Studies on the basic building blocks of light-harvesting complexes using ultrafast two-dimensional electronic spectroscopy

Muhammad Faisal Bin Khyasudeen

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**NANYANG
TECHNOLOGICAL
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**Studies on the Basic Building Blocks of Light-Harvesting
Complexes Using Ultrafast Two-Dimensional Electronic
Spectroscopy**

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

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

2019

Statement of Originality

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Assoc. Prof. Tan Howe Siang

Authorship Attribution Statement

This thesis contains material from one paper published in the following peer-reviewed journal where I was the corresponding author.

Chapter 3 is published as Nowakowski, P. J.; Khyasudeen, M. F.; Tan, H.-S. The Effect of Laser Pulse Bandwidth on the Measurement of the Frequency Fluctuation Correlation Functions in 2D Electronic Spectroscopy. *Chem. Phys.* **2018**. <https://doi.org/10.1016/j.chemphys.2018.06.015>.

The contributions of the co-authors are as follows:

- Assoc. Prof Tan Howe Siang provided the initial project direction and edited the manuscript drafts.
- I and Dr. Pawel Jacek Nowakowski performed the 2DES experiment and interpreted the data.
- Dr. Pawel Jacek Nowakowski prepared the manuscript drafts. The manuscript was revised by me and Assoc. Prof Tan Howe Siang.

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Abstract

This dissertation describes the development of ultrafast two-dimensional electronic spectroscopy (2DES) using pump-probe geometry and its application in interrogating the underlying femtosecond to picosecond dynamics of basic building blocks in plant light-harvesting complexes (LHC). In the second chapter of this thesis, we provide the description and development of 2DES using a partially collinear geometry. The procedure for post analysis such as conversion of raw data into a purely absorptive 2D data was included in this part of the thesis.

The application of 2DES is divided here into three main chapters. In chapter 3, we aim to investigate the effect of finite bandwidth of the interaction pulses in retrieval of the FFCF from 2D spectrum using three different methods (CLS_{ω_1} , CLS_{ω_3} , ellipticity). Although all of the methods show correct values in broad excitation bandwidth, our results show that it does not hold true when the excitation bandwidth becomes narrower than the studied absorption band. We used Chl *a* molecules to test and show that with the help of simulation of the 2D spectra, it is possible to recover the FFCF using any of these methods.

Chl *a* and Chl *b* are major constituent pigments in LHC complexes. The primary roles of Chl molecules is to absorb light and transfer the energy on a sub-picosecond timescale to the reaction center for the light-chemical energy conversion. It is well established that proteins surrounding Chl molecules play a significant role in optimizing this process. Therefore, understanding the effect of local environment on Chls electronic transition is an important subject to study. 2DES provides a remarkably sensitive tool to study the solute-solvent interaction

with high spectral and time resolution. Accompanied by the center line slope (CLS) analysis, in chapter 4, we elucidate the spectral diffusion dynamics of Chlorophyll *a* (Chl *a*) and Chlorophyll *b* (Chl *b*) in various chemical environments. 2DES was used to measure the frequency fluctuation correlation function (FFCF) of Chl *a* and Chl *b* electronic transition. Three time scales and amplitudes of the frequency fluctuations were recovered for the lowest excited state of Chl ranging from hundreds of femtoseconds to picosecond timescales and assigned as the solvation dynamics and spectral diffusion. By measuring them in various solvents, our results revealed significant differences in the extent of inhomogeneous broadening depending on the solvent used, with the biggest contribution of inhomogeneous broadening being due to the polar hydrogen bond solvent and smallest due to the nonpolar solvents. Interestingly, by comparing the results between Chl *a* and Chl *b*, our measurements indicated an effect of substituent group in porphyrin ring at position 7 on the rate of relaxation dynamics from an initially inhomogeneous broadening becoming more homogeneous at later T_w (population time). Such evolution was found to be faster for Chl *a* than Chl *b* as described in the chapter 4 of this thesis.

In the last part of this study (Chapter 5), we utilized 2DES to observe the mechanism of population transfer from the Q_x band (S_2 state) to the Q_y band (S_1 state) in Chl *a* molecule. An ultrafast relaxation from Q_x to Q_y band was observed to take place in less than $T_w=150$ fs. Furthermore, observing the cross peak after excitation of the Q_x band reveals the type of correlation between the two transition dipole moments. Our results indicate that the Q_x and Q_y band exhibit minimal correlation even at very short population times. We suggest a possible mechanism explaining lack of the correlation that is based on the fact that the Q_x

and Q_y transition dipole moments are orthogonally oriented with respect to each other.

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studies. In addition, I owed to my family members, especially my parents who have been motivating me emotionally when there are hard times during my research studies. Last but most important of all is my supervisor Assoc. Prof Tan Howe Siang, without whom this thesis would not be possible. He introduced me into the exciting world of ultrafast spectroscopy which now I find hard to abandon. His continuous encouragement and countless discussions have changed the way I think not only in the scientific world but also life in general.

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List of Abbreviation

2DES	Two-dimensional electronic spectroscopy
2DEV	Two-dimensional electronic-vibrational spectroscopy
2DIR	Two-dimensional infrared spectroscopy
2DOS	Two-dimensional optical spectroscopy
2Q2D	Two quantum two-dimensional electronic spectroscopy
3PEPS	Three photon echo peak shift
ABS	Absorption
AOPDF	Acousto-optic programmable dispersive filter
B band	Soret band
Bchl	Bacteriochlorophylls
CCD	Charge couple device
Chl	Chlorophyll
Chl-e6	Chlorin-e6
Chp	Chopper
CLS	Centre line slope
DCM	Chirp mirror

DPPG	1,2-Dipalmitoyl-phosphatidyl-glycerole
DPTAP	1,2-dipalmitoyl-3-trimethylammoium-propane
EET	Excitation energy transfer
EMI	Emission
ESA	Excited state absorption
FFCF	Frequency fluctuation correlation function
FID	Free induction decay
FL	Focusing lens
FT	Fourier transform
FWHM	Full width half maximum
FXCF	Frequency fluctuation cross correlation function
GVD	Group velocity dispersion
H ₂ PC	Metal-free phthalocyanine
HPLC	High performance liquid chromatography
HWHM	Half width half maximum
IRF	Instrument response function
LD	Linear Dichroism
LHC	Light- harvesting complexes
MCD	Magnetic circular dichroism

MeOH	Methanol
Mg	Magnesium
MgMP-HRP	Magnesium-mesoporphyrin horseradish peroxidase
MgOEP	Magnesium octaethylporphyrin
NA-ESMD	Non-adiabatic excited-state molecular dynamics
ND	Neutral density
NOPA	Non-collinear optical parametric amplifier
NPQ	Non-photochemical quenching
PM	Parabolic mirror
Pol	Polariser
Py	Pyridine
QM	Quantum mechanical
S1/Q _y	1 st excited state of Chlorophyll
S2/Q _x	2 nd excited state of Chlorophyll
SPM	Self phase modulation
TAS	Transient absorption spectroscopy
THF	Tetrahydrofuran
Et ₂ O	Diethyl Ether
TPP	Tetraphenylporphyrin

T_w	Population time
W_d	Wedge
WLC	White light continuum

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Chapter 1

Introduction

1.1 Chlorophyll in plants photosynthesis

Chlorophyll (Chl) is a primary photosynthetic pigment and has an important role in Light-Harvesting Complexes (LHC). Its main function is to capture the light energy from the Sun and transfer the excitation energy to the reaction center where light-chemical energy conversion process occurs. In each subunit of light-harvesting complex II (LHC II) trimer, there are 14 Chl molecules embedded in the protein scaffold from which eight of them are chlorophyll *a* (Chl *a*) and six are chlorophyll *b* (Chl *b*). The positions of these Chl molecules in LHC II trimer have been successfully determined at atomic resolution by X-ray diffraction studies [1,2]. As depicted in Figure 1-1 (A and B), Chl pigments assemble in a tightly packed arrangement in a group of 2-4 molecules. This crowded arrangement establishes strong coupling between the Chls and thus enable the fast excitation energy transfer (EET) to occur before the deactivation of the excitation energy by the decay of lowest excited state (fluorescence lifetime of Chl in LHCII oligomers is between 0.2 and 1 ns [3]). As reported by van Grondelle *et al.* and Akhtar *et al.* , the process of EET via incoherent Förster energy transfer occurs in hundreds of femtosecond to 20 picosecond which is remarkably faster than the fluorescence lifetime of Chl [4,5].

