Applications of ultrafast multidimensional electronic spectroscopy to the study of plant light harvesting systems and colloidal quantum dots

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Applications of Ultrafast Multidimensional Electronic Spectroscopy to the Study of Plant Light Harvesting Systems and Colloidal Quantum Dots

Zhang Cheng

School of Physical and Mathematical Sciences

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Abstract

This dissertation focuses on the development of ultrafast multidimensional electronic spectroscopy using a pump probe geometry and its application in discerning the energy transfer mechanics and band structure analysis on plant light-harvesting systems and colloidal quantum dots.

In green plants, the first step of light absorbing processes is carried out and implemented by the light-harvesting complex II (LHCII). The trimeric and aggregated LHCII exhibit the unquenched and quenched excitonic states of Chlorophyll (Chl), respectively. Two-dimensional electronic spectroscopy (2DES), which allows direct observation of correlation of excitation and emission energy polls, enables the mapping of the pathways and dynamics from Chl *b* to Chl *a* to give insights into the mechanism of non-photochemical quenching that protects the system away from photodamage. Long-lived intermediate Chl *a* states are present in trimers, while in aggregates, the population decay of these excited states is significantly accelerated, suggesting that, overall, the energy transfer within the LHCII complexes is faster in the aggregated state. In addition, 2DES experiments under conditions free from singlet-singlet annihilation and anisotropic decay are done in the following study. The energy transfer between the different domains within the Chl *a* manifold is investigated and found to proceed on time scales ranging from hundreds of femtoseconds to five picoseconds, before reaching equilibration. The bidirectional (uphill and downhill) energy transfer of the equilibration process between excited states are clearly observed in experiments. Furthermore, exciton equilibration and excitation trapping in intact Photosystem I (PSI) complexes as well as core complexes isolated from *Pisum sativum* are studied.

Due to the flexibly tailored band structure and optical properties of semiconductor nanocrystals, colloidal semiconductor quantum dots (QDs) become the new black in modern industry. Along with the enormous application in sensing, detecting and lasing *etc.*, understanding of the discrete-like band-edge structure caused by the quantum confined effects and eventually manipulating the corresponding optical properties become an key interest by the physicists and chemists in related field. With the above mentioned points in mind, ultrafast transient absorption (TA) measurements are performed on CdSe core type QDs and subsequently a model to explain the features of the band-edge spectra is developed and described. From our fits of the experimental TA spectra, biexcitonic binding energies for the three lowest energy transitions are obtained. Subsequently, room temperature 2DES measurements are performed on the CdSe QDs accompanying with the nodal line slope (NLS) analysis. This 2D lineshape study indicates unique determination of the contributions from different linewidth broadening sources. NLS method is much more sensitive compared with the 1D histogram TEM analysis of the size dispersion inhomogeneity. Adopting the lineshape model from TA spectroscopy and additional simulation on 2DES spectra, quantitative determination of the linewidth information for the involved the band-edge lowest three energy levels X_1 , X_2 and X_3 are available.

For a pulse-shaper assisted pump-probe geometry 2D spectroscopy setup, apart from third order nonlinear optical signals, it is also possible to obtain fifth-order nonlinear optical signals. With appropriate phase-cycling schemes, the fifth-order optical signals can be measured. We report on the development of fifth-order two-quantum two-dimensional electronic spectroscopy (2Q2DES). The implementation of this 2Q2DES allows the detection of double quantum coherence, so that multi-excitonic behaviour can be probed. Chl *a* molecules are used to test the validity of the technique and system. Preliminary results on colloidal CdSe QDs are reported as well to gain insights into biexciton dynamics and behaviour.

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List of Abbreviation

2DES	Two-dimensional Electronic Spectroscopy
2Q2DES	Two-quantum Two-dimensional Electronic Spectroscopy
AOPDF	Acousto-optic Programmable Dispersive Filter
ATP	Adenosine Triphosphate
Chl	Chlorophyll
DAS	Decay-associated Spectra
DSFD	Double-sided-Feynman-Diagram
EET	Excitation Energy Transfer
ESA	Excited-state Absorption
FFCF	Frequency-frequency Correlation Function
FWHM	Full Width at Half Maximum
GSB	Ground State Bleaching
IR	Infrared
LHCI	Light-harvesting Complex I
LHCII	Light-harvesting Complex II
NC	Nanocrystal

NLS	Nodal Line Slope
NPQ	Non-photochemical Quenching
OPA	Optical Parametric Amplifier
PSI	Photosystem I
PSII	Photosystem II
QD	Quantum Dot
RC	Reaction Centre
RMSE	Root-mean-square-error
SE	Stimulated Emission
TA	Transient Absorption

Chapter 1

Introduction

1.1 General overview

In recent decades, the development of a theoretical description of nonlinear spectroscopic measurements by Mukamel [1] has boost interest in experimental development of ultrafast multi-dimensional spectroscopy. Among these, third-order two-dimensional (2D) optical spectroscopy has become a well-established technique to gain insights into the dynamics of molecules [2–5] and mechanism of excited state processes [6–9].

Two prevailing approaches had been applied to accomplish this multidimensional optical spectroscopic signal. One typically method is based on the boxcar geometry approach, where two separate sets of data (rephasing and non-rephasing) are summed to generate the purely absorptive 2D spectra [10–12]. However, slight inaccuracy in determining the relative time delays of these two sets of data will produce distortions to the 2D peak shapes that may then be falsely assigned to non-existing processes [13]. Another way to perform 2D optical spectroscopy is to use a pulse-shaper assisted pump-probe geometry, in which the measured 2D spectra are automatically purely absorptive [14, 15] without having to worry about the inaccurate “phase” problem.

A treatment of turning a conventional pump-probe instrument into a multidimensional ultrafast optical spectroscopy has been described generally by Zanni *et. al.* [16, 17] at infrared region, followed by Ogilvie at visible frequency [15]. This pulse-shaper assisted

method allows easy transformation from an ultrafast transient absorption spectroscopy into a multidimensional optical spectroscopy. The pulse-shaper generates a double-pulse train with accurate control of the relative phases and time delays. By fourier transfer over the first coherence time, the mapping over the pump frequency can be achieved so that correlation of excitation energy and emission energy is accomplished. Our work is built on the basis of such treatment of ultrafast optical spectroscopy - so called two-dimensional electronic spectroscopy (2DES).

In natural photosynthetic light harvesting systems, the ability of exploiting light from the sun with remarkable near unify quantum efficiency has attract much attention in the scientific world. This photosynthetic process is initiated in the light absorbing and energy transfer by the pigment-protein complex antenna in the thylakoid membranes in plant cells [18]. The excitation is then channelled to the reaction center (RC) where it drives photochemistry [19]. All these dynamics happen on ultrafast time scales ranging from tens of femtoseconds to nanoseconds. On the other hand, synthesized semiconductor nanomaterials stand out due to their great flexibility in the chemical and physical properties controlled by the nano-scale size and shape. The application in solar energy conversion [20] *etc.* also attract enormous attention from physicists and chemists. With the above mentioned perspective in mind, by fine tuning the ultrafast 2DES experimental parameters to coincide with the absorption and emission band of studied systems and carefully calibration and analysis of dynamics and peakshape of the 2DES spectra, information on energy transfer and kinetic evolution of plant photosynthetic systems can be obtained. Additional lineshape properties and multi-exciton behaviours of colloidal nanocrystals are studied.

1.2 Scope of this dissertation

The arrangement of this thesis is presented as follows:

Chapter 2 first presents the technical development of the ultrafast multidimensional optical spectroscopy used in our work. A quick overview of the natural photosynthetic complexes and synthesized nanoscale semiconductor materials is introduced in the following section of this chapter.

Chapter 3 applies our third-order 2DES technique to gain insights into the energy transfer and dynamic in the plant photosynthetic systems. Firstly, the trimeric and aggregated light-harvesting complex II (LHCII) are carefully measured and compared to reveal detailed information on mechanics of the non-photochemical quenching (NPQ) in photosynthetic process. Then the evidence of bidirectional equilibrium of Chlorophyll (Chl) *a* in LHCII at physiological temperature are probed and fully reported. Lastly, photosystem I (PSI) in green plants are investigated by comparing the PSI-LHCI super-complex with their core moiety so that hypothesis of equilibration with the “red” Chls states are modeled to gain insights into the energy transfer and charge separation in PSI.

Chapter 4 uses ultrafast transient absorption (TA) spectroscopy - also known as pump-probe spectroscopy to study the lineshape features in colloidal CdSe quantum dots (QDs) beyond the bandedge exciton states. Double-sided-Feynman-daigrams (DSFDs) were commonly used to facilitate understanding different physical processes quantum mechanics. By enumerating DSFDs representing different processes and analysing the weights relative to each other, a model is established to simulate the TA spectrum in good agreement with experimental data and previous literature study.

Chapter 5 extends the lineshape study on CdSe QDs into multidimension. We perform nodal line slope (NLS) analysis on our experimental 2DES. This method provides information on contributions from distinguished line-broadening effects i.e. spectral diffusion, homogeneous broadening and inhomogeneous broadening. Analysed results determines that detailed linewidth values can be achieved from our simulation scheme, and the proposed approach is much more sensitive compared with the 1D histogram transmission electron microscopy (TEM) analysis on the size dispersion inhomogeneity.

Chapter 6 introduce fifth-order 2DES reaching the higher level excitations. Theoretical derivations and experimental implementations are demonstrated. Proper phase-cycling scheme for retrieving desired fifth-order signals is discussed. This chapter introduces the necessary simplified model to understand the fifth-order 2DES signals. Experiments are conducted on Chl *a* molecules and CdSe QDs. Results of Chl *a* sample is used to prove the validity of such low-intensity high-order 2D signal. These results also provide information on the biexciton dynamics on quantum confined semiconductor nanocrystals.

Concluding remarks and works in future direction are listed in **Chapter 7**.

1.3 Major contributions of this dissertation

The major contributions and importance of work presented in this dissertation are listed as follows:

- A robust ultrafast two-dimensional optical spectrometer based on a partially collinear geometry is developed with improved performance. This pulse-shaper assisted pump-probe geometry enables precise and flexible control over the relative phase and time delay within the excitation pulse train, which minimizes the requirement for opto-mechanical modification while turning a pump-probe apparatus into a multidimensional spectroscopic setup.
- Light-harvesting systems including LHCII and PSI are carefully investigated by 2DES. Energy transfer and equilibration process in different domains and manifolds in LHCII are revealed, which give insights into the mechanism of non-photochemical quenching that protects the system away from photodamage. Furthermore, exciton equilibration and excitation trapping in intact PSI complexes as well as core complexes isolated from *Pisum sativum* are studied, which offers remarkable evidence to the “red reaction centre” model.

- A detailed description of absorption spectrum and spectroscopic features of TA spectrum measured with CdSe QDs are well described based on the double-sided Feynman diagrams (DSFDs). Meanwhile, the biexciton binding energy shifts can be determined from the fitting. In addition, 2DES investigation of CdSe QDs is presented in terms of spectral diffusion, inhomogeneous broadening, and homogeneous broadening by analysing the NLS values of 2DES spectra. This NLS method suggests a much higher sensitivity to the size dispersion inhomogeneity.
- Fifth-order 2DES technique are developed based on the partially collinear third-order 2DES. This higher-order multidimensional electronic spectroscopy enables the detection of double quantum coherence, so that multi-excitonic behaviour can be probed. Preliminary results indicate that our collected and post-processed 2Q2DES spectra are real fifth-order signal without cascading. The coupling between one-quantum and two-quantum states can be well visualized in the fifth-order 2D spectra.

1.4 Personal contributions of the author

In order to avoid redundancy of exposition, here I briefly clarify the personal contributions of the work presented in this thesis.

For the ultrafast 2DES study on LHCII trimers and aggregates comparison reported in Section 3.1 of this thesis, the author joined the experiments conducted by Dr. Miriam M. Enriquez and actively participated in modification of the experimental setup and improvement of data acquisition. About the equilibration and dynamics investigation in LHCII and PSI in Chapter 3, all experiments were done by the author with the help of Ms. Parveen Akhtar, who is our close collaborator together with the scientist Dr. Petar H. Lambrev from Hungarian Academy of Sciences in Szeged. Parveen, a PhD candidate as well, was the one who isolated and fabricated the plant photosynthetic complexes with

high quality used in Chapter 3. The simulation models built to clear out the contribution of singlet-singlet annihilation and the equilibration processes in 2DES spectra were built and accomplished by Peter in Matlab.

As to the research on the colloidal CdSe semiconductor QDs in Chapter 4 to 6, all experiments and data collection were designed and conducted by the author. The QDs samples were provided by our collaborators in National University of Singapore. The simulation work to fit and explain the lineshape features in TA spectra in Chapter 4 was mainly done by Thanh Nhut Do on the extension of the DSFDs which were enumerated by the author under the guidance of their supervisor. The NLS analysis in Chapter 5 was improved and modified by the author and all post data processing were carried out as well. Finally, the author developed and implemented the fifth-order two-quantum (2Q) 2DES. Those improvement includes enabling the pump wavelength to tune between different experiments, extending the phase-cycling scheme to retrieve coherent fifth-order 2Q2D signals and developing models to interpret the three-level system and two-state system with experimental results.

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Chapter 2

Technical Preparation and Background Information

In this chapter, necessary concepts, theoretical background and techniques that will be used in this thesis are introduced. Motivations of conducting ultrafast multidimensional spectroscopy on targeted systems will be presented.

2.1 Experiment methods

2.1.1 Ultrafast transient absorption spectroscopy

The motivation of developing ultrafast science is based on the desire in observing ultrafast processes and in various physical, chemical and biological systems. Since the first laser came into being in 1960 [21], with the rapid development of pulse lasers and mode-locking techniques, ultrafast science has experienced picosecond(ps, 10^{-12} s) [22], femtosecond(fs, 10^{-15} s) [23], and proceed to attosecond(as, 10^{-18} s) [24] period. Time-resolved spectroscopy equipped with ultrashort laser pulses enables us to observe ultrafast chemical, atomic and electronic dynamics which can not be captured by the streak camera due to its limited time resolution. Time-solved transient absorption (TA) spectroscopy - so called “pump-probe” spectroscopy - involves excitation on the sample with one pump pulse and probing with another pulse at a variable delay. The change in the transmitted probe light versus the delay time is measured to evaluate the ultrafast processes.

2.1.1.1 Experimental Setup

Figure 2.1: *Schematic representation of ultrafast transient absorption spectroscopy setup.*

A schematic illustration of a pump-probe experiment setup is plotted in Fig. 2.1. The optical pulses centered at 800 nm with 42 fs pulse duration and 820 μ J pulse energy are provided by a Ti:sapphire crystal amplified laser system (Legend Elite, Coherence) with a repetition rate of 1 kHz. A small proportion of this light beam (5%) was focused on a thin Ti:sapphire window (2 mm) to generate white light continuum and hereafter pass through a delay stage, acting as the probe light as well as a local oscillator. On the other hand, the majority of the fundamental 800 nm laser pulse train pumps a home-built, double-stage optical parametric amplifier (OPA) in order to generate a near-infrared output (1200-1400 nm). This near-IR light is subsequently frequency-doubled to 428-500 THz (600-700 nm) by a BBO crystal. This tunable light is then modulated by a commercially available, acoustic-optic programmable pulse shaping unit (Dazzler, Fastlite), to remove the chirp and get the pulse nearly transform-limited. The shaped pump light and the delayed white light are overlapped on the sample unit. Whereafter, a spectrometer (Acton SP2300) assembled with a CCD (PIXIS 100B) is adopted to resolve frequency information from the transmitted signal in the probe direction with a 1 kHz

readout rate. The chopper is synchronized at a rate of 500 Hz to take care of the scattered light induced by the pump light away from the signal. The reference light path is designed to normalize the fluctuation induced by the laser system. The collected TA signal is the absorption difference spectrum

$$\begin{aligned}\Delta A &= -\log \frac{I_{pumped}}{I_{unpumped}} \\ &= -\log \left(\frac{I_{pumped,unchopped}^{sig} - I_{pumped,chopped}^{sig}}{I_{unpumped,unchopped}^{sig} - I_{unpumped,chopped}^{sig}} / \frac{I_{pumped,unchopped}^{ref} - I_{pumped,chopped}^{ref}}{I_{unpumped,unchopped}^{ref} - I_{unpumped,chopped}^{ref}} \right).\end{aligned}\tag{2.1}$$

where $I_{pumped,unchopped}^{sig}$ and $I_{pumped,chopped}^{sig}$ are the signals in the probe light path with and without chopping respectively. $I_{unpumped,unchopped}^{sig}$ and $I_{unpumped,chopped}^{sig}$ are the counterparts in the reference light path. Shutter control of the pump is realized by the pulse-shaper acting as a chopper.

2.1.2 Two-dimensional electronic spectroscopy

Multi-dimensional optical spectroscopy has been used widely in the study of molecule structure and dynamics for the past few decades. It distinguishes itself from other ultrafast spectroscopy by providing one more dimension along the excitation frequency to reveal information of coupling states and molecular structure and dynamics. In 2D spectroscopy, the molecular system interacts with three pulses and the resultant nonlinear polarization gives a nonlinear response, which is then heterodyne-detected by a local oscillator.

Several experimental approaches have been applied to generate and measure this multidimensional ultrafast spectroscopic signal. In the conventional way, a fully non-collinear geometry, also known as the boxcar geometry (in Fig. 2.2), is adopted in nonlinear optical spectroscopy [10–12, 25], which means three pulses interact with the sample from different directions. The targeted rephasing and non-rephasing third-order nonlinear signals emit under different phase matching conditions, so they are collected

separately. The purely absorptive spectrum cannot be directly achieved because it needs the summation of the rephasing and non-rephasing signals. As a result, this box-car geometry approach, which requires precise control of the relative time delay, suffers from the inaccurate “phase” and distortion issue.

Figure 2.2: *Schematic representation of 2D spectroscopy with a boxcar geometry.*

An alternative way to directly obtain pure absorptive spectrum is a partially collinear pump-probe geometry [15, 17, 26–29], that means the first two excitation pulses are collinear while the probe pulse is non-collinear with them (shown in Fig. 2.3). The signal emits in the same direction as the third pulse which at the same time acts as a local oscillator to heterodyne detect the signal [14]. No phasing is therefore necessary and the ambiguity of whether the peak shapes are distorted due to imperfect phasing is not an issue. This partially collinear geometry benefits from the rapid development of pulse shaping techniques in the optical region [30–33], which enables a precise control in the relative phases and delays (t_1) between excitation pulses. Therefore, our existing pulse-shaper assisted pump probe setup can be “easily” developed into a 2D spectroscopy with almost no opto-mechanical modifications. The double-pulse train used for excitation in 2D experimental can be generated with a high quality by a programmable pulse-shaper.

Figure 2.3: *Schematic illustration of 2D spectroscopy with a pump probe geometry.*

In the formalism of the perturbative nonlinear polarization theory and diagrammatic perturbation theory, the collected 2D signal is viewed as the third-order polarization which can be expressed by

$$P^{(3)}(\mathbf{k}, t) = \int \int \int dt_3 dt_2 dt_1 R^{(3)}(t_3, t_2, t_1) E_3(\mathbf{k}, t - t_3) E_2(\mathbf{k}, t - t_3 - t_2) \times E_1(\mathbf{k}, t - t_3 - t_2 - t_1), \quad (2.2)$$

here t_1 is the coherence time between first two excitation pulses; t_2 , known as T_w , is the waiting time between the second pump pulse and the probe, known as T_w in Fig. 2.3. $R^{(3)}(t_3, t_2, t_1)$ is the response function, including all the third-order perturbation terms which contribute to the molecular polarization. The electric field in 2D measurement with a pump-probe geometry can be expressed as

$$\begin{aligned} E(t) = & A_1(t + t_2 + t_1) \exp(-i\omega_L t + i\phi_{21} + i\mathbf{k}_{\text{pump}} r) \\ & + A_2(t + t_2) \exp(-i\omega_L t + i\mathbf{k}_{\text{pump}} r) \\ & + A_3(t) \exp(-i\omega_L t + i\mathbf{k}_{\text{probe}} r) + c.c. \end{aligned} \quad (2.3)$$

where ϕ_{21} is the relative phase between the two pump pulses.

The third-order response includes rephasing and non-rephasing terms under different phase matching conditions. They can be expressed as

$$R_R(t_3, t_2, t_1) = - \left(\frac{i}{\hbar} \right)^3 \mu^4 \Theta(t_3) \Theta(t_2) \Theta(t_1) e^{-i\omega_0(t_3-t_1)} F_R(t_3, t_2, t_1), \quad (2.4)$$

$$R_{NR}(t_3, t_2, t_1) = - \left(\frac{i}{\hbar} \right)^3 \mu^4 \Theta(t_3) \Theta(t_2) \Theta(t_1) e^{-i\omega_0(t_3+t_1)} F_{NR}(t_3, t_2, t_1), \quad (2.5)$$

where R_R and R_{NR} are the rephasing and nonrephasing response functions, respectively. Θ is the Heavyside step function, ω_0 is the resonant frequency of the two-level system, and F is the peak shape function that contains the dephasing, population decay details of the system [1]. In a pump-probe phase matching geometry, the rephasing and the non-rephasing are emitted collinearly and are automatically phased resulting in the desired absorptive spectrum.

In impulsive limit, we assume the pulse are Dirac delta functions. The frequency resolved measurement with a square-law detector can be described as

$$\tilde{S}(\omega_3, t_1) = (R_R^*(\omega_3, -t_1) + R_{NR}(\omega_3, t_1)) I_{pump}(\omega_1) I_{probe}(\omega_3). \quad (2.6)$$

Finally, the purely absorptive 2DES spectrum as the sum of rephasing and nonrephasing signals is heterodyne-detected using a local oscillator pulse and then Fourier transformed over t_1

$$\tilde{S}(\omega_3, \omega_1) = (R_R^*(\omega_3, -\omega_1) + R_{NR}(\omega_3, \omega_1)) I_{pump}(\omega_1 - \omega_L) I_{probe}(\omega_3 - \omega_L). \quad (2.7)$$

2.1.2.1 Phase-cycling scheme for multi-dimensional spectroscopy

As we know from the linear absorption spectrum, the fundamental frequency of a molecular system is usually high, which requires detection with high response speed. Partially rotating frame which means shifting the emitted frequency by a reference frequency were used to reduce the sampling points with larger decay step size [17]. In our experiments,

data collection was performed in a rotating frame with the reference wavelength ω_{ref} . The detuning frequency $\Delta\omega = \omega - \omega_{ref}$ is always set at ~ 50 THz, which determined the Nyquist limit to be ~ 10 fs. Analogues to collinear nuclear magnetic resonance (NMR) spectroscopy [2, 33], 2D spectroscopy with a pump probe geometry is necessary to perform phase-cycling in order to retrieve desired spectrum [11, 14, 34, 35]. The relative time delay (t_1 , sometimes known as τ as well) and phase (ϕ_{21}) between the first two excitation pulses are precisely scanned by the pulse-shaper to perform phase-cycling scheme. The t_1 delay is scanned with an increment of 3 fs over a range of 150 fs, which satisfies the Nyquist sampling limit. And the relative phase is cycled for every t_1 .

What we aim is to directly obtain the purely absorption signal which is a sum of the rephasing and non-rephasing signals. Absorptive spectra are frequently used in multidimensional experiments because they have the highest frequency resolution and lack phase twist which can distort spectra [17]. The total collected signal in the probe direction is a function of ϕ_{21} . Experiments need to be done repeatedly with different phases in order to obtain a specific coherence term from the summation of all order of signals. For $1 \times L$ phase-cycling scheme, the relative phase ϕ_{21} is taken along $0, 2\pi/L, \dots$ till $2(L-1)\pi/L$.

2.1.2.2 Global Analysis

Time-resolved spectroscopy is widely used in the study of photosynthetic systems [36–43]. One of the key focus of the study is the chromophore dynamics and its evolvement affected by the neighbour chromophores and proteins. In terms of the data analysis and interpretation, global analysis is a way that simultaneously analyses all the measurements, which offers an effective evaluation and model of the data.

The global analysis procedure relies on the assumption that the diagonal peaks as well as the cross-peaks in the 2D spectra follow the kinetics of the excited state population in the system, which can be described as a sum of exponential components [44–46]. The time-dependent 2D signal is described as a sum of exponential components:

$$\Delta A(\lambda_1, \lambda_3, T_w) = \sum_{i=1}^n \text{DAS}_i(\lambda_1, \lambda_3) \cdot e^{-\frac{T_w}{\tau_i}}. \quad (2.8)$$

$\Delta A(\lambda_1, \lambda_3, T_w)$ is the difference absorption spectrum at a certain waiting time T_w . The lifetimes τ_i and the pre-exponential amplitudes DAS_i (2D DAS, decay-associated spectra) were determined by non-linear least squares fitting to the experimental 2D decay kinetics, corrected for group-velocity dispersion (chirp) of the probe. The lifetimes as well as the DAS were unconstrained free parameters. The lifetimes were non-linear parameters, whereas the DAS amplitudes were determined by linear least-squares fit at every iteration of the algorithm[44]. The algorithm minimizes the normalized squared sum of residuals.

In a typical 2D DAS spectrum, negative and positive peaks indicate decay and rise of ground state bleaching (GSB) or stimulated emission (SE) signal, respectively. In addition, positive amplitudes may also represent the decay of excited-state absorption (ESA). The resulting lifetimes indicate that a certain process is happening on that specific time range.

2.2 Systems under study

2.2.1 Photosynthetic systems in green plant

Photosynthesis, as the primary source of energy and oxygen for almost all life, is one of the most important biological process on earth occurring in plants, algae and some types of bacteria [47]. In plants, there are four stages happening in the overall processes of photosynthesis to transfer sunlight into chemical energy in and around the thylakoid membranes [48] (Fig. 2.4): 1) light absorption by antenna systems and energy transfer to reaction center (RC) which happens on femtosecond to picosecond scale; 2) electron transfer in reaction centre that induces chemical changes (picosecond to nanosecond scale); 3) Adenosine triphosphate (ATP) synthesis (microsecond to millisecond); 4) Carbon fixation in chloroplast (millisecond to second). From the ultrafast dynamics mentioned above, we foresee that ultrafast TA spectroscopy and multidimensional spectroscopy equipped with fs to ps time resolution apparatus is a promising technique to study the energy transfer

and dynamic of photosynthetic process especially for the chromophore-protein antenna systems and the flow to RC.

