{3D}^{(5)}(\omega_t, \omega'_\tau, \omega_\tau) &= \alpha_{3D}^{(5)}(\omega_t) \alpha_{3D}^{(5)}(\omega'_\tau) \alpha_{3D}^{(5)}(\omega_\tau), \\
 \alpha_{3D}^{(5)}(\omega_t) &= \tilde{\mathcal{E}}_5(\omega_t - \omega_L), \\
 \alpha_{3D}^{(5)}(\omega'_\tau) &= \tilde{\mathcal{E}}_4^*(\omega'_\tau - \omega_L) \tilde{\mathcal{E}}_3(\omega'_\tau - \omega_L), \\
 \alpha_{3D}^{(5)}(\omega_\tau) &= \tilde{\mathcal{E}}_2^*(\omega_\tau - \omega_L) \tilde{\mathcal{E}}_1(\omega_\tau - \omega_L).
 \end{aligned} \tag{3.50}$$

Figure 3.6 presents the real parts of the 3D spectral response function (a), the approximate (b) and the exact (c) 3DOS spectrum obtained from Eqn. (3.38), Eqn. (3.50) and Eqn. (3.40), respectively. The 3D spectral response function in spectrum (a) is the 3DOS spectrum obtained using δ -pulses. In the approximate 3DOS spectrum (b), the first and the second pulse spectra are responsible for $\alpha_{3D}^{(5)}(\omega_\tau)$ and the spectrum is altered along the first coherent dimension ω_τ . Similarly, the third and the fourth pulses produce $\alpha_{3D}^{(5)}(\omega'_\tau)$ and the last fifth pulse contributes to $\alpha_{3D}^{(5)}(\omega_t)$. Since the local oscillator is taken as the white light continuum, the approximate 3DOS spectrum is mostly elongated along ω_t . The comparison of the approximate (b) and exact (c) spectra re-

veals that the approximate expression of Eqn. (3.46) performs well as is evidenced by the fact that these two spectra look almost identical.

3.7 Conclusion

In this study, we investigate the effects of finite pulse durations on fifth-order 2Q-2DOS and 3DOS spectra. Instead of directly calculating the time domain polarization, the approach from frequency domain significantly simplifies the calculations. Yet, due to the complication of fifth-order signals, we still need to make some assumptions during the theoretical derivations to obtain totally analytical expressions for the signals.

In general, within the regimes of our assumptions, our simplified expressions work well. In situation A (where the pulses are short on the timescale of vibrational wavepacket dynamics and electronic dephasing) for 2Q-2DOS, the expression in Eqn. (3.28) reproduces almost exactly the 2Q-2DOS spectrum as the one calculated by triple integration. Unfortunately, Eqn. (3.28) still contains one layer of integration and has to be evaluated numerically. On the other hand, in situation B (the pulses are short on the timescale of vibrational wavepacket dynamics, but long on the timescale of electronic dephasing), the simplified expression of Eqn. (3.34) is totally analytical. However, the simulations of 2Q-2DOS spectra in situation B show that the approximate analytical formula somewhat underestimates the phase factors. The approximate expression of Eqn. (3.46) for 3DOS spectra is also fully analytical and reproduces well the spectral features of the exact spectra.

The results of this work aim to contribute to the needs of the higher-order nonlinear optical spectroscopic community. As the computational cost increases drastically with the order of signal, our approximate expressions can be efficient alternative ways for simulating fifth-order spectra. With the knowledge of how the finite durations of the pulses can alter the spectra, we can explore the hidden information by disentangling the finite pulse duration effects.

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Chapter 4

Temperature dependence of the energy transfer in LHCII studied by 2DES

4.1 Introduction

Light-harvesting complexes of plants play an important role in photosynthesis. They harvest solar energy and transfer it to the reaction centre of photosystems, where conversion into chemical energy takes place. Light-harvesting complex II (LHCII) is the main light-harvesting antenna complex of plants, associated with Photosystem II (PSII). LHCII presents in high amounts in the chloroplast membranes of plants, making it the most abundant membrane protein on Earth [1, 2]. The major, trimeric form of LHCII binds eight Chlorophylls (Chls) *a*, six Chls *b* and four xanthophylls as pigment cofactors per monomeric subunit [3, 4]. Their relative orientations, determined in large part by the protein structure and binding sites as described in Chapter 1, provide the framework for efficient, ultrafast excitation energy transfer (EET). The Chls are vertically distributed in two layers – one closer to the stromal side and the other closer to the luminal side of the membrane – and form several groups of strong electronic dipole-dipole, or excitonic, interactions. The stromal layer contains two strongly coupled Chls *a* domains, comprising of pigments *a*610-611-612 and *a*602-603, respectively. The luminal layer contains one excitonic Chls *a* pair (*a*613-614) and Chl *a*604, which are surrounded by Chls *b* (*b*605, *b*606, and *b*607).

A number of time-resolved spectroscopic studies have revealed the dynamics of EET between Chls within or between the monomeric subunits in LHCII [5, 6] occurring on timescales from tens of femtosecond to tens of picosecond. Briefly, the majority of excitations on Chl *b* are transferred to Chl *a* within few hundred femtoseconds. Exciton relaxation between strongly coupled Chl *a* pigments can be very fast (< 100 fs) but the kinetics is limited by slower transfer between Chl *a* domains especially from one layer to another, and the slowest timescales are attributed to inter-monomer EET. A powerful technique to elucidate EET pathways especially between states of similar energy and overlapping spectra, such as the Chl *a* exciton states in LHCII is two-dimensional electronic spectroscopy (2DES), which correlates the excitation and detection frequencies [7, 8]. Several groups have applied 2DES to investigate different aspects of the exciton dynamics in LHCII at cryogenic temperature [9, 10], as well as room temperature (RT) [11–13]. Up to now, despite the wealth of information recorded by 2DES, there has been no complete reconciliation of the data with exciton models of LHCII.

Structure-based theoretical modelling has provided deeper insight into the specific pathways and rates of EET [14–16]. Modelling is greatly aided by fitting to experi-

mental time-resolved data, especially collected at cryogenic temperatures, mainly because of the restricted pigment and protein motions that reduce disorder and spectral linewidth [17, 18]. In this way, the calculations can be based on a static protein conformation closer to the crystal structure, compared to the modelling results based on the observables obtained at room temperature. However, there are several problems in relating low-temperature data to the physiological state. There is extensive experimental and modelling evidence that structural fluctuations in LHCII can significantly change the Chls transition energies and excitonic couplings leading to a different excited-state energy landscape at different temperatures. Simulations of optical spectra for LHCII appear to fit the experimental data well only up to about 120 K [19, 20]. A study by Vrandečić et al. using combination of quasielastic neutron scattering and optical spectroscopy showed a strong temperature dependence of the transition energies and linewidths in LHCII attributed to dynamic disorder of the protein [21], which is later confirmed by Golub et al. [22]. Consequently, the dynamics of EET is also temperature-dependent, with slower components in the range of 10-30 ps observed at and below 77 K [23] but not at RT [13]. However, the discrepancy can also be partly due to singlet-singlet annihilation decay. Savikhin et al. reported a slowdown of the anisotropy decay by a factor of 4-5 at 13 K and considered the spectral line broadening and uphill EET pathways as factors contributing to the temperature dependence [24].

In our earlier 2DES investigation of LHCII at RT [13], the slowest exciton relaxation timescale was found to be about 5 ps leading to complete thermal equilibration between all states and disorder realizations. These data showed that overall exciton equilibration is faster than predicted by current models. This earlier study also demonstrated that 2DES is well suited to resolve uphill and downhill EET processes which are difficult to resolve in transient absorption experiments. In this chapter, we use 2DES to elucidate the temperature dependence of the uphill and downhill EET processes. We explore the kinetics of exciton relaxation in LHCII as a function of temperature in the range of 77 to 300 K using 2DES in a low-excitation regime with high signal-to-noise ratio and negligible singlet-singlet annihilation. This yields high-resolution kinetic data that can be used for detailed modelling.

4.2 Material and methods

LHCII trimers were isolated by solubilizing PSII-enriched membrane fragments isolated from dark-adapted leaves of green-house grown pea (*Pisum sativum*), with 0.7% *n*-dodecyl β -maltoside (β -DM) followed by sucrose density gradient ultracentrifugation [25] as described in more detail in ref. [13].

2DES measurements were performed using a femtosecond laser system in a partially collinear pump-probe beam geometry setup, described elsewhere [26]. Briefly, pairs of

Figure 4.1. Diagram for 2DES experimental setup. The abbreviations in the figure are show as follow. BS: beam splitter, ND: neutral density filter, P: polarizer, Sapphire: 2-mm sapphire window, short-pass: short-pass filter with 720 nm cut-off wavelength.

excitation pulses of 35-fs duration with controllable interpulse delay time and phase were obtained by passing the output of a non-collinear parametric amplifier (TOPAS White, Light Conversion) through an acousto-optic programmable pulseshaper (Dazzler, Fastlite). The excitation pulse spectrum was centred at 675 nm, coinciding with the Chl *a*'s Q_y absorption band of LHCII (see Fig. 4.2). Whitelight continuum generated by a 2-mm sapphire window was used as a probe (and reference) beam after being compressed with ten round trips on a pair of chirped mirrors (DCM9, Laser Quantum). The cross-correlation width of the excitation and probe pulses was 70 fs (FWHM). The polarization of the probe pulse was set as 'magic angle' (54.7°) relative to the pump pulse polarization to eliminate anisotropic decays. The delay T_w between pump and probe pulses was controlled with a retroreflector mounted on a motorized translation stage in the probe beam path. After the probe interacts with the pump pulse pair, the probe and reference beams were spectrally resolved in a spectrograph (Acton SP2300) by dispersing on a CCD array (Pixis100) to obtain the intensity spectrum $I^{sig}(\omega_t)$ and $I^{ref}(\omega_t)$, respectively. The time delay τ between the two pump pulses was scanned between 0 and 378 fs with 6-fs steps and background-free 2D photon echo signals were recorded by utilizing a 2×1 -phase cycling scheme [27] applying a partially rotating frame. The τ -dependent transient absorption signal $\Delta A(\tau, T_w, \omega_t)$ was obtained as

$$\Delta A(\tau, T_w, \omega_t) = -\log \left[\frac{\frac{I_{pump-on,whitelight-on}^{sig}(\omega_t) - I_{pump-on,whitelight-off}^{sig}(\omega_t)}{I_{pump-off,whitelight-on}^{sig}(\omega_t) - I_{pump-off,whitelight-off}^{sig}(\omega_t)}}{\frac{I_{pump-on,whitelight-on}^{ref}(\omega_t) - I_{pump-on,whitelight-off}^{ref}(\omega_t)}{I_{pump-off,whitelight-on}^{ref}(\omega_t) - I_{pump-off,whitelight-off}^{ref}(\omega_t)}} \right]. \quad (4.1)$$

In Eqn. (4.1), the terms having superscripts *sig* and *ref* indicate the intensity spectra resolved from the probe and the reference beams, respectively. The subscript *pump-on* and *pump-off* represent the terms obtained when the pump pulse is on and off by the pulseshaper. Similarly, the *whitelight-on* and *whitelight-off* terms are obtained when the whitelight continuum is on or off by the mechanical chopper spinning at 500 Hz. The chopping of whitelight can help to eliminate the scattered light from the pump. The normalization by dividing signals retrieved from the probe and the reference beam is useful to reduce the noise caused by fluctuation in the whitelight spectrum. The $\Delta A(\tau, T_w, \omega_t)$ signals were then Fourier-transformed along τ to obtain 2D electronic spectra $S(\omega_\tau, T_w, \omega_t)/S(\lambda_\tau, T_w, \lambda_t)$ in the frequency/wavelength domain. The waiting time (T_w) between the excitation and probe pulses was scanned from -100 fs to 100 ps in a quasi-logarithmic progression.

For 2DES measurements, the LHCII samples were diluted in degassed buffered medium (pH 7.8) containing 60% (v/v) glycerol, 20 mM tricine and 0.03% β -DM. The

Figure 4.2. Time-resolved fluorescence of LHCII. (a) LHCII in buffer without glycerol; (b) LHCII in buffer with 60% glycerol. Measurements were done by time-correlated single photon counting as described in ref. [28]. The figures show decay-associated emission spectra obtained from global multiexponential fit of the fluorescence decays at different detection wavelengths (excitation at 633 nm). The average lifetime of LHCII in glycerol is decreased by 15%.

solution was contained in a cell between two sapphire windows separated by a 0.5-mm Teflon spacer shielded with cryogenic grease and placed in a liquid-nitrogen-cooled vacuum cryostat (Janis Research) with BaF₂ windows. The optical density of absorption was adjusted to 0.3 at the Chl Q_y maximum. The experiments were performed at a series of temperatures from 77 to 295 K. Before each 2DES experiment, the relative transient absorption signal at the Q_y maximum was measured at delay time 100 ps while varying the excitation power (as reported in ref. [13]), to verify that there is no quenching from singlet-singlet annihilation.

A series of time-resolved fluorescence experiments were done in different concentration of glycerol. The fluorescence data show that higher concentration of glycerol (above 60%) causes some aggregation of the complex which may introduce additional excitation decay components. In 60% glycerol the average fluorescence lifetime of LHCII is reduced by approximately 15% (see Fig. 4.2). This mild quenching is reflected in the 2DES experiments in 60% glycerol as well.

4.3 Results

We performed 2DES experiments at different temperatures in the range of 77 to 295 K with excitation pulses roughly covering the wavelength region from 650 to 700 nm [see Fig. 4.3(a)]. Our goal was to record the Chl excited-state kinetics undistorted by singlet-singlet or singlet-triplet quenching (annihilation). This necessitated the use of low excitation pulse energies, on one hand to avoid the generation of multiple excitations in the same complex and, on the other, to prevent the accumulation of long-lived

Figure 4.3. (a) Absorption spectrum of LHCII recorded at 77 K, normalized at the maximum, and spectrum of the excitation laser pulses used in 2DES (shaded area). (b) Absorption spectra of LHCII at five different temperatures. LHCII was diluted in 60% glycerol, placed in an optical cryostat and the absorption spectrum was recorded using an OceanOptics spectrophotometer. The laser excitation spectrum was recorded using the CCD camera of the 2DES instrument

states (triplets) in the case where the sample cannot be refreshed (at low temperatures). The absence of quenching was rigorously tested and confirmed by excitation density dependence studies similar to our prior experiments at RT [13], apart from a minor decay component always present in glycerol-containing medium.

4.3.1 2DES at 77K

We will first qualitatively describe the results of the 2DES measurements performed at 77 K. Selected 2D electronic spectra at different waiting times T_w are plotted in Fig. 4.4. The colour code indicates the purely absorptive 2DES signal amplitude – negative amplitudes indicate ground-state bleach (GSB) and stimulated emission (SE) and positive amplitudes present excited-state absorption (ESA). The signal reflects the conditional population of excited states absorbing at detection wavelength λ_t (along the horizontal axis), upon initial excitation at wavelength λ_τ (vertical axis). Off-diagonal signals originate from exciton coupling (delocalized excitations) or EET between states at λ_τ and λ_t . At low temperature EET proceeds primarily from shorter- to longer-wavelength Chls, resulting in the appearance of off-diagonal bleaching signals (cross-peaks) at $\lambda_t > \lambda_\tau$.

Due to the significantly narrower lineshape in general, the 2D spectra are more structured and individual features are better resolved at 77 K compared to RT. At early waiting times ($T_w = 100$ fs), although the signal is largely along the diagonal, off-diagonal signals are also observed, e.g. at $670 \rightarrow 675$ nm ($\lambda_\tau \rightarrow \lambda_t$) and $673 \rightarrow 678$ nm, as a result of exciton couplings as well as very rapid exciton relaxation, as

Figure 4.4. Representative purely absorptive 2D electronic spectra of detergent solubilized LHCII trimers recorded at 77 K with excitation pulses centred at 670 nm, at selected waiting times T_w as indicated. The symbols λ_τ and λ_t indicate excitation and detection wavelengths, respectively.

previously shown [9]. In the time between 100 fs and 1 ps, short-wavelength diagonal signals decay in favour of off-diagonal signals. However, evidently the decay is faster for states at around 670 nm, which lose about 50% amplitude, than at around 665 nm, where the amplitude loss is only about 20%. After 10 ps practically no diagonal signal is detected at wavelength shorter than 672 nm, showing that all excitations have been transferred to lower-energy Chls, at detection wavelengths 674–682 nm. The spectral changes between 10 and 100 ps are smaller but firmly resolved – a continued rise of the off-diagonal bleach at λ_t equal or longer than 677 nm (e.g. compare amplitude at 670 → 677 nm) indicates slow downhill transfer to the lowest energy states. It must be noted that at this long waiting time the spectral shape along λ_t is invariable at excitation wavelengths below 675 nm. However, for λ_τ in the range of 675–682 nm, there is a pronounced correlation between λ_τ and λ_t – the bleaching signal peaks along the diagonal indicating inhomogeneous broadening (see below).