Figure 1-1 The LHC II trimer from a top view (A) of the stromal side and side view (B). Grey, cyan, green and orange color indicate polypeptide Chl *a*, Chl *b*, and carotenoid, respectively. Reproduced with permission from [1]

On top of the process of EET, Chl engage in plants regulatory mechanism by dissipating the excessive amount of light as heat. The process known as non-photochemical quenching (NPQ) is believed to be the main mechanism responsible for the protection of the delicate photosynthetic apparatus from any photo cellular damage [6]. In strong light condition, the NPQ mechanism will quench the excited state lifetime of Chl and prevent the formation of triplet state from the singlet oxygen species[7]. This triplet state is believed to be hindered

by the modification of the LHC conformational structure and variation in the strength of Chl-Chl and Chl-carotenoid interactions. [8,9]

In addition to the pigment-pigment interaction, pigment-protein interaction also has a prominent role in plant photosystems. The interaction between Chl and protein allows conformational control of Chl and therefore offers some degree of flexibilities for its structural and spectral properties modification. Recent attention has been given to the discovery of long-lived coherence in the LHC which is purported to enhance the EET process. Despite at a non-optimum condition such as low temperature, the system still maintained its function efficiently [10]. This suggests that the survival of these processes is governed by the involvement of protein scaffolding providing an adaptation to the modulated fluctuation. Figure 1-2 shows the energy diagram and overall process of Chl *a* and Chl *b* in term of Jablonski diagram.

Figure 1-2 Jablonski diagram of Chl *a* and Chl *b* molecules. The purple and light red upward arrow indicates the absorption of photon from the ground state to excited states (S_1/S_n). The downward arrow indicates the radiationless energy dissipation as heat energy, fluorescence, and phosphorescence which compete with the photosynthesis process.

One of the main challenges for researchers, especially in the engineering and scientific fields, is to integrate and mimic the plant's energy conversion process into a transducer device that can be used as an alternative renewable energy resource. While the overall efficiencies of PV in solar energy conversion is considered higher than the photosynthetic organism (based on Blankenship *et al.* suggestion that the efficiencies of PV and photosynthetic organism should be compare between the H₂O splitting efficiencies (10-11%) and energy efficiencies of photosynthesis (<1%) which is define as energy content (heat of combustion of glucose to CO₂ and H₂O) of biomass divided by solar irradiance over the same area [11]), there are ideas to incorporate PV with other highly efficient fundamental processes occurs in plants such as transfer of energy, electron, photoisomerization, quantum coherence and redox chemistry as possible ways to improve the current efficiencies of PV systems. Another approach is to bio-inspired the basic aspects of plant apparatus such as their basic building blocks either in vivo or in vitro. Thus, it is one of the primaries aims of this dissertation, to better understand the spectral characteristic of basic building blocks of plants namely Chl *a* and Chl *b* using the recent technique called ultrafast two-dimensional electronic spectroscopy (2DES).

1.2 Chemical structures of chlorophylls

The spectral properties of Chl are strongly influenced by its chemical structure. The difference in the side chain group of porphyrin ring, for example, shifts the spectrum of Chl either to the red or blue side of the spectrum. A straightforward example is Chl *a* and Chl *b*. As shown in Figure 1-3, the change of methyl to a formyl group at position R₇ (According to IUPAC numbering) shifts the lowest excited state of Chl (Q_y band) to the blue side of the spectrum, from 665 nm in Chl *a* to 650 nm in Chl *b*. Aside from Chl *a* and Chl *b*, there are also various other types of chlorophylls that exist in nature. In different photosynthetic organisms there are pigments such as Chl *c*, Chl *d*, and Chl *f* (in the order of their discovery) which differ by the substituent group of the side chain at either position R₂, R₃ or R₇. Where Chl *a* and Chl *b* can be found commonly in most green plants, algae and cyanobacteria, Chl *c* and Chl *d* were reported to be found in brown and red algae respectively [12,13]. For Chl *d* and *f*, they were discovered in cyanobacterium *Acaryochloris marina* and stromatolite of Cyanobacteria from Shark Bay off the coast of Western Australia [14,15]. In addition, there are also Chl analogs that were discovered in bacteria or artificially synthesized such as Bacteriochlorophylls (BChl), Magnesium octaethylporphyrin (MgOEP), Chlorin-e6 and Tetraphenylporphyrin (TPP). In Figure 1-3 and Figure 1-4, we present the structure of Chl *a* and Chl *b* with their linear absorption spectrum compared to their analogs spectrum obtained from literature [15,16].

Figure 1-3 The structure of Chl *a* and Chl *b* with their respective linear absorption spectrum in common solvent. Reproduce with permission from

[15]

B

Figure 1-4 (A) Structures of BChl, MgOEP, Chl-e6, and TPP and (B) their respective linear absorption spectrum. The linear spectrum for BChl *a*, MgOEP and TPP is reproduced from Photochemcad software[16].

Although each of the Chls has their own distinct properties, a common structural feature is the presence of the macrocyclic π conjugated electron box or also known as the porphyrin ring. According to Govindjee and Mauzerall [17,18], the porphyrin ring is an important prerequisite for a molecule to act as the role of Chl in photosynthesis. The properties of porphyrin ring allow the Chls to have an asymmetric, large molecular structure and more importantly, conformational flexibilities. The motion of the porphyrin ring such as bending, twisting and stretching (Figure 1-5) around the metal centre is particularly important for Chl to control its physicochemical properties such as tuning their absorption spectrum, larger Stoke shift and shorter lifetime of the first excited state [7].

Figure 1-5 Bending, twisting and stretching motion of the porphyrin ring (arrow showing out of the plane movement). Reproduce with permission from [19]

In term of structural stability, Chls in LHC are reported to have a good stability due to multiple possible binding sites that they can form with the protein matrix [20]. For example, the presence of formyl group at position R_{13}^1 in Chl *a* and R_7 in Chl *b* allows the hydrogen bond formation with the protein group such as Leucine and Glutamine [2,21]. Besides that, the magnesium metal center can form a donor-acceptor complex interaction in penta-coordination form with the amino acid sidechain and water as the fifth ligand. Moreover, the hydrophobic interaction can be formed by the interaction between the phytol tail group of the Chl with the reaction center of protein [22]. Although some of Quantum Mechanical (QM) simulations excluded the phytol tail from the calculation (due to insignificant effect to the electronic state), it is believed that the phytol tail may indirectly affect the spectral properties by maintaining the pentacoordinated structure through metal-ligand interaction [23]. With these and others electrostatic interaction, it is thus not surprising that Chl has been able to manage and survive in non-optimum conditions and a fluctuating environment.