Figure 2.4: A model of the photosynthetic membrane of plants [48]. There are four major multi-protein complexes in the thylakoid membranes: Photosystem II (PSII-LHCII), Photosystem I (PSI-LHCI), the cytochrome b_6f ($cytb_6f$) and the ATP synthase.

2.2.1.1 Light-Harvesting Complex II

In plant photosynthetic systems, the key players are photosystem I and II (PSI/PSII) supercomplexes containing numerous proteins and pigments shown in Fig. 2.4. The first step of light absorbing and energy transfer happens in these high density pigments, called light-harvesting complexes antenna systems. In PSII, the corresponding antenna system is LHCII. One unique function of LHCII antenna apart from light-harvesting is that it involved the non-radiative dissipation of excess excitation energy so that it can protect the organisms against photodamage [49]. This process refers to as non-photochemical quenching (NPQ) of Chl fluorescence [50–52]. LHCII complex exists as a trimeric structure with three LHCII monomers. The detailed structure view of LHCII trimer and monomer (shown in Fig. 2.5). Each LHCII monomer contains eight Chl a and six Chl b . The energy immigration and light quenching processes is of key interest on LHCII complex in scientific research.

2.2.1.2 Photosystem I

As mentioned in Section 2.2.1.1, as the main player in photosynthesis, PSI as well as PSII is a multi-subunit supercomplex with plenty of proteins and pigments. PSI has trimeric and monomeric structure according to its living organisms (in cyanobacteria or in higher plant counterparts, respectively). In higher plant, PSI exists as a monomeric complex containing 17 protein subunits and more than 200 cofactors [54]. It has two distinct and loosely associated moieties with a deep cleft between them shown in Fig. 2.6 [54]. The RC core complex and LHCI antenna system locate in these two different moieties. The connection and energy transfer pathways between and within these two parts have triggered the interest in the ultrafast spectroscopy study and we will apply 2DES to the investigations on these matters.

Figure 2.6: *PSI structure from the stroma view* [55].

2.2.2 Colloidal semiconductor quantum dots

Semiconductor nanomaterials are central to both fundamental and applied research and development nowadays due to its unique physical and chemical properties that makes themselves significantly different from its bulk counterparts. The quantum confinement

effects in nanomaterials, especially for nanocrystals (NCs) or quantum dots (QDs) which exerts a profound size and shape dependence allows scientists certain control over the optical and electronic properties [56–58]. The finite structure puts restrictions on the wave vectors allowed to propagate within the energy bands in semiconductors. The size and shape determines a set of discrete states available in each band.

QDs whose particle size is comparable to or even smaller than the Bohr radius have the ultimate confinement in energy levels because of the zero-dimension degree of freedom in quantum structure. The size, shape and surface characteristics affects the optical properties and functional applications such as optical detectors [59], solar energy conversion [60, 61], biological sensing [62] and lasers [63] *etc.*

In particular, as to one of the many applications - solar energy study, the process of multiple exciton generation (MEG) is of interest because it relates to the development of optical gain and boosts the efficiency of photoconversion [61, 64]. Although the single excitation consisting of one bound electron/hole pair has been well studied by time-resolved TA or time-resolved photoluminescence measurements for recent two decades, MEG is still under investigation in early stage [64–66]. Biexciton is the simplest MEG representative, which involves two electron/hole pairs. The confinement effects make the biexciton strongly bounded and correlated with the single exciton than that in bulk semiconductors. The biexciton-ground state coherences generally do not radiate owing to the prohibition of corresponding two-quantum transitions [67]. The traditional one-dimension linear spectroscopy cannot provide clear interpretation due to the crowded peaks with strong overlapping in 1D spectra. As a result, by utilizing our proposed 2Q2DES, high-order coherences, biexciton generation process, its correlation with single exciton can be isolated spectrally using direct detection. In addition, not only the observation of the bound exciton pairs can be achieved, the bound energy strength and dynamics of biexciton state can be also easily resolved from our method. Therefore, this method is particular attractive for MEG studies in QDs and many other researches where process of multiexciton or many-body correlation is non-negligible.

Chapter 3

Energy Transfer and Kinetics in Plant Photosynthetic Systems Probed by Two-Dimensional Electronic Spectroscopy

3.1 Energy transfer dynamics in trimers and aggregates of light-harvesting complex II

In green plants, the major and most abundant antennas are the trimeric light-harvesting complex II (LHCII) located on the periphery of the reaction center of Photosystem II (PSII). In addition to the light-harvesting function, LHCII is involved in the non-radiative dissipation of excess excitation energy, a process collectively referred to as non-photochemical quenching (NPQ) of chlorophyll (Chl) fluorescence [50–52], a key mechanism for protection against photodamage under excess radiation levels. *In vitro*, significant quenching manifested in shortening of the Chl *a* excitation lifetime [68–71] and development of a farred emitting state [72, 73] are observed upon protein aggregation of LHCII. Aggregation of LHCII in solution by means of removal of detergent provides a good *in vitro* model of LHCII in the quenched state. The molecular mechanism behind NPQ has so far not been unequivocally established [74] and there may, in fact, be several mechanisms acting in parallel, such as decay via the S_1 state of lutein [75, 76] and via a

Chl-Chl charge transfer state [72, 73, 77]; other mechanisms have been proposed as well [78–80].

While a great amount of effort has been centered on explaining the precise mechanism of quenching the excess excitation energy of Chls, in-depth analyses on examining the Chl energy transfer process in the quenched state of LHCII are scarce. Femtosecond TA spectroscopy studies on LHCII aggregates previously using Chl *a* excitation have led to proposals of models and dynamics of energy flow to the intermediate quencher state, be it either a carotenoid S_1 state [76] or a Chl-Chl charge transfer state [77]. On the other hand, the presence of Chl-Chl and possibly Chl-Car charge-transfer states has been recently confirmed by Stark spectroscopy [81] and spectral hole-burning [82]. Regardless of the mechanism of dissipation, there is ample evidence that structural changes within the LHCII protein complex activate the quenching, although an agreement does not seem to exist in the literature on this question either. No studies on the energy transfer dynamics and excitonic coupling between Chls *a* and *b* in LHCII aggregates have been reported so far. In this work, we aimed to verify whether and how aggregation alters the pathways and energy transfer dynamics within the complexes when compared to the unquenched, detergent-solubilized state.

Chl energy transfer pathways and dynamics in the unquenched trimeric LHCII have been extensively studied using pump-probe spectroscopy [39, 83–85]. However, in the case of spatially unresolved Chl excitonic states, pump-probe spectroscopy alone cannot directly provide information on which Chl excitonic states are coupled. Fourier transform two-dimensional electronic spectroscopy (2DES) [2, 8, 9, 86] provides a more detailed picture of the precise correlations of coupled Chl excitonic states by studying evolution in the cross peak signal at different time delays. The two frequency axes in a 2D spectrum enable direct correlation of the excitation and emission energies of coupled states, thus revealing excited state population transfer from one state to another. Multiple energy transfer routes and couplings in LHCII have been analyzed using 2DES at low temperature [19, 87]. More recently, 2DES has been used to study the kinetics and pathways of

energy transfer from Chl *b* to Chl *a* in LHCII trimers at physiological temperature, by selectively exciting the entire Chl *b* band [46]. As a follow-up to this study, we applied the same excitation to both LHCII trimers and aggregates and monitored the excitation energy transfer from Chl *b* to Chl *a* by the same 2DES technique. We examine how the change in the environment and possibly conformation of the LHCII complex upon aggregation affects the energy transfer routes and excitonic couplings on the ultrafast time scales. While the general characteristics of Chl energy transfer remain in the aggregated state, specific changes in the dynamic population of intermediate-energy Chl *a* states can be identified.

3.1.1 Experimental details

2DES was carried out using a femtosecond laser system in a partially collinear pump-probe beam geometry setup as previously described [14, 34, 35]. Briefly, a Ti:sapphire regenerative amplifier (Legend Elite, Coherent), seeded by mode-locked pulses from a Ti:sapphire oscillator (Micra, Coherent), produces pulses centered at 800 nm at 1 kHz repetition rate. The output laser pulse, having 0.8 mJ pulse energy and 40 fs pulse duration, is used to pump a two-stage, home-built optical parametric amplifier (OPA). Near infrared signal generated from the OPA is frequency doubled to obtain 650 nm pump pulse with 15 nm bandwidth to provide excitation to the entire Chl *b* Q_y region. The pump excitation spectrum overlaid with the linear absorption spectra of LHCII trimers and aggregates in the Chl *b* Q_y region is illustrated in Fig. 3.1.

The excitation beam is directed to an acousto-optic programmable dispersive filter (AOPDF) pulse-shaper [88, 89] (Dazzler, Fastlite) to generate the first two collinear interaction pulses at varied coherence time delays t_1 and relative phases. Compression by the pulse-shaper yielded pump pulses with 55 fs pulse duration. The first two excitation pulses were attenuated to 15 nJ energy per pulse and scanned from 0 to 150 fs t_1 delay with increments of 3 fs steps. Data collection over t_1 was taken using a partially rotating

frame with reference wavelength set at 730 nm. Background-free 2D signals were acquired using a 1×2 phase-cycling scheme as previously discussed [14, 35]. To investigate for the presence and effect of singlet-singlet annihilation process in the measurements, 2D spectra of the samples were also recorded using laser excitation energies of 10 and 65 nJ per pulse.

Figure 3.1: *Normalized linear absorption spectra in the Chl Q_y region of LHCII trimers in aqueous buffer solution containing 0.03% dodecyl maltoside and of aggregates in detergent-free buffer, overlaid with the spectrum of the excitation pulses used for 2DES measurements. The energy level positions of Chl a (solid lines) and Chl b (dashed lines) exciton states in trimeric LHCII are included in the plot [90].*

The probe pulse is generated from a white light continuum produced by focusing a small fraction of the amplified 800 nm beam on a 2 mm sapphire window. The excitation dynamics is recorded by controlling the time delay of the probe pulse with respect to the second excitation pulse, referred as waiting time t_2 , with a motorized delay stage. The probe beam is spatially overlapped with the pump beam on the sample and sent to a spectrometer (Acton SP2300, Princeton Instruments) equipped with a CCD detector

(Pixis 100B, Princeton Instruments) to spectrally resolve the signal. LHCII samples were recycled through a flow cell of 1 mm optical path length to ensure that the pump beam excites different portions of the sample during data collection. 2D spectra were recorded up to $t_2 = 800$ ps. Linear absorption spectra were taken before and after measurements to check for the sample integrity. Correction for dispersion of the probe light and global fitting analysis of the 2D spectra were carried out in MATLAB using a method described in Ref. [46].

LHCII trimers were isolated and purified by sucrose density gradient ultracentrifugation using PSII-enriched membrane fragments (so called BBY particles) isolated from pea (*Pisum sativum*) as starting material according to Caffarri *et al.* [91]. Aggregates were prepared by removing the detergent from the solubilized LHCII solution with the absorbing beads (BioBeads SM-2, Bio-Rad). LHCII suspension at 30-50 g/ml Chl in 20 mM tricine buffer was incubated at room temperature with 80-90 mg/ml Bio-Beads for at least 2 h with continuous stirring. Sample solutions of LHCII trimers and aggregates were adjusted to an optical density equivalent to 0.2 at the Chl *b* Q_y band (650 nm) in a 1 mm path length.

3.1.2 Results

3.1.2.1 Purely absorptive 2D spectra

2DES provides direct correlations of electronic couplings between pigments within the excitation coherence during time t_1 and the emission coherence during time t_3 . Fourier transformation along t_1 produces the pump excitation frequency axis. Here, the electronic signals from the measurements are plotted in terms of excitation wavelength λ_1 on the vertical axis and detection wavelength λ_3 on the horizontal axis. The purely absorptive 2D spectra of LHCII trimers and aggregates recorded at waiting times t_2 of 300 fs, 3 ps, and 30 ps are presented in Fig. 3.2. The 2D spectra are plotted using identical contour and color intensity scale for convenience of comparison. In the 2D spectra, the peaks

Figure 3.2: *Purely absorptive 2D spectra of LHCII trimers (left column) and aggregates (right column) recorded in the Chl Q_y region at 300 fs, 3 ps, and 30 ps waiting times (t_2). The 2D spectra are plotted in terms of excitation wavelength λ_1 and detection wavelength λ_3 .*

along the white dashed line (diagonal peaks) correspond to the linear absorption position of Chl *b* Q_y band, which is within the spectral range of the excitation pulse, whereas the peaks in the off-diagonal region (cross peaks) result from couplings and energy transfer between Chl excitonic states. Since our first two excitation pulses did not cover the spectral range of the Chl *a* Q_y band (Fig. 3.1), neither diagonal peaks nor quantum coherences [92, 93] due to Chl *a* were observed in the 2D spectra; hence, the range of λ_1 wavelengths above 665 nm is not included in the plots. Although certain overlap exists between the vibronic Q_y band of Chl *a* and the main Q_y absorption band of Chl *b*, the intensity of the vibronic band is much lower; hence, direct excitation of Chl *a* states is negligible.

Photosynthetic light-harvesting systems usually follow a downhill excitation population transfer probed along $t_2 > 0$. The cross peaks in the 2D spectra provide information on the connectivity of excitons from initially excited Chl *b* to Chl *a* states. Due to large spectral overlap of exciton transition bands in the spectrum, we cannot unambiguously assign the peaks corresponding to a single exciton state. However, the cross peak signals provide substantial information on which Chl spectral regions are energetically coupled.

The decrease of the diagonal peak signal along the white dashed line during time evolution is accompanied by an increase of the cross peak signal corresponding to Chl *a* bleaching, peaking at around $\lambda_1 = 680$ nm. The rise of Chl *a* bleaching is indicative of energy transfer taking place between the coupled Chl pigments. At ultrafast timescale of 300 fs in Fig. 3.1, the cross peaks in both LHClI trimers and aggregates are noticeably asymmetrical with respect to the excitation wavelength λ_1 . The asymmetry is due to the higher intensity found at the intersection $\lambda_1 = 655$ nm, $\lambda_3 = 665$ nm. The negative signal at intersections $\lambda_1 \rightarrow \lambda_1$ generally indicates energy flow from initially excited Chl *b* states at wavelength λ_1 to acceptor Chl *a* states at λ_3 . The signal intensity is more pronounced at the cross peak intersection $655 \rightarrow 665$ nm, than at $645 \rightarrow 665$ nm, suggesting that for both trimers and aggregates the deactivation of Chl *b* excited states proceeds through

different pathways. Excitation energy originating from Chl *b* states at $\lambda_1 = 645$ nm directly populates low-lying Chl *a* states around $\lambda_3 = 680$ nm, whereas energy from Chl *b* states at $\lambda_1 = 655$ nm flows through intermediate energy states at $\lambda_3 \geq 660$ nm.

For both LHCII preparations, the Chl *b* diagonal peaks are nearly undetectable in the 2D spectra at delay time of 3 ps, while the intensity of the main Chl *a* cross peak is increased, indicating that excitation energy from Chl *b* has been transferred to Chl *a* over this time period. However, there is still pronounced intensity at the $655 \rightarrow 660$ nm intersection, showing that energy transfer from the 660 nm state to lower energy Chls is markedly slower. Significant differences between the detergent-solubilized trimers and aggregated LHCII were not observed in these 2D spectra. At 30 ps waiting time, the cross peak shape is almost symmetric revealing the (quasi) equilibrium population of Chl *a* states. The signal intensity in LHCII aggregates is obviously lower owing to the faster decay to the ground state in those quenched samples.

3.1.2.2 Global analysis

In order to gain insight on the temporal evolution of the 2D spectra and hence on the kinetics of energy transfer and relaxation process of Chl excitonic states in LHCII, global lifetime analysis of the time series of 2D absorptive spectra [46] was performed in LHCII trimers and aggregates. The global fitting was carried out for 2D spectra recorded from 150 fs to 800 ps. For both samples, four kinetic decay components were required to obtain satisfactory fits to the 2D spectra. An additional component, compared to previous analyses [46], was necessary due to the broader delay time range used here. The results of the global analysis provide characteristic lifetimes of the system kinetics and the 2D decay-associated spectra (2D DAS) describe the correlation between the Chl excitonic states actively participating in the kinetics at a given lifetime. 2D DAS and the respective lifetimes are compared for LHCII trimers and aggregates in Fig. 3.3. The 2D DAS reveal characteristic spectral profiles of intermediate states that are not readily identified just by

Figure 3.3: 2D decay-associated spectra (2D DAS) of LHCII trimers (a-d) and aggregates (e-h) resulting from global lifetime analysis of a series of 2D spectra at t_2 from 150 to 800 fs with four exponential decay components. The decay lifetimes are indicated on the plots.

inspecting the 2D spectra alone. The absorptive 2D signal is dominated by the negative ground-state bleaching and consequently the 2D DAS amplitudes show dynamic changes of the bleaching signal. Negative 2D DAS amplitude, represented by red color, thus indicates decay of the bleaching signal, whereas positive 2D DAS amplitude, coded in blue color, indicates that the amplitude of the bleaching grows with the given lifetime.

The first 2D DAS of trimers (Fig. 3.3a) and aggregates (Fig. 3.3e), corresponding to lifetimes of 270 and 240 fs, respectively, are very similar. Excitation energy transfer between strongly coupled Chl *b* and Chl *a* excitonic states is evident on this time scale. The negative ($\lambda_3 = 640 \rightarrow 665$ nm) and positive ($\lambda_3 = 670 \rightarrow 690$ nm) signals correspond to the decay of Chl *b* and rise of Chl *a* bleaching signal, respectively. The second 2D DAS (Figs. 3.3b and f) reflect slower energy transfer pathways between Chls, occurring on a time scale of ~ 2.8 ps. In contrast to the first 2D DAS (Figs. 3.3a and e), the slow energy transfer from Chl *b* to Chl *a* is mediated by intermediate energy Chls which are evident by the appearance of negative cross peak intensity around $\lambda_3 = 660 \rightarrow 675$ nm. In solubilized trimers, the intermediate intensity is found between $\lambda_3 = 660$ and 665 nm and strongly coupled to lower-energy Chl *b* absorbing at $\lambda_1 = 654 \rightarrow 656$ nm. In aggregates, however, additional negative amplitude can be observed in the range $\lambda_3 = 665 \rightarrow 675$ nm, testifying that lower-energy Chl *a* exciton states relax on this time scale in aggregates. Comparing the amplitude of the negative diagonal peak signal of Chl *b* in the first and second 2D DAS, majority of the excitation energy from Chl *b* is transferred to Chl *a* at the more rapid time scale of 240-270 fs. Fig. 3.4 below will provide more details of the results of the second 2D DAS.

The third 2D DAS of trimers (Fig. 3.3c) and aggregates (Fig. 3.3g) decays around 14-15 ps and is characterized by a broad negative cross peak at the detection wavelength of 665-690 nm, signifying decay of the Chl *a* Q_y bleaching signal. The cross peak in trimers (Fig. 3.3c) is noticeably broader on the short-wavelength side, in the region of 655 $\rightarrow$ 668 nm intersection, indicating the presence of long-lived intermediate exciton

Figure 3.4: *Horizontal slices at selected excitation wavelengths λ_1 of the second 2D DAS component (2.8 ps) of LHCII trimers (a) and aggregates (b) from Figs. 3.3b and f, respectively.*

states. In contrast, the aggregate 2D DAS (Fig. 3.3g) does not have sizeable amplitude in the region of 655 $\rightarrow$ 668 nm, since the population of the corresponding energy levels decays on a faster time scale, as evidenced by the 2.8 ps 2D DAS.

The 14-15 ps 2D DAS component can be attributed to a combination of energy equilibration of a portion of excited state population from weakly connected Chls to the lowest Chl excited state, and singlet-singlet annihilation, occurring when two migrating excited states collide within their lifetime [39, 94, 95]. The third 2D DAS component has significantly larger amplitude in aggregates. This is most likely because in aggregated LHCII excited states can jump over adjacent complexes migrating over a much larger domain size [95, 96], thus increasing the likelihood for annihilation. For LHCII aggregates, the effective domain size is about 20 $\times$ larger than the trimers, hence the much greater 15 ps contribution due to the singlet-singlet annihilation.

The final 2D DAS reflects the slow decay of the lowest energy Chl a exciton states to the ground state. Accordingly, in trimers the decay lifetime was found around 3 ns (Fig. 3.3d), whereas in the quenched aggregates it was reduced to $\sim$ 170 ps (Fig. 3.3h). Thus

the final lifetimes are consistent with the bulk of experimental data on aggregated and trimeric LHCII [68, 69, 71, 72, 76, 77].

It is evident from the 2D DAS that, apart from the well-known shortening of the overall excited-state lifetime, LHCII aggregates are characterized by a change in excited-state dynamics of Chl *a* occurring on the picosecond time scale, evidenced by a change in the shape of the 2.8 ps 2D DAS. The spectral shape of the 2.8 ps 2D DAS is illustrated in Fig. 3.4 in the form of a set of slices at different λ_1 and compared with solubilized trimers. The intermediate negative peak for trimers is positioned near $\lambda_3 = 665$ nm and is only distinctly pronounced for $\lambda_1 > 654$ nm. In aggregates, on the other hand, the spectra show a strong negative peak at $\lambda_3 = 670$ nm which is correlated with $\lambda_1 = 650$ -656 nm and a weaker positive amplitude at 680 nm, representing the rise of lower-energy Chl *a* state population.

3.1.2.3 2D spectra obtained with high power excitation pulses

In order to evaluate the contribution of singlet-singlet annihilation to the kinetics of excited-state decay, 2D spectra of aggregates and trimers were also measured using lower and higher excitation pulse energies (10 nJ and 65 nJ). The probability for multiple excitation within the pulse duration grows with the pulse energy and so does the probability for annihilation. The same global analysis procedure with four kinetic components was applied to the 2D spectra. While lower energies yielded qualitatively similar 2D DAS (Appendix A.1), there were notable differences at 65 nJ (Fig. A.1). Differences in the spectral profiles are evident in the longer lifetime components, particularly the second 2D DAS (Figs. A.1b and f). At high pulse energies, the 2D DAS for both trimers and aggregates reveals decay in a broad range of wavelengths between 660 and 680 nm, whereas the 2D DAS for low-power measurements (Figs. 3.3b and f) showed a clear rise in the Chl *a* excited-state population. Another apparent difference is that the relative amplitude of the 21-22 ps 2D DAS at high power (Figs. A.1c and g) is significantly higher, compared

Figure 3.5: 2D decay-associated spectra of LHCII trimers (a-d) and aggregates (e-h) resulting from global analysis of the 2D spectra obtained using excitation pulses of 65 nJ energy.

to the relative amplitude of the 14-15 ps 2D DAS at low power (Figs. 3.3c and g). Even in solubilized trimers, this is the predominant pathway for excitation energy decay at high pulse energies. It can be concluded that singlet-singlet annihilation is one reason for the observed excited state decay on the time scales of 2-3 ps and especially 15-20 ps in the high power excitation measurements.

3.1.3 Discussion

In the present work we investigated the energy transfer pathways and dynamics in LHCII trimers and aggregates using 2DES. Rather than focusing on the lowest-lying energy states responsible for quenching, we directed our attention to the overall energy transfer dynamics starting from excited Chl *b*. Since Chl *b* molecules interact with neoxanthin on the luminal side of LHCII, and neoxanthin is known to undergo conformational changes upon aggregation [97], it is reasonable to expect that energy transfer from Chl *b* will be also affected, especially since aggregates display marked changes in their circular dichroism spectra in the Chl *b* region [98–100]. However, our 2DES results did not reveal any gross changes in the time scale of energy transfer or Chl *b* - Chl *a* couplings in aggregated compared to solubilized LHCII, which both display similar spectral evolution on the time scale of 0.2-0.3 ps (compare the 2D DAS in Figs. 3.3a and e). Furthermore, the intermediate energy 2D cross-peak (Fig. 3.3b), reported earlier for trimers [46], is also observed in aggregates (Fig. 3.3f), similarly found at $\lambda_3 = 660\text{-}665$ nm and coupled to low energy Chl *b* states at $\lambda_1 = 654\text{-}656$ nm.

3.1.3.1 Two long-lived intermediate energy states

In our experiments, an even longer-lived Chl *a* intermediate state was resolved, absorbing at around 670-675 nm, which equilibrates with the lowest-energy exciton states on the time scale of 15 ps (the value is probably not exact since this lifetime is primarily dominated by singlet-singlet annihilation). Exciton energy transfer models based on Redfield / modified Redfield theory predict generally faster equilibration times [83] but slow energy

transfer between Chl *a* states, on the time scale of tens of picoseconds, have been estimated by Renger *et al.* [101] applying generalized Förster theory. Based on the comparative global analysis of the 2D spectra of trimers and aggregates, we propose that the long-lived intermediate state at 670-675 nm decays faster in aggregates, on time scale of ~ 3 ps, as opposed to 15 ps in trimers. This conclusion is mainly based on the pronounced negative amplitude in the 2.8 ps 2D DAS of aggregates at the intersection $650 \rightarrow 670$ nm (Figs. 3.3f and 3.4b) and, respectively, the lack of the respective cross-peak amplitude in the 15 ps 2D DAS (Fig. 3.3g). It must be noted that this intermediate state (670-675 nm) has a different identity than the intermediate state at 660-670 nm (Fig. 3.3b, discussed also in earlier works on LHCII [46, 87, 102]). The long-lived state is not only distinguished by its longer lifetime (in trimers) and lower energy but also because it has stronger coupling to Chl *b* states absorbing at ~ 650 nm than at ~ 655 nm. The latter characteristic is the reason why this state is readily resolved by 2DES.