For two selected excitation wavelengths – 668 and 674 nm – the spectral evolution

Figure 4.5. Horizontal slices of the 2D electronic spectra of LHCII at 77 K at selected waiting times (top row) and T_w -dependence of the 2D echo signal at selected detection wavelengths (bottom row) plotted for two excitation wavelengths, 668 nm (left) and 674 nm (right). In the lower panels, the symbols represent the measured data points and the lines are obtained by global multiexponential fitting.

over time is shown in greater detail in Fig. 4.5, in terms of horizontal slices of the 2D spectra and kinetic traces at selected detection wavelengths. Upon excitation at 668 nm, already after 100 fs, the horizontal slices exhibit an intense bleaching band at 674 nm, indicating strong coupling between states absorbing at these wavelengths. The initial bleach at 668 nm decays in the first ps and nearly vanishes after 3 ps. At longer delay times, the amplitude of the main bleaching band increases and its maximum shifts to the red, as excitations are transferred to lower-energy states. This cascade is clearly illustrated by the kinetic traces in the first 1 ps, the diagonal 668 signal decays, while the 668 → 674 nm cross-peak amplitude rises. At times longer than 1 ps, the intermediate 668 → 674 nm decays in turn, giving rise to the 668 → 678 nm signal. It is worth noting that the overall bleaching intensity continues to increase up to 10 ps, which clearly indicates the absence of singlet-singlet annihilation.

The slow relaxation in the long-wavelength region (674-680 nm) is well illustrated

by the horizontal slices and kinetic traces upon 674 nm excitation. In this case, the spectra show only very small change in the first 1 ps apart from the disappearance of a shoulder at 668 nm (reaffirming that bands around 668 and 674 nm belong to the same exciton pair). The kinetic traces show that the decay of the diagonal signal at 674 nm is significantly slower than at 668 nm. Spectral evolution is observed on a timescale of 110 ps, where the bleaching maximum is red-shifted from 674 to 678 nm. Even slower dynamics is observed in the kinetic traces – the 674 $\rightarrow$ 678 nm trace decays while the 674 $\rightarrow$ 680 nm rises between 10 and 50 ps.

4.3.2 Global analysis

For a semi-quantitative overview of the excitation dynamics, the time-resolved 2D electronic spectra were subjected to global multi-exponential analysis [11]. The 2D decay-associated spectra (2D DAS) resulted from four-exponential fitting of the kinetics at 77 K are plotted in Fig. 4.6. Negative or positive amplitudes primarily indicate that the GSB/SE signal decays or rises, respectively, on a given timescale. EET can be followed in the first three 2D DAS (with respective lifetimes of 0.4, 2.7 and 15 ps) by the decaying diagonal states and corresponding rise of cross-peaks, mainly at longer detection wavelengths. The final spectrum (>1 ns) represents decay to the ground state.

The 2D DAS illustrate the observations discussed above, namely: 1) fast EET (0.4 ps timescale) from Chl *a* states absorbing around 670 nm to lower states and from Chl *b* to states around 674 nm; 2) slower EET (mostly 2.7 ps DAS) from the region 660-668 nm with corresponding positive cross-peaks at $\lambda_t = 675$ -678 nm; 3) even slower relaxation (2.7 and 15 ps timescales) in the low-energy Chl *a* region 675-680 nm. Additional insight can be drawn from well-resolved negative cross-peaks in the 2D DAS, notably the 670 $\rightarrow$ 674 nm cross-peak in the 15-ps DAS, which shows the decay of Chl *a* at 674 nm, populated via fast relaxation from upper-lying states (670 nm). Another feature of interest in the 2D DAS is the presence of positive cross-peaks below the diagonal ($\lambda_t < \lambda_\tau$) indicating uphill EET to shorter-wavelength states. For example, a cross-peak at 680 $\rightarrow$ 675 nm in the 2.7-ps DAS mirrors the ‘downhill’ cross-peak 675 $\rightarrow$ 680 nm. The uphill pathways of EET will be discussed in the following section. The shape of the final 2D DAS is practically identical to the 2D electronic spectrum at $T_w = 100$ ps (see Fig. 4.4) and shows a pronounced correlation between excitation and detection wavelengths in the red-most region. The inhomogeneous width of the lowest-energy peak is around 85 cm^{-1} , estimated from the diagonal width (HWHM) of the 2D DAS spectrum and the homogeneous width (antidiagonal HWHM) is 30 - 40 cm^{-1} . The inhomogeneous width is larger compared to the value of 35 cm^{-1} determined at 4.2 K by hole burning spectroscopy [29, 30]. Although the inhomogeneous linewidth is expected to be broader at higher temperature, our result may be somewhat overestimated

Figure 4.6. 2D decay-associated spectra (2D DAS) of LHCII at 77K, obtained from four-component global lifetime analysis of the 2DES data. Red/blue colors represent negative/positive amplitudes (decay/rise of the negative absorptive signal).

here because of overlap between diagonal and cross-peak signals.

4.3.3 Temperature dependence

It is evident that not only the absorptive spectral lineshape of the Chl Q_y transitions, but also the dynamics of EET recorded at 77 K considerably differ from those measured at RT [13]. While cryogenic temperatures provide a great advantage in resolving spectral components and modelling the excitation dynamics, ultimately it is the goal to gain knowledge on the function of the light-harvesting antenna under natural physiological conditions.

We performed 2DES measurements at intermediate temperatures to follow the changes in spectral lineshape and exciton dynamics in LHCII. For comparison, 2D electronic spectra and horizontal slices (at $\lambda_\tau = 668$ nm) are plotted in Fig. 4.7 for selected waiting times. Control measurements at RT were done as well, with results very similar to the previously published [13]. Several changes in the 2D spectra can be observed with the increase of temperature:

- The lineshape along both λ_τ and λ_t gradually broadens.

- The maximum along λ_t shifts to the red.
- Partly because of the larger transition bandwidth, the initial population of excited states upon short-wavelength excitation (e.g. $\lambda_\tau = 668$ nm) is detected over a wider wavelength range – effectively as if excitations were instantly transferred to lower-energy states.
- The spectra at 10 ps show higher amplitudes below the diagonal indicating uphill energy transfer ($\lambda_t < \lambda_\tau$).
- The correlation between $\lambda_t < \lambda_\tau$ is gradually lost and at 230 K (as well as at RT) the shape of the 10-ps spectrum along λ_t is virtually independent of λ_τ . In other words, a final equilibrium state is reached within 10 ps, regardless of the initial excitation. In contrast, the final spectra show inhomogeneous broadening at lower temperature.
- The final quasi-equilibrated state is reached at shorter waiting times – very little spectral evolution can be observed beyond 3 ps at 230 K (compare the horizontal slices).

TABLE 4.1. EET lifetimes determined by global analysis of the 2DES data at different temperatures.

Temperature (K)	τ_1 (ps)	τ_2 (ps)	τ_3 (ps)	τ_4 (ns)
77 [†]	0.34±0.05	2.5±0.2	14.2±0.9	>1
110 [‡]	0.2±0.2	2.0±1.1	9.7±5.8	>1
150 [‡]	0.2±0.1	1.6±0.8	7.4±3.1	>1
230 [‡]	0.2±0.1	1.7±0.7	6.3±2.1	>1
295 [‡]	0.2±0.4	0.9±2.8	5.4±13.2	>1

[†]The error bars of 77 K results are the standard deviations from four independent experiments.

[‡]The error bars of these results are calculated from the Jacobian $\mathbf{J}$ and the mean-squared error MSE of the fits as $\sigma = \sqrt{MSE(\mathbf{J}^T \mathbf{J})^{-1}}$.

We analysed the 2DES data at all temperatures by four-exponential fitting as with the 77 K data. The obtained lifetimes are summarized in Table 4.1 and the 2D DAS are plotted in Figs. 4.8 and 4.9. The second and the third decay lifetime shortened with temperature – from 2.5 and 14.2 ps at 77 K to 0.9 and 5.4 ps at RT, respectively. Undoubtedly, exciton equilibration occurs on faster timescales at higher temperatures. A comparison of the final 2D DAS representing decay to the ground state is plotted in Fig. 4.9. The spectra show inhomogeneous broadening (static disorder) below 230 K – this is clearly illustrated in the normalized horizontal slices in Fig. 4.10. On the other hand, at 230 K and higher temperature, the final spectrum no longer depends on the excitation wavelength.

Figure 4.7. 2D electronic spectra of LHCII recorded at 77, 110, 150 and 230 K. From top to bottom: 2D electronic spectra at $T_w = 100$ fs and 10 ps, horizontal slices of the 2D spectra along $\lambda_t = 668$ nm for different waiting times.

Figure 4.8. 2D DAS resulting from the global analysis of the 2DES data recorded at temperatures 77, 110, 150 and 295 K. The respective lifetimes are indicated in the plots. For each temperature, the spectra are plotted on a fixed color scale to facilitate comparison of the amplitudes. The final decay spectra (in the nanosecond range) are plotted in Fig. 4.9.

Figure 4.9. Final 2D DAS showing the decay of the quasi-equilibrated excited states at different temperatures

Figure 4.10. Horizontal slices (along detection wavelength) of the final 2D DAS at selected excitation wavelengths for different temperatures. The curves are normalized to their negative maxima for easier comparison. A clear dependence of the final spectrum on excitation wavelength is observed below 230 K.

Figure 4.11. Average lifetimes of the dynamics at selected excitation and detection wavelengths as a function of temperature. (a) Average decay lifetime calculated from the diagonal 2D DAS amplitudes at 662, 668 and 672 nm. (b) Average rise time of the off-diagonal amplitude at $\lambda_t = 678\text{-}681$ nm. See text for details on the calculation. The maximal standard deviation of the reported values is 8% at 77 K (from four independent experiments).

To estimate the overall acceleration of EET, we can compare the average lifetimes for diagonal and off-diagonal signals (see Fig. 4.11). The average decay lifetimes of the diagonal signals in Fig. 4.11(a) were calculated according to the formula $\tau_{avg} = \sum_{i=1}^3 a_i \tau_i / \sum_{i=1}^3 a_i$ (the nanosecond decay to the ground state is not considered). The lifetime of the longest-lived signal, at 662 nm, was 5.8 ± 0.4 ps at 77 K and decreased to 4.3 ps at RT. At 668 nm, the decay time changes from 3.6 to 2.7 ps and at 672 nm – from 4.3 to 3.3 ps. It is interesting to point out the peculiar temperature dependence of the diagonal peak at 662 nm. The marked drop in the lifetime between 77 K and 110 K may indicate an abrupt change in the EET dynamics. This behaviour is not mirrored by the signal at 668 nm.

Figure 4.11(b) shows the average rise times of off-diagonal signals, calculated by normalizing the rise-term amplitudes to the amplitude of the decay (nanosecond) component: $\tau_{avg} = - \sum_{i=1}^3 a_i \tau_i / a_4$. Together these results show that as temperature increases, the overall time needed for excitations to reach the lowest-energy state progressively decreases. For example, the rise time of the 662 → 680 nm signal decreases from 4.5 to 1.2 ps between 77 and 295 K.

The normalized cross-peak amplitudes presented in Fig. 4.12 show some obvious trends. Most notably, as temperature increases, the total rise amplitude becomes a smaller fraction of the decay amplitude, which is a result of the temperature-dependent spectral broadening. The middle-lifetime rise component is also diminished; however, the redistribution of amplitudes to some extent compensates for the change in the re-

Figure 4.12. Temperature dependence of the 2D DAS amplitudes, at the indicated wavelengths ($\lambda_\tau \rightarrow \lambda_t$), of the three EET lifetime components ($\tau_1 = 0.2\text{-}0.3$ ps, $\tau_2 = 1.8\text{-}2.4$ ps, $\tau_3 = 6\text{-}14$ ps) relative to the respective amplitude of the nanosecond decay component.

spective lifetimes. Therefore, to interpret the results in terms of change in EET rates, one must consider the spectral overlap as well as kinetic overlap, i.e., representation of complex dynamics with a small set of (four) lifetimes.

4.4 Discussion

4.4.1 EET dynamics at 77 K

An abundance of experimental evidence can be found in the literature that temperature significantly affects not only the spectroscopic properties of LHCII such as peak positions and widths in the absorption and fluorescence spectra, but also the excitation dynamics. The slowest components of exciton equilibration at RT have been found to occur on timescales of about 5 ps by transient absorption [23, 31, 32], three-pulse photon echo [33] and 2DES [13]. Decay components in the 10-20 ps at RT have been ascribed to singlet-singlet annihilation and may reflect isoenergetic intermonomer EET [23, 34], which is corroborated by theoretical calculations [15]. On the other hand, the 77 K results presented here are in line with several transient absorption and time-

resolved fluorescence studies that reported Chl *a*-Chl *a* EET timescales of 12-36 ps at temperatures below 80 K [5, 35–39]. Savikhin et al. measured the anisotropy decay at temperatures between 50 and 100 K and determined that it is strongly temperature-dependent at 680-682 nm, whereas a weak dependence was found at 650 nm [24]. Thus, it can be stated with high degree of certainty that Chl *a*-Chl *a* EET slows down at low temperature. However, there is considerably less certainty regarding the specific Chls and EET pathways involved. Even the spectral shapes and relative amplitudes of the different decay components vary largely among experiments. To some extent this can be due to the varying degree of singlet-singlet and singlet-triplet annihilation, which affects mostly components in the tens of ps range.

Figure 4.13. EADS obtained from the 77 K data analysed separately for excitation wavelengths 662, 670, 675 and 676 nm.

To facilitate comparing the results in this work with literature data we ‘simulate’ transient absorption spectra by applying a narrowband envelope centred at a given excitation wavelength to the 2D electronic spectra and integrate the spectra. For selected excitation wavelengths the 77 K data are analysed applying a sequential kinetic model

and the resulting evolution-associated decay spectra (EADS) are plotted in Fig. 4.13. The lifetimes as well as the spectra for 662 nm, 670 nm and 674 nm excitations are closely comparable to the data reported by van Grondelle's group [6, 37–39]. Considering the different excitation wavelengths together as well as the 2D DAS, we can make a more confident analysis of the EET pathways at 77 K and compare with existing models.

4.4.2 Long-live intermediate states

An outstanding question exists regarding the Chl states in the intermediate wavelength region 660–670 nm that act as a bridge between Chl *b* and Chl *a*. For example, Visser et al. observed decay lifetimes of 2.4 and 12 ps upon 661 nm excitation [37] (reproduced in the present study – cf. the 2.2 and 13 ps EADS in Fig. 4.13). Novoderezhkin et al. attribute these lifetimes to two Chls, *b*605 and *a*604, respectively, which, according to their exciton model, contribute mainly to the dynamics in the 660–670 nm region [15, 40]. In contrast, Renger's *ab initio* calculations assign a far lower energy level of Chl *a*604 which places it outside of the intermediate region [16]. It must be noted though that their calculated EET rates do not reproduce the slow dynamics observed experimentally at 660–670 nm. While our data confirm that states around 665 nm decay relatively slowly (on a timescale of 2–3 ps), the 2D DAS indicate that their population has almost no contribution to the dynamics beyond 10 ps, in contrast to the very slow population decay of Chl *a*604 calculated by Novoderezhkin et al. Interestingly, the temperature dependence of the signal in this region (see Fig. 4.11) may hint at a conformational switch that alters the energy of the 'bottleneck' state (see Section 4.4.5 below).

The 12–18 ps lifetimes obtained from analysis of the 2DES data (see Figs. 4.6 and 4.13) are almost exclusively associated with EET among low-energy Chls *a* – in the 675–680 nm region. These can be assigned to EET between the three Chl *a* exciton clusters, especially between the luminal (Chls *a*613–614) and the stromal clusters (Chls *a*602–603 and *a*610–611–612) in the same monomeric subunit. Alternatively, the slow dynamics could represent excitation migration between monomers. This would be in line with calculated inter-monomer transfer timescales between the stromal clusters and stromal-luminal clusters in the 10–20 ps range [15]. Comparative 2DES study of LHCII trimers and monomers could in principle resolve this ambiguity.

In summary, the 2DES results at 77 K largely confirm the knowledge of LHCII exciton dynamics gathered over the past decades but also provide additional clues that can potentially refine the available exciton models. To realize this potential, we have performed model fitting of the 2DES data, which will be presented in Chapter 5.