1.3 Electronic properties of Chl *a*

Starting from the early 1950s, the first spectroscopy measurement to determine Chl electronic transition was carried out by Linschitz and Sarkanen [24] in which they have reported the steady-state absorption spectra of Chl *a* and Chl *b*. As new techniques in spectroscopy emerge, a vast number of spectroscopic experiments are being conducted on Chl systems including linear and circular dichroism [25,26], fluorescence upconversion [27], pump-probe [28], and a more recently ultrafast two-dimensional electronic spectroscopy (2DES)[29–31].

According to the Gouterman's model [32,33], there are four main bands in Chl labeled as Q_y band (1st excited state), Q_x band (2nd excited state), and at a higher energy side of the spectrum, B band (B_x and B_y). In addition, there are also first vibronic replica of the Q_x and Q_y band labeled as Q_{x1} and Q_{y1} . Even though one may think that the assignment of the two lowest excited states in Chl *a* is rather simple, the electronic assignment of Q_x and Q_y band is still being debated and controversial [34].

Figure 1-6 Linear (left) and Magnetic circular dichroism (MCD) spectrum of Chl *a* dissolved in Pyridine with only the Q_x, Q_y, Q_{y1} and B band labeled.

MCD spectrum is reproduced with permission from [34]

Currently, two different assignments of the Q_x band coexist being designated as “traditional” and “modern”. The idea of different assignments arises because the experimental results were contrary to one another especially on the assignment of the Q_{y1} and the Q_x band [35]. In the modern assignment, as depict in Figure 1-6, the Q_x band is assigned as the lower energy position compared to the Q_{y1} band, being roughly at 640 nm for Q_x and 600 nm for Q_{y1} (when measured in pyridine). For the traditional assignment, however, Q_x and Q_{y1} are assigned with Q_x band positioned at higher energy than Q_{y1} band. The experiment identifying the traditional assignment in early 1960’ was based on absorption(ABS) and emission(EMI) spectroscopy[36]. The results of the experiment showed an asymmetry between ABS and EMI spectra due to the decrease in the emission spectrum of the lower energy band (600nm) assigned as Q_x band. Using high-resolution polarized fluorescence excitation spectra at 4.5 Kelvin, Rebane and Avarmaa assigned the lower band as Q_x origin which support the traditional assignment [37]. Meanwhile, experiments on linear and circular dichroism of Chl *a* carried out by Umetsu *et al.* in align with the modern assignment based on positive and negative peaks found at 640nm and 670 nm respectively [25,38]. Moreover, it was corroborated by the semi-empirical calculation from Scherer *et al.* which reported that Q_x and Q_y bands essentially possess mutually perpendicular transition dipole moment [39], which would result in Q_x band and Q_y band being negative and positive, respectively, in dichroism experiment (as shown in Figure 1-6 (right)). Recently, Reimers *et al.*

revisited these assignments and argued that if the Q_x band would be located further from Q_y band as in the traditional assignment, it should not have a significant influence on decoherence and exciton transfer processes contrary to what has been observed in photosynthesis [34]. Although it is tempting to discuss more on the different assignment of the Q_x band in Chl *a*, our studies related to the Q_x and Q_y band assignment as presented in Chapter 5 followed the recently favored “modern assignment” consistent with the assignment from LD, MCD results and line narrowing optical spectra [35,40].

The position of Q_x and Q_y bands can be influenced by the coordination number of the Mg metal in the porphyrin ring. In physiological conditions, the pentacoordinated structure, where the nucleophilic site of one solvent molecule bind to the Mg atom, is preferably formed similarly to what has been reported in protein-bound LHC [41]. However, in a strong nucleophilic solvent [42], high pressure[43] or low temperature [44] condition, the metal center tends to form a hexacoordinated structure, where two of the solvent molecules bind to a Mg atom. For example, it has been reported from Raman spectroscopy studies that in lower polarity solvents such as pyridine, the hexacoordinated structure is predominantly formed [45].

In terms of the excited state lifetime of Chl *a*, studies by Shi and co-workers using fluorescence depletion method has reported an ultrafast internal conversion from the B band to Q_y band which occur in the range of 146 fs to 260 fs depending on the solvent used. While the transfer from Q_x to Q_y state occurred in the range of 100 to 226 fs. Furthermore, the internal conversion time is reported to increase with an increase of the dielectric constant of the solvents [27]. These results were then corroborated by the computational simulation

studies on Chl *a* using non-adiabatic excited-state molecular dynamics (NA-ESMD) by Zhao *et al.* and vibronic coupling model by Reimer *et al.* [34,46]. The lowest excited state or the Q_y band in solution was reported to decay as fluorescence to the ground state in ~6.0 nanosecond as measured by fluorescence spectroscopy studies by Scheer *et al.* [47].

1.4 Solvation dynamics

Solvation dynamics is a measure of solvent response dynamics when the chromophore in solution changes its dipole moment between the ground and excited state. After the photoexcitation of chromophore, solvent molecules will reorganize themselves by moving and rearranging to find a new equilibrium condition. In transient absorption or fluorescence upconversion spectroscopy, the dynamics of solvent response can be manifested by the Stokes shift which is measured by taking the difference between the position of maximum of the emission at particular population time and maximum of emission at infinite time (or fluorescence emission at steady state). The normalized form of the solvent response function is given by the following equation.

$$C(T) = \frac{\omega(T) - \omega(\infty)}{\omega(0) - \omega(\infty)}$$

where $\omega(T)$ is the frequency of emission at particular population time T , $\omega(\infty)$ is the frequency of emission at infinite time and $\omega(0)$ is the emission frequency at time zero. The Stokes shift emission can be visualized by the illustration in Figure 1-7 which shows the fluorescence emission decrease in energy as the population time T proceeds.

Figure 1-7 Schematic energy diagram showing the solvation dynamics process. As time proceeds, the energy of the solute decreases and giving rise to the Stokes shift in fluorescence spectrum.

The typical time scale of the solvent response is in the order of femtosecond to nanosecond. The first few tens to hundreds of femtoseconds are usually assigned as the intramolecular relaxation. Few hundred femtosecond to nanosecond time scale solvation dynamics are often associated with the random diffusive motions caused by the rotation and translation of the bulk solvent [48]. In case of solvation dynamics of Chl, studies by Martinsson *et al.* and Shi *et al.* performing transient absorption spectroscopy on Chl *a* in ethanol and Chl *b* in both acetone and pyridine revealed a time constants of ~ 100 fs which was attributed to the thermal fluctuation of the solvent molecules and 1.7 ps to 20 ps assigned to the dielectric relaxation of the solvent molecule [28,49].