The 2D DAS show that the intermediate state population decays but do not necessarily reveal the fate of the excitation energy. There can be several reasons for the accelerated decay in aggregated LHCII. The excitations could decay by ultrafast internal conversion to the ground state, in which case this intermediate state would be an effective quencher, or, more likely, by relaxation to another Chl *a* exciton state, carotenoid molecule, or a mixed (charge-transfer) state. Another possibility that needs to be considered is that the apparent decay is due to singlet-singlet annihilation in the aggregates.

3.1.3.2 Contribution of singlet-singlet annihilation to the excited-state decay

Previous time-resolved studies on LHCII aggregates [77, 103] have demonstrated that annihilation is mostly relevant on a time scale of tens to hundreds of picoseconds but due to its non-exponential kinetics, it may influence the shorter-lived lifetime components as well [77]. The 2DES measurements performed at high excitation energy (65 nJ) clearly demonstrate that singlet-singlet annihilation has significant impact on the kinetics even

on a time scale of 2-3 ps. Rather than rising, the population of low-energy Chl *a* states decays with a 2-3 ps lifetime in aggregates and, to a lesser extent, trimers. It is thus a viable hypothesis that the enhanced 3 ps decay found in aggregates at 15 nJ pulse energy is also simply due to inter-trimer exciton migration and annihilation. To evaluate this hypothesis we compare the 2DES results obtained at lower energy excitation of 10 nJ (Appendix A.1) from the same samples and with the same experimental conditions (other than pulse energy) as the 65 nJ results. The results, having lifetimes and 2D DAS features very similar to those obtained at 15 nJ, are useful for quantitative comparison of the contribution of singlet-singlet annihilation to the kinetics. Consequently, we obtained that under the condition of low power measurement, there is no contribution of annihilation on the trimeric sample and only minimal contribution to the corresponding 2D DAS of aggregates.

3.1.3.3 On the identity of the intermediate states

Based on the results presented here, we can only speculate about the possible causes for the accelerated decay of the intermediate Chl *a* state. Mostly because of its fast lifetime, it can be argued that the underlying process is not related to quenching by a specific molecular species, such as *e.g.* a zeaxanthin or other carotenoid with low-lying energy level, but is exciton or exciton-charge-transfer state equilibration in which the donor is an intermediate-energy (~ 670 - 675 nm) Chl *a* exciton state. Recent structure-based calculations of site energies of Chl molecules in LHCII by Müh and Renger [104] predicted red-shift of site energies of specific Chl *a* molecules upon aggregation of solubilized trimers. A 3 nm red-shift of site energies was observed for Chls *a*603 and *a*610 which absorb at 676 nm and 679 nm, respectively, in the aggregated state. A 1-2 nm red-shift in energy is also observed for Chls *a*611, *a*612 and *a*613 with site energy positions between 670 to 676 nm. The additional Chl intermediate states observed at 665-675 nm in the 2.8 ps 2D DAS of aggregates (Fig. 3.3f) may be due to the effective lowering of site

energies of specific Chl *a* pigments in the aggregated state. These Chl *a* pigments are within the vicinity of the two lutein pigments which may have undergone reorientations upon protein aggregation thereby inducing environmental changes which affect the Chl site energies [104].

3.1.4 Conclusion

In summary, in this work we have uncovered a long-lived intermediate state exciton state in LHCII trimers, which becomes strongly coupled to lower-lying states and respectively short-lived upon aggregation. While this change does not necessarily have a major influence on the overall excited-state dynamics, it does signify that the change in the environment of LHCII can affect not only the dynamics of the lowest-lying energy states but also the pathways and dynamics of energy transfer between higher intermediate states. Our case study also demonstrates that minor changes in the energy-transfer dynamics of a highly complex system can potentially be detected by room-temperature 2DES, which would be of great importance, for instance, in engineering photoactive pigment-protein complexes for performing specialized functions or improving their efficiency.

3.2 Chlorophyll *a* excited-state equilibration in LHCII

The excitation energy transfer (EET) dynamics in LHCII has been studied extensively over the past decades using various time-resolved spectroscopic techniques and modelling approaches [105]. Yet, despite the wealth of literature data, there is no complete consensus on the dynamics and pathways of EET, particularly in the equilibration process in the manifold of Chl *a* exciton states. Upon photoexcitation, due to the narrow spread in energy of the manifold of Chl *a* exciton states, significant uphill and downhill EET will occur concurrently, leading eventually to a dynamic thermal equilibrium. A wide range of lifetimes for this equilibration process have been reported for LHCII monomers and trimers. At temperatures of 77 K or lower, excitation on the blue side of the Q_y band of

Chl *a* is followed by downhill EET with time constants from hundreds of femtoseconds to about 20 ps [37, 38, 84, 106]. At physiological temperatures, 5-7 ps lifetime components assigned to Chl *a* exciton equilibration have been observed by transient absorption [36, 39, 94], fluorescence [40] and three-pulse photon echo [41, 107]. However, significantly longer lifetimes, in the range of tens of picoseconds and more, have also been reported [36, 108, 109]. The slowest lifetimes have been assigned to one Chl *a* pigment absorbing in the region of 665-670 nm [38, 87]. This has been referred to as the bottleneck state and, in agreement with structure-based modelling, assigned to Chl *a*604 [83, 87] in the crystal structure of Liu *et al.* [53]. The assignment has been challenged by more recent modelling [110] that incidentally also predicts slow Chl *a* - Chl *a* equilibration lifetimes in the tens of picoseconds region [101]. Thus, at present neither the timescales nor the molecular pathways of exciton equilibration in the Chl *a* domain are unambiguously established.

Two-dimensional coherent electronic spectroscopy (2DES) has advantages over conventional pump-probe by providing separate spectral information of donor and acceptor molecules participating in EET [42, 111]. 2DES has been used to examine kinetics and pathways of EET from Chl *b* to Chl *a* in isolated, detergent-solubilized LHCII at low temperature [43, 87] and recently at physiological temperature [46, 49, 112, 113]. In order to clearly observe such dynamics, purely absorptive 2D spectra need to be obtained and analyzed without compromising the spectral resolution and precision, time range, or dynamic range (signal-to-noise ratio).

In this section, 2DES experiments are performed aiming to resolve the room-temperature dynamics of exciton equilibration in the Chl *a* manifold in LHCII trimers, with excitation pulses centered at 675 nm, covering the Chl *a* absorption band. To avoid singlet-singlet annihilation, we used excitation pulse energies low enough to virtually eliminate the possibility for multiple simultaneous excitations in the complexes. In addition, the first two excitation pulses were plane-polarized at the magic angle relative to the third interaction

pulses to measure only the population dynamics of the system. The data are also of sufficiently high dynamic range to perform global multi-component kinetic analysis to obtain quantitative values for the energy equilibration dynamics.

3.2.1 Experimental method

Figure 3.6: Normalized linear absorption spectrum in the Chl Q_y region of LHCII trimers in aqueous buffer solution containing 0.03% dodecyl maltoside overlaid with the spectrum of the excitation pulses used for 2DES measurements.

Fourier-transform femtosecond 2DES was performed in a partially collinear pump-probe geometry [14, 35]. The first two interaction pulses (excitation pulses) were tuned to a central wavelength of 675 nm with 18 nm bandwidth, matching the Q_y absorption band of Chl a in LHCII (Fig. 3.6), 45 fs temporal width and energy of 0.5-0.8 nJ per pulse. An acousto-optic programmable dispersive filter pulse-shaper [89] (Dazzler, Fastlite) was used to scan the coherence time delay t_1 from 0 to 150 fs with 3 fs increments in a 1×2 phase-cycling scheme. White-light supercontinuum generated in a 2 mm sapphire window was used as the third interaction pulse (probe). The probe light was plane-polarized at the magic angle (54.7°) with respect to the polarization of the excitation pulses. The

time delay between the second excitation pulse and the probe pulse (population waiting time T_w) was varied from -0.3 ps to 600 ps in a logarithmic progression.

Trimeric LHCII samples were isolated according to the procedures given in Caffarri *et al.* [91]. For 2DES, LHCII samples were diluted in degassed buffer solution containing 20 mM Tricine (pH 7.8) and 0.03% β -DM to obtain absorption of 0.33 at the excitation wavelength (675 nm), in a 1 mm optical cell. In this regime, due to propagation effects, there will be slight broadening of the 2D peakshapes along the detection wavelength [114]. During the 2DES experiments the sample was passed through a flow cell and continuously refreshed.

3.2.2 Results and discussion

Representative 2D spectra for selected waiting times T_w are plotted in Fig. 3.7. A spectrum at coordinates (λ_1, λ_3) reflects the conditional differential absorption detected at wavelength λ_3 after excitation at λ_1 . The negative signal at detection wavelengths above 660 nm is contributed mainly by ground state bleaching (GSB) and stimulated emission (SE) from the first singlet excited state of Chl *a*. The appearance of cross peaks in the 2D spectra mainly reflects redistribution of excitation energy (EET) during the waiting time T_w . Because of preferentially downhill EET, the signal gradually red shifts. If the system of coupled chromophores has reached a thermally equilibrated state, the signal becomes independent of excitation wavelength (Fig. 3.7f). Thus, we can conclude that, firstly, part of the excitations from higher-energy Chl *a* are transferred to lower-energy states within 150 fs (Fig. 3.7a) and, secondly, by noticing that there is no change between 2D spectra at $T_w = 12.5$ and $T_w = 32$ ps (Figs. 3.7c, d, respectively), equilibration among all Chl *as* is complete after about 10 ps.

At physiological temperatures, the energy gaps between several exciton states in LHCII are within the thermal energy (the Boltzmann term kT spans about 10 nm in this region); for the equilibration process, both forward (downhill) and reverse (uphill)

Figure 3.7: Purely absorptive 2D spectra of LHCII trimers recorded in the Chl Q_y region with excitation pulses at 675 nm. (a-d) - contour plots of the 2D spectra recorded at the indicated waiting times (T_w), plotted in terms of excitation wavelength λ_1 and detection wavelength λ_3 . Contour lines mark areas of equal absorptive signal intensity given in the color scale bars. (e,f) - horizontal slices at excitation wavelengths of 665, 675 and 685 nm extracted from the 2D spectra at waiting times 0.1 ps (b) and 30 ps (d). The slices are normalized at their negative maxima for easier comparison.

EET are expected to occur [107, 113]. This is clearly seen in Fig. 3.8, which shows the T_w dependence of the 2D signal amplitude at the high-energy (665 nm) and low-energy side (685 nm) of the Q_y absorption band. In Fig. 3.8a (Fig. 3.8b) we see the spectrum peak gradually red shifts (blue shifts) with increasing T_w due to downhill (uphill) EET. The peak position in both Figs. 3.8a and b converges to 679 nm by ~ 10 ps.

Figure 3.8: *Contour plots showing the waiting-time dependence of the LHCII 2D signal at two fixed excitation wavelengths $\lambda_1 = 665$ nm (a) and 685 nm (b). The plots can be understood as analogous to pump-probe experiments with pump wavelengths, corresponding to λ_1 - i.e. on the blue and red side of the Q_y absorption band of LHCII.*

Furthermore, following the overall signal amplitude as the spectral evolution progresses (Fig. 3.8), it can also be concluded that low-energy Chl *a* states (at detection wavelengths near 680 nm) have larger dipole strength, i.e. they are superradiant. This is likely because the states are delocalized over excitonically coupled pigments with favorably oriented transition dipole moments [115]. The observation is in agreement with the nonlinear polarized fluorescence study of Schubert *et al.* [116] who estimated that the emitting state in LHCII is superradiant, having two-fold larger dipole strength compared to monomeric Chl.

We performed global analysis on the 2D spectra at T_w from 100 fs to 300 ps to obtain a quantitative estimation of the spectral equilibration kinetics. Three 2D decay-associated

spectra (2D DAS) with lifetimes 0.54 ps, 4.7 ps, and 3.2 ns were necessary and sufficient to obtain a good fit (Fig. 3.9). The errors in the first two lifetimes did not exceed 30% in repeated experiments, but were larger (up to 160%) for the final lifetime as it is beyond the measurement range.

Negative and positive amplitudes indicate decay and rise of GSB/SE signal, respectively. Positive amplitudes can also represent decay of excited-state absorption (ESA). EET is identified as a negative diagonal peak ($\lambda_1 = \lambda_3$) at the absorption wavelength of the donor spectral form and a positive peak at λ_1 matching the donor and λ_3 matching the acceptor. For reversible EET in the equilibration dynamics, two antisymmetric positive/negative pairs are observed in the 2D DAS. The downhill (uphill) EET produces a cross-peak above (below) the diagonal, at $\lambda_3 > \lambda_1$ ($\lambda_3 < \lambda_1$). In a recent paper Duan *et al.* [113] have reported such above-/below-diagonal 2D DAS cross-peaks, which they attributed to EET between Chl *a* and *b* occurring on a timescale of 1.1 ps. We note that, although the forward and reverse EET rates may be different, depending on the energy gap and the size of the pools, the observable spectral changes always occur with the same equilibration rate (timescale). This relationship makes the 2D DAS a powerful means to identify EET pathways. Here, antisymmetrically positioned positive/negative peaks are evident in the first two 2D DAS (Fig. 3.9); the latter one (4.7 ps) shows reversible EET between 667 nm and 680 nm spectral forms. The shape of this 2D DAS is reminiscent of the 1.1 ps 2D DAS reported by Duan *et al.* [113]. The amplitude ratio (~ 0.36) of the 667 $\rightarrow$ 680 nm and the 680 $\rightarrow$ 667 nm cross-peaks, after adjustment for the excitation pulse spectrum, reflects the forward/reverse rate constant ratio [113]. Thus, we have clearly resolved and characterized the bidirectional thermodynamic equilibration between Chl *a* excited states coupled by reversible EET. Whereas the 4.7 ps 2D DAS appears to involve a single pathway between two Chl pools, the 0.54 ps component features more than one EET pathway with a major contribution from spectral forms lying in the middle of the Chl *a* region (672 $\rightarrow$ 675 nm). This faster equilibration component was not observed by

Figure 3.9: 2D decay-associated spectra (2D DAS) of LHCII trimers, resulting from a three-exponential fit of the transient 2D signals from 150 fs to 64 ps with best-fit lifetimes of 0.54 ps, 4.7 ps, and 3.2 ns. (a,c,d) contour plots of the 2D DAS (amplitude in the color scale bars); (b,e,f) horizontal slices of the corresponding 2D DAS at three excitation wavelengths, normalized at their negative maxima.

Duan *et al.* [113]. The final 2D DAS (3.2 ns) does not describe any EET - the spectral shape is the same at any excitation wavelength and features a main negative GSB/SE peak and a small valued positive band at $\lambda_3 < 660$ nm attributed to ESA. The DAS represents decay of the completely equilibrated state and is consistent with the documented excited-state lifetime of LHCII [69, 96].

While the measured lifetimes for the equilibration dynamics (0.54 ps and 4.7 ps) are in good agreement with numerous previous studies, we do not observe any slower spectral equilibration processes. We contend that lifetimes in the range of 10-20 ps observed previously [36, 49, 108, 109] are contributed by singlet-singlet annihilation, which is often evident because of the non-conservative shape of the DAS, indicating excitation decay rather than EET, and has been acknowledged in earlier works as discussed by Connelly *et al.* [39]. Crucially, if we normalized the 2D spectra to the total integrated intensity at every delay time, thus eliminating the decay, global analysis resulted in only two components with lifetimes and 2D DAS very similar to the annihilation-free data (Figs. 3.9c and e). With increasing excitation rate, progressively faster annihilation kinetics would distort the exciton equilibration components [49].

To validate the interpretation of the 2D DAS and reveal the spectral identities of the EET species, simulated 2D spectra with a phenomenological model consisting of four exciton states, or pools, connected by reversible (Boltzmann-factored) EET, were presented in Appendix A.2.

3.2.3 Conclusion

We can conclude that spectral equilibration in LHCII is kinetically limited by EET occurring on a 5 ps timescale between two Chl *a* pools. The participating spectral forms (667 nm, 680 nm) can be unambiguously resolved in the 2D spectra, especially owing to the capability of 2DES to follow simultaneously energetically uphill and downhill EET. It is reasonable to assume, in accordance with other works, that the longer lifetime represents

EET from the luminal side of the complex to the lower-energy Chls on the stromal side (Chl *a* 613/614 to 610/611/612 [53]). Faster EET processes on sub-ps timescale probably represent equilibration within the luminal and stromal Chl *a* pools. We estimate that the slow equilibration time has up to 40% contribution to the kinetics, whereas 25-35% of excitations on upper exciton states are transferred within 150 fs and 25-35% are transferred on the timescale of 500 fs (see Appendix A.2). The slower components in the range of 20 ps, detectable by singlet-singlet annihilation, may reflect additional spatial equilibration between same-energy exciton states on different monomers in the trimer (possibly Chl *a* 602/603 [85, 117]), in agreement with numerous earlier works (reviewed in ref. [118]). Thus, the 2DES results presented here, while consistent with earlier data, separate the kinetics of spectral equilibration from inter-monomer transfer and, combined with new structure-based modelling, enable a more accurate and detailed understanding of the light-harvesting dynamics.

3.3 Excitation transfer and trapping kinetics in plant Photosystem I

Photosystem I (PSI) of green plants encompasses more than 200 pigments and cofactors, bound in a 650 kDa pigment-protein complex consisting of up to 18 protein subunits, performing a photocatalytic reaction with a quantum efficiency of nearly unity [54]. It possesses two physically distinct structural moieties. The core complex, which is strongly conserved among all oxygen-evolving photosynthetic organisms, binds 98 Chl *a*, including the reaction centre (RC) Chls, 22 β -carotene molecules and all carriers of the electron-transport chain between the external electron donor and acceptor, plastocyanin (or cytochrome) and ferredoxin [119, 120]. The second moiety is the peripheral antenna, light-harvesting complex I (LHCI), consisting of four Chl *a/b*-binding proteins, Lhca1-4.

A distinct feature of PSI of almost all organisms is the presence of long-wavelength Chl spectral forms - “red” Chls - that have lower energy levels than the RC Chl P700

[121]. To utilize the energy absorbed by the “red” Chls, excitations must be transferred energetically uphill to the RC [122]. Despite that uphill excitation energy transfer (EET) slows down the overall trapping, the quantum efficiency of PSI remains very high [123]. Depending on species, the PSI core complex of cyanobacteria contains several such “red” Chls. However, in plants they are located primarily, or exclusively in the peripheral antenna, LHCI, showing broad absorption and emission bands with maxima around 715 and 735 nm, respectively [124, 125].

The processes of EET and trapping in PSI of plants, photosynthetic bacteria and algae are extensively studied by various time-resolved spectroscopy techniques, including fluorescence and femtosecond transient absorption. Briefly, spectral equilibration in the core antenna has been found to occur on a timescale from 100-150 fs to several ps. Several studies have resolved spectral changes with lifetimes in the range of 2-6 ps in cyanobacteria [123, 126–129], ascribed to EET between the bulk Chls in the core and “red” Chls. Overall trapping in the core complex occurs by one major phase around 20 ps but the lifetime depends on the amount and energies of the “red” Chls [123]. In the PSICLHCI complex of green plants, trapping shows an additional phase between 50 and 130 ps [122, 130–132].

The complexity of the PSI antenna, the large number of pigments and spectral overlap makes it difficult to unambiguously interpret the spectroscopy data and distinguish between different kinetic models. Two-dimensional electronic spectroscopy (2DES) has distinct advantages in following the kinetics of EET. 2DES is a third-order nonlinear optical spectroscopic technique that measures a frequency-frequency correlation map linking the electronic excited state frequencies of coupled transitions. We have recently shown that exciton equilibration can be explicitly resolved in the excited-state kinetics of light-harvesting complex II by room-temperature 2DES [133], reducing the uncertainty in ascribing kinetic lifetimes to EET processes (see in Section 3.2 as well). Previously, 2DES was performed on PSI complex from cyanobacteria demonstrating the dynamics of

fast EET [134] on the timescale up to several hundred femtoseconds. However, no 2DES studies have been reported on PSI from eukaryotic organisms and only very limited femtosecond spectroscopy data overall are available for higher plant PSI.

With this in mind, we performed room-temperature 2DES and time-resolved fluorescence studies of isolated PSI core complexes and intact PSI-LHCI complexes from *Pisum sativum* (pea) to extract information on the dynamics of EET and charge separation in plant PSI from the femtosecond to nanosecond timescales. Analysis of the 2DES results confirmed, with high degree of accuracy, lifetimes and spectral features of PSI. We were able to unequivocally resolve uphill and downhill spectral equilibration components in the core complex on timescale of 0.5-4 ps and slower (tens of ps) EET associated with LHCI. This would further gain insight into the interpretation of kinetic models.

3.3.1 Experimental details

Figure 3.10: *Normalized linear absorption spectrum of PSI core and PSI-LHCI complexes overlaid with the spectrum of the excitation pulses used for 2DES measurements.*

Fourier-transform femtosecond 2DES was carried out in a partially collinear “pump-probe” geometry using an experimental laser system described earlier [14] with the follo-

wing modifications. The first two interaction pulses (excitation pulses) were tuned to a central wavelength of 680 nm with 18 nm bandwidth, matching the Q_y absorption band of Chl *a* in PSI (Fig. 3.10, 45 fs temporal width and energy of 0.5-0.8 nJ per pulse. An acousto-optic programmable dispersive filter pulse-shaper (Dazzler, Fastlite) was used to scan the coherence time delay t_1 from 0 to 150 fs with 3 fs increments in a 1×2 phase-cycling scheme. White-light supercontinuum generated in a 2 mm sapphire window was used as the third interaction pulse (probe) and local oscillator. The probe beam is spatially overlapped with the pump beam on the sample and sent to a spectrometer (Acton SP2300, Princeton Instruments) equipped with a CCD detector (Pixis 100B, Princeton Instruments) to spectrally resolve the signal. The probe light was plane-polarized at the magic angle (54.7°) with respect to the polarization of the excitation pulses. The time delay between the second excitation pulse and the probe pulse (population waiting time T_w) was varied from -0.3 to 600 ps in a logarithmic progression.

Starting material was PSI-enriched stromal membrane vesicles isolated from pea leaves according to the protocols of Peters *et al.* [135]. Both samples PSI-LHCI and PSI core used for 2D experiments exhibited 0.3 optical density at the Chl *a* Q_y band (680 nm) in a 1 mm path length and contained 20 mM sodium ascorbate and 60 μ M phenazine methosulfate to ensure rapid recovery of the RC. The samples were continuously circulated during the measurement to avoid the encounter of a complex with the same laser pulse twice. The absorption was recorded before and after the experiment to control the sample integrity.

3.3.2 Results and discussion

The 2DES experiments were performed with excitation pulses centred at 680 nm that spectrally overlap the Q_y absorption region of Chl *a* (Fig. 3.10) to reveal the dynamics of excitation energy redistribution in PSI-LHCI and PSI core on a femtosecond-nanosecond timescale. The maximal transient absorbance signal (at 680 nm) for all experiments was

no more than 2 mOD. Based on the excitation pulse energies, as well as on the magnitude of the transient signal, we estimate, on average, less than one excitation in a PSI complex per pulse (Appendix A.3). Therefore, singlet-singlet annihilation has negligible effect on the kinetics.

3.3.2.1 Purely absorptive 2D spectra

Figure 3.11: *Representative purely absorptive 2D spectra of PSI-LHCI (a) and PSI core (b) recorded in the Chl Qy region with excitation pulses at 680 nm at the indicated waiting times (T_w). The spectra are plotted in terms of excitation wavelength λ_1 and detection wavelength λ_3 . Contour lines mark areas of equal absorptive signal intensity given in the color scale bars. All 2DES are plotted on the same intensity scale.*

Representative purely absorptive 2D spectra of PSI core complexes and intact PSI-LHCI for selected waiting times T_w are plotted in Fig. 3.11. The vertical axis represents the excitation wavelength λ_1 and the horizontal axis represents the detection wavelength λ_3 . Spectra were recorded at different delay (waiting) times T_w following the dynamics of the system after the initial excitation. At detection wavelengths longer than 670 nm

the transient absorptive signal is contributed mainly by Chl *a* GSB and SE. In principle, the intensity of the signal at coordinates (λ_1, λ_3) reflects the concentration of excited states absorbing at λ_3 , which have been populated via EET from initially excited states absorbing at λ_1 . In the absence of EET or spectral diffusion, the 2D signal is found along the diagonal line, $\lambda_1 = \lambda_3$. On the other hand, excitonic coupling and EET between two states give rise to cross peaks at λ_1 and λ_3 corresponding to the donor and acceptor state transition energy, respectively. In addition to EET, cross-peak signals can also originate from spectral diffusion, *i.e.* fluctuations in the excited-state energy levels. Due to the large spectral overlap and very small energy gaps in the Chl *a* Q_y region, cross peaks are not resolved in the 2D spectra. Nevertheless, the time evolution of the 2D spectra holds information regarding the pathways and dynamics of EET.

In a system of interacting chromophores with varying excitation energy levels, EET will occur from higher-energy to lower-energy states, a thermodynamically favourable direction causing a shift of the 2D signal to longer detection wavelengths 3, *i.e.* to the left of the diagonal line. This fact is very well represented by 2D spectra of PSI-LHCI (Fig. 3.11). Inspecting the 2D spectrum at $T_w = 0.15$ ps, it is clear that within this time a part of the energy is already transferred from initially excited states absorbing at $\lambda_1 = 675\text{-}685$ nm to lower energy states at $\lambda_3 = 690\text{-}700$ nm. The evolution of the 2D spectrum from 0.15 ps to 5 ps shows the progressive shift to long-wavelength states (> 700 nm), concomitant with a decrease in the overall signal intensity due to trapping of excitations by the RC. The 2D spectrum at short waiting times (T_w) shows pronounced correlation of excitation and detection wavelength but at longer waiting times, as the system becomes thermally equilibrated, this correlation is progressively lost and the spectral shape becomes independent from excitation wavelength. Because of this relationship, 2DES gives a definitive time course of thermal equilibration, which appears to be virtually complete within 5 ps in both PSI core and PSI-LHCI. The most obvious difference between the spectra of the two preparations is the extended tail at

$\lambda_3 > 700$ nm in the PSI-LHCI supercomplex, which is due to the additional “red” states in the peripheral antenna. The appearance of these long-wavelength components in the supercomplex is clearly visible within a picosecond, signifying a rapid EET to the “red” Chls in LHCI.