4.4.3 Static disorder and spectral diffusion

The long-delay 2D electronic spectra in Fig. 4.4 and the final 2D DAS at 77 K in Fig. 4.6 have two distinct regions – an off-diagonal region, where the spectral shape is mostly independent of the excitation wavelength and the region around the diagonal line, where the signal shows marked dependence on λ_r . The fact that the final relaxed state at 77 K is different depending on where the initial excitation was, is in stark contrast to the RT result, wherein any such dependence is lost and the final state is thermally equilibrated within a few ps [13]. There can be different causes for the non-equilibrated shape at low temperature. One cause is the inhomogeneous broadening, or static disorder, of the final state. In other words, the lowest energy level varies among LHCII units, locked in slightly different protein conformations. Interpreted in this way, we estimate that the final state at 77 K has an inhomogeneous width of around 85 cm^{-1} (HWHM), which persists for the entire measurement period of 100 ps without signs of spectral diffusion. At higher temperatures, thermal fluctuations diminish or completely lose the correlation between the excitation and detection wavelength (spectral diffusion). Another possible cause for the excitation dependence is that at 77 K there exist at least two distinct sink states in the same protein complex, hence at least two pools of Chls, which are not connected by EET. In favour of this possibility are the findings of Vrandečić et al. that the lowest-energy levels and fluorescent states are temperature-dependent – at RT and down to 150 K fluorescence is emitted mainly from Chl *a*612 but other states also contribute to the emission below 150 K [21]. This can be understood if at low temperatures there is effectively no EET between, e.g. the luminal Chl *a* pool (Chls *a*604, *a*613, *a*614) and the stromal-side Chls *a* (including Chl *a*612). Only single-molecule spectroscopy can distinguish with certainty whether different sinks co-exist in the same complex or the different energy levels are only a result of structural heterogeneity of the ensemble. In either case, the 2DES results provide further independent evidence that supports the notion that the exciton energies are strongly related to protein motions [21, 22, 41]. The static excitation-detection correlation, or inhomogeneous broadening of the final state is distinctly observed at temperatures 110 and 150 K, whereas spectral diffusion appears to occur at temperatures of 230 K and above. This behaviour roughly coincides with the glass phase transition of the LHCII protein at around 240 K [21].

4.4.4 Uphill/downhill EET

In our previous study of LHCII dynamics at RT, we made the case that 2DES provides advantage over one-dimensional transient absorption in its ability to simultaneously resolve and quantify uphill and downhill EET pathways [13]. One of the goals of the present work was to further verify the quantitative relationship by determining the ratios of the downhill/uphill cross-peak amplitudes as a function of temperature. Uphill EET

Figure 4.14. Total redistribution 2D difference spectra at different temperatures. For a given temperature, the redistribution difference spectrum is the sum of the 2D DAS for all components except the last decay (to the ground state). The total uphill EET signal decreases with lowering the temperature.

is suppressed at low temperature because the available thermal energy falls below the energy gaps between exciton states. The detailed-balance condition dictates that the ratio of forward/reverse (uphill/downhill) rate constants should follow the Boltzmann distribution function [42]. Downhill/uphill EET is followed by the cross-peak intensity at λ_t greater/less than λ_τ . The overall redistribution of energy in downhill and uphill pathways is depicted by the sum of all EET 2D DAS, plotted in Fig. 4.14 for different temperatures. The figure shows that, as the temperature gets lower, the ‘uphill cross-peaks’ amplitude is reduced, and the effect is more pronounced at detection wavelengths farther away from the diagonal (where the energy gap is larger). At 77 K the uphill cross-peak is only a few nm from the diagonal line. The same tendency can also be observed on any given timescale in the respective 2D DAS as shown in Fig. 4.8.

It is useful to quantify this result in terms of rate constant ratios and compare with the detailed balance. However, because of the spectral overlap (e.g. between diagonal and cross-peaks) and especially because the linewidth is temperature-dependent, such

Figure 4.15. Temperature dependence of the uphill/downhill cross-peak amplitude ratios of the final 2D DAS representing the decay equilibrium in LHCII. The cross-peak ratio λ_a/λ_b (wavelengths indicated in the plot) is calculated as $(\lambda_a \rightarrow \lambda_b)/(\lambda_b \rightarrow \lambda_a)$. Dashed lines – estimated equilibrated population of the respective states, from the Boltzmann distribution: $\exp\left(\frac{E_a - E_b}{k_B T}\right)$.

calculation can only be done correctly with an appropriate spectral model. Alternatively, the uphill/downhill ratio can be estimated from the 2D electronic spectrum after equilibration is complete (at $T_w > 30$ ps). Figure 4.15 shows the ratio of the amplitudes of the final 2D DAS component (nanosecond decay) at selected ‘uphill’ wavelengths (e.g. $678 \rightarrow 664$ nm) relative to the ‘downhill’ counterpart amplitude ($664 \rightarrow 678$ nm) as a function of temperature. The uphill/downhill amplitude ratios increase with temperature, depending on the energy gap between the two wavelengths. The uphill/downhill cross-peak intensity ratios appear to match the expected Boltzmann equilibrium population of the respective states. It must be noted that this is also only a rough approximation, not accounting for the temperature-dependent spectral overlap. Another source of error is the ESA, which may have a different contribution to the signal depending on the wavelength (the values in Fig. 4.15 are calculated assuming constant ESA background). The error should be larger at low temperatures, where uphill EET is less pronounced and the cross-peak signal is dominated by ESA. A spectral model would be necessary to extract more precise thermodynamic parameters from the data.

4.4.5 Temperature dependence of EET

We established that exciton relaxation in LHCII is overall slower and the final excited state is heterogeneous due to static disorder or unconnected Chl pools, whereas at RT faster EET and spectral diffusion result in rapid thermal equilibration of excitations. These two extremes fall in different regimes of protein dynamics. While the protein is mostly frozen at 77 K and soft or melted above 240 K (glass transition), at temperatures

110-230 K we are in the intermediate regime of limited conformational dynamics. Conformational changes of the protein environment can in turn alter excited-state dynamics which depend on the pigment-protein interactions (affecting the Chl site energies), exciton coupling and EET in the Förster regime.

When quantifying the EET dynamics, we must be aware of the distinction between the observed timescale of spectral evolution and the underlying intrinsic EET rate constants and the fact that changes in the former are not necessarily grounded in changes in the latter. It is well established that temperature affects the position and lineshape of the absorption/emission bands in LHCII [43]. At RT, initial excitations are distributed over a broader range of exciton levels merely because of their enhanced spectral bandwidth. This is partly the cause for the apparent shortening of the average rise time of the lowest-energy states [see Fig. 4.11(b)]. The spectral broadening per se enhances the overall efficiency of the light-harvesting antenna under physiological conditions. On the other hand, changes in the transition energies and spectral lineshapes will inevitably affect the EET rate constants as well, which is reflected in the global lifetime analysis of the 2DES data. The outcome is general acceleration of exciton relaxation with temperature, as the decay and rise lifetimes of the diagonal and cross-peak signals, respectively, as well as the lifetime amplitudes at different wavelengths show. A significant contribution to this is the strong temperature dependence of energetic uphill pathways. In an inhomogeneous energy landscape, excitations are dynamically trapped and detrapped via uphill transfer. As these routes become inaccessible at lower temperature, EET slows down.

The different temperature dependences of the decay lifetimes (Table 4.1, Fig. 4.6) and cross-peak amplitudes of the 2D DAS (Fig. 4.8), for example compared between 662 nm and 668 nm excitation, hint that exciton states may have a different temperature response. It is tempting to speculate that the average site/exciton energy of at least some states is temperature-dependent, changing the EET pathways. The peculiar drop of the 662-nm diagonal peak lifetime, for example, could be indicative of a temperature-dependent conformational switch causing red shift of the ‘bottleneck’ Chl *a*604 above 77 K [44]. However, we must reiterate that spectral decomposition with a suitable model is crucial to extract the full information encoded in the 2DES data. Quantifying the temperature dependence of EET rates requires a microscopic quantum mechanical model. To fully describe the EET dynamics, one must consider that different protein conformations become accessible at different temperatures, which will affect the spectral density function and exciton landscape [22]. At intermediate temperatures, the dynamics of spectral diffusion become comparable to the dynamics of EET between exciton states, both of which affect the spectral evolution in time. The temperature-dependent 2DES data, in combination with suitable temperature-dependent dynamic-structure quantum modelling, could in principle reveal transitions from one to another

EET regime.

4.5 Conclusion

The 2DES results showed that exciton equilibration in LHCII is temperature-dependent. Two key observations in our experiments reported here are: (1) strong temperature dependence of the cross-peak signals assigned to uphill EET, upholding the earlier interpretation of the cross-peaks in the 2D DAS [13], and (2) considerable slowdown of EET at cryogenic temperatures. The close correspondence between the measured uphill/downhill cross-peak amplitudes and the expected ratios from the detailed balance reinforces the applicability of 2DES to identify EET pathways and model EET with thermodynamic constraints.

Confirming our earlier measurements, we find that spectral equilibration occurs on timescales not longer than about 5 ps at RT, but progressively slower dynamics, up to tens of ps, can be observed at lower temperatures. From the excitation-wavelength dependence of the amplitudes of the slowest kinetic components in the low-temperature 2DES data, it can be concluded that the rate-limiting EET steps involve low-energy Chl *a* exciton states (675-680 nm). Contrary to current models of the LHCII excitation dynamics, the relaxation of intermediate ‘bottleneck’ states (around 665 nm) occurs primarily on a time scale of ~ 2 -3 ps and has little contribution to the dynamics beyond 10 ps. While the data suggest that the EET rates between exciton pairs might have different temperature responses, further data analysis/modelling will be necessary to ascertain this.

At the temperature of 230 K and higher, because of exciton equilibration as well as spectral diffusion, the final excited state is independent of the initial excitation. This is not the case at lower temperatures, where static energetic disorder is manifested in the 2D spectra as a non-decaying correlation between the excitation and detection wavelength. Energy redistribution in LHCII is overall more rapid and efficient at physiological temperature. Thermal protein motions facilitate this in several ways: by lineshape broadening, increasing the spectral overlap and resonance EET, enabling additional uphill pathways and avoiding excitation traps.

The experimental data reported here encode a wealth of information regarding the energetic and dynamic properties of LHCII, including electronic dephasing times, inhomogeneous broadening, EET rates, etc., and their temperature dependence. While some of these parameters can be directly read from the data, others can be determined through modelling. Thus, these results will help in refining the current exciton model of LHCII and in better understanding the photophysics of this and similar light-harvesting complexes. In Chapter 5, we will report on the determination of spectral and kinetic parameters by phenomenological kinetic modelling of the 2DES data.

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Chapter 5

Revealing the excitation energy transfer network of LHCII by the phenomenological analysis of 2DES

5.1 Introduction

During the first step of photosynthesis, incident photons from sunlight are harvested by light-sensitive pigment-protein antenna complexes, where the excitation energy is equilibrated before being transferred to the photosynthetic reaction center. The arrangement of antenna complexes around a reaction center is such, that nearly every photon absorbed drives photochemistry [1]. Light-harvesting complex II (LHCII), the peripheral antenna of Photosystem II (PSII), is the most abundant antenna complex in the biosphere [1]. In nature, LHCII mostly exists in a trimeric form, in which each monomeric unit is comprised of eight chlorophylls (Chls) *a*, six Chls *b* and four xanthophylls [2]. These pigments are non-covalently bound within the three trans-membrane α helices. The protein environment of LHCII optimizes the arrangements of the pigment molecules that enable the ultrafast excitonic energy transfer (EET) that normally occurs on femtosecond to picosecond timescales [1].

The dynamic of the EET process in LHCII has been studied intensively using various spectroscopic techniques [3]. The general conclusions from these studies are that EET from Chl *b* to Chl *a* takes place rapidly, with sub-ps characteristic times in the range of 0.15-0.6 ps at room temperature and 0.3-0.6 ps at low temperature [4–11]. This fast phase is followed by slower (several ps) transfers from Chls absorbing in an intermediate region (660-670 nm) between the major Chl *b* and Chl *a* Q_y bands. The information about the electronic energy level structure of the excited states which is largely contributed by interactions between Chls and Chl-protein is key to understanding the ultrafast dynamics of the EET network.

With the determination of the high-resolution crystal structure of trimeric LHCII [2, 12], several theoretical studies have been performed in an effort to establish a comprehensive picture of the EET network in LHCII. The proposed models employ various levels of theory like Förster energy transfer [13], Redfield and modified Redfield [14, 15], and combined modified Redfield-generalized Förster [16, 17]. The most advanced models proposed by Novoderezhkin et al. [16] and Renger et al. [17] that use modified Redfield-generalized Förster theory grouping the Chls in the complex into a few strongly coupled clusters. EET within each cluster is treated with modified Redfield theory and between different clusters with the generalized Förster formalism. These two models have achieved consistent results with the experimental observations mentioned above. While there are similarities between these two advanced models, there

are also important differences between them. In the Redfield-Förster picture proposed by Novoderezhkin et al. [16] the weak excitonic coupling between any vibronic transitions of the pigments plays an important role and results in exciton delocalization. The model of Renger et al. [17] assumes that the protein vibrations can destroy any coherence and lead to exciton localization. As a result of these different approaches, the EET rates obtained by Novoderezhkin et al. are relatively faster.

In recent years, two-dimensional electronic spectroscopy (2DES) has been employed to characterize ultrafast processes in photosynthetic systems [18–26]. With one more spectral dimension compared to transient absorption (TA) spectroscopy, 2DES enables the detection of spectrally resolved features of the donor-acceptor pairs participating in the EET processes. Several 2DES studies have been conducted on LHCII at both physiological [20–22, 25] and cryogenic temperatures [27–29]. At physiological conditions, the 2DES study conducted by Wells et al. revealed different transfer pathways between the high and low energy Chls *b* in LHCII [20]. The EET from high energy Chl *b* directly to Chl *a* was detected with a sub-ps rate. The low energy Chls *b* show multi-step dynamics with sub to several ps transfers to intermediate states followed by few-ps transfers to the final Chl *a*. The equilibration between the Chls *a* manifold was resolved by Akhtar et al., and found to occur within a few picoseconds [25]. At 77 K, the 2DES measurement of LHCII conducted by Schlau-Cohen et al. reported sub-100 fs relaxation rates between the strongly coupled Chls and a few to tens of picosecond rates of inter-cluster transfer processes [27]. By observing quantum beats in non-rephasing 2D electronic spectra, Calhoun et al. could determine 14 excitonic energy levels in LHCII at 77 K [29]. More recently, 2DES has been applied to probe the energy transfer in LHCII in a range of temperatures from 77 K up to room temperature [30] with substantial improvement in the resolution of spectral components at 77 K. Although valuable spectroscopic information has been unveiled with 2DES, a complete characterization of the EET network of LHCII with one to one mapping between the excitonic levels and pigment identities remains elusive. In this chapter, we report on our approach to extract physical parameters and mechanistic information about the exciton states, EET pathways and dynamics from 2DES measurements by phenomenological model fitting. This approach was applied to model the 2DES data of trimeric LHCII obtained at 77 K and propose tentative EET schemes that compares well with the modified Redfield-generalized Förster models mentioned above. Without a substantial a priori knowledge about the complex, the phenomenological approach can construct reasonable EET schemes with the determination of three lowest energy sinks absorbing at 675, 677-678 and 680-681 nm with no significant EET connecting them. The energy equilibration between these three lowest energy states occurs via uphill energy flow to higher states, which is partially the reason for the observed temperature dependence of the energy equilibration process [30]. Although the complete EET scheme

cannot be determined uniquely from the available experimental data, the phenomenological approach can provide a simple but potentially powerful tool to investigate the EET network in photosynthetic complexes, whose atomistic structures have not been resolved.

5.2 Model

Here we present the procedure to obtain a phenomenological description of the EET network in LHCII from 2DES data. The principles of the method were proposed by Dostál et al. [31] and were implemented to analyze the EET network of Fenna-Matthews-Olson (FMO) complex of green sulfur bacteria by Thyryhaug et al. [24]. In that work, the fitting procedure is conducted to extract the excitonic energy levels, spectral line-shape of each exciton and the transfer-rate matrix based on the 2D electronic spectra. However, spectral features of the FMO complex are well-separated and individual exciton states are identifiable in the 2D spectra. In contrast, LHCII exhibits highly congested 2D electronic spectra; hence, the criterion of good discernibility of spectral peaks [31] is not satisfied and the modelling approach needs to be modified to resolve the EET network.

The parameters of the model are excitonic energy levels, the spectral linewidth of each exciton, the transfer-rate matrix containing information about the EET between excitons, a matrix containing information of the cross-peaks at zero waiting time (for example arising due to exciton coupling) and a phenomenological excited-state absorption (ESA) spectrum. A non-linear least-square fitting routine is implemented to fit the modelled 2D electronic spectra to the experimental data. In this way, we can obtain valuable information about the excitons and energy transfer network in LHCII.