Additional to transient absorption and upconversion fluorescence, other techniques such as hole burning, and three-pulse photon echo peak shift (3PEPS) can be used to measure the solvation dynamics. In the transient hole-burning experiment the solvation dynamics are monitored by exciting the inhomogeneous frequency distribution of an electronic state with a narrow bandwidth excitation pulse. The response of the solvent is manifested by the time-dependent broadening of the hole [50]. 3PEPS uses three pulses to monitor the peak shift between two echo signals in the phase matching direction of $-k_1 + k_2 + k_3$ and $k_1 - k_2 + k_3$ as a function of the population time [51]. Another recent method that allows the study of solvation dynamics with high sensitivity is 2DES. 2DES provides additional information to the conventional transient absorption spectroscopy by resolving the pump frequency into another axis, allowing to observe a correlation between ω_{Pump} (excitation frequency) and ω_{Probe} (detection frequency). By doing so, one can uncover couplings that would be obscured in congested linear spectra, studying electronic coherences and measure the spectral diffusion processes. Spectral diffusion measures the ensemble average frequency fluctuation of a transition as a function of population time and can be quantify using frequency fluctuation correlation function (FFCF). FFCF is sensitive to change in the solvation environment and its normalized form is given by the following equation

$$C(T) = \frac{\langle \delta\omega(T) \delta\omega(0) \rangle}{\langle \delta\omega(0) \delta\omega(0) \rangle}$$

Where $\delta\omega(T) = \omega(T) - \langle \omega \rangle$

Figure 1-8 Schematic diagram of the spectral diffusion process. FFCF measure the probability that $\omega(T)$ is same as $\omega(0)$ averaged over all starting frequencies.

Wells *et al.* have quantified FFCF using linear absorption and 2DES on Chl *a* in methanol. Their results shows that the FFCF occur over four time scales; the shortest time scale of 65 fs was attributed to the inertial motion of the solvent, 500 fs and 7 ps were assigned to solvent reorganization process and >1 ns was attributed to residual inhomogeneity [52]. More recently, Moca *et al.* have implement 2DES to study the effect of solvent such as polarity, viscosity and protic solvent (hydrogen bonding) on the extent of inhomogeneity of Chl *a*. They concluded that the extent of inhomogeneity is a strong function of solvent being the most affected by viscosity and protic solvent and to a lesser degree, polarity [53]. In the next following chapters, we will describe analytical and experimental methods to extract FFCF from 2DES measurements and further explore solute-solvent interaction of Chl *b* in addition to Chl *a* molecules.

1.5 Scope of work

This dissertation outlines the development and application of 2DES in elucidating the electronic properties of Chls. In the second chapter of this thesis, we provide the description of 2DES using a step-by-step approach for those who would want to convert their working transient absorption setup into a partially collinear 2DES. Furthermore, the procedure for post analysis such as conversion of raw data into a purely absorptive 2D data is included in this part of the thesis.

In chapter three, investigation of the effect of finite excitation bandwidth on the retrieved FFCF values is presented. Several methods such as ellipticity and center line slope (CLS) was compared and tested to find the most robust way of extracting the FFCF in spite of narrow bandwidth of the interaction pulses that commonly occur in 2DES experiments. In addition, we performed a 2D simulation to further corroborate the experimental results and thus establish methods to retrieve the full FFCF despite the limitation on the laser excitation bandwidth.

In chapter four, we study the spectral diffusion of Chl *a* and Chl *b* in various chemical environments. The influence of polarity and protic type of solvent on the degree of inhomogeneity and dynamics of spectral diffusion were explored.

Lastly, in chapter five, we investigate the ultrafast time scale of the correlation dynamics between the two lowest excited states of Chl *a*. We have derived the so-called frequency fluctuation cross-correlation function (FXCF) that reveals the information about the type and amount of correlation between

two transitions and used it to look at the correlation of the Q_x and Q_y band of Chl *a* in pyridine.

1.6 References

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Chapter 2

Converting a Transient Absorption

Spectroscopy to 2DES

2.1 Introduction

Two-dimensional electronic spectroscopy is a powerful technique that enables the measurement of the femtosecond dynamics of various physical, chemical and biological systems. It can be considered as an ‘upgrade’ to the conventional pump-probe or transient absorption spectroscopy (TAS) experiment[1]. In TAS, the detection or ‘probe’ spectrum is resolved and presented as the transient absorption spectrum, however, the excitation or ‘pump’ is spectrally unresolved within the pump pulse spectral bandwidth. In 2DES, frequency resolution can be achieved along the ‘pump’ frequency dimension. The resulting data is usually presented as a two-dimensional contour plot with the excitation or ‘pump’ frequency as one axis and the detection or ‘probe’ spectrum as the second axis. This provides the ability to measure a correlation between the excitation and detection frequencies in an ultrafast spectroscopy measurement enabling the observation of couplings or energy transfers. Various experiments have been carried out using the 2DES technique, ranging from the infrared to the ultraviolet-visible region, studying photosynthetic energy transfer

[2–7], photochemical reactions [8], and quantum dots nanoparticle [9,10], among others.

A TAS experiment involves a ‘pump’ pulse exciting the system followed by a ‘probe’ pulse after a delay time of T_w . The transient absorption is then measured as the difference spectrum of the ‘probe’ pulse with and without the ‘pump’, $S_{\text{TAS}}(T_w, \omega_t)$, where ω_t denotes the detection frequency. The schematic of a TAS experiment is illustrated in Figure 2-1. In order to transform a TAS into 2DES setup, one introduces an additional pulse before the ‘pump’ pulse with an interpulse delay τ (Figure 2-1). For every delay, T_w , experiments are repeated by stepping through a series of τ steps. Similar to TAS, the difference spectrum of the probe is then collected to generate a dataset $S_{2\text{D}}(\tau, T_w, \omega_t)$. Subsequently, a Fourier transformation is performed on $S_{2\text{D}}(\tau, T_w, \omega_t)$ along the τ axis to generate the 2DES spectrum $S_{2\text{D}}(\omega_\tau, T_w, \omega_t)$.

2DES spectroscopy belongs to a family of 3rd order nonlinear optical spectroscopies [11–13] and relies on the interaction of the system with three pulses to generate a photon echo signal which is then heterodyne detected. In the above paragraph, although 2DES is described based on TAS spectroscopy, there are several other ways to implement a 2DES experiment and can be categorized according to the spatial arrangement of the three interacting pulses, namely, collinear, non-collinear and partially collinear. Collinear 2DES [14–17] has all three interacting pulses positioned along a single beam. Non-collinear 2DES [1,18] has all three interacting pulses arriving from different directions. The method described in this chapter is considered a partially collinear 2DES [19–21], with the first two interactions arriving along one beam direction and the third pulse (the probe beam) along another beam direction (Figure 2-1b). These 2DES

experiments performed using different beam geometries have their benefits and drawbacks [22]. For the partially collinear or pump-probe geometry setup, we describe here some major advantages. The first advantage is that the pump-probe geometry allows facile measurement of 2DES spectra with purely absorptive peaks. This is due to the fact that the first two interacting pulses by being collinear can have their inter-pulse delay and phase controlled to extremely high precision and stability by means of a pulse shaper. The second advantage is that a TAS can be easily converted into a partially collinear 2DES experiment by adding a programmable acousto-optic assisted pulse shaper on the pump beam pathway. There is no other major optomechanical adjustment that needs to be made. After the conversion, the system can both function as a TAS as well as a 2DES. As TAS is in widespread use, the pump probe (partially collinear) 2DES approach is potentially the path of least resistance for labs with TAS capability who wish to ‘upgrade’ their system and obtain the 2DES capability. The aim of this section is to assist research groups who are interested to make such an upgrade.