Figure 3.12: *Waiting time dependence of the 2D signal at three fixed excitation wavelengths $\lambda_1 = 675$ nm 682 nm and 690 nm. The plots can be understood as analogous to imaginary pump-probe experiments with pump wavelengths corresponding to λ_1 C i.e. on the blue and red side of the Q_y absorption band of PSI-LHCI (top). Time traces of the absorptive 2D signal at selected wavelength intersections, indicated in the plots as λ_1/λ_3 (bottom).*

Fig. 3.12 shows the waiting time dependence of the signal at fixed excitation wavelengths (λ_1) of 675 nm, 682 nm and 690 nm. Hence, the plots represent the dynamic spectral evolution, on a logarithmic time scale, for the case when initial excitation is on the high, middle, or low energy side of the Q_y absorption band, respectively. Excitation on the blue side ($\lambda_1 = 675$ nm) leads to gradual spectral shift to longer wavelengths

(downhill EET), whereas excitation in the red tail ($\lambda_1 = 692$ nm) is followed by a blue shift (uphill EET). The peak position converges for both excitation wavelengths at times longer than 10 ps. The spectral shifts reveal exciton equilibration in the Chl *a* manifold (both downhill and uphill) taking place on the time scale of hundreds of fs to about 10 ps. The same downhill/uphill EET processes are illustrated by the selected time traces in the bottom panel of Fig. 3.12. The time courses show energy redistribution occurring in the first few ps after excitation - the diagonal traces (675/675, *etc.*) rapidly decay, whereas off-diagonal traces show a lag-phase (*e.g.* 675/690) or rise of the bleaching signal amplitude (682/700) that follows the population of states via EET. Note that EET can also be uphill (*e.g.* 690/675 trace). The plots also show that a component absorbing at *sim*700 nm is populated at times 1-10 ps and decays afterwards. A similar trend was observed in isolated core complexes (data not presented).

3.3.2.2 Global analysis

For a quantitative description, the transient 2DES data were subjected to global lifetime analysis, as previously described [46]. Good fit of the PSI core data in the time window 0.15-300 ps was obtained with three unconstrained exponential decay components (with lifetimes 0.49, 3.5 and 19 ps) and a non-decaying component. The lifetimes are in good agreement with those obtained from pump-probe spectroscopy experiments of cyanobacteria and green algae [126–128, 136–141] .

The pre-exponential amplitudes at different λ_1/λ_3 form two-dimensional decay-associated spectra (2D DAS), plotted in Fig. 3.13 for isolated core complexes. The 2D DAS amplitude shows if a 2D cross peak rises or decays with a certain lifetime and is useful to identify EET pathways. Generally, EET from state A to B is reflected in the 2D DAS as a positive (rise) cross peak at (A/B) and a corresponding negative (decay) diagonal peak at (λ_A/λ_A). Conversely, the reverse EET produces a cross peak at (λ_B/λ_A) and a diagonal peak at (λ_B/λ_B), if the excitation pulse can access λ_B . Thus, the symmetric

Figure 3.13: 2D decay-associated spectra of PSI core, resulting from a four-exponential fit of the transient 2D signals from 150 fs to 300 ps with best-fit lifetimes of 0.47 ps, 3.5 ps, 19 ps a non-decaying component. Negative amplitudes (red) represent decay and positive amplitudes - rise of the bleaching signal.

pairs of cross/diagonal peaks in the 2D DAS can identify donor-acceptor pairs coupled by reversible (energetically downhill/uphill) EET [133]. The first 2D DAS on Fig. 3.13 (0.49 ps lifetime) features a positive cross peak above the diagonal at excitation wavelengths 670-680 nm and a symmetric cross peak below the diagonal at 680-690 nm. Therefore, the 2D DAS strongly suggests that exciton equilibration between states in the 670-690 nm wavelength region occurs on a 0.5 ps timescale. Furthermore, the slanted ellipsoid shape of the cross peaks shows that multiple states of different energies are engaged in EET. In this respect, exciton equilibration in PSI is reminiscent of spectral diffusion of an inhomogeneously broadened band, which would produce the same characteristic 2D DAS shape [133].

The second 2D DAS (3.5 ps lifetime) exhibits a negative peak around $\lambda_3 = 685$ nm and a broad positive cross peak at $\lambda_3 = 704$ nm. This component can describe well the net downhill EET from the bulk Chls to a pool of Chl *a* with an absorption band around 700 nm. Because the excitation pulse spectrum does not extend to 700 nm, it is not possible to observe uphill cross peaks in this region. EET processes with a very similar spectral signature and lifetimes in the range of 2-3 ps been observed in PSI from cyanobacteria [123, 126, 128, 136, 142, 143] and *C. reinhardtii* [137, 138, 141]. The spectral changes were usually attributed to equilibration between bulk antenna excited states and low-energy red Chls. Here we show that the plant PSI core, which contains less, if any, red Chls [54, 124], exhibits the same kinetic behaviour. The 2D DAS spectrum is not conservative, i.e. the negative peak amplitude is larger than the positive peak amplitude. The stronger decay amplitude suggests loss of excitations on this timescale. We can thus argue that initial trapping already occurs on the timescale of 3 ps. The negative peak is slanted along the diagonal (inhomogeneously broadened or slow spectral diffusion), whereas the positive cross peak lacks any such correlation, which means, first, that the exciton energies are not (yet) randomized and, second, that there is minimal correlation between the donor and acceptor state energies.

Figure 3.14: *Horizontal slices of the 2D DAS of PSI core complexes at excitation wavelengths 670, 680, 690 nm.*

The 19 ps 2D DAS has only negative amplitudes, except at detection wavelengths under 670 nm, where it shows Chl excited state absorption. The majority of excitations in the PSI core decay on this timescale. In contrast to the previous 2D DAS, the peak shape of the trapping component is completely independent from excitation wavelength, which can be confirmed by comparing horizontal slices of the 2D DAS at different λ_1 (Fig. 3.14). From analysis of the shape of the 2D DAS we can conclude that excitations migrate rapidly between Chls in the PSI core antenna so that a thermal equilibrium of excited states is reached in a time much shorter than 19 ps. The main charge separation process (trapping) in the PSI reaction centre, occurs on a time scale of 19 ps.

The final 2D DAS corresponds to a state that does not decay within our experimental T_w range. It shows two distinct bleaching peaks - around 680 and 700 nm. It represents the spectrum of the oxidized RC (P700+) [144, 145] and probably long-lived excitations in a small number of uncoupled Chls. Horizontal slices of the 2D DAS (Fig. 3.14) are very similar to the P700+ transient absorption spectrum, reported for example in [146]. It features a negative peak at approximately 700 nm, a positive peak at 690 nm and a small negative peak at 680. These features are well explained with one broad negative band at 700 nm, and a narrower positive band at 690 nm [147]. The negative band corresponds to the P700 state, which is broad due to its charge-transfer character. The positive band corresponds to the other Chl in the P700 dimer which is not oxidized. The difference spectrum is also affected by electrochromic shifts of neighbouring Chls sensing the electric field of the P700+ cation. In our experiments the 680 nm peak appears to be more intense than in the published P700+ spectrum, and the non-decaying spectra are as reported in Melkozernov *et al.* [148]. The additional intensity at 680 nm has been attributed to the presence of some free (uncoupled) excited Chls in the sample, which do not decay during the measurement (free Chls have excitation lifetime of 4-5 ns). The non-decaying spectrum is of much lower amplitude than the rest, indicating that some RC remain closed between excitation cycles. The non-decaying amplitude can

also be reduced because of singlet-singlet annihilation and other quenching processes, for example singlet quenching by PMS [149]. For these reasons, the amplitudes of the DAS, and consequently SAS, are not an accurate estimate of the concentrations of the respective species.

Figure 3.15: *2D decay-associated spectra of PSI-LHCI, resulting from a five-exponential fit of the transient 2D signals from 150 fs to 300 ps with best-fit lifetimes of 0.51 ps, 3.2 ps, 14 ps, 53 ps and a non-decaying component.*

Global analysis of the 2DES kinetics of PSI-LHCI required five components - four decay lifetimes (0.51, 3.2, 14 and 53 ps) and a non-decaying component. The resulting 2D DAS are plotted in Fig. 3.15. The first two (0.5, 3 ps) and the final (non-decaying) 2D DAS are essentially the same as the respective 2D DAS for the isolated PSI core. The main excitation decay lifetime was consistently found to be shorter (14-16 ps), compared to the isolated PSI core (18-20 ps). An additional 53 ps lifetime, not present in the core, is associated with the decay of “red” Chls in the peripheral antenna, absorbing at wavelengths 700-710 nm. The 14 and 53 ps 2D DAS show that trapping in the PSI supercomplex, as in the isolated core, occurs from a thermally equilibrated antenna. A comparison of the spectral shape of the main decay components for PSI-LHCI (14 ps) and PSI core (19 ps) reveals a marked difference on the long-wavelength side. It can be concluded that the 14 ps lifetime in PSI-LHCI reflects both trapping of excitations and equilibration with “red” Chls in the peripheral antenna. Population of “red” states results in reduced net DAS amplitude in the far-red range.

In summary, global analysis of the 2DES data of isolated PSI core complexes and the intact supercomplexes reveals spectral equilibration in the main Chl *a* Q_y band with a 0.5 ps and equilibration with “red” spectral forms at 700 nm on a 3-4 ps timescale. The main trapping lifetime in the core complex is 20 ps and additional 53 ps lifetime is associated with “red” Chls in the peripheral antenna.

3.3.3 Conclusion

The 2DES measurements of plant PSI, reported here for the first time, allowed us to probe the exciton energy transfer and charge separation kinetics in a subpicosecond-to-nanosecond time range. Owing to the very low excitation energies used, we were able to record the excited-state dynamics without distortions from singlet-singlet annihilation. Consequently, fitting the kinetics measured by 2DES resulted in almost the same physical parameters. Therefore, we have demonstrated that detailed artifact-free kinetics of the

most complex systems, such as PSI, can be reliably extracted from 2DES experiments, in parallel with additional unique information that the 2D spectra provide.

Probably one of the strongest advantages of 2DES is its ability to resolve intraband exciton relaxation and identify downhill and uphill energy pathways. Our data unequivocally identify exciton relaxation in the PSI core antenna occurring mainly on a timescale of 500 fs but minor intraband equilibration (or spectral diffusion) in the 680-690 nm region is also observed on a timescale of 3 ps. Conversely, the 2D spectra can readily report if all exciton states are in thermal equilibrium. The latter is possible thanks to highly purified PSI samples (97% on Chl basis) and the reliable 2D lineshapes measured in “pump-probe” geometry 2DES. From comparison of the lineshapes along the excitation wavelength axis we conclude that thermal equilibration in the Chl Q_y band (< 690 nm) is complete in about 5 ps, *i.e.* well before the main trapping phase.

The most significant feature of the plant PSI kinetics, which has not been reported previously, is the 3-4 ps equilibration with a 700 nm spectral component. In this respect, the spectral evolution and trapping kinetics of PSI are very similar among all groups of oxygen-evolving photosynthetic organisms, despite the fact that they differ in the Chl exciton energies, specifically regarding the so-called “red” states.

Chapter 4

Understanding the Features in the Ultrafast Transient Absorption Spectra of CdSe Quantum Dots

4.1 Introduction

Semiconductor nanocrystals (NCs) have been an important research focus in recent decades due to the great flexibility in their optical and electronic properties. They have been shown to have promising applications in optical detectors [59], solar energy conversion [60, 61], biological sensing [62] and lasers [63]. Among the various types and shapes of NCs are, nearly spherical nanoparticles (quantum dots, QDs) [150], elongated nano-sized crystals (quantum wires or quantum rods) [151] and nanostructures with other shapes such as quantum wells [152]. Semiconductor QDs which are in strongly spatial confinement lead to the discrete distribution of energy levels and result in the rise of interests from researchers both experimentally and theoretically.

Previous works on QDs have provided the static information such as size, shape and surface to reveal structural and optical properties including linear and nonlinear absorption. Recent studies are more focused on the charge carrier dynamics, exciton relaxation or combination pathways and multi-exciton generation and interaction effects by means of varying nonlinear optical techniques [66]. Using time-resolved photoluminescence spectroscopy, single and multi-exciton emission energies and decay dynamics of CdS/ZnSe

core/shell type QDs is analyzed to control property of type II QDs with regards to the thickness of the shell [153]. Multi-channel hot exciton relaxation dynamics was reported on colloidal CdSe quantum dots by adopting femtosecond state-resolved pump-probe experiments to study the cooling dynamics and distinguish Auger recombination rate [64, 154]. More recently, multi-dimensional electronic spectroscopy was applied to study the size and state dynamics [155] and excitonic quantum coherence has been studied [156, 157].

The energy structure in QDs has been described in different level of theories. Ekimov *et al.* [158] reported the model of solving the Hamiltonian of CdSe QDs system with concern given to the degeneracy of valence band and Coulomb interactions between the electrons and the holes. With the model, the three lowest energy states of the holes were determined as $1S_{3/2}$, $1P_{3/2}$ and $2S_{3/2}$, which were well-resolved in the linear absorption spectra of CdSe QDs of various sizes. The study was later shown to be in good agreement with the experimental photoluminescence measurements by Norris *et al.* [159]. In addition, a dark-bright exciton model was put forward to study the temperature dependence of the single-exciton radiative lifetimes [160–162], in which the band-edge exciton splitting was described as a result of the strong electron-hole exchange interactions, the asymmetry of the quantum dots and the anisotropies associated with the crystal field. Ultrafast optical spectroscopy was also performed to explore the biexcitonic dynamics of QDs. However the various prominent features of the TA spectra of QDs have not been adequately explained.

The linear spectrum of the CdSe QDs is dominated by the three lowest energy transitions. Shown in Fig. 4.1a is the linear spectrum of CdSe QDs with approximately 65 Å diameter, with the positions indicated of the bands of $1S_e - 1S_{3/2}$, $1S_e - 2S_{3/2}$ and $1P_e - 1P_{3/2}$ transitions. These transitions have been previously assigned as X_1 , X_2 and X_3 , respectively and we adopt the notation of the Caram *et al.* [155] to denote the electronic states of the CdSe QDs, as summarized in the Fig. 4.1b. Overlaid in Fig. 4.1a is a TA

Figure 4.1: (a) Linear spectrum of CdSe QDs (blue line) overlaid with excitation laser pulse (green line) and TA spectrum at $T = 100$ ps (orange line); (b) Electronic energy levels of CdSe QDs.

spectrum of the same QDs at 100 ps delay. The negative features of A and B replicates the features of the X_1 and X_2 transition in the linear spectrum. However, at about 2.1 eV, the spectra changed sign and assumes a positive feature C, before going negative again at about 2.22 eV.

Although the ultrafast TA spectra have previously been studied, the main attention was paid to the band edge, which is associated with the X_1 transition. Klimov used the dynamics of band-edge signal to determine rates of Auger recombination [163]. Sewall *et al.* analyzed the biexcitonic binding energy of the band-edge peak to observe the cooling dynamics of hot excitons [154]. In his study, the transient absorption (TA) spectrum of only the band-edge X_1 transition of the CdSe QDs was discussed.

To the best of our knowledge, we have not come across any articles that attempt to explain the features beyond the band-edge peak (X_1 transition). In this work, we describe a model to explain the positive and negative features in the TA spectra. It is arranged as follows: In Section 4.2, we present the experimental details of the pump-probe measurement to obtain the TA spectra of the CdSe QDs. In Section 4.3, we explain in detail the model that we have used to model the TA spectra. Section 4.4 presents the fit to the experimental data using the model developed in section 4.3, and the parameters recovered from the fit. The article ends with a conclusion.

4.2 Experimental method

The experimental setup is detailed in former work from our group [14]. 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 (OPA). After the OPA, a near-infrared output was obtained and frequency-doubled to 630 nm by a BBO crystal. This output is used as our pump pulses and coincide with the band-edge transition of our targeted QDs (Fig. 4.1). This excitation light is compressed by a commercially acousto-optic programmable pulse shaping

unit (Dazzler, Fastlite) to a pulse duration of 56 fs (1.1 times transform-limited) with 10 nJ energy per pulse. The probe beam then passes through a computer controlled delay stage to generate the delay T . The pump beam and the probe beam then overlaps on the sample sealed in a cuvette with 1mm path length. Thereafter, a spectrometer (Acton SP2300) with a CCD detection array (PIXIS 100B) is used to measure the spectrally resolved TA spectrum of the probe beam.

CdSe QDs samples were synthesized according to a procedure previously reported by Chakraborty *et al.* [164]. With a suitable injection temperature and reaction time, nearly monodisperse QDs with band-edge absorption at ~ 630 nm were synthesized. The nanoparticles were processed from growth solution via three cycles of precipitation in methanol and re-dispersion in toluene. The processed sample of QDs was then further diluted with toluene to obtain an optical density of 0.2 at the band edge transition.

4.3 Model

We first model the linear absorption spectra using multi-Gaussian components, based on the theory proposed by Norris [159]. The remaining unresolved absorption continuum, which was considered as the Rayleigh scattering, can be described using a polynomial background term [159]. As indicated in Fig. 4.1, the linear spectrum is dominated by the three lowest electronic transitions of the CdSe QDs, which are $1S_e - 1S_{3/2}$, $1S_e - 2S_{3/2}$ and $1P_e - 1P_{3/2}$ transitions denoted as X_1 , X_2 and X_3 respectively. Mathematically, the linear absorption is modelled as

$$A(\omega) = \sum_{i=1}^3 \alpha_i \exp \left[- \left(\frac{\omega - \omega_i}{\gamma_i} \right)^2 \right] + p_1 + p_2\omega + p_3\omega^2, \quad (4.1)$$

here α_i are the weighted coefficients which are proportional to the absolute square of the transition dipole moments of the respective transitions, ω_i are the center of the Gaussian peaks, γ_i are the factor controlling the Gaussian widths and p_1 , p_2 and p_3 are the polynomial coefficients used to model the background terms.

We next discuss the model to describe the TA spectrum. The TA signal spectrum consists of contributions by the ground state bleach (GSB), stimulated emission (SE) and excited state absorption (ESA) processes associated with the X_1 , X_2 and X_3 transitions:

$$\Delta A(\omega) = -GSB(\omega) - SE(\omega) + ESA(\omega), \quad (4.2)$$

where $\Delta A(\omega)$ is the TA spectrum. On the right hand side of the equation, the first two terms carry the negative sign and the last term carries the positive sign.

The spectral components of GSB and SE are usually taken to be the same as the linear absorption of the particular transition of interest [154, 165]. The ESA depends on the transition from one excited state to another higher-order excited state (for molecular systems) or multiexcitonic absorptions (for semiconductor systems). In the case of QDs, the ESA corresponds to the transition from a single excitonic state to a biexcitonic state. Due to Coulombic attraction arising from the excited electron-hole pair in the single excitonic state, the energy gap for the consecutive transition, which is the ESA transition in our case, will be redshifted. This redshift is referred to as the biexcitonic binding energy shift [64]. The ESA spectrum can therefore be modeled on the basis of the linear absorption spectra redshifted according to the biexcitonic binding energies. Based on this understanding, the transient absorption spectrum of CdSe QDs is simulated in Sewalls work [154] as

$$\Delta A(\omega) \propto -2A(\omega) + A(\omega - \Delta_{XX}), \quad (4.3)$$

where the former term is twice the linear absorption $A(\omega)$ implying that each transition in the linear spectrum comprises two processes SE and GSB. In Sewalls work, this model was used to model the TA of the band edge which is due to the X_1 excitation only. However, as we will point out below, Eq. 4.3 provides an overly simplistic picture, and cannot describe the TA associated with the X_2 and X_3 transitions.

Based on state-filling model presented by Klimov [163], we treat each hole state as spin-doubly degenerated. Moreover, the distinguishability between spin-up and spin-

down electrons are taken into consideration. The detailed denotations for amended electronic system are presented in Fig. 4.2.

Figure 4.2: (a) The schematic electronic energy levels of CdSe QDs with each hole state treated as spin-doubly degenerated state; (b) The denotations for electronic transitions with distinguishable spin-up (darker color) and spin-down (lighter color) electrons.

In our experimental condition, the pump pulse is comparatively narrow in the spectral domain (see Fig. 4.1) and we predominantly excite the band edge peak which falls only in the X_1 transition, while the probe pulse using a white light continuum is able to probe a much bigger spectral band. Furthermore, it may be assumed that even if the pump has initially excited some parts of the X_2 or X_3 spectral band, after a delay, on the order of 10 ps [64], the population would have decayed to the lowest $1S_e - 1S_{3/2}$ electron-hole state X_1 due to carrier relaxation.

The GSB, SE and ESA processes can be enumerated using Double-sided Feynman Diagrams (DSFDs). The DSFDs are shorthand notations that represent terms from a time dependent perturbative treatment of nonlinear optical response functions. These terms can be calculated and summed up to give the TA spectrum [166]. Below, we will

systematically enumerate the DSFDs for the processes associated with the X_1 , X_2 and X_3 transitions. This will then be used to explain the features in the TA spectrum of the QDs.

The TA or pump-probe spectroscopy belongs to the family of third order nonlinear optical spectroscopies. In the language of time dependent perturbation theory that is used to describe such nonlinear optical spectroscopies, the "pump" pulse interacts twice with the sample, and after a delay (waiting time) of T_w , the "probe" pulse interacts once. This sets up a third order polarization which then manifest as the signal that emanates in the same direction as the probe pulse. This signal is then heterodyned detected by the "probe" pulse and frequency resolved detected as the transient absorption spectrum using a spectrometer.

In the DFSDs (Figs. 4.3, 4.4 and 4.5), time flows from the bottom to the top, and the density matrix elements are tracked in the center column. The first two interactions are from the pump pulse and the third interaction is from the probe pulse. These interactions are represented by solid line arrows. The last arrow (dashed line) denotes the signal field. Listed in Figs. 4.3, 4.4 and 4.5 are DSFDs for the "rephasing" processes, where the evolution between the first and second interactions involves the off diagonal density matrix element $|g\rangle\langle X_i|$. There is another set of the same number of DSFDs that belongs to the "non-rephasing" processes where the evolution between the first and second interactions involves the off diagonal density matrix element $|X_i\rangle\langle g|$, which are not depicted in Figs. 4.3, 4.4 and 4.5.

We first consider the DFSDs of the GSB, SE and ESA processes for the X_1 transition in Fig. 4.3. In the first row of Fig. 4.3, we depict the processes that lead to a GSB signal. The second row depicts the processes that lead to a SE signal and the third row enumerates the processes that lead to a ESA signal. Following the sign rules of the DSFDs for the third order signals, GSB and SE processes are proportional to $-\mu_{g \rightarrow X_1}^4$ carrying the negative sign while ESA processes are proportional to $|\mu_{g \rightarrow X_1}|^2 |\mu_{X_1 \rightarrow X_1 X'_1}|^2$

Figure 4.3: X_1 emission group: DSFDs of all possible rephasing coherence transfer pathways for the GSB, SE and ESA processes associated with the X_1 transition.

which carries a positive sign. $\mu_{g \rightarrow X_1}$ is the transition dipole moment for the transition to a single exciton state ($g \rightarrow X_1$) while $\mu_{X_1 \rightarrow X_1 X'_1}$ is the transition dipole moments for the transition to a biexcitonic state ($X_1 \rightarrow X_1 X'_1$). Assuming simplistically that the values of $\mu_{g \rightarrow X_1}$ and $\mu_{X_1 \rightarrow X_1 X'_1}$ to be the same, all the DSFDs will have similar amplitudes. Taking these into consideration, one can conclude that there is a ratio of 2 to 1 between the negative (GSB and SE) and positive signals (ESA).

Figure 4.4: X_2 emission group: DSFDs of all possible rephasing coherence transfer pathways for GSB and ESA processes associated with the X_2 transition.

We now discuss the TA signal contributed by the X_2 transitions (shown in Fig. 4.4). In the first row, the GSB signals are similar to the X_1 case as presented in Fig. 4.3. However, there is no SE signals. This is due to the fact that there is no excited population in the X_2 state. For the ESA signal, we need to take into consideration the Pauli exclusion principle. The X_1 ($1S_e - 1S_{3/2}$) and X_2 ($1S_e - 2S_{3/2}$) transitions share the same electron state. Therefore, after the “pump” excitation, if the electron state $1S_e$ is populated with a spin-up electron of the $1S_e - 1S_{3/2}$ transition (denoted as X_1), the “probe” can only excite the spin-down electron of the $1S_e - 2S_{3/2}$ transition (denoted as X_2) and vice-versa.

Essentially we can only reach the biexcitonic states $|X_2X'_1\rangle$ or $|X'_2X_1\rangle$. This restriction leads to fewer number of DSFDs for the ESA signals as shown in Fig. 4.4. One can then conclude that the ratio between negative (GSB) and positive signals (ESA) is still the same as the X_1 case, namely 2 to 1.

Figure 4.5: X_3 emission group: DSFDs of all possible rephasing coherence transfer pathways for GSB and ESA processes associated with the X_3 transition.

We now discuss the TA signal contributed by the X_3 transitions (shown in Fig. 4.5). Analogous to transitions associated with X_2 , there is no SE signal. However, in this case, the X_1 ($1S_e - 1S_{3/2}$) and X_3 ($1P_e - 1P_{3/2}$) do not share the same electron state. Subsequently, the "probe" pulse can excite a total of four combinations between spin-up and spin-down electrons of X_1 and X_3 transitions described by 4 DSFDs in the second row of Fig. 4.5. Taking this into consideration, there is two more ESA DSFDs compared to the X_2 case. This results in the ratio of negative (GSB) to positive (ESA) signals to be 1 to 1.