According to the study of Dostál et al. [31], provided the system meets a set of criteria, the corresponding 2D electronic spectra are proven to be fully described by

$$\mathbb{S}_{2D}(\omega_t, T_w, \omega_\tau) = L_3 \text{diag}(\boldsymbol{\mu}^2) \mathbf{F} \exp(\mathbf{K} T_w) \text{diag}(\boldsymbol{\mu}^2) L_1^T, \quad (5.1)$$

where L_1 and L_3 are the matrices containing information about the lineshape along ω_τ and ω_t dimensions, respectively, $\text{diag}(\boldsymbol{\mu}^2)$ is the diagonal matrix with elements equal to the squared excitonic transition dipole moments, $\mathbf{K}$ is the transfer-rate matrix with an explicit form presented later and the matrix $\mathbf{F}$ defines the species-associated spectra in a way that the elements $\mu_i^2 F_{ij}$ characterize the net TA signal detected at $\omega_t = \omega_{0-i}$ caused by the unit population present at the j th exciton. Dostál et al. present the explicit form of matrix $\mathbf{F}$ as [31]

$$\mathbf{F} \propto \mathbb{I} + \mathbf{U} + \mathbf{F}_{ESA}, \quad (5.2)$$

where $\mathbb{I}$ is the identity matrix corresponding to the stimulated emission (SE) signal, $\mathbf{U}$ is a matrix containing all elements equal to one representing ground-state bleach (GSB) and $\mathbf{F}_{ESA}$ is a matrix describing the ESA signal. In this study, due to the small contribution of the ESA compared to the GSB and SE signals, we do not include the $\mathbf{F}_{ESA}$ into the matrix $\mathbf{F}$. We also alter the form of our matrix $\mathbf{F}$ as

$$\mathbf{F} \propto \mathbb{I} + \mathbf{X}, \quad (5.3)$$

where $\mathbb{I}$ is the identity matrix corresponding to the diagonal SE and GSB signals and $\mathbf{X}$ is a matrix with off-diagonal elements only, describing the off-diagonal GSB at zero waiting time.

Using Eqn. (5.1), the previous studies described 2D electronic spectra with the totally uncorrelated lineshape, i.e., the 2D lineshape is factorizable into the linear spectral lineshape matrix L_1 and L_3 [24, 31]. In this work, we remove the lineshape factors L_1 and L_3 in Eqn. (5.1) and we can obtain an expression of the so-called intensity 2D matrix as

$$\mathbf{I}^{2D}(T_w) = \text{diag}(\boldsymbol{\mu}^2) \mathbf{F} \exp(\mathbf{K}T_w) \text{diag}(\boldsymbol{\mu}^2), \quad (5.4)$$

which, for a system of N excitons, is an $N \times N$ matrix describing the intensity of each individual 2D peak in the 2D electronic spectrum at waiting time T_w . The 2D lineshape function $S_{ij}^{2D}(\omega_t, \omega_\tau)$ is described by the bivariate 2D Gaussian distribution controlled by the linewidths of the i th and the j th exciton as

$$S_{ij}^{2D}(\omega_t, \omega_\tau) = \frac{1}{2\pi\sigma_i\sigma_j\sqrt{1-\rho^2}} \times \exp\left(\frac{-1}{2(1-\rho^2)} \left[\frac{(\omega_\tau - \omega_i)^2}{\sigma_i^2} + \frac{(\omega_t - \omega_j)^2}{\sigma_j^2} - \frac{2\rho(\omega_\tau - \omega_i)(\omega_t - \omega_j)}{\sigma_i\sigma_j} \right]\right), \quad (5.5)$$

where $\sigma_i = \text{HWHM}_{inh,i}/\sqrt{2\ln 2}$ is the linewidth of the i th exciton with $\text{HWHM}_{inh,i}$ is the half-width at half-maximum of the inhomogeneous spectral lineshape, ω_i is the excitonic level of the i th exciton and ρ is the correlation degree which is 0 for the cross-peaks and varies between 0 and 1 for the diagonal peaks.

For cross-peaks, we assume that there is no correlation between any excitonic states, the 2D peakshape function becomes

$$S_{ij}^{2D}(\omega_t, \omega_\tau) = \frac{1}{2\pi\sigma_i\sigma_j} \exp\left(\left[\frac{(\omega_\tau - \omega_i)^2}{2\sigma_i^2} + \frac{(\omega_t - \omega_j)^2}{2\sigma_j^2} \right]\right). \quad (5.6)$$

For diagonal peaks, we need to introduce the non-zero value of ρ to describe the

elongated 2D peakshape in the long wavelength region ($\lambda_\tau > 675$ nm). The 2D peakshape function for the diagonal peak of the i th exciton becomes

$$S_{ii}^{2D}(\omega_t, \omega_\tau) = \frac{1}{2\pi\sigma_i^2\sqrt{1-\rho_i^2}} \times \exp\left(\frac{-1}{1-\rho_i^2} \left[\frac{(\omega_\tau - \omega_i)^2 + (\omega_t - \omega_i)^2 - 2\rho_i(\omega_\tau - \omega_i)(\omega_t - \omega_i)}{2\sigma_i^2} \right]\right). \quad (5.7)$$

Due to the non-zero value of ρ_i , the HWHM of homogeneous linewidth is calculated as

$$\text{HWHM}_{hom,i} = \sigma_i \sqrt{2 \ln 2} \sqrt{\frac{1 - \rho_i^2}{2 + 2\rho_i}}. \quad (5.8)$$

With the expression in Eqn. (5.4) and the 2D lineshape functions of the $N \times N$ spectral peaks, the 2D electronic spectra of an N -chromophore system can be calculated and a non-linear least square fitting routine can be constructed to obtain the kinetic information of the system contained in the transfer-rate matrix $\mathbf{K}$.

The fitting routine is constructed as follows. We describe the Chl pigments in LHCII by N excitonic levels and the input parameters of the fit take the form as

$$\begin{aligned} \mathbf{E} &= [E_1 \ E_2 \ \dots \ E_N]^T, \\ \boldsymbol{\mu} &= [\mu_1 \ \mu_2 \ \dots \ \mu_N]^T, \\ \boldsymbol{\sigma} &= [\sigma_1 \ \sigma_2 \ \dots \ \sigma_N]^T, \end{aligned} \quad (5.9)$$

where $\mathbf{E}$, $\boldsymbol{\mu}$ and $\boldsymbol{\sigma}$ are vector arrays with elements E_i , μ_i and σ_i corresponding to the excitonic energy level, the transition dipole moment and the linear linewidth of the i th exciton, respectively.

In addition, the $\mathbf{X}$ matrix representing the relative cross-peak intensities at zero waiting time [Eqn. (5.10)], the phenomenological ESA spectrum $\mathbf{S}_{ESA}^{2D}(\omega_t, \omega_\tau)$ and the transfer-rate matrix $\mathbf{K}$ are also necessary inputs to simulate the 2D electronic spectra.

$$\mathbf{X} = \begin{pmatrix} 0 & X_{12} & \cdots & X_{1N} \\ X_{21} & 0 & \cdots & X_{2N} \\ \vdots & \vdots & \ddots & \vdots \\ X_{N1} & X_{N2} & \cdots & 0 \end{pmatrix}. \quad (5.10)$$

The values of elements X_{ij} of the $\mathbf{X}$ matrix are constrained between -0.5 and 0.5. These cross-peaks originate from the excitonic coupling between pigments creating the off-diagonal GSB signals at zero waiting time. Due to the limited time resolu-

tion of the experiment and the lack of reliable experimental 2D spectrum at $T_w = 0$, cross-peaks arising as a result of very fast exciton relaxation (sub-hundred femtosecond transfers) can also be indistinguishably modelled by $\mathbf{X}$. In such case, the absolute value of X_{ij} elements can be higher than 0.5 but the model is not strictly physically realistic. Therefore, we keep the limits of ± 0.5 for the X_{ij} element and treat it as an indication for fast exciton relaxations that remains underestimated in the model. Furthermore, because the early-time 2D spectra ($T_w < 300$ fs) do not show any significant cross-peaks in the uphill region ($\lambda_t < \lambda_\tau$), we only include the lower-half (downhill) region of the $\mathbf{X}$ matrix into the fit, while setting uphill X_{ij} elements to zero.

The transfer-rate matrix $\mathbf{K}$ containing all information about the EET rates takes the form of Eqn. (5.11), where the rate constant k_{ij} is the EET rate constant of the transfer from the j th exciton to the i th exciton..

$$\mathbf{K} = \begin{pmatrix} -\sum_i k_{i1} & k_{12} & \cdots & k_{1N} \\ k_{21} & -\sum_i k_{i2} & \cdots & k_{2N} \\ \vdots & \vdots & \ddots & \vdots \\ k_{N1} & k_{N2} & \cdots & -\sum_i k_{iN} \end{pmatrix}. \quad (5.11)$$

The ratio between downhill and uphill EET rates is fixed by the Boltzmann factor $\frac{k_{ij}}{k_{ji}} = \exp\left(\frac{E_i - E_j}{k_B T}\right)$, where E_i and E_j are the excitonic energies of the i th and j th excitons, k_B is the Boltzmann constant and T is the absolute temperature. This constraint arises from the detailed-balance condition that ensures that the ratio of forward/reverse (uphill/downhill) rate constants are dictated by the Boltzmann distribution function [32]. The diagonal element of $\mathbf{K}$ is the population relaxation rate of the i th exciton which includes a fixed 2-ns decay k_{ii} to the ground state.

With the input parameters shown in Eqns. (5.9)-(5.11), the intensity 2D matrix $\mathbf{I}^{2D}(T_w)$ can be calculated using Eqn. (5.4). With the properly calculated 2D peak-shapes, the 2D electronic spectrum $\mathbf{S}^{2D}(\omega_t, T_w, \omega_\tau)$ at any population time T_w is simulated as

$$\mathbf{S}^{2D}(\omega_t, T_w, \omega_\tau) = \sum_{i=1}^N \sum_{j=1}^N I_{ij}^{2D}(T_w) S_{ij}^{2D}(\omega_t, \omega_\tau) + \mathbf{S}_{ESA}^{2D}(\omega_t, \omega_\tau), \quad (5.12)$$

where $I_{ij}^{2D}(T_w)$ and $S_{ij}^{2D}(\omega_t, \omega_\tau)$ are elements of the intensity 2D matrix calculated in Eqn. (5.4) and the corresponding 2D peakshape calculated by Eqns. (5.5)-(5.7), respectively. The $\mathbf{S}_{ESA}^{2D}(\omega_t, \omega_\tau)$ term is the phenomenological ESA spectrum calculated from the linear absorption, fluorescence and TA spectra.

The ESA spectrum along the detection axis ω_t is estimated by subtracting the pump-

probe spectrum $\text{Pup}(\omega_t)$ at late waiting time (100 ps) by the fluorescence spectrum $F(\omega_t)$ and the ground-state bleach spectrum $\text{GSB}(\omega_t)$ estimated by a Gaussian function. Along the excitation axis ω_τ , the ESA spectrum is scaled by the excitation spectrum $I_{exc}(\omega_\tau)$ and the linear absorption $A(\omega_\tau)$. The phenomenological ESA spectrum is time-independent and calculated as

$$\mathbf{S}_{ESA}^{2D}(\omega_t, \omega_\tau) \propto [I_{exc}(\omega_\tau)A(\omega_\tau)]^T [\text{Pup}(\omega_t) - 0.5(F(\omega_t) + \text{GSB}(\omega_t))], \quad (5.13)$$

where $\text{Pup}(\omega_t)$ is the pump-probe (TA) spectrum at late waiting time (100 ps), $F(\omega_t)$ is the fluorescence of LHCII at 77 K and $\text{GSB}(\omega_t)$ is a Gaussian centering at 676.5 nm. The subtracted spectrum is then fitted with two Gaussian functions to estimate the shape of the ESA spectrum phenomenologically.

Figure 5.1. The estimation of ESA phenomenologically based on Eqn. (5.13) (circles) and the fit using two Gaussian functions (orange line).

We note that the precise form of $\mathbf{S}_{ESA}^{2D}(\omega_t, \omega_\tau)$ is not very important as the intensity of ESA is very small compared to the GSB and SE signals. Without subtracting the ESA spectrum, all signals will gradually decay to zero and cannot change into positive values. However, some of the experimental traces (for $T_w > 10$ ps) change sign from negative to positive, due to the contribution of the ESA. Therefore, it is justifiable in adding the phenomenological ESA spectrum to make the kinetic traces fit better.

So far, we have not considered finite-bandwidth effects of the excitation pulse spectrum. From the experimental EET timescales resolved from global analysis (0.4, 2.7 and 15 ps as shown in Section 5.3.4), the excitation pulse duration of 50 fs is shorter than the observed dynamics. Therefore, the effect of finite bandwidth in 2D electronic spectra can be incorporated using the approximated expressions derived by Do et al. as

[33]

$$\mathbb{S}^{2D}(\omega_t, T_w, \omega_t) = I_{exc}(\omega_t) \mathbf{S}^{2D}(\omega_t, T_w, \omega_t), \quad (5.14)$$

where the $I_{exc}(\omega_t)$ is the intensity spectrum of the excitation pulse, needed to incorporate finite-pulse effects [33]. We do not need the spectral dependence of the probe pulse along ω_t (detection wavelength) because the finite-pulse effects of the probe are automatically eliminated with phase cycling (similarly, the differential absorbance in conventional TA measurements is independent of the probe intensity, within a range).

We used a non-linear least squares optimization procedure minimizing the χ^2 statistic to fit the model described above to the 2D electronic spectra having $T_w = 100$ fs to 100 ps. A series of fits were conducted to determine the number of excitonic levels that are necessary for a reasonable goodness of fit. Next, to determine the uniqueness of the best-fit solution and to ensure reaching a global χ^2 minimum, the optimization was repeated 1600 times with random initial values of the model parameters. The fitting results were sorted based on the residuals from the best fit (lowest residual) to the worst fit (highest residual) and the best 120 fits are chosen to be further interpreted.

5.3 Results and Discussion

5.3.1 Excitonic states

Figure 5.2. Distributions of the excitonic energy levels obtained from the best 120 fitting results (histograms) overlaid with the experimental linear absorption spectrum of LHCII at 77 K (thick blue line) and the laser excitation spectrum used in the 2DES measurements (grey shaded area). The thickness of the bars in each histogram is proportional to the uncertainty of the corresponding excitonic level.

Fitting the experimental 2D electronic spectra with varying numbers of exciton

Figure 5.3. Inhomogeneous linewidth (half-width at half-maximum – HWHM) distributions of the eight excitonic levels obtained from the best 120 fits in the unit of cm^{-1} .

states evidently results in solutions that greatly differ in the goodness-of-fit, evaluated by the χ^2 statistic, the amplitude of the residuals and the qualitative likeness of the fits and experimental data. We determined that a minimum of eight exciton states was necessary for an adequate description of the data. Increasing the number of excitons did improve the fit, however, at the expense of a sharp increase in the correlations between fit parameters, especially in the transfer-rate matrix, and number of solutions, which makes the results difficult to interpret. Therefore, we restrict the analysis to eight states.

A histogram of the exciton levels of the 120 best fits (lowest χ^2), selected among 1600 eight-exciton fits with random starting parameters, is shown in Fig. 5.2. The relatively low excitation intensity below 660 nm (respectively lower signal-to-noise level in the data) and the short Chl *b* lifetimes preclude accurate modelling of Chl *b* dynamics. Hence, this region was modelled as a single exciton level with a fixed position at 655 nm that serves as representative state for the Chl *b* manifold. The remaining seven exciton levels were free fit parameters. In Fig. 5.2, we can see that excitonic states are well-defined with narrow distributions, especially for the five lowest levels with the centers at 670-671, 673, 675, 677-678 and 680-681 nm. The two higher-energy exciton states at 662-664 nm and 666-668 nm are resolved with relatively lower accuracy.

TABLE 5.1. Comparison of the excitonic levels of LHCII in the unit of cm^{-1} (unshaded columns) and nm (shaded columns) determined in this work with the ones reported by the optical spectroscopic studies [21, 27, 29] and structure-based calculations [16, 17, 34].