Figure 2-1 Schematic diagram of experiment in pump probe (a) and 2DES

(b)

Much valuable information can be obtained from the T_w dependent behaviour of the peakshapes in a purely absorptive 2DES spectra. The purely

absorptive peakshapes details the degree of inhomogeneous versus homogeneous broadening of a transition. Between these two limits, there is a slow process occurring on a subpicosecond to picosecond time scale known as spectral diffusion [23–25]. Spectral diffusion can be quantified by the frequency fluctuation correlation function (FFCF) of the transition and measured using 2DES. There are several methods used to extract FFCF from a purely absorptive 2DES spectra, such as ellipticity [26], eccentricity[24] or center line slope method (CLS)[25,27]. In CLS method, one takes each slice parallel to the pump (CLS_{ω_1}) or probe (CLS_{ω_3}) frequency axis in 2D spectra and fit a linear line to the maxima of all slices. The value of the gradient of the fitted line at different T_w equates the normalized FFCF. One of the main advantages of the pump-probe 2DES is its ability to unambiguously measure purely absorptive spectra. We will demonstrate the usage of pump probe 2DES to measure the purely absorptive 2DES and obtain the CLS_{ω_1} of the Q_y transition of chlorophyll *a* (Chl *a*) in the next chapter.

Figure 2-2 Linear Spectrum of chlorophyll *a* (black) overlapped with the pump spectrum (red) (451 THz)

2.2 Experimental Setup

2.2.1 Ultrafast Transient Absorption Spectrometer (TAS)

The TAS setup can be converted to 2DES by adding a pulse shaper on the pump pathway. Here, we briefly outline the specifications of the requisite TAS spectrometer. In doing so, we list the details of our setup for the convenience of the reader. However, we note that setup with comparable specifications will work as well.

Laser pulses from a commercially available Ti:sapphire regenerative amplifier laser system (Coherent, Legend), operating at 1 kHz centered at 800nm with 120 fs in pulse duration were used in the experiment as shown in Figure 2-3.

The wedge was set up to split the laser beam in two. The major portion of the light is directed into a non-collinear optical parametric amplifier (NOPA) to generate a pump pulse (centered at 450 THz), while a smaller portion was used to generate white light continuum (WLC) acting as a probe pulse. A curved mirror was used to focus pump and probe beams and overlapped at the focal point on the sample. The residual pump beam after the sample was blocked and the propagating probe beam was collimated using a matching curved mirror which was then directed to the detector. A waveplate and polarizer were placed in the probe pathway set at magic angle, 54.7 degrees relative to the polarization of the pump pulse to eliminate contributions from orientational dynamics. To impose a delay T_w of the probe pulse with respect to the pump pulse, motorized translation stage with the precision of femtosecond time step was used in the experiment (Figure 2-3). To facilitate data collection in the experimental setup, we implement two choppers in the experiment:

- the first chopper (CH1) is set to have the chopping frequency equal to quarter of the laser's frequency (250 Hz) in the pump beam pathway, in order to measure the change in absorption (excited sample (pumped) – non-excited sample (unpumped)) (note: Dazzler Fastlite AOPDF was used as chopper);
- the second chopper (CH2) at half of the laser frequency (500 Hz) at the probe pathway, in order to remove background noise (bn) and scattered light (sca) from the pump.

As a detection system, we use a monochromator (HORIBA Jobin Yvon, TRIAX 190) with a CCD detector (Princeton Instruments, Pixis 100) to disperse and record the intensity of the probe beam, respectively. The CCD camera was

adjusted to get 500 X 100 active pixels and be triggered by the regenerative amplified laser system at 1 kHz to collect

$$\Delta A(\omega_t) = -\log \left(\frac{I(\omega_t)_{pumped+sca+bn} - I(\omega_t)_{sca+bn}}{I(\omega_t)_{unpumped+bn} - I(\omega_t)_{bn}} \right). \quad 2.1$$

Note: Commonly, the data is collected in wavelength (λ_t), but for ease of explanation, the frequency unit (ω_t) will be used for the descriptions and explanations below.

A group of four shots was collected in a loop to calculate the absorption difference spectrum. In the equation above, the first part of the nominator, $I(\omega_t)_{pumped+sca+bn}$ denote both pump and probe beams being unblocked, while the second part $I(\omega_t)_{sca+bn}$ is the signal when only the pump beam is unblocked. In the denominator part, the $I(\omega_t)_{unpumped+bn}$ denotes the signal when only the probe beam is unblocked and $I(\omega_t)_{bn}$ is signal in the case when both pump and probe beams are blocked. We used a LabVIEW program with a data acquisition device (NI USB-6212 BNC, National Instrument) to control the delay time between pump and probe pulses (T_w) by the motorized delay stage and the recorded data was saved into a file. To obtain a transient absorption spectrum, $S_{TAS}(T_w, \omega_t)$, we repeat the sequence with a series of T_w .

Figure 2-3 Schematic representation of two-dimensional electronic spectroscopy setup. Wd: Wedge Window; PM1-4: Parabolic Mirror $f = 150\text{mm}$; DCM: Chirp Mirror (DCM 11 Laser Quantum Novanta Inc); Chp: Optical Chopper; Pol1 and Pol2: Polarizer; WP: Half waveplate; FL: Focusing Lens $f = 50\text{mm}$; ND Filter: Neutral Density Filter

2.2.2 Conversion of the Transient Absorption Spectrometer (TAS) into a two-dimensional electronic spectrometer (2DES).

To convert the TAS into a 2DES, we insert the Dazzler Fastlite unit [28], an acousto-optic programmable dispersive filter (AOPDF) in the TAS spectrometer's pump pulse beam path. By applying a specific acoustic wave from the waveform generator to the TeO_2 crystal, the AOPDF is able to shape the spectral phase and amplitude of the laser pulses. This is then used to generate two pulses with controllable relative delays (τ) and phase (ϕ_{12}). Figure 2-5 shows the shaped spectrum in the frequency domain corresponding to $\tau = 100\text{ fs}$ with 0 phase (left) and π phase (right). Additionally, the AOPDF can be used to compress the output pump pulses. Note: The phase-matched shaped pulses produced from AOPDF will exit at an orthogonal polarization and at small diffracted angle to the incoming light field.

In order for the AOPDF to shape the laser pulses according to the parameters specified in the software, the laser pulse needs to enter the Dazzler perpendicular to the input face plane of the crystal. To ensure that, we attached the AOPDF unit to a manual rotation stage which can then tune the angle parallel with the table plane. In the AOPDF software, a reference frequency within the pulse spectral band was chose. Subsequently, the AOPDF position was calibrated by first using the AOPDF software to create a spectral hole at the specific reference frequency and measure pump spectrum using the spectrometer. The position of AOPDF was then adjusted using rotation stage until the hole in the measured spectrum matches the frequency specified in the software (as shown in Figure 2-4).

Figure 2-4 Pump beam spectrum with and without a spectral hole generated by the AOPDF unit. Here, the spectral hole is set at 451 THz with a 3 THz width.

We perform a two-step phase cycling scheme by carry out measurements with a relative pulse phases (φ_{21}) of 0 and π radians to obtain sets of data $S(\varphi_{21};$

τ, T_w, ω_t). More on the theory of phase cycling schemes can be found in ref [20,29,30]. The waveform was set to load twice consecutively to count for pump and scattered pump spectrum

Figure 2-5 Shaped spectrum of the first two interaction ‘pump’ pulses with $\phi_{12} = 0$ (left) and $\phi_{12} = \pi$ (right) at delay $\tau = 100$ fs. The red line depicts the reference frequency, (ω_{ref}) set at 425 THz.