Here we use the numerical modelling of the TA spectrum as described in Eq. 4.2. For the GSB signals for X_1 , X_2 , and X_3 , we use the Gaussian functions obtained from the fit

of linear spectrum as described in Eq. 4.1

$$GSB(\omega) \propto \sum_{i=1}^3 \alpha_i \exp \left[- \left(\frac{\omega - \omega_i}{\gamma_i} \right)^2 \right]. \quad (4.4)$$

The SE pathways which only appears in X_1 emission group (Fig. 4.3) is expressed as

$$SE(\omega) \propto \alpha_1 \exp \left[- \left(\frac{\omega - \omega_1}{\gamma_1} \right)^2 \right]. \quad (4.5)$$

The ESA signal can be interpreted as arising from the single excitonic to biexcitonic transition ($X \rightarrow XX$), which is redshifted from single excitonic transitions ($g \rightarrow X$) by a biexcitonic binding energy, $\Delta_i = E_{X_1} + E_{X_i} - E_{X_1X_i}$, where E_{X_1} is the energy of the X_1 state, E_{X_i} is the energy of the X_i state and $E_{X_1X_i}$ is the energy of the biexcitonic X_1X_i state. This biexcitonic binding energy arises from Coulomb interactions caused by the excited electron and hole pair. With the ratio of GSB and SE signal to ESA derived earlier, the contribution of the ESA spectrum can be expressed as

$$\begin{aligned} ESA(\omega) \propto & \alpha_1 \exp \left[- \left(\frac{\omega - \omega_1 + \Delta_1}{\gamma_1} \right)^2 \right] + \frac{1}{2} \alpha_2 \exp \left[- \left(\frac{\omega - \omega_2 + \Delta_2}{\gamma_2} \right)^2 \right] \\ & + \alpha_3 \exp \left[- \left(\frac{\omega - \omega_3 + \Delta_3}{\gamma_3} \right)^2 \right]. \end{aligned} \quad (4.6)$$

The first term in Eq. 4.6 represents the X_1 emission group and it is the same as the term in SE and GSB signals because there are equal number of accessible ESA processes compared with GSB and SE processes. However, in the X_2 emission group (Fig. 4.4), the ESA processes are only half of the GSB pathways due to Pauli exclusion principle. So a factor of $\frac{1}{2}$ appears in the second term in ESA contribution of Eq. 4.6. Similarly, the last term, which represents the X_3 emission group, shares the same weight as the respective GSB processes. Taking into consideration all the terms in Eq. 4.4, 4.5 and

4.6, the transient absorption spectrum of CdSe QDs is modelled using the formula below

$$\begin{aligned} \Delta A(\omega) \propto & - \sum_{i=1}^3 \alpha_i \exp \left[- \left(\frac{\omega - \omega_i}{\gamma_i} \right)^2 \right] - \alpha_1 \exp \left[- \left(\frac{\omega - \omega_1}{\gamma_1} \right)^2 \right] \\ & + \alpha_1 \exp \left[- \left(\frac{\omega - \omega_1 + \Delta_1}{\gamma_1} \right)^2 \right] + \frac{1}{2} \alpha_2 \exp \left[- \left(\frac{\omega - \omega_2 + \Delta_2}{\gamma_2} \right)^2 \right] \\ & + \alpha_3 \exp \left[- \left(\frac{\omega - \omega_3 + \Delta_3}{\gamma_3} \right)^2 \right]. \end{aligned} \quad (4.7)$$

where the first negative summation represents the GSB spectrum and the second negative term stands for the SE signals, while the next three positive terms are ESA signals.

Figure 4.6: (a) TA lineshape for features A and B of Fig. 4.1a with the ratio between negative and positive signal being 2 to 1; (b) TA lineshape for feature C with the ratio between negative and positive signal being 1 to 1.

Qualitatively, we can now explain the trends of the TA spectrum lineshape. As depicted in Fig. 6, the negative peaks represent GSB and SE signals and the positive peaks are ESA signals. In Fig. 4.6a, with the 2 to 1 ratio of negative and positive signals, the TA lineshapes of features A and B are demonstrated. With the negative peak representing GSB and SE centered at the linear absorption maximum and the

positive ESA peak redshifted by the biexcitonic binding energy, the TA lineshape is an asymmetric negative peak approximately replicating the linear absorption peak. On another hand, if the amplitudes of negative and positive signals are equal as in the case for the X_3 emission group (Fig. 4.5), the resultant TA lineshape has a "dispersive" profile as depicted in Fig. 4.6b. The feature C in TA spectra shown in Fig. 4.1a is the positive half of this dispersive line. The other negative half is not resolved due to the limitation of probe pulse spectral range.

4.4 Results and discussion

We apply our proposed model to the experimental collected linear absorption spectrum. The results are plotted in Fig. 4.7 with $R^2 > 0.99995$ and the root-mean-square-error (RMSE) around 3.97×10^{-4} . We can see that the simulated spectrum is a reasonable fit to the experimental data. The linewidth of each transition increases with the growth of energy from X_1 to X_3 . This broadening is consistent with previous works done by Norris [159], Bawendi [167], and Hoheisel [168]. From the fitting results, we can determine that the transition energies of the X_1 , X_2 and X_3 are 1.96, 2.03, and 2.21 eV, respectively.

Although there is an overlap between the pump excitation spectrum and the X_2 state (Fig. 4.7), the resultant excited population in the X_2 transition is much smaller than the one in the X_1 transition (see Appendix A.4). Furthermore, through carrier relaxation, all the population would have decayed to the $1S_e - 1S_{3/2}$ electron-hole state and spectral diffusion has resulted in the redistribution over the whole of the X_1 band (see Appendix A.4) by $T_w = 10$ ps [64], therefore we are only including TA spectra for $T_w > 10$ ps in our studies here.

Referring to the analysis in the Model section, the TA spectrum of CdSe QDs with $T_w = 100$ ps is fit with Eq. 4.7. The results of the fit are depicted in Fig. 4.8 with $R^2 = 0.9238$ and $RMSE = 0.0060$. We can see the obvious deviation between the experimental and the simulation results. In addition, the fitting results return the values of biexcitonic

Figure 4.7: *Modelling results of the linear absorption spectrum for CdSe QDs.*

binding energy of $\Delta_1 = 7.45 \pm 4.21$ meV, $\Delta_2 = 78.43 \pm 14.89$ meV, and $\Delta_3 = 41.84 \pm 4.55$ meV, which are not physically reasonable because previous studies in Ref. [154], [163] and [169] indicated that biexcitonic binding energy cannot be as large as 70 meV.

Hitherto, we have assumed that the transition dipole moments $\mu_{X_1 \rightarrow X_1 X'_1}$ for the transition to a biexcitonic state ($X \rightarrow XX$) to be the same as the transition to a single exciton state ($g \rightarrow X$) $\mu_{g \rightarrow X_1}$. We relax this condition and allow the ESA processes to undergo the transitions with different transition dipole moments. In this case, we modify the model for transient absorption spectrum to become

$$\begin{aligned} \Delta A(\omega) \propto & - \sum_{i=1}^3 \alpha_i \exp \left[- \left(\frac{\omega - \omega_i}{\gamma_i} \right)^2 \right] - \alpha_1 \exp \left[- \left(\frac{\omega - \omega_1}{\gamma_1} \right)^2 \right] \\ & + \alpha_1 m_1 \exp \left[- \left(\frac{\omega - \omega_1 + \Delta_1}{\gamma_1} \right)^2 \right] + \frac{1}{2} \alpha_2 m_2 \exp \left[- \left(\frac{\omega - \omega_2 + \Delta_2}{\gamma_2} \right)^2 \right] \\ & + \alpha_3 m_3 \exp \left[- \left(\frac{\omega - \omega_3 + \Delta_3}{\gamma_3} \right)^2 \right]. \end{aligned} \quad (4.8)$$

Figure 4.8: *Comparing the simulated transient absorption spectrum with the experimental transient absorption spectrum.*

where m_i is the ratio between the absolute squares of the transition dipole moments $m_i = \left| \mu_{X_1 \rightarrow X_1 X'_1} \right|^2 / \left| \mu_{g \rightarrow X_1} \right|^2$. These parameters account for the change of transition dipole moments caused by the perturbation of excitation. Using the modified model, the transient absorption spectrum at $T_w = 100$ ps is fit with non-linear-least-square method.

Figure 4.9: Comparing the experimental with the simulated transient absorption spectrum using the modified model on CdSe QDs.

The resultant fitting is much better than the previous fit, with $R^2 = 0.9962$ and $RMSE = 0.0013$. The resultant biexcitonic binding energy values are $\Delta_1 = 3.179 \pm 0.512$ meV, $\Delta_2 = 9.18 \pm 2.27$ meV, and $\Delta_3 = 28.09 \pm 2.27$ meV. This results is consistent with previous studies [154], [163] and [169]. The fit for the m_i values are all greater than 1 (See Table 4.1) implying that the transition dipole moment is greater for the transition to a biexcitonic state ($X \rightarrow XX$) than the corresponding transition to a single exciton state ($g \rightarrow X$). This can be rationalize by the increasing of overlap between electron and hole wavefunctions in the biexcitonic states [170, 171]. Our values are consistent with the study conducted by Takagahara [170] which concludes that the oscillator strength

(which is proportional to the absolute square of transition dipole moment) ratio between biexcitonic and monoexcitonic transition has a typical value around 1 to 2.5. We also conduct the global fitting for all data having T_w ranged between 10-900 ps. All TA spectra within this T_w range are normalized to eliminate the population decay dynamics. The fitting is conducted over the entire data set and the summarized results are presented below.

Figure 4.10: *Normalized global fitting results of TA spectra with T_w ranging from 10 ps to 900ps.*

The values obtained from the range of delays from 10 ps to 900 ps are very similar. The trends of the biexcitonic binding energies and transition dipole moments values obtained from our fits are in broad agreement with the values reported in the literature (from several meV to ~ 20 meV [154, 163, 169]). However as the optimization of the values are interdependent in our fit, the set of values recovered for biexcitonic binding energies and transition dipole moments ratio values may not be unique. Additional studies, such as the theoretical calculations of the biexcitonic binding energies and transition dipole moments values will help us narrow the range of values for the fit. Further experiments using

Table 4.1: *Summarized fitting results of TA spectra.*

Model	Fitting Results	R^2	RMSE
Original model	$\Delta_1 = 7.45 \pm 4.21$ meV		
	$\Delta_2 = 78.43 \pm 14.89$ meV	0.9238	0.0060
	$\Delta_3 = 41.84 \pm 4.55$ meV		
Modified model	$\Delta_1 = 3.179 \pm 0.512$ meV		
	$\Delta_2 = 9.18 \pm 2.27$ meV		
	$\Delta_3 = 28.09 \pm 2.27$ meV	0.9962	0.0013
	$m_i = \left \mu_{X_1 \rightarrow X_1 X'_1} \right ^2 / \left \mu_{g \rightarrow X_1} \right ^2 \neq 1$ $m_1 = 1.689 \pm 0.042$		
	$m_2 = 1.267 \pm 0.088$		
	$m_3 = 1.175 \pm 0.036$		
Global Fitting	$\Delta_1 = 3.422 \pm 0.084$ meV		
	$\Delta_2 = 9.716 \pm 0.358$ meV		
	$\Delta_3 = 28.25 \pm 0.35$ meV	0.9956	0.0318
	$T_w = 10\text{-}900$ ps $m_1 = 1.693 \pm 0.007$		
	$m_2 = 1.290 \pm 0.013$		
	$m_3 = 1.180 \pm 0.006$		

technique such as 2DES will be able to help us clarify the contribution of homogeneity, inhomogeneity and spectral diffusion to the spectral features [172].

4.5 Conclusion

Using a simple model, we were able to explain the negative and positive features in the TA spectrum (1.9 eV to 2.2 eV) of CdSe quantum dots with 65 Å diameter. We provide a model to fit the TA spectra. This is done by a careful enumeration of all GSB, SE and ESA processes associated with the three lowest energy transitions $1S_e - 1S_{3/2}$, $1S_e - 2S_{3/2}$ and $1P_e - 1P_{3/2}$. We then introduced global fitting in our model to fit our experimental TA spectra. We obtain the biexcitonic binding energies, corresponding to the $1S_e - 1S_{3/2}$, $1S_e - 2S_{3/2}$ and $1P_e - 1P_{3/2}$ states as 3.4, 9.7 and 28 meV, respectively. These results are in general agreement with literature values.

Due to the strong spatial confinement in semiconductor NCs, especially in QDs, the strength of electron-hole Coulomb interactions are significantly enhanced compared with their counterparts in bulk semiconductor [163]. Therefore, it is of great importance to determine multi-exciton binding energies since it is a direct indicator on the strength of Coulomb interactions contributions. It is hoped that this model enables us to gain better information of the contributions from above mentioned processes.

Chapter 5

Understanding the Peakshapes of Two-dimensional Electronic Spectra of Colloidal CdSe Quantum Dots

5.1 Introduction

Ultrafast multidimensional optical spectroscopy is a promising technique with great capabilities in the study of condensed matter. There has been various applications over a whole range of spectral regions. These include the mid infrared (MIR) region to probe vibrational systems and structural dynamics [173–176] and visible [7, 46, 49, 133] and ultraviolet (UV) [177] regions for studying natural and artificial light-harvesting systems. Ultrafast two-dimensional electronic spectroscopy (2DES) has also been applied to the study of colloidal quantum dots (QDs). Scholes *et al.* used 2DES to probe the exciton-exciton superposition states of CdSe QDs and measured a decoherence time of 50 fs [178]. Size and electronic state structure and dynamics in CdSe QDs were explored and investigated by Engel *et al.* using 2DES as well [155, 179, 180]. One of the key capability of 2DES is its ability to distinguish and quantify the homogeneous, inhomogeneous and spectral diffusion contributions to the spectral lineshapes of the measured system. These quantities are particularly important in the characterization of QDs. The spectral broadening mechanisms in QDs can be mainly interpreted from three sources: size and

shape inhomogeneity [159, 181, 182], spectral diffusion [183, 184] and phonon broadening which contributes to the homogeneous broadening [185, 186].

In 2DES measurement, three ultrafast visible light pulses interact with the sample. The interactions generate a third order polarization in the sample which is then heterodyne detected by the local oscillator. The generated signal are mapped along the excitation and emission energies, showing certain spectral shape and amplitude features. By carefully investigating the amplitude, peak position, line shape, peak shape and dynamic evolution of the 2DES spectra, various information on molecular dynamics and structure would be revealed and determined in detail.

Over the years, much effort has been spent on the analysis of the dynamics and shape 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 give detail of the inhomogeneous and homogeneous widths, respectively [1, 166, 187]. This principle is being used by a recent work by Turner *et al.*, which studied the size dependence of CdSe QDs with homogeneous linewidths decreasing as the radius increase by characterizing the antidiagonal linewidths of band-edge 2DES spectra [188] which represents the $1S_{3/2}$ to $1S_e$ transition.

An important quantity that characterizes homogeneity, inhomogeneity and spectral diffusion is the frequency-frequency correlation function (FFCF) [1, 189]. A variety of efforts has been explored with certain limitations to retrieve the FFCF from 2DES spectra. These methods include three pulse photon echo peak shift (3PEPS) experiment [186, 190–192], central line slope (CLS) analysis [193, 194], ellipticity [195, 196] and eccentricity [197] *etc.* All above methods have been documented to be linearly proportional to FFCF under certain assumptions and limitations. 3PEPS experiment requires crucial criteria for the excitation pulses parameters due to the fact that the first two pulses overlap in short time region resulting in complication of the intrinsic contributions. CLS analysis is able to successfully extract FFCF without the need of complicated response function

calculations. This CLS method used the slope of the line that connects the maximum points of the cuts through the 2DES spectra parallel to the probe frequency. It tracks the spectral diffusion dynamics over the measured waiting time window. However, CLS analysis works well with well resolved peaks of 2DES spectra while it may not be applicable to 2DES spectra with overlapping peaks, such as the overlap of $|0\rangle$ - $|1\rangle$ and $|1\rangle$ - $|2\rangle$ bands in vibrational systems. In addition, finite bandwidth of the pump pulses would affect the determination of CLS as well [193, 194]. When it comes to ellipticity and the eccentricity, which requires linewidth characterization of diagonal or anti-diagonal cuts through the 2D spectra, the interference between the $|0\rangle$ - $|1\rangle$ and $|1\rangle$ - $|2\rangle$ bands may dramatically influence the results [194].

Another related technique to recover information on the homogeneity, inhomogeneity and spectral diffusion of a system is the nodal line slope (NLS) analysis [198]. This technique takes advantage of the interference of positive value bands and negative value bands in 2D spectra. These features are very common in colloidal semiconductor QDs ensembles, they typically generate such redshifted signals with the opposite sign of the conventional negative going bleaching signals in transient absorption (TA) and 2DES measurement due to the excitation to the biexciton states leading to the intrinsic excited state absorption signals [64, 65, 178, 198–200]. The NLS method becomes a promising observable for quantitative determination of contributions from different line-broadening sources in colloidal QDs ensembles. The NLS slope value indicates the initial contributions from different broadening effects, while the dynamics within the observing window reflect the corresponding dynamical line-broadening, mainly spectral diffusion.

In a recent paper, Jonas and co-worker have used the NLS separating positive and negative peaks to analyze the band-edge inhomogeneity and homogeneity in colloidal PbSe QDs [200]. The results are compared with those measured by advanced transmission electron microscopy (TEM) methods to facilitate the determination of inhomogeneous linewidths. This may suggest that the NLS analysis can also be straightforwardly applied

to the analysis of CdSe QDs. However, the nodal line features in a 2DES spectrum of CdSe differs from that of PbSe significantly. In PbSe case, the NLS analysis concerns the lowest $1S_h$ - $1S_e$ transition. On the other hand, for the CdSe case, as we will show later, the NLS analysis if it were to be applied to CdSe will have to include transitions other than the lowest transition.

The absorption spectrum for colloidal CdSe QDs systems is a broad band with quantum confined behavior due to the nano-scale size effect. The exciton manifolds have been well documented by Ekimov *et al.* [158] and clearly resolved by linear absorption spectrum and photoluminescence measurements by Norris *et al.* [182]. However, size and shape inhomogeneity and other effects broadens the absorption band of each exciton state and it is usually the case that the bands from different transitions overlap with each other. Furthermore, the biexciton binding energy shift for QDs system varies from a few meV to hundreds of meV. This will naturally introduce the nodal planes in the spectra of nonlinear optical experiments, such as TA spectroscopy and multidimensional ultrafast electronic spectroscopy, when ESA to the biexciton state is taken into consideration. As a consequence, the analysis of the NLS for colloidal CdSe QDs ensemble systems can be complicated.

To provide a model to explain the NLS results and to use it to characterize the inhomogeneity and homogeneity in a sample of colloidal CdSe QDs, we need a full account of the contributions of the spectral positions of the ground state bleaching (GSB), stimulated emission (SE) and excited state absorption (ESA) components that make up the 2DES spectra. In a previous article, we have explained the features of the ultrafast transient absorption spectra, including the features that contributed to the three lowest transitions [201]. We have enumerated all the processes that contribute to the features in the transient absorption spectrum. Here, we expand on this approach to explain the various features of the 2DES spectra of CdSe, including the lowest three transitions. By doing so, we are able to explain the NLS that appears in the 2DES spectra of

CdS and recover the hidden information out of the NLS analysis from experimental 2D spectra. The article is arranged in the following manner. In Section 5.2, the theoretical preparation regarding the NLS would be demonstrated. Then Section 5.3 will present the experimental method of 2DES and related experimental results. In Section 5.4, a simulation workflow which helps to quantify the line shape analysis would be developed in detailed explanation, followed by a full discussion on simulation results in Section 5.5.

5.2 Theoretical background

2D signal can be described as a 2D Fourier transform of third order nonlinear response function. This nonlinear response is related to the FFCF $\langle \delta\omega(t)\delta\omega(0) \rangle$ and the lineshape function $g(t)$ [5]. Kubo introduce a lineshape function as a sum of two components describing the homogeneous and inhomogeneous dynamics [202]

$$g(t) = t/T_2^* + \frac{\Delta\omega^2}{2}t^2, \quad (5.1)$$

with a pure dephasing time T_2^* and the fluctuation amplitude $\Delta\omega$.

Within such assumption, the linear absorption spectrum is expressed as a Voigt profile which is a convolution of a Lorentzian lineshape with the Gaussian distribution [203]

$$A(\omega) \propto \frac{1}{(\omega - \omega_{01})^2 + (f_L/2)^2} \otimes e^{-\frac{(\omega - \omega_{01})^2}{(f_G/2\sqrt{\ln 2})^2}}, \quad (5.2)$$

where f_L and f_G are the full width at half maximum (FWHM) of the Lorentzian lineshape and the Gaussian distribution respectively.

From Eq. 5.2, the linewidth information for the homogeneous and inhomogeneous dynamics can be easily retrieved from the linear absorption spectrum. However, simply fitting the linear absorption spectrum using the Voigt profile can be distorted due to the presence of experimental background noise [204] and spectral diffusion effects [5]. Given such consideration, careful 2D lineshape analysis is indeed of great necessity to characterize different line-broadening dynamics in a targeted system. In a three state

system i.e., $|0\rangle$, $|1\rangle$, $|2\rangle$, where $|2\rangle$ state is the biexciton state for the case of semiconductor QDs. Three processes are associated with third order nonlinear signal in both pump probe and 2DES spectroscopy, namely, ground state bleaching (GSB), stimulated emission (SE) and excited state absorption (ESA). The first two terms share the same negative sign, while the last term shows the opposite sign with a redshift referred as the biexciton binding energy shift [64, 201]. The resulting peakshape is a pair of positive value peak to negative going peak in the corresponding transition positions. The nodal plane appearing in between the pair of signals is the key observable in NLS analysis. It is sensitive to the presence of the inhomogeneity and the tilt of the nodal line is obvious and quantifiable [205].

Here the NLS $S(T_w)$ is the inverse of the slope of the nodal plane that connects the positive going peak to the negative going peak in 2D spectrum. Kwac and Cho have analytically proven that the NLS is linearly proportional to the FFCF [198]

$$S(T_w) = \frac{\langle \delta\omega(T_w) \delta\omega(0) \rangle}{\langle \delta\omega(0) \delta\omega(0) \rangle}. \quad (5.3)$$

The NLS value $S(T_w)$ varies from 0 to 1 with the increasing contribution from the inhomogeneously broadened Gaussian component. This behavior is analogous to the CLS analysis introduced by Kwac *et al.* [193, 194]. However, CLS method suffers from the limitation of the finite pulse bandwidth, especially when the bandwidth is much narrower than the absorption spectrum [194]. On the contrary, the NLS is immune to the finite bandwidth because the nodal points retrieved from the cuts through the 2DES spectra that are parallel to the probe axis are not affected by the finite bandwidth of pump pulse. In addition, for systems with more complex energy levels such as QDs, CLS can be ill-conditioned especially when peaks are not well frequency-resolved.

5.2.1 NLS in the inhomogeneous or homogeneous limit

We present the numerically calculated 2DES spectra representatives using the theoretical Voigt profile lineshape function to show the unique determination of contribution from

Figure 5.1: Numerically calculated representatives of 2DES spectra (a) in inhomogeneous limit and (b) in homogeneous limit.

inhomogeneous line-broadening. By varying the ratio of inhomogeneous linewidth to homogenous linewidth within a fixed Voigt profile, characteristics of 2DES spectra in inhomogeneous and homogeneous limits are presented in Fig. 5.1 and the corresponding NLS is performed as well. For the sake of simplicity, equal weightage is set for both the positive peak and negative peak. Fig. 5.1a shows the illustration of 2D spectrum when the inhomogeneous linewidth is 5 times broader than the homogeneous linewidth. Therefore, the peak shape is dominated by the Gaussian distribution according to Eq. 5.1 and aligned along the diagonal axis. The nodal plane in this inhomogeneous limit shows a large tilt which is very close to 1. For the homogeneous case shown in Fig. 5.1b, where the linewidth of inhomogeneity versus homogeneity is 1 is to 5, the inverse of the slope value is 0.05. Now the nodal plane is almost vertically oriented and the 2D peaks result in a more Lorentzian-like lineshape. The change of the contributions of inhomogeneity and homogeneity is apparent in the change of orientation of the nodal line and the NLS is a clear and sensitive observable for these line-broadening effects.

5.2.2 NLS in QDs ensembles

We now consider a QDs system with its two lowest energy states excited. The pump spectrum is considerably narrow and it predominantly cover the lowest band-edge energy state X_1 , while the third pulse has a rather broad spectral range to probe not only the X_1 transitions but also the X_2 transitions. The schematic illustration of the energy states and corresponding transitions is shown in Fig. 5.2a. Under such excitation condition, both X_1 and X_2 transitions would generate a pair of peaks with the opposite signs, leading to two nodal lines indicating the related line-broadening contributions. In addition, there would be a third nodal plane in between the X_1 and X_2 related pairs. This nodal plane is influenced by both X_1 line-broadening effects and X_2 line-broadening effects. Fig. 5.2b-d show the uniquely determined NLS results with the increasing contribution from the inhomogeneous line-broadening. The inverse of the slope values change from ~ 0.2 to almost 0.6 when the linewidth ratio of inhomogeneity versus homogeneity increases from 0.5 to 1.5. Again, for simplicity purposes, although the relative amplitude for X_1 and X_2 transitions are set to be different to take into account the difference in transition dipole moments, here, the transitions for positive signal and negative signal for the same state is maintained to be the same. This amplitude weightage would affect the peak shape and the NLS analysis to a large extent. When one amplitude of a certain peak was considered weak comparing to others, the corresponding peak may be compensated or overwhelmed by the neighboring ones. This situation is more likely to happen if the peak shift is small. Careful determination of peak positions, the relative amplitude weightages and the biexciton binding energy shifts are of great importance in the simulation. All these parameters have been achieved with great accuracy in our previous study in TA measurements on CdSe QDs [201].

Figure 5.2: (a) Illustration of energy levels and transitions for QDs system with the lowest two exciton states involved; (b)-(d) Numerically calculated representatives of 2DES spectra with the ratio of inhomogeneous linewidth versus homogeneous linewidth varying from 0.5 to 1.5 for QDs system with the lowest two exciton states involved.