This work		Schlau-Cohen et al.	Calhoun et al.	Duan et al.		Novoderezhkin et al.		Renger et al.		Zucchelli et al.	
		15763	634.4	15510	644.7	15416	648.7	15430	648.1	15504	645
		15720	636.2	15480	646.0	15383	650.1	15399	649.4	15408	649
		15688	637.4	15430	648.1	15361	651.0	15340	651.9	15326	652.5
15265	655	15619	640.3	15360	651.0	15237	656.3	15293	653.9	15232	656.5
		15557	642.8	15290	654.0	15193	658.2	15251	655.7	15221	657
		15539	643.5	15210	657.5	15184	658.6	15147	660.2	15152	660
15060-15105	662-664	15367	650.7	15130	660.9	15011	666.2	15009	666.3	15015	666
		15315	653.0	15030	665.3	14985	667.4	14968	668.1	14993	667
14970-15015	666-668	15251	655.7	14990	667.1	14893	671.4	14927	669.9	14948	669
14900-14925	670-671	15247	655.9	14910	670.7	14869	672.5	14919	670.3	14848	673.5
14860	673	15155	659.8	14880	672.0	14791	676.1	14807	675.4	14804	675.5
14815	675	15103	662.1	14810	675.2	14787	676.3	14779	676.6	14771	677
14750-14770	677-678	15049	664.5	14770	677.0	14775	676.8	14769	677.1	14706	680
14685-14705	680-681	14996	666.8	14700	680.3	14667	681.8	14716	679.5	14641	683

In Table 5.1, we present the excitonic levels of LHCII obtained from our proposed model compared with the previous literature values. The reported values are determined by spectroscopic studies, such as the numerical fitting of the Hamiltonian with the 2D electronic spectra by Schlau-Cohen et al. [27], the determination of 14 exciton levels from the quantum beats observed in the non-rephasing 2D spectra by Calhoun et al. [29], and the global analysis fitting and molecular modelling of Duan et al. [21]. Our results are also compared with the ones determined from the structure-based calculations, e.g., the Redfield-Föster picture of Novoderezhkin et al. [16], the ab initio electrostatic quantum calculation of Renger et al. [17], and the analysis of Chl macrocycle deformations by Zucchelli et al. [34].

As shown in the Table 5.1, the lowest five excitonic levels obtained by our model are highly consistent with the determination by Calhoun et al. based on coherence beating with an error margin of $\pm 10 \text{ cm}^{-1}$ [29]. In structure-based calculations of Novoderezhkin et al. [16], there is no exciton absorbing at 673 nm. Instead, there are two excitons located at 670-671 nm and two others at 677-678 nm region. On the other hand, the ab initio calculation of Renger et al. predicts only three absorption levels above 670 nm: *a*610 pigment at 679 nm, *a*602 and *a*613 are degenerate at 675 nm and *a*604 with *a*603 absorb at 673 nm [17].

Figure 5.3 shows the distributions of inhomogeneous linewidths (half-width at half-maximum – HWHM) of the eight fitted excitonic levels. In the low-energy region ($>670 \text{ nm}$), most states have linewidths ranging between 30 and 55 cm^{-1} , except for the lowest state at 681 nm having $\sim 80 \text{ cm}^{-1}$ linewidth. The broadening of the lowest state could be due to several reasons. On one hand, the lineshapes are modelled as Gaussian, which could be inaccurate in the real system – the low-energy tail of the spectrum seems to deviate from a Gaussian. On the other hand, the lowest energy state has quite low absorption cross-section leading to weak signal in 2D electronic spectra, which could be affected by the ESA.

The significantly broader lineshape of the two states below 670 nm ($100\text{-}200 \text{ cm}^{-1}$) is an indication of the existence of additional exciton levels in this region in the real system, which are omitted in the model. Therefore, the spectral and kinetics information at the shorter wavelength region is less detailed and interpretation of the EET pathways obtained by the fit needs to reflect this.

The homogeneous linewidth is an important information that cannot be determined by conventional TA spectroscopy. In our model, the homogeneous linewidths of the exciton states are independent unconstrained fit parameters describing the 2D peakshapes of the respective diagonal peaks. Figure 5.4 presents the distributions of the homogeneous linewidths (HWHM) of the eight excitons. For the six lowest-energy states, the homogeneous linewidth is roughly within $20\text{-}35 \text{ cm}^{-1}$. The 673 nm state is slightly narrower than others ($\sim 16\text{-}22 \text{ cm}^{-1}$). The two higher energy states at 655 nm and 664

Figure 5.4. Homogeneous linewidth (anti-diagonal HWHM of the 2D peakshapes) distributions of the eight excitonic levels obtained from the best 120 fits in the unit of cm^{-1} . The notations are the same as Fig. 5.3

nm are broad due to the high uncertainty in fitted results as discussed above. Compared with the reported homogeneous linewidth (HWHM) of Chl *a* in LHCII of $<1.5 \text{ cm}^{-1}$ obtained by single molecule spectroscopy at 1.8 K [35] and 65 cm^{-1} obtained from 2DES measurement at room temperature [21], we can interpolate to estimate the homogeneous linewidths at 77 K around 18 cm^{-1} , which is in the range of our results. Thus, the modelling approach reliably retrieves the homogeneous linewidths for, at least, the lowest six excitons.

5.3.2 Variation in the fitted kinetic parameters

The dynamics of exciton relaxation is described by the transfer-rate matrix $\mathbf{K}$. To investigate the robustness of the model, we compare the transfer rate constants obtained from the 120 best fits (Fig. 5.5). Because of the large number of fit parameters and limited spectral resolution and dynamic range, the optimization can reach multiple solutions having equivalent goodness-of-fit (within numerical noise). However, the discrete distributions of $\mathbf{K}$ elements show that in practice, only a small number of different solutions exists (within the given eight-exciton parameter space). Moreover, all solutions share common characteristics, for example, the fit parameters (both spectral and dynamic) of the lowest three excitons are very similar. We conducted k-means clustering analysis applying the Lloyd algorithm [36] to group the 120 best-fit $\mathbf{K}$ matrices into the clusters of similar solutions. As a result of the k-means analysis, the rate constants in Fig. 5.5 are grouped into two clusters (indicated by red and blue colors). We note that if we increase the number of excitons into 9 states, the k-means clustering analysis cannot

be performed successfully due to the very high heterogeneity of the obtained solutions. Therefore, within our signal-to-noise ratio, an 8-exciton model is the most reasonable choice.

One cluster, indicated in blue, is highly homogeneous, that is, the rate constants (as well as the rest of the model parameters) have virtually identical values among all solutions. The second cluster (red) also exhibits high degree of overall homogeneity except for some elements of the $\mathbf{K}$ matrix, which vary among solutions. The variability is most pronounced for the 655 nm and 664 nm excitons (the two left columns in Fig. 5.5) and, to some extent for the 668 nm exciton, whereas the differences among the rest of the $\mathbf{K}$ matrix elements are largely inconsequential. Thus, there is ambiguity in the model regarding the high-energy excitons, which can be understood considering the lower excitation intensity in this range combined with the fact that the states are relatively short-lived. Overall, we find that the randomized serial optimization routine returns two distinct clusters of highly homogeneous solutions. Further, we select representative solutions from the red and blue clusters and refer to them as ‘model A’ and ‘model B’, respectively. We next compare and contrast the fitting results from models A and B together with the experimental data with the aim to extract the major dynamics information in LHCII.

5.3.3 Simulated 2D electronic spectra and spectral evolution kinetics

Figure 5.6 compares 2D electronic spectra calculated using model A and the corresponding experimental ones at selected waiting times $T_w = 0.2, 2$ and 100 ps. The fitting quality of model B is very similar, and not presented here. The fit qualitatively reproduced the main spectral features of the experimental spectra. In the experimental 2D spectrum at early waiting time $T_w = 200$ fs, the main spectral features concentrate at the lower energy region ($\lambda_\tau > 670$ nm) and within this region, the simulated spectrum replicates the experimental result very well. The main difference between the simulated and experimental spectra is in the region $\lambda_\tau < 665$ nm. The diagonal feature at 660 nm and the off-diagonal signals at $(\lambda_\tau, \lambda_t) = (660, 676)$ nm are underestimated in the simulated 2D spectrum. This deviation is an indication that the model lacks some states absorbing in this short-wavelength region (below 660 nm), evidently because the weak intensity signals (due to the lower excitation intensity) have less statistical weight.

The 2D spectrum at $T_w = 2$ ps is very well reproduced by the fit. The cross-peak signal at $(\lambda_\tau, \lambda_t) = (660, 676)$ nm is comparable between the fit and the experimental spectra. The elongated diagonal feature at 662-670 nm in the experimental spectrum is still slightly underestimated in the fit, further suggesting that there are one or more additional excitonic states in the real system within this spectral region. The diagonal signal is well discerned after 4-5 ps in agreement with the existence of a long-lived

Figure 5.5. Transfer-rate matrix **K** grid showing the transfer rate constant values of the 120 lowest-residual fits. The vertical axis shows the EET rate values in unit of ps^{-1} . The horizontal direction of each graph shows the increasing of χ^2 . The residual variation within the best 120 fits is negligible ($< 1/1000$ compared to the highest residual fit). Rows and columns in the grid represent the donor and acceptor states, respectively, and the diagonal cells (shaded) show the total decay rates of the corresponding excitonic levels. The values are grouped in two clusters, indicated by the symbol color, based on k-means clustering analysis.

Figure 5.6. Comparison between the simulated 2D electronic spectra obtained from the representative fit of the model A (left) vs. the corresponding experimental ones (right) at waiting times $T_w = 0.2, 2$ and 100 ps. The experimental details are presented in Chapter 4.

‘bottleneck’ state in this wavelength region revealed in numerous earlier studies [16, 17, 27]. Another difference between the fit and the experimental spectra at $T_w = 2$ ps is the absence of off-diagonal feature around $(\lambda_\tau, \lambda_t) = (674, 680)$ nm. This missing feature causes a ‘kink’ in the contour lines of the fitted 2D spectrum which is not visible in the experimental one.

At $T_w = 50$ ps and longer, EET has virtually completed, which is evidenced by the invariant shape of the 2D electronic spectra (not shown). All spectral features are vertically aligned at the emission wavelength of 680 nm and the fit can reproduce this feature very well. The diagonal peaks in the 675-680 nm region are still elongated along the diagonal, which is apparent from the asymmetric spacing of the contour lines around this

Figure 5.7. The representative kinetic traces for selected diagonal peaks (left) and for cross-peaks (right). The legend indicates the coordinates of the peaks as $(\lambda_\tau, \lambda_t)$ nm. The model A and model B fit results are shown as continuous curves and dashed curves, respectively.

region. It is possible that this spectral feature stems from the inhomogeneously broadened lineshape. At 77 K, the protein can be frozen in multiple conformations, resulting in a static energetic disorder, i.e., variation in the absorption frequencies, manifested as diagonally elongated 2D peakshape. A separate possibility is that, at this temperature, some EET pathways are shut down creating disconnected pigment pools wherein excitations relax to the respective pools local energy sink only.

Next, we compare the fitting results of the kinetic traces between the two models. The kinetic traces are more important as they are directly connected to the EET network. The analysis of the EET kinetics is based on a comparison of the model and experimental time courses at different excitation/detection wavelengths. Figure 5.7 presents the fitting results for representative kinetic traces obtained from model A (continuous curves) and model B (dashed curves) for selected diagonal (left panel) and off-diagonal (right panel) wavelengths. The simulated traces capture the main kinetic features, especially the traces of diagonal peaks in the range of 664-674 nm. For the 674 nm diagonal trace, the modelled signal intensity at $T_w < 1$ ps is slightly underestimated by both models. The decay of the long-wavelength (in the range of 674-678 nm) diagonal signals at $T_w > 30$ ps is represented better by model B, whereas model A produces traces that overestimate the decay, with very slow decay lifetimes (>100 ps), which will be discussed later.

The cross-peak dynamics is also reproduced quite well within the experimental noise level, especially between the time of $T_w = 0.5$ -20 ps. At very short times, some cross-peak amplitudes are underestimated in the models (e.g. blue and red traces in Fig. 5.7, right panel). The deviation indicates that the models omit some very fast relaxation processes or cross-peaks at zero waiting times, or both. Additionally, model B deviates from the experimental dynamics at $(\lambda_\tau, \lambda_t) = (673, 675)$ nm, colored in yellow, in the 1-2 ps time range. On the other hand, as mentioned above, model B describes better the

dynamics at $T_w > 30$ ps. Therefore, from the rough comparison, we can recognize the trade-offs in models A and B, which result in better representation of the dynamics at shorter and longer time range, respectively.

5.3.4 Global analysis

A typical method to extract the major dynamics timescales from 2D electronic spectra is by using global analysis. The whole 2DES data set is described by a sum of two-dimensional decay associated spectra (2D DAS) as

$$\mathbb{S}^{2D}(\omega_t, T_w, \omega_\tau) = \sum_{i=1}^n \mathbb{A}_i(\omega_t, \omega_\tau) \exp(-T_w/\tau_i), \quad (5.15)$$

where $\mathbb{A}_i(\omega_t, \omega_\tau)$ is the i th 2D DAS with the corresponding decay lifetime of τ_i and n is the number of lifetimes can be resolved. Within the experimental noise, four decay lifetimes are enough to reasonably fit the data (see Fig. 5.8). In our sign convention, the negative (positive) amplitude in 2D DAS indicates the decay (raise) of GSB and SE signals or the raise (decay) of ESA signal.

From the fitting results of models A and B, we reconstruct the whole set of 2D spectra and subject them into the global analysis as Eqn. (5.15) and the results are shown in Fig. 5.8. Qualitatively, the 2D DAS of model A and model B are quite similar as the experimental ones. The first two lifetimes are also reproduced quite well in both models.

In the first sub-picosecond lifetime, the experimental 2D DAS shows a transfer from 670 nm to 675-677 nm region. In both models A and B, the main energy transfer feature is well replicated. There is additional diagonal decay signal around 655-665 nm which is also virtually resolved in the experimental 2D DAS but not very distinguishable from the noise.

The second experimental 2D DAS exhibits a main diagonal decay around 675 nm and another less pronounce decay extending up to 662 nm with the lifetime of 2.7 ps. The corresponding simulated 2D DAS from model A can replicate all these spectral features nicely, although the obtained lifetime of 4.5 ps is slightly slower. On the other hand, the 4-ps 2D DAS obtained from model B underestimates the diagonal decay feature along 662-668 nm. The off-diagonal rise (positive 2D DAS signal) at $(\lambda_\tau, \lambda_t) = (665-676, 678 \text{ nm})$ is also reproduced better in model A. Additionally, there is a small signature of uphill energy transfer around $\lambda_\tau = 680 \text{ nm}$, which is a negative diagonal peak at 680 nm and a positive cross-peak at $(\lambda_\tau, \lambda_t) = (680, 675 \text{ nm})$. This spectral feature of uphill EET is resolved clearer in the 4.5-ps 2D DAS of model A, while it seems to be underestimated in model B results.

The slowest energy transfer timescale resolved in the experimental 2D DAS is 15

Figure 5.8. Left, middle and right columns show the 2D DAS obtained from global analysis of the experimental, model A and model B 2D electronic spectra, respectively.

ps showing the slow equilibration between the 675-678 nm excitons with the lowest state at 680-681 nm. In this timescale, the model B performs better both spectrally and dynamically. Comparing with the experimental timescale, model B can retrieve a much better result of 12 ps, compared to the 153 ps of model A. In the spectral aspect, beside the main diagonal decay at 675 nm, there is a significant contribution from the higher energy diagonal region (662-668 nm), which is lacking in the 153-ps 2D DAS of the model A, whereas in model B, this feature is replicated nicely. The uphill energy transfer feature is also more pronounced in model B.

The global analysis results reaffirm the point that due to the lesser number of excitons compared to the real system, the fitting results exhibit a trade-off between two models A and B. The faster dynamics (<10 ps) is described better in model A and the slower one is reproduced better by model B.

5.3.5 Exciton state connectivity and dynamics

TABLE 5.2. Fitting result for the transfer-rate matrix **K** obtained from (a) model A and (b) model B. The entries are arranged similarly to Fig. 5.5. The orange and yellow shading cells indicate strong and weak couplings reflected by **X** matrix elements

(a)	655.0	664.0	668.0	670.3	672.6	675.1	677.5	681.0
655.0	-0.43							
664.0		-2.3		0.15				
668.0	0.39		-0.25		0.01	0.01		
670.3		2.1		-3.7		0.28	0.08	
672.6			0.10		-1.03	0.01		0.03
675.1			0.15	2.0	0.03	-0.37	0.03	
677.5				1.5		0.07	-0.11	
681.0	0.04	0.19			0.98			-0.03

(b)	655.0	664.0	666.9	670.4	672.5	675.0	677.1	679.6
655.0	-2.0	0.01						
664.0	0.32	-0.18		0.01				
666.9			-56		5.5			
670.4	0.49	0.16		-0.49		0.06	0.01	
672.5	1.2		56		-8.2	0.37	0.06	0.07
675.0				0.38	1.0	-0.50	0.03	
677.1		0.01		0.10	0.40	0.07	-0.10	
679.6					1.3			-0.07

The kinetics of the system is described by two matrices – the **X** matrix of zero-time cross-peak amplitudes and the transfer-rate matrix **K**. The best-fit values of **K** obtained from model A and model B are presented in Table 5.2 with the shading colors indicating the corresponding elements of the **X** matrix. Due to the limited experimental time resolution, the fast EET processes occurring between strongly coupled pigments on the timescale of tens of femtoseconds [16] are not resolved in the 2DES data. These

TABLE 5.3. Population amplitudes (weighted eigenvectors) table of the transfer-rate matrix **K** obtained from (a) model A and (b) model B. The first row represents the dynamics lifetimes in unit of picosecond calculated from the reciprocal of eigenvalue of the corresponding transfer-rate matrix **K**. The first column is the fit results of excitonic level in nm.