In order to reduce the number of time steps along τ , and thus reduce the experimental time, data are typically collected in a partially rotating frame. In a non-rotating frame experiment, the signal $S(\phi_{21}; \tau, T_w, \omega_t)$ oscillates along τ corresponding to the separation of the states. For example, the Q_y band of Chl *a* with a separation frequency of 451 THz corresponds to an oscillation period of ~ 2.2 fs. According to Nyquist sampling limit (sampling limit is less than half the period of oscillation) the data will need to be acquired with a sampling step size smaller than 1.1 fs. However, by using a pulse shaper, we can shift the experiment into a rotating frame which decreases the oscillation of the measured signal. The frequency variable in a rotating frame is specified as $\Delta\omega = \omega - \omega_{\text{ref}}$, where the

reference frequency ω_{ref} can be specified in the AOPDF software. In a partial rotating frame case, a reference frequency is selected that lies between zero frequency and the transition frequency. In other words, partially rotating frame refers to a partial downshift of the observed transition frequency to a value slightly more than zero frequency and thus having a longer Nyquist period. In contrast, a fully rotating frame refers to choosing a reference frequency that is near or equal to the transition frequency (or the laser pulse center frequency) $\omega_{\text{ref}} = \omega_{01}$, and the non-rotating frame as choosing the reference frequency as zero frequency $\omega_{\text{ref}} = 0$. Here we choose $\omega_{\text{ref}} = 425$ THz, which result in the measured frequency of ~ 26 THz, and a signal oscillating at ~ 20 fs [16,21,29]. In Figure 2-5 shows the shaped pump spectra with indicated reference frequency (red line) at 425 THz. Note that the reference frequency can be set outside of the pump frequency spectrum, as is the example here.

The appropriate waveforms were then uploaded to the AOPDF and signals are collected for delay τ from 0 to 140 fs in step sizes of 3 fs (140 fs correspond to period where the oscillation of the coherence or free induction decay (FID) has sufficiently decayed to background noise level). To control the synchronization of loaded waveforms, a 1 kHz trigger was connected from the laser to “trigger/counter” section on the NI card and to the AOPDF. The waveforms are then continuously changed at a rate of 1 kHz in a looped sequence. An electronic output (TTL signal) will be generated by the AOPDF labeled as S2. A BNC cable was used to connect S2 to the NI card at the “analog in” section. S2 generates a signal on every first waveform. For each loaded waveform, the CCD detector will collect and store 200 sets of data in a single run, then it will be stored in the RAM before being subsequently saved onto a hard disk. An average of 1000

samples was collected for each 0 and π phase to obtain a good signal to noise ratio. After scanning τ , motorized translational stage on the probe beam pathway was used to change T_w and obtained the signal for certain population time. The collected data sets can then be summarized as $S(\varphi_{21}; \tau, T_w, \omega_t)$.

2.3 Data analysis

In order to get purely absorptive 2DES spectrum, we first perform weighted summation for the collected data at different interpulse phases [14] obtaining spectrum as shown in Figure 2-6a. For 1 x 2 phase cycling this is:

$$S(\tau, T_w, \omega_t) = \frac{1}{2} (S(\varphi_{21} = 0) + S(\varphi_{21} = \pi) \exp(-i\pi)) \quad 2.2$$

Subsequently, a Fourier transform of the summed signal $S(\tau, T_w, \omega_t)$ along τ was performed to resolve the excitation frequency. In order to increase the spectral resolution along the pump axis, we perform zero padding before the Fourier transform. Zero padding adds zeroes to the signal leading to a smaller frequency spacing between adjacent frequency points in the FFT spectrum. The resulting signal is free from the phase independent strong pump-probe signal, however, because the 1 x 2 phase cycling cannot discriminate between weighting factor $\exp(-i\pi)$ and $\exp(i\pi)$ the real part of the obtained 2D spectrum consists of two identical purely absorptive peaks. These peaks are symmetrical about zero and positioned at $\Delta\omega_\tau = \omega_{01} + \omega_{\text{ref}}$ and $\omega_\tau = -(\omega_{01} - \omega_{\text{ref}})$ as shown in Figure 2-6b.

A final 2DES spectrum was obtained by extracting the half of the spectrum for $\omega_\tau > 0$, and the ω_τ axis was readjusted by adding the reference frequency ω_{ref} to the pump axis. In this way, the spectrum in Figure 2-6b is transformed to the 2DES spectrum of Figure 2-7a. With that, the 2DES spectrum is ready to be presented or further analyzed. The MATLAB codes to process the 2D data are provided in supplementary section of this thesis.

2.4 Representative Results:

Figure 2-6a depicts a typical set of data $S(\tau, T_w, \omega_t)$ for chlorophyll *a* molecule in Tetrahydrofuran solvent collected in a partially rotating frame with the reference frequency set at 425 THz. The vertical axis in Figure 2-6a, τ , is obtained by delaying the first two pulses using the AOPDF, while the horizontal axis is the probe axis resolved by the spectrometer (ω_t). After performing Fourier transform along τ , one will get a pump frequency axis as shown in Figure 2-6b.

Figure 2-6 2DES spectrum shown in a: time domain and b: frequency domain.

The two pure absorptive peaks are found at (25 THz, 450 THz) and (-25 THz, 450 THz). The lower half part corresponding to the aliased signal can be discarded while the upper half part will be used for presentation and analysis.

This aliased signal can be removed by using higher step phase cycling scheme such as 1 x 3, where the phases are stepped through $\varphi_{21} = 0, 2\pi/3$ and $4\pi/3$ radians [20]. For most experiments performed in a partially rotating frame, where the peaks appear away from the aliased counterpart, it is sufficient to collect data using 1 x 2 phase cycling scheme and neglect the aliased signal in the analysis.

After the adjustments of the axis by discarding the aliased signal and adding the reference frequency of 425 THz, the resulting 2DES spectra are presented in Figure 2-7. Here we show a series of 2D spectra at different T_w .

To demonstrate the quality of the purely absorptive peakshape, we use the T_w series of 2D spectra to study the spectral diffusion of the transition. Here, the 2DES spectra demonstrate an elongated peak along the diagonal at initial T_w times. As T_w increases, the 2DES evolves from elliptical to become more circular in shape. The red line in 2DES spectra indicates the linear fit to the maxima from each slice along the pump frequency axis, as described before. The region of 2DES spectra for fitting was limited to the range from 444 THz to 457 THz pump probe axis, at which maxima could be clearly evaluated. The value of the slope extracted from the linear fit corresponds to the value of the $\text{CLS}\omega_3$, which was shown to be equivalent to the normalized FFCF [25]. Each of the $\text{CLS}\omega_3$ data as T_w progresses was plotted together with the fitting curve of triexponential decay function in Figure 2-8. From here, the first component with 0.2857 ± 0.0090 contributions was assigned as the thermal fluctuations of the solvent molecules

occurring with a decay time of ~ 0.21 ps. The two next components, ~ 1.9 ps and ~ 48 ps were ascribed to the inertial solvation of the chromophore with 0.1281 ± 0.0060 and 0.0263 ± 0.0030 contributions respectively. The final decay component shows the residual inhomogeneity, which lasts for the time exceeding the delay times measured in this experiment with the contribution of 0.0177 ± 0.0010 . Due to the presence of the coherent artifact in 2DES, we have excluded the fitting and extraction of the FFCF from the data for the first 110 fs.