5.3 Experimental results

The 2DES measurement is carried out on a conventional pump probe apparatus with programmable pulse-shaper in a partially collinear manner which was previously described in Ref. [14]. Briefly, in order to map the excitation frequency for an additional dimension, the single pulse train used in conventional pump probe setup is replaced by a double-pulse train with controllable relative phase and time delay. To avoid truncation artifacts which would result in distortion on the Fourier-transformed 2DES peak shapes, data along t_1 coherence time are taken until 120 fs to make sure that the dephasing signal has sufficiently decayed to below noise level. The excitation light is tuned to be centered at 625 nm to coincide with the band-edge $1S_{3/2} - 1S_e$ (X_1) transition of our CdSe QDs. These pump pulses hold a pulse duration of 57 fs with 2 nJ energy per pulse, which provide an excitation density of $\sim 2 \times 10^{14} \text{ cm}^{-2}$. The probe light is a white light continuum and covers the three lowest bands of $1S_{3/2} - 1S_e$ (X_1), $2S_{3/2} - 1S_e$ (X_2) and $1P_{3/2} - 1P_e$ (X_3) transitions as assigned in our previous conventional pump probe study [201]. CdSe QDs samples were synthesized and processed according to the protocols previously reported by Chakraborty *et al.* [164] with band-edge absorption at 1.984 eV. The CdSe QDs in the sample has an average diameter of 4.9 nm and a size distribution of about 11% according to the TEM image analysis (Appendix A.5.1). The processed CdSe QDs with diameter of 4.9 nm was further diluted with toluene to obtain an optical density of 0.2 at the band edge transition in a 1 mm cuvette so the corresponding average number of excitons per QD of about 0.24. Fig. 3 summarizes the linear absorption spectrum overlaid with excitation pulse together with the scaled transient absorption (TA) spectrum at population time $T_w = 100$ ps. In the TA spectrum, two negative features at ~ 1.98 eV and 2.06 eV respectively correspond to GSB and SE signals of the X_1 and X_2 transition, respectively. However, the positive feature at about 2.2 eV represents the redshifted ESA peak of X_3 transition by the biexciton binding energy.

Figure 5.3: *Linear spectrum of CdSe QDs (blue line) overlaid with excitation laser pulse (pink area) and TA spectrum at $T_w = 100$ ps (red line) which is scaled 20 times for visualization purpose.*

We then perform 2DES measurement using the same apparatus. A typical 2DES spectrum collected at $T_w = 100$ ps is depicted in Fig. 5.4a as an example. The vertical axis represents the excitation energy (pump frequency) while the horizontal axis represents the emission energy (probe frequency). As discussed above, the states and transitions are described in Fig. 5.4b. The excitation pulse predominantly covers the X_1 transition, consequently there is only one diagonal peak in the 2D spectrum. It is a sum of the GSB and SE signals of X_1 transition as explained in its counterpart in pump probe spectrum in Fig. 5.3. As viewed along the probe dimension, there is a negative cross peak showing at the X_2 position. Due to broadening effects especially at room temperature, this cross peak is not clearly definable and appears as a spread from the diagonal peak along the probe axis. Further away from the diagonal peak, the positive cross peak arises from the redshifted ESA of X_3 transition. These peak positions agree with the results from TA spectrum.

Figure 5.4: (a) 2DES spectrum of 4.9 nm CdSe QDs collected at $T_w = 100$ ps at room temperature. The diagonal line marks where $\Omega_1 = \Omega_3$. The solid white line shows the nodal line; (b) Illustration of energy levels and transitions for CdSe QDs with X_1 , X_2 , and X_3 states involved.

In order to retrieve detailed lineshape information from this broad 2DES spectra, NLS analysis introduced in Section 5.2 is performed over a series of 2DES spectra at a series of T_w delays. As mentioned above, the CdSe QDs ensembles were excited by a narrow band pump but probed by a broad white light continuum. The transitions covers the band-edge three lowest energy levels, X_1 , X_2 and X_3 states. Since the three lowest states are overlapped to a certain extent in the spectral domain and the biexciton binding energy shift is small [154, 163, 169], especially for X_1 state (a few meV), the positive peaks corresponding to X_1 and X_2 ESA processes were sufficiently offset by the dominance of the negative signals, thus rendering it unobserved. As a result, the apparent nodal plane shows up only in the position between the X_2 negative peak and the X_3 positive peak and the NLS analysis was conducted right onto this line. Each one-dimensional cut through the 2DES spectrum parallel to the probe axis is fitted using a polynomial function so that accurate nodal plane can be determined. The resulting characterized nodal points can be fitted linearly within the pump excitation region. The solid white line in Fig. 5.4 shows the resulting nodal line fit from the experimental data in the vicinity of pump maximum.

Fig. 5.5 depicts the measured NLS value versus the population time T_w . Despite the fluctuations caused by the experimental background noise, these data points tend to follow a constant value of 0.47 over the observation window of 0.3 to 900 ps. Since the NLS value is proportional to the same FFCF as the CLS analysis, this conserved behavior shows that there exists negligible T_w dependence in CdSe QDs ensembles so that there is no obvious spectral diffusion occurring on the time scale of our measurement. This agrees well with former studies which state that the spectral diffusion process for CdSe QDs happens only either on fast sub-picosecond scale [155] or in much longer sub-second time scale [183] and it is not thermally activated [206].

Figure 5.5: *The NLS data as the population time T_w progresses.*

In principle, the NLS value varies from 0 to 1. It reflects the degree of inhomogeneity over homogeneity of the ensemble. In ideal cases, as the targeted ensemble has only homogeneous broadening, the peak shape of the cross peaks is more round and thus the NLS value will be close to 0. While in the case of purely inhomogeneous line-broadening, 2DES peak shapes show clear elongation along the diagonal axis, which will result in the NLS value approaching 1. Therefore, the more inhomogeneity in the ensemble, the bigger

will be the NLS value. In CdSe QDs, size and shape inhomogeneity is a well-known inhomogeneous broadening effect. From the experimental data, we observe the NLS value to be 0.48, which means both homogeneity and inhomogeneity play a significant role in our CdSe QDs. However, how much does the homogeneous linewidth and the inhomogeneous linewidth contribute to the NLS value in our CdSe QDs ensemble? This question will be answered with the help of a systematical simulation analysis on the experimental data which will be presented in the next section.

5.4 Simulation development

Figure 5.6: *Schematic workflow for 2DES peak shape simulation development.*

In order to quantitatively characterize the inhomogeneous and homogeneous line-broadening contributions from the 2DES spectra, a systematic simulation workflow was developed. As demonstrated in Section 5.2.2, we employ the information achieved from fitting of experimental data on linear absorption and transient absorption study so that carefully determination of total linewidths, transition dipole moments, weightage of different processes and so on was performed. Finally, with these known information derived from former experiments, we use the Kubo lineshape function as the foundation for 2D peak shape study and manually adjust the ratio of inhomogeneous linewidth versus homogene-

ous linewidth within the total linewidth limitation to characterize the NLS value. From the parameters used to achieve the fit, the linewidth contributions can be quantified.

5.4.1 Linear absorption fitting

The ensemble line-broadening effects in colloidal QDs arise mainly from three mechanisms: size and shape inhomogeneity, spectral diffusion, and phonon broadening. Among these broadening effects, the phonon coupling contributes to the homogeneous broadening and is often not the dominant contribution to the transition linewidth even at room temperature [207]. Considering that the spectral diffusion effect is negligible in CdSe QDs on our targeted time scale, inhomogeneity is the main line-broadening contribution in the CdSe ensembles. Given that consideration, we use multi-Gaussian components using standard nonlinear least-squares methods to fit the linear absorption spectrum. As indicated in Fig. 5.7, the band-edge linear spectrum is dominated by the three lowest X_1 , X_2 and X_3 electronic transitions of the CdSe QDs. Mathematically, the linear absorption spectrum is fit as

$$A(\omega) = \sum_{i=1}^3 \alpha_i \exp \left[- \left(\frac{\omega - \omega_i}{\gamma_i} \right)^2 \right] + N, \quad (5.4)$$

here, N is a constant term to monitor remaining unresolved absorption continuum, which was considered as the Rayleigh scattering. The fitting results are summarized in Tab. A.1.

Table 5.1: *Summarized fitting results of Linear Absorption spectrum on 4.9 nm CdSe QDs.*

	α_i			γ_i (meV)			ω_i (eV)		
Parameters	α_1	α_2	α_3	γ_1	γ_2	γ_3	ω_1	ω_2	ω_3
Values	0.1215	0.1098	0.2226	44.51	98.08	121.9	1.983	2.050	2.285

Figure 5.7: *Fitting results of the linear absorption spectrum on 4.9 nm CdSe QDs.*

5.4.2 Transient absorption fitting

As documented in our previous study on CdSe QDs ensembles using TA spectroscopy [201], by carefully enumerating all possible double-sided Feynman diagrams (DSFDs) accounting for the corresponding GSB, SE and ESA signals and combining the hole state spin-doubly degeneracy condition, the TA signal can be simulated with the expression below

$$\begin{aligned} \Delta A(\omega) \propto & - \sum_{i=1}^3 \alpha_i \exp \left[- \left(\frac{\omega - \omega_i}{\gamma_i} \right)^2 \right] - \alpha_1 \exp \left[- \left(\frac{\omega - \omega_1}{\gamma_1} \right)^2 \right] \\ & + \alpha_1 m_1 \exp \left[- \left(\frac{\omega - \omega_1 + \Delta_1}{\gamma_1} \right)^2 \right] + \frac{1}{2} \alpha_2 m_2 \exp \left[- \left(\frac{\omega - \omega_2 + \Delta_2}{\gamma_2} \right)^2 \right] \\ & + \alpha_3 m_3 \exp \left[- \left(\frac{\omega - \omega_3 + \Delta_3}{\gamma_3} \right)^2 \right], \end{aligned} \quad (5.5)$$

where m_i are the ratios between the absolute squares of the transition dipole moments for biexciton versus single exciton $m_i = \left| \mu_{X_1 \rightarrow X_1 X'_1} \right|^2 / \left| \mu_{g \rightarrow X_1} \right|^2$. The determined fit

parameters including the biexciton binding energy shift are summarized in Tab. A.2. The fit TA spectrum at $T_w = 100$ ps overlaid with the experimental data is depicted in Fig. 5.8.

Figure 5.8: *Fitting results of the TA spectrum on 4.9 nm CdSe QDs.*

Table 5.2: *Summarized fitting results of TA spectrum on 4.9 nm CdSe QDs.*

	Δ_i (meV)			m_i		
Parameters	Δ_1	Δ_2	Δ_3	m_1	m_2	m_3
Values	15.04	9.32	44.18	1.08	0.37	0.88

5.4.3 2DES simulation

With the combined information of the electronic state energy, relative transition dipole moment, total linewidth, weightage of different physical processes and biexciton binding energy shift of each energy band, ensemble 2DES spectrum is simulated by

$$\tilde{S}(\omega_t, \omega_\tau) = (R_R^*(\omega_t, -\omega_\tau) + R_{NR}(\omega_t, \omega_\tau)) I_{pump}(\omega_\tau - \omega_L) I_{probe}(\omega_t - \omega_L), \quad (5.6)$$

where $R_R(\omega_t, \omega_\tau)$ and $R_{NR}(\omega_t, \omega_\tau)$ are the third order nonlinear response functions for rephasing and nonrephasing signals accordingly with peakshape information defined by $g(t)$, the Kubo lineshape function with a Voigt profile in Eq. 5.2. $I_{pump}^*(\omega_\tau - \omega_L)$ is the spectrum of excitation pulses while $I_{probe}^*(\omega_t - \omega_L)$ is the one for white light continuum (treated as a constant function). A product peakshape 2D spectrum is calculated for each state first and then 2D spectra with different band-edge states are added. Eq. 5.6 is applicable as for a system whose response function does not vary with T_w , such as this case where the response function has no measurable spectral diffusion, the 2DES spectrum is a multiplication of the Voigt profile and excitation and emission spectra.

Figure 5.9: (a) Simulated 2DES spectrum with NLS; (b) Experimental 2DES spectrum at $T_w = 10$ ps with NLS.

We simulate the 2D spectrum at $T_w = 10$ ps by maintaining the total linewidth parameter obtained from linear absorption spectrum and varying the ratio between inhomogeneous linewidth and homogeneous linewidth. In this way, the line-broadening contributions from inhomogeneity and homogeneity can be evaluated. Thus, the electronic state, relative transition dipole moment, weightage of different physical processes and biexciton binding energy shift of each energy band are all fit parameters in the convolution model. Eventually, the best simulated result is the 2D spectrum when the linewidth

ratio is set to be 1.4 to 1 shown in Fig. 5.9 with NLS value of 0.47 which is a close match to the experimental data. The linewidth parameters of inhomogeneous broadening and homogeneous broadening from the final fits are listed in Tab. A.3.

Table 5.3: *Resulting inhomogeneous and homogeneous linewidths for X_1 , X_2 and X_3 states.*

	f_G (meV)			f_L (meV)		
States	X_1	X_2	X_3	X_1	X_2	X_3
Values	51.6	113.8	141.4	36.9	81.3	101.0

5.5 Discussion

From the 2D simulation, we see that for the band edge X_1 peak, our samples have a static inhomogeneous linewidth of 51.6 meV and a homogeneous linewidth of 36.9 meV. These values are considered smaller compared with the ones reported in previous literature for CdSe QD ensembles with slightly different sizes. For reference, Salvador *et al.* reported that CdSe QDs with diameters ranging from 30 Å to 40 Å and have inhomogeneous linewidths of 80 meV to 120 meV. The corresponding homogeneous linewidth is determined as 100 meV [192]. In 2006, they obtained the static inhomogeneity of 74 meV at 4 K for CdSe QDs [186]. We see that our fitted FWHM of the total linewidth for band-edge X_1 state, which is 74.11 meV, is considered narrow when comparing with the values reported above. It is not surprising because the average linewidths of QDs ensembles measurement have certain variations from batch to batch [208]. Besides, the bigger QDs they are, the smaller homogeneous linewidths will be [209]. Our results from the simulation model indicate that the contributions from inhomogeneity and homogeneity are comparable and for the first time the linewidth information is revealed not only for the band-edge X_1 state but also for the X_2 and X_3 states. Linewidth values increases with the higher level of electronic energy states. And the NLS value provides a single

indicator to quantify the homogeneous and inhomogeneous line-broadening contribution to the total linewidth.

When we intend to compare the inhomogeneity from 2DES spectra with the distribution of effective diameters from TEM image, there seems to be a disagreement between them two values. Our sample exhibit a 0.52 nm diameter dispersion as obtained from TEM (Fig. A.7 in Appendix A.5.1. Using theoretical sizing curve [163], this 4.9 nm CdSe QDs and 11% dispersion have a band-edge inhomogeneity with a FWHM of about 130 meV. This value is different from the 74 meV linear absorption linewidth (total linewidth), even without any homogeneous line-broadening contribution, not to mention the inhomogeneous linewidth obtained from 2DES. Such difference is not unique as similar phenomena and results have been presented in PbSe nanocrystals [200, 210, 211]. Spherical QDs are likely to appear elongated and non-symmetric properties, while normal TEM analysis interpret the size dispersion in 1D histogram which may be inadequate or misleading. Given such consideration, Jonas's group performed three sets electron microscopy measurements, including low-resolution TEM, annular dark field-scanning TEM and high resolution TEM, and grazing incidence small angle X-ray scattering measurement on spherical PbSe QDs to compare and determine the reasonable size and shape distribution for bandgap inhomogeneity [200]. With careful analysis, the size and shape inhomogeneous linewidth is determined as 81-86 meV which is comparable with the results of about 85 meV from 2DES spectra. Therefore, it is also likely that the 1D histogram TEM image analysis on CdSe QDs will not be an accurate determination of the size and shape dispersion.

As comparison, we perform the same experiments and NLS analysis on another batch of CdSe QDs ensembles with a slightly bigger average diameter of 5.9 nm and a smaller bandgap energy of 1.975 meV. All the measurement data and simulation results are listed in Appendix A.5.2. We see from the basic TEM analysis that this batch (batch II) of QDs share the very similar size and shape distribution of 11% indicating the same inhomogeneity with the batch of QDs described and discussed earlier (batch I). However, the

comparably small slope value of 0.35 shows apparently different line-broadening contributions between these two batches of QDs. From the linear absorption fitting, we learn that the batch II dots has narrower linewidths especially for X_2 states. This is clearly reflected in the NLS measurement and simulations. The 2DES spectrum simulations suggest that for batch II dots contributions from inhomogeneous and homogeneous line-broadening effects are still comparable whereas the homogeneous linewidth prevail slightly over the inhomogeneous linewidth. The linewidth ratio 0.82:1 leads to the inhomogeneity of the lowest three energy levels being 39.9, 57.4 and 110.8 meV while the homogeneity being 48.7, 70.0 and 135.1 meV respectively. The NLS behaves as a sensitive indicator for the inhomogeneity determination whose difference might be easily hidden by the basic TEM 1D histogram measurement.

5.6 Concluding remarks

In conclusion, we reported here that the NLS retrieved from 2DES spectra provides a unique determination of contributions from spectral diffusion, inhomogeneous and homogeneous line-broadening effects for band-edge energy levels on colloidal CdSe QDs ensembles. By carefully and accurately characterizing parameters such as total linewidths and biexciton binding energy shifts *etc.* from linear absorption and pump probe measurements, the indirect known information helps to analyze the 2DES NLS value so that further simulation and precise determination of band-edge inhomogeneity and homogeneity can be accomplished. We measure negligible spectral diffusion effects from the CdSe QDs within our measured window. Meanwhile, line-broadening contributions from inhomogeneity and homogeneity are comparable with each other at room temperature. In addition, the inhomogeneous linewidths vary to a certain extent from batch to batch as well as the single dot linewidth measurement reported in literature. The NLS value is a much more sensitive indicator compared with the 1D histogram TEM analysis of the size dispersion inhomogeneity. Furthermore, with the development of simulation method, it

can quantitatively determine the linewidth information of the lowest three energy levels, X_1 , X_2 and X_3 . The difference between the TEM size dispersion measurement and NLS method from 2DES suggests that improvement need to be made in the TEM image analysis of QD size dispersion.

Chapter 6

Development of Fifth-order Two-quantum Two-dimensional Electronic Spectroscopy

6.1 Introduction

Most 2D spectroscopy performed can be classified as third-order nonlinear optical spectroscopies. However, there is also 2D spectroscopy that belongs to fifth-order signals including two-quantum coherence information [12, 175, 212–215]. These fifth-order signals generated from five interactions with pulse sequences provide direct measurement of the energy transfer to higher excited states and reveal hidden couplings for multilevel systems. One approach is using five independently controllable excitation pulses with certain delays and relative phases as seen in Fig. 6.1a. When using a pump-probe geometry, the first four pulses act as excitation and the fifth one probes the population of energy levels. The resultant signal can then be Fourier transformed over coherence time t_1 and t_3 , so the purely absorptive 3D spectrum can be achieved [214, 216]. It is obvious that the independent control over the five pulses increases the complexity of the system and makes it hard to maintain a high level of stability of the pulse sequence. A much less complex approach to achieve fifth-order 2D spectrum is to conduct experiments on the basis of third-order 2D optical spectroscopy in which only three independent electric fields are required and multiple interactions for each pump pulse are allowed to reach two-quantum coherence.

As depicted in Fig. 6.1b, the first two excitation pulses both interact twice with the sample which leads to double-quantum coherences. Especially when a pump-probe geometry is adopted, a completely absorptive spectrum can be directly retrieved by the means of phase-cycling. Thus, theoretically, the third-order 2D nonlinear spectroscopic techniques can be extended to fifth-order two-quantum 2D electronic spectroscopy (2Q2DES) with minimal adjustment.

Figure 6.1: *Schematic layouts for fifth-order nonlinear spectroscopy. (a) five pulse interaction (b) three pulse interaction.*

6.2 Theory

In this section, we demonstrate the theoretical preparation for 2Q2DES on three-level system model and two coupled two-level system model respectively.

6.2.1 Three-level system

Following the logic of 2DES experiment, we present here the nonlinear phase-matching method of measuring the fifth-order signals for a three-level system. In perturbation theory, the material polarization can be expanded according to the number of interactions with the electric fields

$$P(\mathbf{k}, t) = P^{(1)}(\mathbf{k}, t) + P^{(3)}(\mathbf{k}, t) + P^{(5)}(\mathbf{k}, t) + \dots \quad (6.1)$$

In a 2Q2D experiment, the sample interacts five times with the incident lights, leading to a fifth-order polarization $P^{(5)}(\mathbf{k}, t)$ which is expressed by

$$P^{(5)}(\mathbf{k}, t) = \int \int \int dt_3 dt_2 dt_1 R^{(5)}(t_1, t_2, t_3) E_3(\mathbf{k}, t - t_3) E_2^2(\mathbf{k}, t - t_3 - t_2) E_1^2(\mathbf{k}, t - t_3 - t_2 - t_1), \quad (6.2)$$

here t_1 also known as τ in Fig. 6.1b, is the coherence time between first two excitation pulses; t_2 , known as T_w , is the waiting time between the second pump pulse and the probe. $R^{(5)}(t_1, t_2, t_3)$ is the response function, including all the fifth-order perturbation terms which contribute to the molecular polarization. This response can be presented using double-sided Feynman diagrams (DSFDs). The coherence transfer pathways are depicted in Fig. 6.2. Arrows labeling the vector $\mathbf{k}$ represent interactions with the light field. The curvy arrows represent the emitting signal, which all propagate in the same direction as the third probe pulse because the first two pump pulses are collinear and share the opposite $\mathbf{k}$ vectors. The sign rules in fifth-order DSFDs are opposite to those in third-order. Fig. 6.2b shows GSB and SE of the first excited state. Fig. 6.2c describes SE from the second excited state to the first excited state and the ESA signal respectively. Fig. 6.2d details the possible processes due to exciton annihilation during T_w .

The response function for the coherence transfer pathways plotted in Fig. 6.2 can be described as

$$R_1^{(5)}(\tau, T_w, t) = +\mu_{01}^4 \mu_{12}^2 \exp[-i(\omega_{01} + \omega_{12})\tau - i\omega_{01}T_w - i\omega_{01}t] F_1(\tau, T_w, t), \quad (6.3)$$

$$R_2^{(5)}(\tau, T_w, t) = +\mu_{01}^4 \mu_{12}^2 \exp[-i(\omega_{01} + \omega_{12})\tau - i\omega_{01}T_w - i\omega_{12}t] F_2(\tau, T_w, t), \quad (6.4)$$

$$R_3^{(5)}(\tau, T_w, t) = -\mu_{01}^2 \mu_{12}^4 \exp[-i(\omega_{01} + \omega_{12})\tau - i\omega_{12}T_w - i\omega_{12}t] F_3(\tau, T_w, t), \quad (6.5)$$

$$R_4^{(5)}(\tau, T_w, t) = -\mu_{01}^2 \mu_{12}^4 \exp[-i(\omega_{01} + \omega_{12})\tau - i\omega_{01}T_w - i\omega_{12}t] F_4(\tau, T_w, t), \quad (6.6)$$

$$R_5^{(5)}(\tau, T_w, t) = -\mu_{01}^4 \mu_{12}^2 \exp[-i(\omega_{01} + \omega_{12})\tau - i\omega_{01}T_w - i\omega_{01}t] F_5(\tau, T_w, t), \quad (6.7)$$

$$R_6^{(5)}(\tau, T_w, t) = +\mu_{01}^2 \mu_{12}^4 \exp[-i(\omega_{01} + \omega_{12})\tau - i\omega_{12}T_w - i\omega_{12}t] F_6(\tau, T_w, t), \quad (6.8)$$

Figure 6.2: (a) Model of three-level system; (b-d) DSFDs of fifth-order 2D double-quantum coherence transfer pathways under the desired partially collinear phase-matching condition; .

μ is the transition dipole moment and $F(\tau, T_w, t)$ is the dephasing and population relaxation function that describes the peak shape. It should be noted that here we only plot the rephasing signals for the sake of simplicity. There also exist their counterparts, the non-rephasing signals. We note that the processes $R_1^{(5)}, R_2^{(5)}$ and $R_5^{(5)}$ will give rise to crosspeaks at $((\omega_{01} + \omega_{12}), \omega_{01})$ in the frequency coordinates for 2Q2D, while the processes $R_3^{(5)}, R_4^{(5)}$ and $R_6^{(5)}$ will give rise to crosspeaks $((\omega_{01} + \omega_{12}), \omega_{12})$. Consequently, the possible appearance of exciton annihilation would compensate the intensity of the signal during time evolution T_w .

To enumerate the processes in three-level systems, here we define that α is the number of arrows pointing to the right minus to the number of those pointing to the left for the first pump pulse in the DSFDs. β is counted in the same rationale in the case of the second pump pulse. Since the pump pulses are collinear and the emission takes place in the probe direction, α and β are not independent [166]. Besides, during the waiting time T_w , the system must evolve in a population state, so we have $\alpha + \beta = 1$. For processes of fifth- and lower orders in a pump-probe geometry system, it constraints the value of α and β to be $|\alpha|, |\beta| \leq 2$ [215].

The light field in our 2Q2D experiment can be expressed by

$$\begin{aligned} E(t) = & A_1(t + T_w + \tau) \exp(-i\omega_L t + i\phi_{21} + i\mathbf{k}_{pump}r) \\ & + A_2(t + T_w) \exp(-i\omega_L t + i\mathbf{k}_{pump}r) \\ & + A_3(t) \exp(-i\omega_L t + i\mathbf{k}_{probe}r) + c.c. \end{aligned} \quad (6.9)$$

where ϕ_{21} is the relative phase between the two pump pulses.