(a)	0.2	0.5	0.9	2.3	3.4	4.4	170	2000
655.0	0.00	0.00	0.00	0.97	0.01	0.02	0.00	0.00
664.0	0.01	1.47	0.00	0.01	-0.03	0.02	0.01	0.01
668.0	0.00	0.01	-0.04	-2.07	0.87	2.64	0.03	0.05
670.3	-0.06	1.68	-0.01	0.12	-0.35	0.31	0.17	0.14
672.6	0.00	0.02	2.41	-0.28	0.03	0.34	-0.13	0.35
675.1	0.04	-1.88	-0.08	1.57	-5.36	5.62	1.19	1.00
677.5	0.02	-1.16	0.02	-0.90	4.92	-7.21	3.40	2.66
681.0	0.00	-0.14	-2.30	0.58	-0.09	-1.74	-4.68	11.10

(b)	0.016	0.4	0.5	1.7	3.3	5.7	13	2000
655.0	0.00	0.00	0.99	0.00	0.00	0.01	0.00	0.00
664.0	0.00	0.00	-0.17	-0.01	-0.02	1.49	0.01	0.01
666.9	0.72	0.06	0.16	-0.01	0.01	0.01	0.00	0.04
670.4	0.00	0.01	-0.26	0.25	0.27	0.91	0.09	0.19
672.5	-0.76	0.59	1.56	-0.06	0.13	0.07	0.03	0.45
675.0	0.01	-0.29	-1.00	-0.38	1.05	1.01	0.33	1.26
677.2	0.00	-0.09	-0.27	0.06	-0.76	-2.69	2.59	3.11
679.6	0.02	-0.29	-1.01	0.15	-0.68	-0.79	-3.04	8.36

processes are partially compensated by the **X** matrix, which essentially provides instantaneous redistribution of the excitation energy between the strongly coupled states. Hence, the transfer-rate matrix **K** alone does not provide a comprehensive picture of EET network and must be analyzed concurrently with the **X** matrix to provide a physically reasonable EET scheme. The decay lifetimes of the system, which are obtained as the reciprocal eigenvalues of the transfer-rate matrix and the corresponding population amplitudes (weighted eigenvectors) are reported in Table 5.3(a) and Table 5.3(b) for models A and B, respectively. Finally, Figure 5.9 shows the simulated population dynamics of the eight excitons after uniform initial excitation.

Strongly coupled exciton states

Inspection of the **X** matrix gives hints about the strongly coupled states, which result in significant cross-peak intensity at early waiting times. It is important to note that the **X** matrix elements are constrained within ± 0.5 . The values of 0.5, represented by the orange shading in Tables 5.2 and 5.3, indicate a strong coupling and relatively fast (< 100 fs) relaxation, between two excitons. Strong coupling is also described by a large transfer rate constant ($> 10 \text{ ps}^{-1}$). Both models A and B contain high-valued off-diagonal **X** elements between 664-667 nm and 670-673 nm and between 667-670 nm and 677-680 nm. In model A, these connections are entirely within the **X** matrix, whereas in model B, there is a fast relaxation between 667 nm and 673 nm. The rapidly relaxing states at 664-667 and 667-670 nm at least partly represent higher exciton states

Figure 5.9. The simulation of population dynamics of eight excitons obtained from model A (left) and model B (right), the initial excitation density is uniform amongst all eight states.

in the strongly coupled Chl *a* domains.

Several other exciton states relax on timescales 0.1-1 ps. In model A, these are at 664 nm (0.5 ps transfer time to 670 nm), 670 nm (0.3 ps transfer to 675-677 nm), and 673 nm (1 ps to 680 nm). In model B, we observe rapid transfer from 673 nm to 675-679 nm. Interestingly, a rate constant of $\sim 1 \text{ ps}^{-1}$ between 673 and 680 nm is consistently determined in all 120 best fits (see Fig. 5.5).

The ‘bottleneck’ intermediate state

Both models A and B predict at least one long-lived state absorbing between 664 nm and 668 nm, which is weakly connected to other Chls and evidently represents the so-called ‘bottleneck’ state, identified in numerous earlier studies [15–17, 27–29]. The total depopulation rate constant is $0.18\text{--}0.25 \text{ ps}^{-1}$. Note that this value is highly consistent among all fits in the respective A and B clusters. The corresponding decay lifetime of the long-lived state is 4.4 ps [Table 5.3(a)] to 5.7 ps [Table 5.3(b)]. A second long-lived state at 670 nm is predicted in model B, which is the main contributor to the 1.7 ps lifetime. In the alternative model A, the 670-nm state decays mainly with a 0.5-ps lifetime. It must be said that both dynamic components can be observed in the experimental 2D DAS (Fig. 5.8), whereas the model is restricted to a single state at 670 nm. Similarly, the long-lived state in both models appears strongly coupled to lower excitons via the **X** matrix, so that 50-60% of the population is instantaneously redistributed. This can be explained if the excitonic level at 664 nm (668 nm in model B) represents two degenerate states, one rapidly and the other slowly relaxing.

Earlier experimental results including the experimental 2D DAS (Fig. 5.8) suggested that the 664-nm state also contributes significantly to the 12-15 ps decay lifetime. However, the present modelling clearly separates the decay of the 664-nm state occurring on a 4-6 ps timescale from the 12-15 ps decay lifetime, which reflects equilibration among the energy sinks (see below).

Energy transfer between the lowest-energy sinks

Figure 5.10. The first, second and third rows show the population evolution of the model A (left column) and model B (right column) after selectively excite the 675, 677-678, and 680-681 nm states, respectively.

All fit results are similar with respect to the lowest three exciton states, 675, 677-678 and 680-681 nm, which act as final energy sinks populated from higher exciton levels mostly within 1 ps (Fig. 5.9) and slowly equilibrate thereafter. A striking result is the lack of significant direct EET among these three sinks. The result is roughly in line with the calculations of Renger et al. [17], who predict rather slow exchanges between the energy sinks. Nevertheless, the excitation energy is slowly equilibrated first via uphill

transfer to higher-energy states, which effectively bridge the different pools so that an equilibrium is established irrespective of the initial excitation. This is demonstrated by the population kinetics with different initial excitations in Fig. 5.10. In this scenario, energetically uphill pathways are critical for spatial EET within the complex and their enhancement at higher, physiologically relevant temperature, will result in overall acceleration of EET, which has been observed experimentally [27]. Bridging pools of low-energy states via uphill transfer to intermediate states is a crucial design principle for photosynthetic complexes, and especially supercomplexes, to ensure successful transfer of excitations irrespective of the photon energy and to avoid excitation trapping.

The 675-nm state equilibrates faster (via 670-673-nm states), on a timescale of 3-6 ps. The energy transfer from the 677-678-nm state is slower and the two fit clusters diverge in the best fit lifetime of 170 ps for model A and 13 ps for model B. However, as it was mentioned earlier, the model A deviates from the experimental kinetics beyond 50 ps, while the 13-ps 2D DAS simulated by model B is qualitatively similar to the experimental 15-ps 2D DAS (Fig. 5.8).

The final state, which is reached in approx. 40 ps in model B (Fig. 5.9), is an equilibrium mainly among the lowest three excitons, with 60% population of the 680-681-nm exciton. This is consistent with the finding that at 77 K there are multiple fluorescence emitting states in LHCII [37–39].

5.3.6 Assignment of exciton states to chlorophylls in LHCII

The phenomenological model described here provides a great deal of insight into the dynamics of EET without requiring substantial a priori knowledge of the system. However, it does not automatically link spectral features with molecular entities (Chls), which is necessary to translate the spectral evolution into the spatial energy transfer. A tentative microscopic model of EET can be proposed (Fig. 5.11) considering the available knowledge of the Chl site energies and exciton couplings, obtained from mutagenesis studies, structure-based quantum modelling and fitting to steady-state and time-resolved spectroscopic data [14–17, 34]. We must stress, however, that the model is only tentative and serves mainly to analyze the experimental dynamics in the framework of detailed structure-based exciton calculations. To create the scheme, we make the following assumptions and considerations:

- The highest exciton states at 655 nm and 664 nm are assigned to Chl *b* and all lower states to Chl *a*.
- The 668-nm state (664-nm in model B) is long-lived, decaying in 4-6 ps via slow branched channels to the main Chl *a* groups. This state can be assigned to Chl *a*604, according to Novoderezhkin [16], but it could also be contributed by a red-shifted Chl *b* [17].

- Exciton states in the model, including the 668-nm state, which are weakly coupled to other states via the transfer-rate matrix **K** (with a total depopulation time of >0.5 ps) but are strongly coupled via off-diagonal **X** matrix elements are considered as representing two degenerate excitons.
- Because of the slow energy exchange between the three lowest exciton states, 675, 677-678 and 680-681-nm states, they are interpreted as each belonging to one of the different Chl *a* exciton domains, encompassing Chl *a*602-603, *a*610-611-612, *a*613-614, and possibly Chl *a*604.
- The lowest-energy state (680-681 nm) and the higher levels strongly coupled to it via the **X** matrix (at 668 nm and 673-678 nm) belong to the Chl *a*610-611-612 group [15–17]. The relaxation from the higher exciton levels is not resolved in the transfer-rate matrix **K**, indicating that it occurs mostly under 100 fs.
- The states at 675 nm and 677-678 nm are then tentatively ascribed to the *a*602-603 and the *a*613-614 groups. The 0.4-0.5 ps decay from 670 nm could be attributed to relaxation within the 670-675-nm exciton dimer.
- The 673-nm level relaxes (1 ps) to the lowest-energy state but is also connected to the 675-nm exciton via **X**. In model B, the relaxation is branched to all lower states. We can interpret this state as the upper level of the 673-678-nm exciton dimer. In this case, by comparing with previous theoretical predictions, it would be reasonable to identify the dimer as the *a*602-603 pair, in which ps relaxation can be expected due to the relatively weak coupling [16, 17, 40]. Alternatively, if the site energy calculation by Renger et al. is considered [17], this state can also be attributed to Chl *a*604.
- Equilibration between the 675-nm and the 677-678-nm pools occurs on a timescale of 2-6 ps. The slowest dynamics involves equilibration between the 677-678-nm and the 680-681-nm pools, occurring on a timescale of 10-15 ps. The latter is consistent with EET between the stromal and luminal pigment groups in Novoderezhkin's model [16].

Without a definitive knowledge of the site energies and exciton couplings of all Chls in LHCII, only speculative assignments can be made. Furthermore, the connectivity between the exciton states is not fully determined by the modelling due to the limited time and spectral resolution of the data. It must be stressed that because of structural heterogeneity (static and dynamic), the real system cannot be fully understood by a one-to-one mapping of absorption bands to individual Chl exciton states, neither can a single matrix of microscopic rate constants fully describe the dynamics. Small conformational changes in LHCII can result in variations in site energies and in exciton couplings that substantially alter the energy landscape and dynamics, which are not resolved in the ensemble-averaged spectroscopy data.

Figure 5.11. EET schemes in trimeric LHCII at 77 K constructed based on fitting results in model A and model B. The red arrows indicate the fast exciton transfer between the strongly coupled domains. The black dash arrows indicate transmembrane EET happening between pigments located on two different membrane sides. Black line arrows indicate the EET happening within the same membrane side. The grey arrows indicate the fast EET happening within Chls *b* manifold or from Chls *b* to Chls *a*. Only the downhill transfers are presented. The colors of excitonic levels indicate strongly coupled domains, except the blue color is used to represent the Chl *b* manifold. The pigment assignments are speculative. See the main text for more details on the interpretations.

5.4 Conclusion

In this study, we construct a phenomenological model describing the EET process in trimeric LHCII at 77 K. We demonstrate that by simultaneously fitting the spectral and kinetic model parameters to the 2DES data along with a robust sampling of the initial parameter space and clustering the fitting results, we can resolve greater details in the spectral composition and the excitation kinetics. In this way, we were able to determine the position of five excitonic levels, at 670-671, 673, 675, 677-678 and 680-681 nm, with very high accuracy. Not only the central wavelengths but also the inhomogeneous and homogeneous linewidths are also consistently determined amongst the five lowest states in the range of 30-55 cm^{-1} and 20-35 cm^{-1} , respectively. The positions and widths for the higher excitonic states are not precisely defined here but can be determined in principle by this approach, provided that higher signal-to-noise data are available in this region. For future studies, where a broadband excitation can be deployed to cover the whole Chl *a* and Chl *b* Q_y bands, the vibronic bands of the Chls should be taken into consideration. In this work, as most of the vibronic bands of Chls *a* are located out of our excitation spectral bandwidth, we have only modelled the lineshape of each exciton level by a Gaussian function.

Within the limits of time and spectral resolution and the signal-to-noise level in the data along with the high dimensionality of the model, it is inevitable that there is no unique solution with respect to the connectivity and dynamics of the system. Clustering analysis classified the fitting results into two groups of alike solutions (A and B). Within one cluster, variations were observed mostly within the highest three exciton states, where signal-to-noise is relatively low and different permutations of kinetic parameters result in similar a goodness-of-fit. Comparatively analysing the two alternative models, we found that model A generally describes better the dynamics at early waiting times and model B performs better for the slower dynamics. Either model omits some details in the kinetics, which is a necessary compromise set by the restriction of eight exciton components. Another limitation of the presently available data is that the dynamics in the first 100 fs and the excitonic states relaxing within this period are not resolved.

Despite these limitations, the phenomenological modelling converges to a reasonable exciton relaxation scheme, which in many aspects agrees with other models constructed by structure-based calculations [16, 17] and time-resolved data [27, 29]. While a long-lived state absorbing in the region 664-668 nm is positively identified by the modelling, its lifetime of 4-6 ps is markedly shorter than earlier predictions. The modelling identifies three energy sinks in LHCII, at 675, 677-678, and 680-681 nm with no significant EET between them. Equilibration between those three Chl *a* pools occurs on timescales from a few ps to tens of ps via upper-lying exciton states and will therefore exhibit pronounced temperature dependence, which is partly the reason for

the faster EET in LHCII observed at room temperature [25]. The final state is a thermal equilibrium among primarily the three sinks, with the lowest state (680-681 nm) having approximately 60% population. Finally, according to the model, this equilibrium is independent of the initial excitation conditions, which is reasonable in the design perspective of the light-harvesting antennas and supercomplexes to ensure the photon-capturing function under different light conditions. However, due to static energy disorder (inhomogeneous broadening), the 2D electronic spectra at 77 K still exhibits a certain degree of correlation between excitation and detection wavelength in the long-wavelength region.

Although the phenomenological modelling is capable of extracting more details from the experimental 2DES data than, e.g., global lifetime analysis, ultimately structure-based quantum modelling together with data fitting is necessary to understand the photophysics of the complex in molecular terms. However, the approach described here can also be applied in the future for different light-harvesting antenna complexes, whose the atomic structural information is not available.

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Chapter 6

Measuring the ultrafast spectral diffusion dynamics of colloidal CdSe nanomaterials

6.1 Introduction

One of the many challenges to the performance of nanomaterial-based applications such as biological imaging [1–5], solid-state lightings [6–8] and lasers [9], is the control of the ensemble emission linewidth [10]. This linewidth is usually assumed to compose of homogeneous and inhomogeneous contributions. The inhomogeneous linewidth is typically associated with the size distribution while the homogeneous condition is dictated by the spectral linewidths due to processes affecting individual particles such as phonon broadening [10, 11].

In the context of the Kubo lineshape theory, we can consider the total lineshape of a material as the ensemble average of the time-dependent transition frequency fluctuation that arises from the environment interactions [12]. In the Kubo lineshape theory, the timescale associated with the homogeneous linewidth is infinitely short as it can be thought of that the individual transition instantaneously fluctuating over a frequency bandwidth. On the other hand, the inhomogeneous linewidth arises from static inhomogeneity and hence is infinitely long in time. This naturally introduces a regime in between these two timescale extremes, which is due to fluctuation on the order of the timescale of the measurement. This is typically known as spectral diffusion. For nanomaterials, the processes happening on the femtosecond to picosecond timescale are very important, as it includes processes like hot exciton relaxation [13] and fast interplane exciton transport in nanoplatelets [14]. These processes affect the ultrafast spectral diffusion dynamics of the materials. Conversely, measuring the spectral diffusion happening on the femtosecond to picosecond timescale allows us an avenue to understand the microscopic details of these important processes.