In measuring $\text{CLS}\omega_1$ from the 2DES spectra, one needs to ensure that the pulses used for the pump excitation are near to the transform limited pulses. This is due to the fact that the chirp/group velocity dispersion (GVD) in the pulse could give a distortion to the 2DES spectra, as reported in reference [31], thus giving an inaccurate interpretation/quantitative data on the spectral diffusion. GVD can be corrected using pulse shaper to obtain pulse duration of less than 1.3 times the transform limited pulse. A successful experiment requires one to obtain a good signal over noise ratio by maximizing the overlap between the pump and probe pulses using relatively low power attenuated to < 100 nJ to prevent sample damage/degradation.

Figure 2-7 Normalized purely absorptive 2DES spectra of Chlorophyll *a* in Tetrahydrofuran solution excited at the Q_y band. The 2DES spectra demonstrate an elongated peak shape along the diagonal at initial T_w= 300 fs evolving to become more circular at later time ~5 ps.

Figure 2-8 CLSω₃ data as a function of T_w (squares) overlaid with the fitted exponential decay (red line).

2.5 Conclusions

In conclusion, the two-dimensional electronic spectroscopy assisted by a pulse shaper is a recent technique that has a vast amount of promising applications. To facilitate potential 2DES users, we have presented details for those who intend to convert Transient Absorption or pump-probe spectroscopy experiment into a 2DES experiment to measure high quality purely absorptive 2DES spectra. We then demonstrate the quality of the 2DES spectra obtained by demonstrating how the FFCF of an electronic transition can be measured using the CLS method.

With only changes in software, the setup for the 2DES using a pulse shaper can be used to also perform higher order 3-Dimensional electronic spectroscopy [32] or two quantum two-dimensional electronic spectroscopy[33].

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Chapter 3

The Effect of Laser Pulse Bandwidth on the Measurement of the Frequency Fluctuation Correlation Functions in 2D Electronic Spectroscopy

3.1 Introduction

Solutes in a non-static environment, be it glass, protein or liquid will experience structural changes due to the solute-solvent interaction. The occurring time-dependent diffusion process causes fluctuation of the transition frequencies, which for distribution of frequencies will manifest itself as a dynamical broadening of an optical absorption spectrum. This spectral diffusion process can be observed as an evolution of the two-dimensional optical spectra (2DOS) [1]. The lineshape of both linear and 2D spectra can be related to the amplitude and time scales of the occurred frequency fluctuations through a frequency fluctuation correlation function (FFCF) [2]. The FFCF describes the ensemble averaged correlation of a certain transition frequency at time zero and later time T_w .

$$C(T_w) = \langle \delta\omega(T_w) \delta\omega(0) \rangle \quad 3.1$$

where $\delta\omega(T_w) = \omega(T_w) - \langle \omega \rangle$.

Extracting the FFCF from experimental 2DOS spectra requires performing a global fitting with a complicated model response function [2–4]. Therefore, in recent years several methods have been proposed to simplify the extraction of the FFCF from experimental 2DOS, such as nodal line slope [5], ellipticity [6,7], center line slope of the slices parallel to the excitation or pump frequency axis (CLS_{ω_1}) [8] or center line slope of the slices parallel to the detection or probe frequency axis (CLS_{ω_3}) [9]. All of these methods are based on the concept that at initial population time T_w the excitation and detection frequencies are perfectly correlated and 2D spectrum is elongated along diagonal due to inhomogeneous distribution, while at infinitely long time the memory of an initial frequency is completely lost resulting in symmetrical, rounded lineshape [10]. With this, the nodal slope method measures the slope of the nodal plane between ground state bleach and excited state absorption features for systems with small anharmonicity and assuming perfect correlation between them [5]. The ellipticity method measures the relationship between diagonal and antidiagonal spectral widths based on an elliptical shape of the 2D feature [6,7]. The CLS_{ω_1} [8] and CLS_{ω_3} [9] methods measure the slope of the line connecting maxima of the slices along the excitation and detection frequency, respectively. All of these methods were shown to give values proportional to FFCF in perfect conditions within certain approximations. However, in the case of a real experimental conditions these values might deviate from ideal. Effects of experimental conditions like apodization, anharmonicity, phasing errors, low signal to noise ratio, fast spectral diffusion or Stokes shift were previously tested and compared between various methods [8,9,11–13]. It was demonstrated that the CLS_{ω_3} method seems to be the most robust when facing mentioned

conditions [9,11,13]. Another experimental condition, which should be addressed especially in case of the two-dimensional electronic spectroscopy (2DES) is the spectral bandwidth of the used interaction pulses. While this might not contribute significantly for two-dimensional infrared spectroscopy, where pulses are mostly much broader than the absorption band spectrum, in 2DES experiments the interacting pulse spectrum is often narrower than the band transition under study. It was shown previously that finite pulse effects can affect, among others, peak position and shape [14,15]. Recently, there have been efforts in devising simple expressions to account for the finite pulse width effect on 2DES spectra [14,16]. These methods include analytical expressions that consider interaction pulses of specific shapes, such as Gaussian [17] and Lorentzian [14]. Another approach uses numerical expressions to generate the experimental 2DES spectra considering interaction pulses of arbitrary shape [16].

In the present article, we present an experimental comparison of three commonly used methods of extracting FFCF from 2DES spectra, i.e. ellipticity, $\text{CLS}\omega_1$ and $\text{CLS}\omega_3$, in terms of their robustness to finite excitation pulse effect. We ascertain that the $\text{CLS}\omega_3$ method works well in minimizing the distortion to the FFCF. We also show that for the ellipticity and $\text{CLS}\omega_1$ methods, accurate FFCFs can still be recovered by applying the simplified expressions for incorporating finite pulses effects in the simulation of the 2DES spectra [16].

We measured a series of 2DES spectra of the lowest electronic transition (Q_y) of Chlorophyll *a* (Chl *a*) in methanol using different bandwidths of excitation pulses. The knowledge of the interaction of chlorophyll pigment molecules with its surrounding is necessary in understanding the efficiency of the ultrafast energy transfer in photosynthetic systems [18]. Its spectral diffusion

dynamics have been previously measured by Wells *et al.* [19] and Moca *et al.* [20] using CLS ω_1 and ellipticity methods, respectively.

3.2 Materials and methods

Chlorophyll *a* sample (Sigma Aldrich) was dissolved in methanol to 0.2 OD at the maximum of Q_y transition in a 1 mm quartz cuvette. All the presented 2DES measurements were performed at the room temperature with a sample constantly flowing through a flow cell with a 1 mm path length and 1 mm quartz walls.