The signal is heterodyne-detected by the probe pulse and spectrally resolved using a spectrometer. We assume that the pulses behaves like impulsive Dirac delta functions, the resultant signal from $R_1^{(5)}$ process can be described as

$$\tilde{S}(\phi_{21}; \tau, \omega_t) \propto \Re \left[\frac{F_R(\tau, \omega_t - \omega_{01}) \exp(i\Delta\omega_{02}\tau) \exp(i2\phi_{21})}{+ F_{NR}(\tau, \omega_t - \omega_{01}) \exp(-i\Delta\omega_{02}\tau) \exp(-i2\phi_{21}) + P} \right]. \quad (6.10)$$

where $\Delta\omega_{02} = \omega_{01} + \omega_{12} - \omega_{ref}$ is the resonant frequency detuned from the reference, which places the data collection in a rotating frame. The P term includes all the lower

order contributions. As only the real part of the measurement is taken, a post-data processing procedure is required to subject the collected data to causality condition [14]. Briefly, inverse Fourier transform is performed and then terms representing non-existed signal before time zero is discarded. Lastly Fourier transform is done again to bring it back to frequency domain. Thus, we get the desired absorptive signal

$$\begin{aligned} \tilde{S}(\phi_{21}; \tau, \omega_t) \propto & F_R(\tau, \omega_t - \omega_{01}) \exp(i\Delta\omega_{02}\tau) \exp(i2\phi_{21}) \\ & + F_{NR}(\tau, \omega_t - \omega_{01}) \exp(-i\Delta\omega_{02}\tau) \exp(-i2\phi_{21}) + P. \end{aligned} \quad (6.11)$$

6.2.2 Two coupled two-level system

Another interesting system is the coupled two-level system which is depicted by the diagram in Fig. 6.3a. The simplest model consists of two two-level systems, which is also termed as two-state system. Examples of such systems can be found in light-harvesting Chl clusters, quantum confined nanomaterials such as QWs and QDs *etc.*

Both the state a and b are one-quantum state with transition frequency of ω_a and ω_b accordingly. First we use matrix representatives to demonstrate the whole flow, which is also useful for simulation study in future work. The time-dependent form of the Schrödinger Equation can be given by a matrix interpretation. The Hamiltonian for the optical response of a system under the semi-classical dipole can be written as [34]

$$\mathbf{H}(t) = \mathbf{H}_0 - \boldsymbol{\mu} \cdot \mathbf{E}(t), \quad (6.12)$$

where $\mathbf{H}_0$ is the molecular Hamiltonian matrix for two two-level systems with bilinear coupling and $\boldsymbol{\mu}$ is the corresponding transition dipole operator matrix. They can be expressed as

$$\mathbf{H}_0 = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & \hbar\omega_b & V & 0 \\ 0 & V & \hbar\omega_a & 0 \\ 0 & 0 & 0 & \hbar(\omega_a + \omega_b) \end{pmatrix} \quad (6.13)$$

$$\boldsymbol{\mu} = \begin{pmatrix} 0 & \mu_b & \mu_a & 0 \\ \mu_b & 0 & 0 & \mu_a \\ \mu_a & 0 & 0 & \mu_b \\ 0 & \mu_a & \mu_b & 0 \end{pmatrix} \quad (6.14)$$

where V represents the bilinear coupling potential.

Figure 6.3: (a) A two-state system consisting of ground state 0 and two one-quantum state a and b, state c represents biexciton state when both a and b are excited; (b) Energy transfer of an uncoupled two-state system and its corresponding transition dipoles; (c) Energy transfer of a coupled two-state system and its corresponding transition dipoles.

We first assume $V = 0$, which means there is no coupling between state a and b . In this case, the Hamiltonian $\mathbf{H}_0$ is diagonalized initially, which means the state itself is the eigenstate basis, so energy transfer and its corresponding transition dipoles can be described by Fig. 6.3b. All the possible coherence transfer pathways can be described by DSFDs in Fig. 6.4. Here we only show rephasing signals, because the nonrephasing counterparts are analogous to the rephasing ones and located in another quadrant in frequency coordinate. In the position of $(-(\omega_a + \omega_b), \omega_a)$ on the 2D spectrum, there are six possible coherent transfer pathways, shown in Fig. 6.4a. It is noticeable that the resultant signals cancel out each other, leading to no peak at all. Similarly, in the position of $(-(\omega_a + \omega_b), \omega_b)$, the emitting signal would compensate themselves as seen in Fig. 6.4b. However considering exciton annihilation which results from population relaxation directly from two-quantum population to one quantum population or even to ground state, three more transfer pathways will be possible (shown in Fig. 6.4c and d). For cases that population relaxation can take place easily and rapidly which will be discussed later in this section, special care must be taken into account.

What happens when bilinear coupling potential $V \neq 0$? The molecular Hamiltonian matrix is no longer eigenstates because $\mathbf{H}_0$ now is not diagonalized. We take advantage of

Figure 6.4: *Feynman Diagrams for two-quantum pathways contributing to 2Q2D signal for uncoupled two-state system (a) with emission in the frequency of ω_a ; (b) with emission in the frequency of ω_b ; (c) with emission in the frequency of ω_a considering exciton annihilation; (d) with emission in the frequency of ω_b considering exciton annihilation.*

matrix operation to transform the matrix $\mathbf{H}_0$ to the eigenstate basis by diagonalization. Thus the transformed Hamiltonian matrix $\mathbf{\Lambda}$ is in eigenstate basis hence contains only on-diagonal element by using transform matrix $\mathbf{C}$. After diagonalization,

$$\mathbf{\Lambda} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & \hbar\omega_- & 0 & 0 \\ 0 & 0 & \hbar\omega_+ & 0 \\ 0 & 0 & 0 & \hbar(\omega_a + \omega_b) \end{pmatrix} = \mathbf{C}^+ \mathbf{H}_0 \mathbf{C}, \quad (6.15)$$

$$\mathbf{C} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos \theta & -\sin \theta & 0 \\ 0 & \sin \theta & \cos \theta & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}, \quad (6.16)$$

here

$$\omega_- = \frac{1}{2}(\omega_a + \omega_b) - \frac{1}{2}[(\omega_a - \omega_b)^2 + 4V^2]^{\frac{1}{2}}, \quad (6.17)$$

$$\omega_+ = \frac{1}{2}(\omega_a + \omega_b) + \frac{1}{2}[(\omega_a - \omega_b)^2 + 4V^2]^{\frac{1}{2}}, \quad (6.18)$$

$$\theta = -\frac{1}{2} \arctan \left[\frac{2V}{\hbar(\omega_a - \omega_b)} \right]. \quad (6.19)$$

Likewise, the transition dipole operator $\boldsymbol{\mu}$ is also transformed to the eigenstate basis by $\mathbf{C}$ and its transpose matrix $\mathbf{C}^+$ when it is real. We obtain the rearranged $\boldsymbol{\mu}'$

$$\boldsymbol{\mu}' = \begin{pmatrix} 0 & \mu_- & \mu_+ & 0 \\ \mu_- & 0 & 0 & \mu_- \\ \mu_+ & 0 & 0 & \mu_+ \\ 0 & \mu_- & \mu_+ & 0 \end{pmatrix}, \quad (6.20)$$

here we assume that $\mu = \mu_a = \mu_b$, $\mu_+ = \mu(\cos \theta + \sin \theta)$ and $\mu_- = \mu(\cos \theta - \sin \theta)$.

It is obvious that the energy level shifted, from $\hbar\omega_a$ to $\hbar\omega_+$, from $\hbar\omega_b$ to $\hbar\omega_-$, while the transition dipoles became μ_+ and μ_- rather than μ_a and μ_b , correspondingly shown in Fig. 6.3c. Fig. 6.5 plots possible coherent transfer pathways. Moreover, for each DSFD in Fig. 6.5a to d, only cases of state + getting excited earlier than state - are considered, but there exist other possible pathways which seem to share the same excitation scheme and still differ from each other with regards to the exact sequence of the one-quantum state + or -. For instance, as depicted in Fig. 6.5e, transition dipole of

Figure 6.5: DSFDs contributing to 2Q2D signal for coupled two-state system: (a) with emission of ω_a ; (b) with emission of ω_b ; (c) with emission ω_a considering exciton annihilation; (d) with emission ω_b considering exciton annihilation; (e) possible pathways which share the same DSFD in diagram(a).

the final signal alters when the order of forming or losing one-quantum or two-quantum coherence changes. Thus proper weights should be placed to take the sequence issue into consideration. Comparing with the result in case of non-coupling, it is obvious to find out that the resultant signal cannot be cancelled out even for the situations without the contribution from exciton annihilation.

Figure 6.6: *Ideal representation of 2Q2D spectrum for two-state system (blue represents positive signal while red indicates negative signal): (a) with neither coupling nor exciton annihilation; (b) with coupling but without exciton annihilation; (c) without coupling but with exciton annihilation; (d) with both coupling and exciton annihilation procedures.*

To sum up, we plot the ideal representation of 2Q2D spectrum for two-state system with or without coupling and with or without excited state annihilation in Fig. 6.6. In the cases of systems with no exciton annihilation processes, when no coupling appears between the two single-quantum states, no signal shows in probe frequency of ω_a and ω_b because all the processes compensate each other, while when there is a bilinear coupling, peaks with opposite signs would come out respectively. On the other hand, in systems that exciton annihilation commonly happens, its natural population relaxation processes will perturb the original system thus even in non-coupling situation, two positive signals will arise in corresponding position. Nevertheless in coupled system, two positive peaks show up with stronger intensity. We can conclude that 2Q2DES can show the difference between coupling system and non-coupling system with clear signatures. What's more,

the existence of annihilation will affect the desired features and make it hard to visualize the coupling character. Then kinetic analysis is of great necessity to justify any verdict.

6.3 Phase-cycling scheme

As we introduced in Section 6.1, our approach to achieve 2Q2DES is to carefully control the pulse sequence and number of interactions with each pump pulse. One straightforward approach is to take advantage of our partially collinear geometry and adopt the phase-cycling technique to extract the 2Q2D signal. Phase-cycling is needed to retrieve desired 2D signals for fully or partially collinear geometry 2D spectroscopic experiments [34, 35]. A 2D spectrum contains a summation of signals from different processes that weighted with different interpulse phase term [14]

$$\tilde{S}(\phi_{21}; \tau, \omega_t) = \sum_{\beta} s(\beta; \tau, \omega_t) \exp(i\beta\phi_{21}), \quad (6.21)$$

here β is +2 for fifth-order rephasing signal and -2 for fifth-order non-rephasing signal, determined by corresponding DSFDs. In order to obtain a specific $s(\beta)$ term, experiments need to be done repeatedly to obtain $\tilde{S}(\phi_{21})$. This process can be phrased as a discrete Fourier transform problem that describes a $1 \times L$ phase-cycling scheme

$$S'_{1 \times L}(\beta; \tau, \omega_t) = \frac{1}{L} \sum_{l=0}^{L-1} \tilde{S}(l \cdot \Delta\phi_{21}; \tau, \omega_t) \exp(-il\beta \cdot \Delta\phi_{21}), \quad (6.22)$$

Where $S'_{1 \times L}(\beta; \tau, \omega_t)$ is the signal obtained from the $1 \times L$ phase-cycling scheme. $\Delta\phi_{21} = 2\pi/L$ is the phase increment step. L is the number of phase sampled which also means the number of data collection processes needed to be done. After the summation of signal, the data is Fourier transformed along τ to achieve the 2D spectrum $S'(\beta; \omega_{\tau}, \omega_t)$.

But when collecting the data, due to aliasing, not only fifth-order polarization but also the other orders would be scanned. And lower-order signals always have much higher intensity, noise induced by fluctuation may easily perturb the less-intense higher-order signals. Certain phase-cycling scheme must be applied to select our desired signals.

6.4 Results and discussion

The experiments are built based on a two dimensional electronic spectroscopy with a partially collinear pump-probe geometry, which is widely used to achieve third order signal, for instance our study in Chapter 3 and 5. The only difference between the fifth order signal conducted here and the normal third order signal in terms of data collection is that both pump pulses interact twice instead once.

6.4.1 Experiments on Chl *a* molecules

In this section, we apply our 2Q2DES approach on chlorophyll(Chl) *a* sample. Relevant research on Chls has been studied to unveil its role played in light-harvesting complexes [217–219]. These Chl *a* molecules have a strong absorption coefficient at ~ 665 nm (15038 cm^{-1}) [220, 221]. They also show obvious excited state absorption below 640 nm (15625 cm^{-1}) [222], so there exists high-lying excited states which hopefully can be probed by 2Q2DES.

Chl *a* was purchased from Sigma Aldrich and dissolved in ethanol to provide an optical density of 0.6 at 650 nm (15385 cm^{-1}) in a 2 mm path length cuvette. Samples were placed in a flow cell with 1 mm optical path length to ensure that the pump beam excites different portions during data collection. All measurements were conducted at room temperature of approximately 298 K. The data collection was performed in a rotating frame with the reference wavelength of 730 nm (411 THz). The detuning frequency $\Delta\omega$ is 50 THz, which determined the Nyquist limit to be 10 fs. The delay τ between the two excitation pulses grew with a step size of 3 fs, over a total delay of 150 fs, satisfying Nyquist sampling.

The black line in Fig. 6.7 shows the linear spectrum of Chl *a* in ethanol overlaid with the pump spectrum. The main peak is assigned to the Q_y transition, within the plane of the porphyrin ring [223]. The peak maximum is centered at 665 nm (451 THz) with a bandwidth of ~ 20 nm (14 THz). The other peaks blue-shifted from the main peak are

generally assigned to a vibronic progression from the Q_y band [46, 224]. The red line represents the pump pulse spectrum with center wavelength at 650 nm (15385 cm^{-1}) and bandwidth of $\sim 20 \text{ nm}$ as well. The pump band is chosen not fully overlapping with the Q_y band because we intended to excite the vibronic features of Chl *a* ($\sim 640 \text{ nm}$) as well.

Figure 6.7: *Linear absorption spectra of Chl a in ethanol (black) with the spectrum of the pump pulse from the OPA (red).*

6.4.1.1 Pure absorptive spectra under different phase-cycling schemes

A. 1×3 phase-cycling scheme

Under 1×3 phase-cycling scheme, experiments need to be repeated through $0, 2\pi/3$, and $4\pi/3$. The data is collected to give

$$S'_{1 \times 3}(\beta = 2; \tau, \omega_t) \propto \tilde{S}(\phi_{21} = 0; \tau, \omega_t) + \tilde{S}\left(\phi_{21} = \frac{2\pi}{3}; \tau, \omega_t\right) e^{-i2 \cdot \frac{2\pi}{3}} + \tilde{S}\left(\phi_{21} = \frac{4\pi}{3}; \tau, \omega_t\right) e^{-i2 \cdot \frac{4\pi}{3}}. \quad (6.23)$$

where $\beta = 2$ is selected. Due to aliasing, when 2Q terms are collected,

$$S'_{1 \times 3}(\beta = 2; \tau, \omega_t) = S'_{1 \times 3}(\beta = (-1) + (+1) \times 3; \tau, \omega_t). \quad (6.24)$$

$S'_{1 \times 3}(\beta = (-1); \tau, \omega_t)$ term which is the 1Q term is collected as well but in the other quadrant.

The results are plotted in Fig. 6.8. The y axis has arbitrary unit here, however in real 2DES spectra there will be a frequency shift to calibrate it. In Fig. 6.8a, the 1Q signal shows in the positive quadrant whereas the simultaneous 2Q term shows in the corresponding negative quadrant. However, in Fig. 6.8b which selects the 2Q term, we see exactly the mirror image of Fig. 6.8a about the $\omega_1 = 0$ axis. And the 1Q signals have much larger intensity.

Figure 6.8: (a) Spectrum of Chl a by choosing $\beta = 1$ under 1×3 phase-cycling scheme; (b) Spectrum of Chl a by choosing $\beta = 2$ under 1×3 phase-cycling scheme.

B. 1×4 phase-cycling scheme

For 1×4 phase-cycling scheme, experiments need to be repeated through $0, \pi/2, \pi, 3\pi/2$. The data is collected to give

$$S'_{1 \times 4}(\beta = 2; \tau, \omega_t) \propto \tilde{S}(\phi_{21} = 0; \tau, \omega_t) + \tilde{S}\left(\phi_{21} = \frac{\pi}{2}; \tau, \omega_t\right) e^{-i2 \cdot \frac{\pi}{2}} + \tilde{S}(\phi_{21} = \pi; \tau, \omega_t) e^{-i2 \cdot \pi} + \tilde{S}\left(\phi_{21} = \frac{3\pi}{2}; \tau, \omega_t\right) e^{-i2 \cdot \frac{3\pi}{2}}. \quad (6.25)$$

when selecting 2Q signals, we simultaneously have the 2Q term showing in the other quadrant because of aliasing.

$$S'_{1 \times 4}(\beta = -2; \tau, \omega_t) = S'_{1 \times 4}(\beta = 2 + (-1) \times 4; \tau, \omega_t). \quad (6.26)$$

The results are depicted in Fig. 6.9. In Fig. 6.9a, the 1Q signal shows in the positive quadrant whereas the simultaneous seventh-order 3Q term shows in the corresponding negative quadrant. But in Fig. 6.9b which selects the 2Q term, both quadrants show the desired 2Q signals.

Figure 6.9: (a) Spectrum of Chl a by choosing $\beta = 1$ under 1×4 phase-cycling scheme; (b) Spectrum of Chl a by choosing $\beta = 2$ under 1×4 phase-cycling scheme.

Now we plot the 1Q2D third-order signal and 2Q2D fifth-order signal spectra in proper proportional coordinates. Fig. 6.10 shows the resultant third-order 1Q spectrum and corresponding fifth-order 2Q spectrum at $T_w = 300$ fs. In third-order 1Q spectrum shown in Fig. 6.10a, strong negative absorption can be seen at 15050 cm^{-1} (664 nm) with 15220 cm^{-1} (657 nm) laser pump, which was contributed by the processes of GSB and SE from the first excited state. The positive peak in Fig. 6.10a represents the excited state absorption. On the other hand, in fifth-order 2Q spectrum, it can be observed that a main positive peak appears at the same position as in 1Q spectrum with 30350 cm^{-1} (329 nm), almost the double frequency of the pump pulse, indicating the processes of GSB and SE from the 1st excited state as the DSFDs depicted in Fig. 6.2b. The negative peak at around 15700 cm^{-1} or above is assigned to be the combined process of excited state absorption (ESA) and SE from the higher excited state to the first excited

state transition as noted in Fig. 3c. It is of great interest that there is another positive sub-peak at 31280 cm^{-1} (320 nm) besides the double frequency excitation. We suspect that it corresponds to one of the manifolds for the higher excited state. However, further investigation is necessary to unveil the mystery.

Figure 6.10: (a) *Third-order 1Q2D spectrum of Chl a at $T_w = 300 \text{ fs}$* ; (b) *Fifth-order 2Q2D spectrum of Chl a at $T_w = 300 \text{ fs}$* .

C. 1×5 phase-cycling scheme

Similarly, for 1×5 phase-cycling scheme, experiments need to be repeated through $0, 2\pi/5, 4\pi/5, 6\pi/5$ and $8\pi/5$. We get

$$\begin{aligned}
 S'_{1 \times 5}(\beta = -2; \tau, \omega_t) &\propto \tilde{S}(\phi_{21} = 0; \tau, \omega_t) + \tilde{S}\left(\phi_{21} = \frac{2\pi}{5}; \tau, \omega_t\right) e^{-i2 \cdot \frac{2\pi}{5}} \\
 &+ \tilde{S}\left(\phi_{21} = \frac{4\pi}{5}; \tau, \omega_t\right) e^{-i2 \cdot \frac{4\pi}{5}} + \tilde{S}\left(\phi_{21} = \frac{6\pi}{5}; \tau, \omega_t\right) e^{-i2 \cdot \frac{6\pi}{5}} \\
 &+ \tilde{S}\left(\phi_{21} = \frac{8\pi}{5}; \tau, \omega_t\right) e^{-i2 \cdot \frac{8\pi}{5}}.
 \end{aligned} \quad (6.27)$$

Same scenarios are applied and showed in Fig. 6.11. Fig. 6.11a have 1Q signal in the positive quadrant and the possible 4Q term in negative quadrant. But in Fig. 6.11b which selects the 2Q term, we believe, in negative quadrant, 3Q signal is collected. The 3Q signal is truncated owing to the limited frequency window we chose.

Figure 6.11: (a) Spectrum of Chl a by choosing $\beta = 1$ under 1×5 phase-cycling scheme; (b) Spectrum of Chl a by choosing $\beta = 2$ under 1×5 phase-cycling scheme.

Figure 6.12: (a) Schematic of the revised three-level system with vibronic structure in its higher excited states; (b) DSFDs involving states $|0\rangle$, $|1\rangle$ and $|2\rangle$ only; (c) DSFDs involving state $|2'\rangle$ as well.

6.4.1.2 Peak interpretation using DSFDs

Under the condition that the Fourier transformed 2D spectrum was properly done and no significant noises perturbed the obtained peaks, we put forward a revised model of aforementioned three-level system to further interpret the processes taking place. The new model consists of four energy levels in which three of them make up a normal three-level system while the remaining one appears as one of the manifolds of the second excited states. I illustrate in Fig. 6.12a that state 0, 1 and 2 are the ground state, first excited state and second excited state respectively and they share the same energy intervals $h\omega_a$. The state 2 represents another high-lying excited state with a larger energy gap $h\omega_b$. All processes are depicted in Figs. 6.10b and c. Fig. 6.10b shows quantum pathways between state 0, 1 and 2. Due to the equal energy gap, processes from R_1 to R_4 overlap at the frequency of $2\omega_a$ and ω_a along pump and probe axes correspondingly. And their summation would give a positive peak with comparatively strong intensity under the assumption that the transition dipole moment μ_{01} is much larger than μ_{12} . Analogous to above analysis, R_5 and R_6 would provide a weak peak with an opposite sign at the position of $(2\omega_a, \omega_b)$ when assuming a rather small value of μ'_{12} . Meanwhile, R_7 and R_8 give rise to the positive sub-peak at $(\omega_a + \omega_b, \omega_a)$ while R_9 and R_{10} account for the extremely weak trough at $(\omega_a + \omega_b, \omega_b)$. Here I draw out the ideal representation of 2Q2D spectrum according to our four-energy-level system model in Fig. 6.13. It is clearly seen that there are four positions showing signals with two crests and two troughs, which is in good agreement with our experimental results. Based on the discussion, we conclude that there exists a high-lying excited state 2' in Chl a molecules which provides ESA signal in higher frequency region.

6.4.1.3 Contribution from third-order signals

In order to examine the authenticity of the signal, a power study was conducted to measure the dependence of the third-order and fifth-order signals on the pump pulse

Figure 6.13: *Ideal representation of 2Q2D spectrum explained by a proposed model of a three-level system with a vibronic structure.*

intensity. Theoretically, in impulsive limit, the third-order and fifth-order signals are proportional to

$$S^{(3)} \propto iR^{(3)}E_1E_2E_3, \quad (6.28)$$

$$S^{(5)} \propto iR^{(5)}E_1^2E_2^2E_3. \quad (6.29)$$

Here in our experiment, the only variables were the light fields of the two pump pulses which are equally intense by altering the energy per pump pulse from 5 nJ to 45 nJ, while the probe pulse energy was left consistent in time. We have

$$S^{(3)} \propto iR^{(3)}E_3I_{pump}, \quad (6.30)$$

$$S^{(5)} \propto iR^{(5)}E_3I_{pump}^2. \quad (6.31)$$

The experimental results were plotted in Fig. 6.14. It is clear from inspection that the third-order signal has a linear dependence on the increasing intensity of the pump. The fifth-order signal rises more rapidly with the increasing pump intensity, which gives

Figure 6.14: *Averaged Intensity of (a) third-order signal and (b) fifth-order signal as a function of pump power from 5 nJ to 45 nJ. 1Q signal gives a linear relationship with the pump intensity. 2Q signal shows a nonlinear quadratic growth.*

a quadratic-like parabolic growth. The mismatch in high intensity region is most likely due to the increasing exciton-exciton annihilation process. But more accurate work need to be performed to make sure no third-order signal can be contaminating. These results which coincide with the above theoretical analysis indicate that the collected 2Q signal should be expected as a fifth-order process.

6.4.1.4 Contributions from cascading signals

Although the power-dependence study confirms the 2Q signal as a fifth-order process, it only ensures the number of interactions with the light fields is five times. There is still doubt about the contribution from the third-order cascading signals. A cascading process involves two third-order excitations, in which an emitted third-order signal is absorbed by another chromophore nearby. These cascading signals have the same number of interactions with the light fields as the fifth-order signal and may satisfy the phase-matching condition as well. As a result, it is necessary to separate these fake fifth-order signals from the real ones.

To clear this doubt, a concentration-dependence study is done to verify the weight of third-order cascades out of our collected signals. As we tell from Eq. 6.29, the desired

Figure 6.15: *Intensity of the fifth-order 2Q signal as a function of sample OD ranging from 0.12 to 0.58 measured in 1 mm cuvette.*

fifth-order signal has a linear relationship with $R^{(5)}$, so it is linearly proportional to the product of concentration and sample path length, cl . However, for third-order cascading signals, the relationship can be described as [175, 213]

$$S_{cascades}^{(5)} \propto i^2 R^{(3)} R^{(3)} E_1^2 E_2^2 E_3. \quad (6.32)$$

Thus the cascading signals follow the scale-up of $c^2 l^2$, which should show a quadratic dependence on the concentration. Fig. 6.15 shows strong evidence of linear dependence, which leads to the conclusion that the major signal is the desired fifth-order spectrum and the cascading term do not contribute to the fifth-order 2QES signal.

6.4.2 Experiments on CdSe QDs

We run 1×4 phase-cycling scheme on colloidal CdSe QDs used in Chapter 5. Measurements are done with T_w ranging from -0.3 ps to 900 ps and all other experimental details can be found in Section 5.3. The collected 1Q2D spectra and 2Q2D spectra at

representative waiting times are depicted in Fig. 6.16. The peak shape features in 1Q2D spectra have been discussed in previous chapters. In brief, the negative signals represent the bleaching of X_1 and X_2 transitions, on top of which overlays the SE signal of the bandedge X_1 transition, and the positive peak in higher frequency position is the ESA signal from X_3 . However, when we look at the corresponding 2Q2D spectra in Figs. 6.16c, d and e, we see very similar features in the same probe energy positions with the opposite sign compared to their 1Q2D counterparts. The spectra experience overall decay dynamics from 0.3 to 900 ps for both 1Q2D and 2Q2D signals without obvious evolution in peakshapes.