Two-dimensional optical spectroscopy such as 2DIR spectroscopy and 2DES has been routinely used to measure the femtosecond to nanosecond spectral diffusion dynamics of various molecular systems [15–17]. In this chapter, we report on results of ultrafast spectral diffusion studies of colloidal CdSe quantum dots (QDs) and nanoplatelets (NPLs). One of the key capabilities of 2DES is its ability to distinguish and quantify the homogeneous, inhomogeneous and spectral diffusion contributions to the spectral line-shapes of the measured system [18]. In 2DES measurements, three ultrashort visible light pulses interact with the sample. The interactions generate a third-order polarization in the sample which is then heterodyne-detected by a local oscillator pulse. The generated signal is mapped along the excitation and detection frequency axes, showing

a certain spectral shape and amplitude features. By carefully investigating the amplitude, peak position, lineshape, peakshape and dynamic evolution of the 2D electronic spectra, various information on molecular dynamics and structure can be revealed and determined in detail. Over the years, much effort has been spent on the analysis of the dynamics and peakshapes of the 2D spectra, both qualitatively and quantitatively. The simplest feature to measure is the diagonal width and the antidiagonal width of the peak describing a single transition, which gives details of the inhomogeneous and homogeneous linewidths, respectively [18, 19]. This principle is being used by a recent work by Turner et al., which studied the size dependence of CdSe QDs' homogeneous linewidths. They observe that the homogeneous linewidth decreases as QD radius increases, by characterizing the antidiagonal linewidths of band-edge 2D electronic spectra [20] which represents the $1S_{3/2} - 1S_e$ transition.

Figure 6.1. (Top row) Linear absorption spectra of CdSe QDs (a) and NPLs (b) samples overlaid with the corresponding excitation spectrum used for the 2DES measurements. (Bottom row) The typical 2D electronic spectra of CdSe QDs (c) and NPLs (d) at the waiting time $T_w = 300$ fs. The dashed lines show diagonal lines $\omega_\tau = \omega_t$.

An important quantity that characterizes homogeneity, inhomogeneity and spectral diffusion is the frequency-fluctuation correlation function (FFCF) [18]. A variety of methods can be utilised to retrieve the FFCF from a 2DES experiment. These methods include the central line slope (CLS) [21, 22], ellipticity [23, 24] and eccentricity [25] methods. All the above methods have been shown to be linearly proportional to the FFCF under certain assumptions and limitations. For the ellipticity and the eccentricity measurements, which requires linewidth characterization of diagonal or anti-diagonal cuts through the 2D spectra, the interference from the overlapping spectral features,

such as the overlap of $|0\rangle \rightarrow |1\rangle$ and $|1\rangle \rightarrow |2\rangle$ bands in vibrational systems, may dramatically influence the results [22]. CLS analysis is able to extract FFCF without the need to calculate complicated response functions. The $\text{CLS}_{\omega_\tau}/\text{CLS}_{\omega_t}$ method uses the slope of the line that connects the maximum points of the cuts through the 2D spectra parallel to the pump/probe frequency. It tracks the spectral diffusion dynamics over the measured waiting time window. However, CLS analysis only works well with distinct spectral peaks while it may not be applicable to the overlapping and congested 2D electronic spectra. In addition, the finite bandwidth of the pump pulses would affect the determination of CLS as well [17, 21, 22]. In the case of CdSe NPLs, the 2D spectra exhibit a very well-defined diagonal peak corresponding to the heavy-hole (HH) transition and the excitation spectrum is much broader than the linear absorption linewidth [see Fig. 6.1(b)]. Therefore, the CLS analysis can be applied for the CdSe NPLs sample.

On the other hand, as shown in Fig. 6.1(a), the 2D electronic spectrum of CdSe QDs sample has a broad diagonal peak (feature A) heavily overlapped with a cross-peak (feature B) and an additional positive feature C. Hence, the CLS analysis is not applicable for this case. We employ another related technique to recover information on the spectral features of 2D electronic spectra, which is called the nodal line slope (NLS) analysis [26]. This technique takes advantage of the interference of positive and negative value bands in 2D spectra. These features are very common in colloidal semiconductor QDs ensembles. They typically generate such red-shifted signals with the opposite sign to the conventional negative going bleaching signals in TA and 2D spectra due to the excitation to the biexciton states leading to the intrinsic excited state absorption signals [13, 27]. The NLS method becomes a promising observable for the quantitative determination of contributions from different line-broadening sources in a colloidal QDs ensemble. The NLS value indicates the contributions from different broadening effects, while the dynamics within the observing window can measure spectral diffusion.

In the following section, we will report the use of NLS and CLS analyses to study the spectral diffusion dynamics of colloidal CdSe quantum dots and nanoplatelets, respectively.

6.2 Theoretical details

6.2.1 Frequency fluctuation correlation function (FFCF)

In a solvated system, the chromophores constantly interact with solvent molecules and the absorption/emission frequencies of the chromophores vary with time. Different interactions can happen on different timescales and this leads to the evolution of the 2D peakshape with respect to the delay time T_w , which is known as spectral diffusion. To describe the dynamics of spectral diffusion, the FFCF is implemented in a form of multi-exponential ansatz, introduced by Kubo [12] and revised by Peter Hamm for

applicability in 2D spectroscopy [18]. In the context of Kubo lineshape theory, the FFCF, which is denoted by $C(T_w)$ from now, takes the form of a multi-exponential function

$$C(T_w) = \sum_i \Delta_i^2 \exp\left(-\frac{T_w}{\tau_{c_i}}\right), \quad (6.1)$$

where Δ_i and τ_{c_i} are the i th frequency fluctuation amplitude and correlation lifetime, respectively. Each component can be originated from a different dynamic process. Hence, they can contribute differently in the 2D spectral peakshape in terms of lifetime and amplitude. The FFCF can be seen as a memory function, which measures the probability that the chromophore can retain its original absorption/emission frequency after a certain delay time. As mentioned before, there are two limiting cases according to Kubo lineshape theory. If the product of fluctuation amplitude and correlation lifetime $\Delta_i \tau_{c_i} \ll 1$, the corresponding component is a homogeneous component, in which the chromophore loses memory about the initial transition frequency quickly and results in a Lorentzian lineshape in the linear absorption spectrum. If the mentioned product $\Delta_i \tau_{c_i} \gg 1$, the component is an inhomogeneous component, where the chromophore maintains memory about its initial transition frequency. Consequently, the linear absorption is the distribution function of the absorption frequency of the ensemble. The intermediate regime, where $\Delta_i \tau_{c_i} \approx 1$, is called spectral diffusion regime and the peakshape evolution is observed within the experimental time window. Equation (6.1) contains all information about the 2D spectral peakshape and the parameters can be obtained from the analysis of 2D spectra such as CLS and NLS methods.

6.2.2 Nodal line slope analysis

The NLS analysis is usually applied to the system with overlapping spectral features. As described before, this technique measures the slope of the nodal line, formed by the overlap between two opposite sign spectral peaks, with respect to the vertical axis. In 2DIR spectroscopy, NLS analysis was used to extract the spectral diffusion lifetimes of vibrational systems such as water [23] and cyanide ions in aqueous solution [28]. Recently, Jonas et al. used the nodal line separating positive and negative peaks in 2D spectra to analyze the bandgap inhomogeneity and homogeneity in colloidal PbSe QDs [29]. The results were compared with those measured by advanced transmission electron microscopy (TEM) methods to facilitate the determination of inhomogeneous linewidths. This suggests that the NLS analysis can also be straightforwardly applied to the analysis of CdSe QDs.

The NLS analysis is conducted as follows. Within the vicinity of the nodal region, where two opposite sign signals partially cancel each other and the nodal line is formed,

Figure 6.2. (Top row) Simulated 2D spectra at indicated delay times T_w of a system having overlapping spectral features. The spectral diffusion lifetime is set as 1 ps. The nodal points and nodal lines are plotted as blue circles and green lines, respectively. (Bottom) The NLS vs. T_w obtained from a series of simulated 2D spectra (circles) and exponential fit (orange line) retrieving the diffusion lifetime of 1 ps.

one-dimensional slices, which is parallel to the probe axis ω_t , are taken from the 2D spectrum. Every slice is then fitted using a polynomial function so that accurate nodal points, where the signal is zero, can be determined. Finally, the nodal points are fitted with a linear function resulting in the nodal line, from which the NLS can be obtained.

To demonstrate the ability of NLS analysis, we conduct the calculation of 2D electronic spectra of an ensemble having two opposite sign spectral peaks representing the $|0\rangle \rightarrow |1\rangle$ and $|1\rangle \rightarrow |2\rangle$ absorption bands and they overlap partially to form a nodal line in between. The FFCFs for both transitions have mono-exponential forms as depicted in Eqn. (6.1) with diffusion lifetime of 1 ps. The result is presented in Fig. 6.2. At early delay time, the NLS is very close to 1 and it decays exponentially at later T_w with the time-constant of 1 ps, which agrees well with spectral diffusion lifetime. Therefore, the NLS analysis can be applied to determine the spectral diffusion lifetimes.

6.2.3 Central line slope analysis

CLS analysis is widely used to investigate the spectral diffusion dynamics in 2D spectroscopy with various theoretical studies [21, 22, 30, 31] and experimental implementations [17, 30–34]. The $\text{CLS}\omega_\tau/\text{CLS}\omega_t$ analysis is conducted by taking one-dimensional slices parallel to the pump/probe (ω_τ/ω_t) axis near the maximum of the 2D peak. Each slice is fitted with an analytical function depending on the model to determine the cen-

Figure 6.3. (Top row) Simulated 2D spectra at indicated delay times T_w of a system having well-resolved peak. The spectral diffusion lifetime is set as 1 ps. The central points and central lines are plotted as blue circles and green lines, respectively. (Bottom) The CLS vs. T_w obtained from a series of 2D spectra (circles) and exponential fit (orange line) retrieving the diffusion lifetime of 1 ps.

tral point, where the intensity of that slice is maximized. The central points are then fitted with a linear function resulting in the central line. The CLS is defined as the slope of the central line with respect to either the probe/detection axis in $\text{CLS}\omega_\tau$ method or the pump/excitation axis in $\text{CLS}\omega_t$ technique. In a recent study, Nowakowski et al. reported that the $\text{CLS}\omega_t$ analysis is more robust as the 2D spectra are usually deformed by the excitation (pump) spectrum, hence the slices parallel to the pump axis ω_τ are easily affected by the spectral shape of the pump [17]. Therefore, in this chapter, we only present the $\text{CLS}\omega_t$ method and henceforth, we shall refer to it as the CLS method.

Simulation results to demonstrate the ability of recovering spectral diffusion lifetimes of CLS method are shown in Fig. 6.3. A series of 2D electronic spectra of an ensemble having one well-resolved spectral peak is calculated. The FFCF has the form of Eqn. (6.1) with one diffusion lifetime of 1 ps. At early delay times, the 2D peakshape is highly elongated along the diagonal line $\omega_\tau = \omega_t$ resulting in the very high initial values of CLS. The 2D peakshape gradually becomes more circular at later delay times and consequently, the CLS values decrease. The simulation results show that CLS analysis can successfully recover the 1 ps diffusion lifetime.

In general, the FFCF is not necessary a mono-exponential decay function. The evolution of CLS vs. T_w can be fitted with a multi-exponential function to retrieve all the diffusion lifetimes and the obtained function is called normalized FFCF $\overline{C}(T_w)$

having the form as

$$\overline{C}(T_w) = \sum_i \mathcal{A}_i \exp\left(\frac{T_w}{\tau_{c_i}}\right). \quad (6.2)$$

In $\overline{C}(T_w)$, the sum of normalized amplitude components $\mathcal{A}_i$ is 1, i.e., $\sum_i \mathcal{A}_i = 1$, and the normalized amplitudes $\mathcal{A}_i$ are related to the fluctuation amplitudes Δ_i as $\mathcal{A}_i = \frac{\Delta_i^2}{\sum_i \Delta_i^2}$. Therefore, from the normalized FFCF $\overline{C}(T_w)$ together with the linear absorption lineshape, we can fully determine the fluctuation amplitudes and correlation (diffusion) lifetimes of the CdSe NPLs sample.

6.3 Experimental details

Figure 6.4. (a-b) The transmission electron microscopy (TEM) images of CdSe QDs (a) and CdSe NPLs (b) samples. (c-d) The corresponding size distributions of CdSe QDs (c) and CdSe NPLs (d).

Spherical monodispersed wurtzite CdSe QDs were synthesized and processed according to protocols previously reported by Chakraborty et al. [35], which yields nanoparticles with band-edge absorption at 1.98 eV. The CdSe QDs have an average diameter of 4.9 nm and a size distribution of about 11% determined from the TEM image as shown in Figs. 6.4(a) and (c). In Fig. 6.1(a), the linear absorption spectrum of CdSe QDs overlaid with the excitation pulse spectrum together with the indications of the three lowest excitonic transitions of $1S_{3/2}-1S_e$ (X_1), $2S_{3/2}-1S_e$ (X_2) and $1P_{3/2}-1P_e$ (X_3).

Five mono-layer (5ML) colloidal CdSe NPLs were prepared according to protocols previously reported by Guzelturk et al. [36]. From the TEM image, the 5ML CdSe NPLs have an average lateral area of $497 \pm 87 \text{ nm}^2$ as determined from the fit in Fig. 6.4(d). The 5ML CdSe NPLs have peaks at 2.25 eV and 2.38 eV corresponding to the heavy-hole (HH) and light-hole (LH) transitions as depicted in Fig. 6.1(b). The excitation pulse spectrum is tuned to cover the HH transition centering at 2.25 eV.

The 2DES measurements are carried out on a 2D electronic spectrometer based on a pulse-shaper assisted pump-probe geometry which was described in previous chapters. Briefly, a commercial Ti:sapphire crystal amplified laser system (Legend Elite, Coherence) with an output pulse centered at 800 nm was used to pump a home-built, double-stage optical parametric amplifier and second harmonic generator, to produce pump pulses tunable in the visible regime. The single pump pulse is passed through an acousto-optic programmable pulse shaping unit (Dazzler, Fastlite) to create a double-pulse train with controllable time delay. These pump pulses have a pulse duration of 60 fs with 2 nJ energy per pulse. The probe light is a white light continuum pulse created by focusing a small portion of the 800 nm fundamental pulse onto a sapphire window. The probe pulse is then detected at waiting (or pump-probe delay) time T_w after the pump pulse and the data is collected and processed as described in Chapter 1.

6.4 Results and Discussions

6.4.1 CdSe Quantum dots

Figure 6.5. (a) The 2D electronic spectrum at $T_w = 300$ fs of CdSe QDs. The dashed line is the diagonal. The green solid line depicts the nodal line separating features B and C. (b) The NLS values (circles) vs. T_w from 300 fs to 900 ps overlaid with a fitted line which correspond to a constant value of 0.4 (orange line).

A 2D spectrum at $T_w = 300$ fs is depicted in Fig. 6.5(a). The vertical axis ω_τ represents the excitation energy (pump frequency) while the horizontal axis ω_t shows the emission energy (probe frequency). There is a negative diagonal peak in the 2D spectrum [feature A in Fig. 6.5(a)] arising from the X_1 transition. As viewed along the probe dimension, there is a negative cross-peak [feature B in Fig. 6.5(a)] associated with the X_2 ($2S_{3/2}-1S_e$) transition. Due to broadening effects, especially at room temperature, this cross-peak is not well resolved and appears as a spread from the diagonal peak along the probe axis. Further away from the diagonal peak, the positive cross-peak [feature C in Fig. 6.5(a)] arises from the red-shifted ESA of the X_3 ($1P_{3/2}-1P_e$) transition. Two negative features (A and B) at 1.98 eV and 2.06 eV correspond to the ground-state bleach (GSB) and stimulated emission (SE) signals of the X_1 and X_2 transition, respectively. The positive feature C at about 2.2 eV represents the red-shifted excited-state absorption (ESA) peak of the X_3 transition by the biexciton binding energy. Due to the strong resemblance between the spectral features in 2D and TA spectra, we adopt the assignment for those features from the interpretations in the previous work describing TA spectra of CdSe QDs [37].

We define NLS as the slope of the nodal line, which connects the nodal points of the horizontal slices of the 2D spectrum, with respect to the vertical axis (ω_τ). The positive peaks corresponding to X_1 and X_2 ESA processes were unobserved due to the small biexciton binding energy shift [27]. As a result, the apparent NLS shows up only in the position between the X_2 negative peak and the X_3 positive peak and the NLS analysis was conducted on this line. The solid green line in Fig. 6.5(a) shows the resulting nodal line fit from the experimental data (blue spots) in the vicinity of the pump maximum as an example. In principle, the NLS value varies from 0 to 1. The inhomogeneity, homogeneity and spectral diffusion of the transitions that give rise to features A, B and C determine the specific value of NLS at any given waiting time T_w .