Figure 6.16: 2DES spectra on CdSe QDs: (a-c) 1Q signals at $T_w = 0.3$, 10 and 900 ps; (d-f) 2Q signals at $T_w = 0.3$, 10 and 900 ps

Considering that our excitation pulse predominately covers the bandedge X_1 peak, we achieve that the calculated average number of excitons per QD $\langle N \rangle$ is ~ 0.24 . The fraction of QDs with multiple excitons follows the Poisson distribution [169, 225]:

$$P_N = \frac{\langle N \rangle^N \cdot e^{-\langle N \rangle}}{N!}. \quad (6.33)$$

where N is the number of excitons, and P_N is the fraction of QDs with N excitons. Therefore, we have the initial populations for our CdSe QDs sample $P_0 = 0.787$, $P_1 = 0.189$, $P_2 = 0.023$ and $P_3 = 0.001$. Bearing these values in mind, we can tell that there is considerable amount of population in single exciton and biexciton states under our experimental excitation density but negligible multiple excitons with $N \geq 3$. With time-resolved 2Q2D spectroscopy, we can foresee the multiple exciton dynamics by analysing kinetics of corresponding peak position.

We list all possible DSFDs for both 1Q2D and 2Q2D coherent transfer pathways in Fig. 6.17). For our partially collinear setup, there are only three and six possible transfer pathways left satisfying the phase matching condition corresponding to 1Q and 2Q signals respectively. Comparing Figs. 6.17b and c, we notice that the 2Q signals containing the kinetics of the GSB ($R_1^{(5)}$), SE of single exciton ($R_2^{(5)}$) and ESA ($R_3^{(5)}$) processes which are the same as those ($(R_1^{(3)}, R_2^{(3)})$ and $R_3^{(3)}$) in 1Q2D. The last three DSFDs ($(R_4^{(5)}, R_5^{(5)})$ and $R_6^{(5)}$) with initial biexciton population during waiting time T_w describe the biexciton dynamics.

We run global analysis on both 1Q2D and 2Q2D spectra. The results are shown in Fig. 6.18. The 2Q2D signals have much less intensity so the spectra seems to have very low signal-to-noise-ratio compared to 1Q2D. The best-fits are achieved with four-exponential decay and three-exponential decay respectively. 2DDAS spectra do not show much valuable information on peakshape evolution but they do show overall decays with different lifetimes. In the case of 1Q2D dynamics, the first 2D DAS on Fig. 6.18a (1.95 ps lifetime) features a positive cross peak below the diagonal at excitation wavelengths 635-640 nm. It infers that exciton equilibration within the excitation region occurs on a 2 ps timescale. It is noticed that the amplitude of this DAS is much smaller compared to the other three DAS spectra, which suggest minor contribution to the overall kinetic. The second DAS (Fig. 6.18b) shows a decay on ~ 30 ps with a comparably small amplitude. Although the experiments are conducted with minimal excitation energy, exciton-exciton

Figure 6.17: (a) Three-level system with state 1 and 2 representing single exciton and biexciton states respectively; (b) DSFDs of third-order 2DES single-quantum coherence transfer pathways; (c) DSFDs of fifth-order 2DES double-quantum coherence transfer pathways under the desired partially collinear phase-matching condition.

annihilation still remain a minor contribution to the kinetic. The third one suggests the Auger recombination rate which happens on hundreds of ps [65]. The final 2D DAS corresponds to a state that does not decay within our experimental T_w range, which is long-lived single exciton lifetime. The errors in the first three(1Q2D) or two(2Q2D) lifetimes did not exceed 35% respectively, but the last component for both fits has a large error (up to 150%) due to the limitation of our measurement range of a maximum of 900 ps.

Now we compare the populated bandedge X_1 peak kinetics in 1Q2D and 2Q2Q spectra and depict the spectral raw data along with the fits for references in Fig. 6.19. The data are taken by averaging over an area of a few nanometers around the maximum peak and are normalized to the intensity of the last time delay point. Such treatment is done because according to the DSFDs in 6.17 at long T_w s 2Q2D signals even with population relaxation involve the same single exciton population state. The 1Q2D and 2Q2D peak kinetics should share the same single exciton lifetime, which is apparently reflected by the overlapping from ~ 600 ps onwards in Fig. 6.19. However, we do observe a clear difference in kinetic comparison at short times.

Now we can measure the biexciton kinetic by simply extracting 1Q2D trace from 2Q2D trace and fit the extracted data using a single exponential decay. Similar treatment has been used in Ref. [65] for pump-dependent study on multi-exciton dynamics of smaller nanocrystals. Here in Fig. 6.20, we use the fitting data from global analysis above (the data that are the sum of several exponential components in Fig. 6.18) rather than the raw data in order to have a much cleaner trace. In this way, we avoid the misleading scattered data points owing to the background noise. The fit gives a lifetime value of 384 ps, which is to our knowledge the first report of biexciton lifetime studying using multi-dimensional electronic spectroscopy.

Figure 6.18: (a-d) Decay-associated spectra of 1Q2D signal, resulting from a four-exponential fit from 0.2 to 900 ps with best-fit lifetimes of 1.95 ps, 31.2 ps, 374 ps and 3727 ps; (g-i) Decay-associated spectra of 2Q2D signal, resulting from a three-exponential fit from 0.2 to 900 ps with best-fit lifetimes of 3.36 ps, 142 ps and 2385 ps; (e,j) horizontal slices at pump maximum extracted from 2DDAS analysis for 1Q and 2Q respectively; (f,k) Kinetic traces and corresponding fits probed at selected position at pump maximum for 1Q and 2Q respectively.

Figure 6.19: *Kinetic traces of 1Q2D and 2Q2D signals with fits.*

Figure 6.20: *Kinetic trace of extracted biexciton dynamics using single exponential decay term.*

6.5 Conclusion

In this chapter, we modify our partially collinear 2DES setup into a fifth-order 2Q2D electronic spectroscopy. The approach enables the system to detect the double-quantum coherence and biexciton population state. Using this technique, multi-exciton behaviour can be probed and analysed. Necessary phase-cycling scheme for retrieving higher-order signals are discussed with the help of the pulse-shaper in the setup and 1×4 phase-cycling scheme is confirmed as the preferred setting. Three-level system and two-state system model are discussed in theory to understand the peakshape features in latter experiments.

We then perform this 2Q2DES experiments on Chl *a* molecules. 2Q2D results indicates that our collected and post-processed signals are real fifth-order terms with no cascading contribution. Furthermore, the same techniques are used on colloidal CdSe QDs samples. Preliminary results show that the kinetics of 2Q and corresponding 1Q peaks indeed differ from each other at early waiting times. Global analysis gives the best-fit on both 1Q2D and 2Q2D kinetic. Biexciton dynamic with lifetime of 384 ps is obtained by extracting 1Q2D kinetics out of 2Q2D kinetic and fitting with a single exponential decay, which is the first report of such multi-exciton dynamics using multi-dimensional electronic technique. Further experiments, analysis and characterization are needed to reveal the full potential of our multi-quantum multi-dimensional electronic technique.

Chapter 7

Conclusions and Future Works

7.1 Conclusions

The motivation of observing ultrafast process and capturing instantaneous moment triggered the desire of developing ultrafast apparatus and techniques in the past half century. In terms of electronic, atomic and molecular motions, most events occurs on femtosecond and picosecond time scale. Among those events, highly efficient light-harvesting processes happening in photosynthetic systems and nano-scale semiconductor materials attract intense attention in biological and physical sciences. Ultrafast optical spectroscopy embedded with femtosecond laser pulses exerts a great potential to investigate the electronic structure and energy transfer and dynamics of these atomic and molecular systems. The work of this thesis is to develop multidimensional spectroscopy equipped with ultrafast femtosecond laser pulses to investigate the energy transfer flow and dynamics of plant photosynthetic systems and band structure analysis of synthesized colloidal semiconductor nanocrystals.

In green plants, LHCII antenna system takes on the very first function of light absorbing processes and has the unique light quenching process without photodamage under excess light radiation levels. 2DES allowing direct observation of correlation of excitation and emission energy polls, is carried out on solubilized trimeric and aggregated LHCII in order to study the non-photochemical quenching mechanics. Energy transfer pathways

and dynamics from Chl *b* to Chl *a* domains are monitored in unquenched and quenched excitonic states. Global analysis of the 2D spectra discerns that energy transfer from Chl *b* to Chl *a* occurs on fast and slow time scales for both LHCII. Owing to slow energy equilibration processes, long-lived intermediate Chl *a* states are present in solubilized trimers, while in aggregates, the population decay of these excited states is significantly accelerated, suggesting that, overall, the energy transfer within the LHCII complexes is faster in the aggregated state. In addition, 2DES experiments under conditions free from singlet-singlet annihilation and anisotropic decay are done in the following study. In this case, the energy transfer between the different domains within the Chl *a* manifold is investigated and found to proceed on time scales ranging from hundreds of femtoseconds to five picoseconds, before reaching equilibration. The uphill and downhill energy transfer of the equilibration process between excited states are clearly observed in experiments. The spectral forms associated with the different energy transfer lifetimes in the equilibration process are identified. Furthermore, exciton equilibration and excitation trapping in intact PSI complexes as well as core complexes isolated from *Pisum sativum* are studied using our room-temperature coherent 2DES. Global lifetime analysis resolved that exciton energy equilibration in the core antenna occurred on a timescale of 0.5 ps. Spectral equilibration component in the core complex with a 3-4 ps lifetime between the bulk Chl states and a state absorbing at 700 nm are further observed. Trapping in the core complex occurred with a 20 ps lifetime, which in the supercomplex split into two lifetimes, 16 ps and 67-75 ps. These data are carefully discussed, with further simulations it can offer remarkable evidence to the “red reaction centre” model.

Owing to the tunable optoelectronic properties of semiconductor nanocrystals and their enormous application in sensing, detecting and lasing *etc.*, understanding of the quantum confined band-edge structure has become an research focus in science and industry. In this thesis, ultrafast TA measurements are performed on CdSe core type QDs and subsequently a model to explain the features of the band-edge spectra is developed and described. The measured TA spectrum consists of contributions by the GSB,

SE and ESA processes associated with the three lowest energy transition of the QDs. The shapes of the GSB, SE and ESA spectral components after fitting the linear absorption are achieved. The spectral positions of the ESA components take into account the biexcitonic binding energy. In order to obtain the correct weightage of the GSB, SE and ESA components to the TA spectrum, we enumerate a series of coherence transfer pathways associated with these processes. From our fits of the experimental TA spectra of 65 Å diameter QDs, biexcitonic binding energies for the three lowest energy transitions are obtained. By carefully and accurately characterizing parameters such as total linewidths and biexciton binding energy shifts *etc.* from linear absorption and pump probe measurements, the indirect known information helps to analysis the 2DES NLS value so that further simulation and precise determination of band-edge inhomogeneity and homogeneity is accomplished. From the experimental NLS value, we obtain negligible spectral diffusion effects from the CdSe QDs on the time scale from sub-ps to $\sim$ ns and the inhomogeneity and homogeneity broadening are comparable at room temperature. In addition, the inhomogeneous linewidths varies to a certain extent from batch to batch as well as the single dot linewidth measurement reported in literature. This NLS method suggests a much higher sensitivity to the size dispersion inhomogeneity.

On the basis of partially collinear third-order 2DES, fifth-order 2DES technique are developed. This higher-order multidimensional electronic spectroscopy enable the detection of double quantum coherence, so that multi-excitonic behaviour can be probed. The implementation of this 2Q2DES is considered “cheap” because opto-mechanical modification is not necessary in our setup and phase-cycling scheme can be achieved by the embedded pulse-shaper in the pump path of the setup. Chl *a* molecules are used to test the validity of the system, indicating that our collected and post-processed 2Q2DES spectra are real fifth-order signal without cascading. Preliminary results on colloidal CdSe QDs show that the kinetics of 2Q and corresponding 1Q peaks indeed differ from each other although it seems that 1Q2D and 2Q2D signals involves the same electronic

states. Careful analysis and characterization of the dynamics is needed to reveal the multi-exciton generation and behaviours.

7.2 Future works

Future directions of this work arises from the improvement of multidimensional electronic spectroscopic technique and the interest of the new candidates of light harvesters. For complex multi-level system, third-order 2DES can only offer indirect information of their multi-step inner energy transfer pathways. Fifth-order 2Q2DES and 3D spectroscopy are promising ways to explore dynamics of complex multi-level molecular systems. Additionally, 2Q2DES will make it possible to gain insights into the two-photon absorption process and dynamics. On the other hand, 2DES with polarization control model of the excitation pulses can improve the ability of the current 2DES apparatus to the next level. This polarization-resolved capability enables the investigation into the anisotropy of chromophores. The different preference along orthotropic polarization would discern valuable structural, organizational or orientational information of large complex systems.

In terms of light harvesters, they always show quite different behaviours under low temperature. Thermal dynamics are suppressed to a large extent under such condition, which makes non-thermal related phenomena clearer to show up in multi-dimensional spectroscopy experiments. Thus, using a cryostat-assisted sampling assembling device allows us to study several other properties, such as the electronic quantum beat and coherence motions, the linewidth study with much less interfering of phonon broadening. For the colloidal semiconductor nanomaterials, nanoplatelets (NPLs) is an interesting new member of the nanomaterial family because of its flexible optoelectronic properties and out-standing monodispersity. Therefore, it will be interesting to look into the special properties of this NPLs using multi-dimensional electronic spectroscopy.

Appendix A

Appendix - Supporting Figures and Methods

A.1 Contribution of singlet-singlet annihilation to the excited-state decay in trimers and aggregates of LHCII experiments

We applied the approach described in the work of Barzda *et al.* [95], of fitting a kinetic model including annihilation to the transient absorption data and determining the second-order kinetic rate constant of annihilation for trimers and aggregates. The inverse rate constants of singlet-singlet annihilation (γ^{-1}) of LHCII were previously found to be in the range of 24-86 ps in solubilized trimers and 16-18 ps in aggregates [95, 103, 226]. The simulated decay traces overlaid on the experimental integrated transients are plotted in Fig. A.2. The estimated inverse annihilation rate constants are $\gamma^{-1} = 60$ ps for solubilized trimers and $\gamma^{-1} = 38$ ps for aggregates. Consistent with previous studies [95, 226], the quenched form of LHCII seems to promote faster annihilation rate, which has been explained as being the result of conformational changes affecting the rate of intermonomer energy equilibration. The fits also accurately model the observation that in trimers, annihilation only occurs within the early timescales (< 100 ps), whereas in the aggregates, owing to their large domain size, annihilation can be observed over the entire timescale of Chl *a* excitation decay. The fits to the annihilation in trimers (based

Figure A.1: 2D decay-associated spectra of LHCII trimers (a-d) and aggregates (e-h) resulting from global analysis of the 2D spectra obtained at waiting times of 0.2-800 ps, using excitation pulses of 10 nJ energy. A narrower bandwidth of the pump pulse (~ 12 nm) is used here, resulting to narrower 2D DAS signals along the excitation wavelength λ_1 compared to the 2D DAS using 15 nJ excitation energy presented in Fig. 3.3. The pump pulse covers the Chl b Q_y region and hence correspondingly describes the energy transfer pathways from Chl b to Chl a.

on $\gamma^{-1} = 60$ ps) have no fast-decaying component around 3 ps, which denotes that there is no contribution of annihilation on this sample. For aggregates, the faster annihilation rate on the aggregates ($\gamma^{-1} = 38$ ps) exhibits a decay component of ~ 3 ps with a small relative amplitude ($\sim 5\%$), suggesting only minimal contribution to the corresponding 2D DAS of aggregates.

Figure A.2: *Experimental kinetic traces (circles) of integrated 2D signals at the Chl a band of trimers and aggregates taken at low and high power excitations, overlaid with fits (lines) resulting from modelling of the annihilation kinetics. The estimated annihilation rates (γ) are 60 ps^{-1} for the trimers and 38 ps^{-1} for the aggregates. The red lines represent the simulated Chl a decay based on time-correlated single photon counting (TCSPC) measurement.*

Another way to test whether annihilation could account for the observed picosecond decay of the intermediate state is simply by inspecting the measured time traces at excitation wavelength of 672 nm and 680 nm and the simulated annihilation decay curve in a 0-5 ps region (Fig. A.3). The simulated curve represents excited-state decay in the absence of energy transfer; hence, it does not rise in the first 2 ps. The curve shows that together annihilation and quenching of the thermally equilibrated excited states can only recover about 10% of the ground state within 5 ps. Evidently, the measured kinetic curve at $\lambda_1 = 672$ nm decays more rapidly (25% decay in 5 ps). Therefore, it can be

confirmed that the intermediate states around 672 nm decay via an intrinsic pathway, which cannot be accounted for by singlet-singlet annihilation.

Figure A.3: Comparison of decay of Chl *a* signals at $\lambda_3 = 672$ and 680 nm in trimers and aggregates. The kinetic traces are integrated signals from the 2D spectra recorded using low power excitation (10 nJ). The red lines represent the simulated Chl *a* decay based on TCSPC measurement.

A.2 Simulation of 2DES spectra and contribution of EET lifetimes to the overall kinetic in Chl a equalibrium of LHCII

The model encompasses a defined number of exciton states and a rate equation matrix describing couplings between them (EET). The exciton states are described by four spectral parameters: transition energy, transition dipole moment, inhomogeneous bandwidth and homogeneous bandwidth. Coupling and population transfer between states (EET) is calculated by solving a rate equation (kinetic) model with the rate constant matrix. Inhomogeneous broadening is simulated by repeatedly calculating the microscopic response of the system for different random realizations sampled from a distribution of eigenstate energies.

The kinetic system, solved analytically, has three EET lifetimes - 0.46 ps, 0.58 ps and 4.8 ps. The first two lifetimes, contributed by EET steps $1 \rightarrow 2$ (663 $\rightarrow$ 667 nm) and $3 \rightarrow 4$ (673 $\rightarrow$ 680 nm), are of similar value and the kinetics can be described well by a single 0.53 ps 2D DAS (Fig. A.4c). The 4.8 ps lifetime (Figure 4d) is due to the slow $2 \rightarrow 4$ transfer, i.e. equilibration between the 663/667 nm pool and the 673/680 nm pool. The simulated 2D DAS bear close similarity to the experimental 2D DAS (Figs. A.4a and c), in terms of cross-peak positions and relative amplitudes. A model incorporating more states with structure-based constraints on the rates and energy levels [83, 101, 109, 113], would be necessary to simulate finer details in the experimental data.

How significant is the slow “bottleneck process with respect to the overall exciton equilibration time? The answer is given by the fractional amplitude of the 5 ps component relative to all spectral equilibration components. However, because of the limited time resolution of the experiment, faster EET components may be unresolved by the global analysis. Despite the limitation, we can approximately estimate the relative contribution of fast EET from a given excited state to the lowest-energy acceptor. Firstly,

Figure A.4: Theoretical model with four coupled states (Chl a), absorbing at 663, 667, 673 and 680 nm. (a) schematic diagram of the model parameters C absorption wavelength (λ), transition dipole moment (μ), EET time constants in ps; (b) simulated linear absorption spectra of the four exciton states; (c,d) contour plots of 2D DAS obtained by fitting two EET lifetimes.

the “EET-free amplitude of a 2D cross-peak is calculated, *i.e.* the amplitude after excitation without any population transfer. To this end, the linear absorption spectrum of LHCII in the Q_y region is decomposed into 14 Gaussian bands (250 cm^{-1} FWHM) and respective higher-energy vibronic sidebands (determined by fitting to the mirrored LHCII fluorescence emission spectrum). Using this model, the EET-free amplitude at any excitation and detection wavelength is calculated, taking into account the experimental frequency resolution on both axes and neglecting coupling between states. If spectral equilibration components are completely resolved by global analysis, the obtained value should be equal to the summed cross-peak amplitudes of the EET 2D DAS. The difference is the contribution of fast EET components not resolved by global analysis. Following this method, we obtained a value of 34% as an upper estimate for the relative contribution of fast ($< 150\text{ fs}$) EET components to the spectral equilibration kinetics upon 665 nm excitation. Consequently, the minimum contribution of the 0.5 ps and the 4.8 ps components to the overall equilibration time is 26% and 40%, respectively.

A.3 Calculation of excitation density in PSI complex

The excitation density in the sample, i.e. the average number of excitations per complex after interacting with the excitation laser pulse, can be approximately estimated from the ratio $\Delta A/2A$, where ΔA is the magnitude of the differential absorption signal induced by the excitation pulses and A is the total (ground-state) absorbance of the sample. The absorbance of the LHCII sample solution at the Chl *a* Q_y maximum was adjusted to 0.3 and the maximal pump-probe signal for data collected at 2 nJ pulse energy (=1 nJ energy per pulse in 2DES) was no more than 2 mOD. Thus, the calculated excitation density is $\sim 0.3\%$ of Chl *a*. In PSI core complex, there exist 98 Chl *a*. Therefore, the excitation density is $\sim 30\%$ in the sample and there is less than one excitation in a PSI complex per pulse.

A.4 Supporting figures of TA study on CdSe QDs

Figure A.5: The overlap between the laser pulse spectrum and the fitted Gaussian peaks for (a) X_1 state (b) X_2 state. The "Spectral overlap" in blue is the multiplication of the laser pulse spectrum and the fitted Gaussian peaks.

Fig. A.5 shows the overlap between the laser pulse spectrum and the fitted Gaussian peaks for X_1 state and X_2 state. As can be seen, there is a considerable difference between the two overlaps (in blue). It is clear that the amount of contributions from X_2 state is much smaller compared with the one from X_1 transition. Besides, there is no overlap between the pump pulse and X_3 transition at all. The ratio of excited X_2 population versus X_1 population is estimated as $\sim 15\%$.

In Fig. A.6, we present the normalized TA spectra for $T = 0.3, 1.0, 10$ and 100 ps. It can be seen that there are differences between the TA spectra $T = 0.3, 1.0$ ps and $T = 10, 100$ ps in the region of 2.03 eV corresponding to the peak of X_2 transition. The former group having a slightly higher amplitude than the latter. This is consistent with the fact that if we assume that some X_2 states are initially excited with the pump pulse, there will be some Stimulated Emission (SE) signal (hence higher amplitude for the $T =$

0.3, 1.0 ps TA signals) which will be depleted after the decay to X_1 after a timescale of $T = 10$ ps (hence lower amplitude for the $T = 10, 100$ ps TA signals).

Figure A.6: *The transient absorption spectra at $T = 0.3, 1.0, 10$ and 100 ps.*

A.5 More results on the NLS analysis of 2DES of CdSe QDs

A.5.1 Size distribution of 4.9 nm CdSe QDs

Figure A.7: (a) TEM image of 4.9 nm CdSe QDs; (b) Corresponding 1D histogram for size distribution from TEM image: the mean diameter is 4.9 nm and the standard deviation of distribution is 0.52 nm.

A.5.2 NLS results on batch II of CdSe QDs

Figure A.8: (a) TEM image of 5.9 nm CdSe QDs; (b) Corresponding 1D histogram for size distribution from TEM image: the mean diameter is 5.9 nm and the standard deviation of distribution is 0.65 nm.

Figure A.9: (a) Fit results of the linear absorption spectrum for 5.9 nm CdSe QDs. (b) Fit results of the TA spectrum for 5.9 nm CdSe QDs.

Figure A.10: (a) Experimental 2DES spectrum at $T_w = 80$ ps; (b) Simulated 2DES spectrum.

Table A.1: Summarized fitting results of Linear Absorption spectrum on 5.9 nm CdSe QDs.

	α_i			γ_i (meV)			ω_i (eV)		
Parameters	α_1	α_2	α_3	γ_1	γ_2	γ_3	ω_1	ω_2	ω_3
Values	0.165	0.1927	0.2699	43.19	62.15	119.9	1.975	2.058	2.252

Table A.2: *Summarized fitting results of TA spectrum on 5.9 nm CdSe QDs.*

	Δ_i (meV)			m_i		
Parameters	Δ_1	Δ_2	Δ_3	m_1	m_2	m_3
Values	9.22	56.89	67.67	1.073	1.072	0.8205

Table A.3: *Summarized fitting results of 2DES spectrum on 5.9 nm CdSe QDs.*

	f_G (meV)			f_L (meV)		
States	X_1	X_2	X_3	X_1	X_2	X_3
Values	39.9	57.4	110.8	48.7	70.0	135.1

Author's Publications

- (i) **Cheng Zhang**, Thanh Nhut Do, Xuanwei Ong, Yinthai Chan, and Howe-Siang Tan. “Understanding the features in the ultrafast transient absorption spectra of CdSe quantum dots”, *Chemical Physics*, **481** (2016): 157-164.
- (ii) Parveen Akhtar, **Cheng Zhang**, Thanh Nhut Do, Gyözö Garab, Petar H. Lambrev, and Howe-Siang Tan. “Two-dimensional spectroscopy of chlorophyll a excited-state equilibration in Light-Harvesting Complex II”, *The journal of physical chemistry letters*, **8** (2016): 257-263.
- (iii) Parveen Akhtar, **Cheng Zhang**, Zhengtang Liu, Howe-Siang Tan, and Petar H. Lambrev. “Excitation transfer and trapping kinetics in plant Photosystem I probed by two-dimensional electronic spectroscopy”, *Photosynthesis Research*, Accepted, Manuscript No. PRES-D-17-00073R2 (2017) DOI:10.1007/s11120-017-0427-2.
- (iv) Miriam M. Enriquez, Parveen Akhtar, **Cheng Zhang**, Gyözö Garab, Petar H. Lambrev, and Howe-Siang Tan. “Energy transfer dynamics in trimers and aggregates of light-harvesting complex II probed by 2D electronic spectroscopy”, *The Journal of chemical physics*, **142**, (2015): 212432.

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