From the experimental data in Fig. 6.5(a) at $T_w = 300$ fs, we measure the NLS value to be 0.4. Figure 6.5(b) depicts the measured NLS value versus the population time T_w . It can be seen that the data points tend to follow a constant value of 0.4 over the observation window of 300 fs to 900 ps. This behaviour shows that there exists negligible spectral diffusion over the time scale of our measurements. This indicates that the line-shape of the CdSe QDs can be adequately described as involving only inhomogeneous and homogeneous contributions. Our observation is consistent with what was observed by Turner and co-workers, where 2DES was performed for colloidal CdSe QDs of sizes ranging from 1.7 nm to 3.0 nm [20]. In the resultant data, the antidiagonal and diagonal width of the peak assigned to the band-edge $1S_{3/2}-1S_e$ (X_1) transition remains constant for $T_w = 75$ fs to 900 ps, thus showing a lack of spectral diffusion over these timescales.

6.4.2 CdSe Nanoplatelets

The 2D electronic spectra of the HH transition of 5ML CdSe NPLs at waiting time $T_w = 150$ fs and 150 ps are presented in Fig. 6.6(a-b). It is clear that there is a T_w dependent variation in the 2D peakshape, with a more elongated shape at shorter T_w to a more rounded shape at longer T_w . This indicates that the HH transition undergoes spectral diffusion over the timescale of the experiment. The fitted CLS from the corresponding 2D spectrum is denoted by the green solid line superimposed onto the plot. The gradient at different values of T_w gives $\overline{C}(T_w)$ [17, 32]. Figure 6.6(c) shows the fitted and the measured $\overline{C}(T_w)$ values from $T_w = 100$ fs to 150 ps. The measured $\overline{C}(T_w)$ can be fitted with three exponential decays (solid line in the bottom graph of Fig. 6.6) with normalized amplitudes $\mathcal{A}_i$ and decay constant τ_{c_i} , $i = 1-3$.

The summary of the CLS fitting results is presented in Table 6.1. Theoretically, $\overline{C}(T_w)$ can bear a value of 1 at $T_w = 0$. In our current data, the normalized amplitudes $\mathcal{A}_i$ add up to 0.90. As our shortest T_w value is 100 fs, it is likely that there is a fast component of < 100 fs that our experiment cannot resolve. We may view this as a homogeneous component with normalized amplitude $\mathcal{A}_0 = 0.10$ as shown in Table 6.1.

Figure 6.6. (a-b) Representative 2D electronic spectra of the heavy-hole transition of CdSe NPLs at waiting time $T_w = 150$ fs (a) and 150 ps (b). The central lines are depicted as the green solid line. (c) The CLS values vs. T_w from 100 fs to 150 ps (circles) overlaid with a fit of a sum of three exponential functions (solid curve).

TABLE 6.1. CLS fitting results of CdSe NPLs.

i	0	1	2	3
$\mathcal{A}_i$	0.10 ± 0.08	0.67 ± 0.07	0.04 ± 0.01	0.190 ± 0.002
τ_{c_i} (ps)	$\ll 0.1$	0.08 ± 0.02	2.5 ± 1.6	ns

From the components of the normalized FFCF $\overline{C}(T_w)$, we can calculate the linear absorption spectrum $A(\omega)$ as

$$A(\omega) = \int_0^{+\infty} dt e^{i(\omega - \omega_0)t} e^{-g(t)}, \quad (6.3)$$

where ω_0 is the center of absorption peak and $g(t)$ is the Kubo lineshape function, obtained from

$$g(t) = \sum_i \Delta_{total}^2 \mathcal{A}_i \tau_{c_i}^2 \left(e^{-t/\tau_{c_i}} + \frac{t}{\tau_{c_i}} - 1 \right), \quad (6.4)$$

and $\Delta_{total}^2 = \sum_i \Delta_i^2$ is the total of squared fluctuation amplitudes of the NPLs. An optimization routine is constructed to fit the lineshape of HH transition from the linear absorption spectrum with the input parameters obtained from $\overline{C}(T_w)$ and the varying parameters are the total of the squared fluctuation amplitudes Δ_{total}^2 and the diffusion lifetime of homogeneous component τ_{c_0} . The fit results are presented in Fig. 6.7. Note that due to the interference of higher absorption states, only the low-energy side of the HH absorption band is considered in the fit. The values of Δ_{total}^2 and τ_{c_0} retrieved from the fit are 23 meV² and 8.5 fs, respectively. From those values, the parameters of FFCF can be fully obtained as shown in Table 6.2.

Figure 6.7. Fit result of the linear lineshape of HH transition from the normalized FFCF $\overline{C}(T_w)$. Only the low-energy side of the HH absorption band is considered in the fit due to the interference of higher absorption states.

TABLE 6.2. FFCF parameters of CdSe NPLs.

i	0	1	2	3
Δ_i (meV)	1.52	3.93	0.96	2.09
Δ_i (THz)	0.367	0.949	0.232	0.505
τ_{c_i}	8.5 fs	76 fs	2.46 ps	ns
$\Delta_i \tau_{c_i}$	0.003	0.07	0.57	$\gg 1$

From Table 6.2, the very fast component of 8.5 fs, which is not directly resolvable from our experimental time window has the product of $\Delta_0 \tau_{c_0} = 0.003 \ll 1$. Hence, it can be assigned as the homogeneous lineshape component. The last component with an amplitude Δ_3 of 2.09 meV can be attributed to the inhomogeneous contribution to the linewidth. In the spectral diffusion regime, we observe two processes happening with different lifetimes. The major component of decay of FFCF is Δ_1 with a timescale of ~ 80 fs. There is a slower and more minor spectral diffusion component (Δ_2) on a

longer timescale of ~ 2.5 ps. Identifying and understanding the microscopic processes that result in the fluctuation in the HH transition frequency, represented by Δ_1 and Δ_2 will be important in a better understanding of the photophysics of CdSe NPLs. Based on the observations that there is no measurable spectral diffusion in the zero-dimensional QDs while spectral diffusion was observed in the two-dimensional NPLs, we may be able to speculate the physical origin of the spectral diffusion observed in the NPLs. The spectral diffusion may have arisen from the excitons moving and sampling different locations on the NPLs plane. In the process, the heterogeneity and unevenness of the NPLs plane cause the fluctuation in transition frequencies of the excitons.

6.5 Conclusion

We performed 2DES measurements on colloidal CdSe QDs and 5ML CdSe NPLs. We measure the ultrafast spectral diffusion dynamics of the $1S_{3/2}-1S_e$ transition of QDs and the heavy-hole transition of the NPLs. In QDs, we observe no spectral diffusion from 300 fs to 900 ps. The conventional analysis of the spectrum of QDs comprising only homogeneous and inhomogeneous contributions is valid. On the other hand, we measure a significant ultrafast spectral diffusion component of ~ 80 fs timescale in the CdSe NPLs. This implies that there is a process of that timescale that is causing the fluctuation of the transition frequency. Identifying this process will be important to achieve a thorough understanding of this type of nanomaterials.

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Chapter 7

Summary and outlooks

7.1 Summary

With the ability to resolve the structure and dynamics of excited-states, ultrafast multidimensional optical spectroscopy (MDOS) has been widely implemented in various studies of physical, chemical and biological systems. Compared to the conventional transient absorption spectroscopy, with higher dimensionality, MDOS is an excellent tool to differentiate various kinetic pathways in complex systems. However, the MDOS spectra usually undergo the deformation caused by the excitation pulses' spectra. Understanding this undesired pulse-shape effect is not always trivial, especially for the higher-order spectra, due to the complicated multi-layer integrals. In this thesis, we presented theoretical studies with the aim of providing simplified expressions describing how the MDOS spectra are shaped by the excitation spectra.

In Chapter 2, by using the time-domain approach, we calculated the third-order 2DOS spectra measured with finite bandwidth pulses. The formalism exactly replicates how the 2DOS spectra are measured experimentally. For the t_2 -independent response functions, which do not exhibit the time evolution of excited population states, we proved that the 2DOS spectrum is the simple multiplication between the 2D spectral response function and the excitation pulse spectra. The obtained formulae are also exact for the quantum beat response functions and exponential decay dynamics, although the expressions are a bit more complicated. In more general cases, where the response function is arbitrarily dependent on population time, the expression is just approximate. However, if the dynamics are slow compared to the pulse duration, the approximation is still very robust [1].

In Chapter 3, we investigated the finite bandwidth effects in fifth-order 2Q-2DOS and 3DOS spectra. The mathematical derivation using time-domain approach to obtain the fifth-order signal becomes tremendous. By switching to the frequency-domain approach, which is also proven to be equivalent with the time-domain one, we could deliver the simplified expressions for 2Q-2DOS and 3DOS spectra under some approximations. For 2Q-2DOS spectra, unfortunately, the exact formula cannot be fully expressed in an analytical form and there are two integrals that need to be numerically calculated. For 3DOS spectra, the obtained results are analogous to the third-order 2DOS spectra. The exact formula for 3DOS spectra including finite bandwidth effects can be obtained for the response functions that do not evolve along two population times t_2 and t_4 . The expression is still a good approximation for the response function varying slowly along t_2 and t_4 . The results of these studies will be important for the nonlinear optical spectroscopy community. As the computational cost rises rapidly with

the increase of the nonlinear order, our simplified expressions provide an easier way to calculate nonlinear optical spectra. In addition, by knowing how the excitation pulse spectra influence the MDOS spectra, with a simple formula, people can eliminate or at least, reduce the undesired effects from the excitation pulse shape [2].

In Chapters 4 and 5, we presented the application of 2DES on studying the excitation energy transfer (EET) dynamics in LHCII – the most abundant light-harvesting antenna on Earth. By performing 2DES measurements on LHCII at various temperatures (77, 110, 150, 230 and 293 K) under annihilation-free conditions, we obtain many insights about the temperature-dependent EET dynamics occurring in Chl *a* manifold. Overall, the EET dynamics slow down when temperature decreases, reflected by the lengthening lifetimes of the 2D decay-associated spectra (DAS). The 2D spectra at low temperature (77, 110 and 150 K) show inhomogeneously broadened lineshapes at the longer wavelength region (> 675 nm). The possible reason can be attributed to the static disorder of the frozen protein environment below the glass transition point [3]. Another possibility is that there are different energy sinks absorbing at this wavelength region and the EET between them are shut down at low temperature. The temperature dependence of uphill/downhill spectral intensity ratio is also confirmed to be in close agreement with the one dictated by Boltzmann distribution [4].

With the aim of extracting more information from the 2DES measurement, we constructed a phenomenological model to fit the 77 K data. Due to the complexity of the biological system and the highly congested 2D spectra even at cryogenic temperature, we have to set some restrictions in our model and hence the consensual EET scheme cannot be determined uniquely. Nevertheless, much valuable information can still be consistently obtained. Within the Chl *a* manifold with the wavelength region > 670 nm, the model can determine five excitonic levels which are in good agreement with the previous theoretical and experimental studies [5–8]. The homogeneous and inhomogeneous linewidths of these excitons are also resolved with the corresponding values of around $20\text{--}35\text{ cm}^{-1}$ and $30\text{--}55\text{ cm}^{-1}$, respectively. The existence of the ‘bottleneck’ state located in the intermediate wavelength region (660–670 nm) is also confirmed by our model, although the lifetime of this state is determined as 4–5 ps, not in the tens of ps timescale as predicted by previous structure-based calculations [5, 6]. Another important finding of the phenomenological model is that there are three terminal energy sinks at 675, 677–678 and 680–681 nm, where the excitation energy is finally distributed regardless of the initial excitation. This is elegant from the design perspective of the light-harvesting antenna as the excitation energy is ensured to be funnelled into the proper spacial locations under different excitation conditions. Consequently, the antenna function can be maintained to ensure the adaptability of plants under different environments.

In Chapter 6, we presented the application of 2DES to study the photophysical prop-

erties of nanomaterials. Due to the quantum confinement, nanomaterials usually exhibit discrete and well-distinct transition states. With the advantage of two-dimensionality, 2DES can extract information about the spectral diffusion from the evolution of the 2D peakshape. CdSe QDs and NPLs are chosen to be the representatives for the zero-dimensional and two-dimensional nanomaterials. The 2D spectra of CdSe QDs show three overlapping features: two negative peaks emitting at X_1 and X_2 transition frequencies, and a positive cross-peak appearing at a red-shifted frequency compared to X_3 transition. Naturally, there is a nodal line arisen from the interference between the negative X_2 and red-shifted positive X_3 peaks. Nodal line slope analysis is employed to track the evolution of 2D peakshape with respect to population time, and a constant slope is obtained. Therefore, in CdSe QDs, there is no significant spectral diffusion occurring within the observed time window (300 fs to 900 ps). For CdSe NPLs, with a very well-resolved distinct diagonal peak in the 2D spectra, corresponding to the HH transition, the central line slope analysis is performed to determine the frequency fluctuation correlation function reflected by the decay of central line slopes with respect to the population time. The CLS analysis reveals four components contributing to the spectral diffusion of the HH lineshape. The homogeneous and inhomogeneous linewidths are determined as 2.3 and 4.4 meV, respectively. Two spectral diffusion components of 15.4 and 0.9 meV happening with the lifetimes of 80 fs and 2.5 ps result in the evolving 2D peakshape within the measured time window (100 fs to 150 ps). By comparing the results from the zero-dimensional CdSe QDs and two-dimensional CdSe NPLs samples, we can speculate that the spectral diffusion happening in the NPLs originated from the exciton movement on the platelets planes and the unevenness of the NPLs thickness introduce the exciton energy fluctuation.

7.2 Outlooks

In this thesis, 2DES has been shown to be an excellent tool with broad applicability to study ultrafast dynamics of various systems from a complicated biological system like LHCII, to artificial nanomaterials like CdSe QDs and NPLs. However, as nothing is perfect and there is always a place for improvement, the presented studies are not exceptions.

The phenomenological model to fit the 2DES data of LHCII can be improved by employing other quantum modelling results. If the Hamiltonian of the whole complex can be determined from the crystal structure together with high-level modelling techniques, we can have a stronger base and much more restricted exciton landscape to construct the EET scheme. The spectral properties of all exciton can be determined with more steady-state observables such as fluorescence, linear and circular dichroism spectra. With the spectral density of the protein environment measured by the inelastic

neutron scattering [9], the exciton-vibrational coupling between the Chls and protein can be taken into account when describing the transfer-rate matrix. As there are many parameters that can vary simultaneously when the temperature changes and the sample solution can undergo phase transition, the temperature-dependent spectral density of the protein and the coupling strength between the protein and Chls at various temperatures are necessary information to investigate the temperature dependence of the EET network in LHCII.

Experimentally, we are limited by our finite bandwidth which can only completely cover the Chl *a*'s Q_y band. Therefore, our 2DES measurements resolve the dynamics of Chl *b* manifold with lower certainty. The limited bandwidth results in the limitation of time resolution. Due to the 100-fs time resolution, we cannot resolve any rapid exciton relaxation between the strongly coupled pigments, which is predicted to be as fast as 20 fs [5, 10]. The ultrabroadband supercontinuum generation using a pressurized noble gas-filled chamber can be the feasible solution. With a reported bandwidth of up to 300 nm and the pulse duration compressed down to 6 fs [11, 12], we will be able to probe the sub-50 fs ultrafast exciton relaxation in LHCII, as well as cover the whole 14 Chls by the broadband excitation.

The CdSe QDs study can be expanded with the further analysis of 2D peakshape to extract more information about the homogeneous and inhomogeneous linewidths. As the excitation pulse spectrum is quite narrow, we excite exclusively the X_1 transition, all the cross-peaks appearing at X_2 and red-shifted X_3 transition frequencies are observed due to the excitonic coupling. Therefore, the 2D peakshape of these cross-peaks should contain the information purely about the cross-correlation function between the X_1 and the X_2/X_3 transitions. A recent study from our group reported the application of 2DES on measuring the frequency fluctuation cross-correlation function between the Q_x and Q_y transitions of Chl *a* [13]. The proposed formalism can be modified and applied to the CdSe QDs sample. By fitting the 2DES data, we can extract more information about the spectral linewidths of this system. For the CdSe NPLs, the results for spectral diffusion can be modelled using some ab initio calculations to provide further details about the mechanism of the excitonic energy fluctuation. In addition, the solvent dependent study can also be performed to provide more information about the spectral diffusion processes. All obtained knowledge is valuable to understand the photophysics of this important system used in various applications in different fields [14–16].

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