The 2DES measurements were performed in a partially collinear (pump-probe) fashion [21,22]. The fundamental laser beam with pulses of around 140 fs centred at 800 nm was obtained from a commercial regenerative amplifier system (Legend, Coherent) operating at 1 kHz. Part of the beam was used to pump a commercial noncollinear optical parametric amplifier (TOPAS White, Light Conversion) providing 35 nm wide pulses centred at 665 nm (maximum of the Q_y transition of Chl *a* in MeOH). This was then sent through an acousto-optic programmable dispersive filter (AOPDF) pulse shaper (Dazzler, Fastlite) creating a collinear two-pulse train serving as the excitation or pump pulses, with a variable delay (τ) and phase between the pulses [23]. Henceforth, we will denote this first two pulses as the ‘pump’ pulses. The AOPDF was also used to compress and spectrally shape pulses to get 35 nm and 10 nm wide (FWHM) pulses with < 1.4 times transform limited temporal length at the sample. A 1 x 2 phase cycling scheme [21,24,25] was used to perform the 2DES experiment. The two excitation pulses were set to 20 nJ per pulse and scanned up to 150 fs delay (τ) in a 3 fs time steps. Data over τ was collected in a partially rotating frame with

a reference wavelength of 705 nm. Another part of the 800 nm fundamental beam was used to generate white light continuum (WLC) probe pulse (which also served as the local oscillator) from a 2 mm sapphire plate. The WLC pulse was passed through a set of chirped mirrors compressing the pulses and directed through a mechanical translation stage providing a delay against the pump pulses (T_w). The polarization of the probe beam was set to 54.7° (magic angle) from the polarization of the pump beam using a half-wave plate and a polarizer. Finally, after being spatially overlapped with the pump beam at the sample, the probe beam was frequency resolved and detected using a spectrometer (TRIAx 190, Horiba) equipped with a CCD detector (PIXIS 100, Princeton Instruments). The T_w delay in the performed experiments was scanned up to 300 ps. However, due to the observed coherent artifact, the signal data was analysed only from $T_w = 130$ fs onwards. The origin of coherent artifact has been long recognized as a common problem in heterodyned pump probe experiment which disturb the analysis of relaxation dynamics near time zero. At around zero T_w delay time, the interference between the two pulses inducing spatial grating in the medium. Coherent artifact is the pump radiation which scatters into the probe beam and thus distort the relaxation of the signal. In the case of 2DES experiment, the coherent artifact overlapping with the signal distort the peak shape of the 2D spectra. This distortion will lead to inaccurate retrieving of FFCF values if the CLS or ellipticity methods were to be used. Removing coherent artifact from signal is complicated due to arbitrary artifact shape which depend on the pump or probe pulse characteristic such as dispersion, pulse duration and stability of each pulses. The subtraction of the coherent artifact by performing an additional 2DES experiment on solvent also does not give good results due to the very long

time needed to acquire each data set and a possible difference of coherent artifact features in case of with or without the sample in the solution.

3.3 Analysis and results

Figure 3-1 shows a few selected 2DES spectra recorded at different delay times T_w with 35 nm and 10 nm bandwidths of the excitation pulses. For the case of the 35 nm pump a trend indicating spectral diffusion process can be clearly observed, where the peak which is initially elongated along the diagonal, becomes more rounded with increasing T_w delay [26]. However, in the case of the 10 nm excitation bandwidth, this becomes diminished by the masking of the Q_y band in the excitation frequency with a narrower pump pulse.

Figure 3-1 2DES spectra of Chl *a* in methanol at different T_w delay times using 10 nm (top row) and 35 nm (bottom row) wide pump pulses. Panels on the left and top show the linear spectrum of Chl *a* (black line) with overlaid pump (left) and probe (top) pulses spectra (red lines) used for each set of experiments

The normalized frequency fluctuation correlation function (FFCF) for the Q_y transition of Chl *a* was recovered from the peak shape analysis of the recorded 2DES spectra using the commonly used methods of $CLS\omega_1$, $CLS\omega_3$ and ellipticity [7–9]. For the $CLS\omega_1$ method, slices through the 2DES spectrum parallel to the pump frequency were fitted with a Gaussian function to determine the maxima frequencies. These maxima, collected for slices in a range of around 200 cm^{-1} around peak maxima in the detection axis (ω_1), were then fitted to a straight line of which gradient is the $CLS\omega_1$. The $CLS\omega_3$ was obtained in the same way as $CLS\omega_1$ with the difference that the slices of 2D spectra were taken parallel to the probe frequency and the $CLS\omega_3$ value is the inverse of the gradient of the line connecting maxima. In the case of the ellipticity method, the diagonal

and antidiagonal slices of the Q_y peak were extracted from the 2DES spectrum and fit to a Gaussian function. The ellipticity value was then obtained from the relation between widths of the fitted to diagonal (w_d) and antidiagonal (w_{ad}) Gaussian functions:

$$Ellipticity = \frac{w_d^2 - w_{ad}^2}{w_d^2 + w_{ad}^2} \quad 3.2$$

Additionally, due to the Stokes shift which is observable in the analysed range in the probe frequency for $CLS\omega_1$, the frequency window under analysis needs to be shifted to match the centre of the Q_y peak. Likewise, for ellipticity method the diagonal and antidiagonal slices have to be taken from the axes of the Stokes shifted peak. Such procedures were however not necessary in the case of $CLS\omega_3$.

Results of the peak shape analysis using the three methods described above, for a number of delay times T_w and different bandwidths of the pump spectrum, are presented in Figure 3-2. All of the performed methods show similar values of the normalized correlation function when the pump spectrum covers the entire Q_y band (Figure 3-2 (Left)). However, for data with a narrower pump, the recovered FFCF values differ depending on the method used (Figure 3-2 (Right)). Both $CLS\omega_1$ and ellipticity methods give values significantly lower than in the case of a 35 nm pump pulse spectrum. However, the $CLS\omega_3$ method recovers similar values for both the 35 nm and 10 nm pump pulses.

Figure 3-2 Normalized correlation function as a function of population decay recovered using described in text CLS ω 3 (black squares), CLS ω 1 (red circles) and ellipticity (blue triangles) methods from data obtained using 35 nm (left) and 10 nm (right)

In order to describe the correlation function, $C(t)$, and model the linear and two-dimensional spectra, we utilize Kubo's stochastic theory of lineshapes [26–28]. Dynamical processes causing the frequency fluctuation are considered here as stochastic perturbations and can be described by a series of exponential decays:

$$C(t) = \sum_i \Delta_i^2 \exp\left(-\frac{t}{\tau_{ci}}\right) \quad 3.3$$

where Δ_i and τ_{ci} are the fluctuation amplitude and correlation time of the i -th component. Double integration of the correlation function gives the lineshape function:

$$g(t) = \sum_i \Delta_i^2 \tau_{ci}^2 \left[\exp\left(-\frac{t}{\tau_{ci}}\right) + \frac{t}{\tau_{ci}} - 1 \right] \quad 3.4$$

which can be then implemented to model linear and 2D spectra using optical response theory [2,29].

Using this, we modelled a linear spectrum and compared it with an experimental data through a least squares fit. Parameters of Δ_i and τ_i for modelling were obtained from a multicomponent exponential fit to the normalized correlation function acquired using CLS ω_3 method, where the normalized amplitude (from Eq. 3.3) is:

$$A_i = \Delta_i^2 / \sum_i \Delta_i^2 \quad 3.5$$

Because the measured population decay time is insufficient for the correlation function to decay to zero we treat an offset from the exponential fit as a residual inhomogeneity (slow modulation limit), where equation 3.4 reduces to $g(t) = \Delta t^2/2$. Additionally, since we do not have information about the correlation function for T_w times shorter than 130 fs, due to the presence of coherent artifact (and short time approximation used in CLS methods[8,9]), we introduced an additional short component with amplitude $A_0 = 1 - \sum_{i=1}^4 A_i$ and correlation time τ_{c0} . The correlation time τ_{c0} together with a sum of the square of fluctuation amplitudes $\sum_i \Delta_i^2$ were set as variables in performed fitting. The parameters obtained this way are presented in Table 3-1, while the resulting linear spectrum obtained from the lineshape function (Eq. 3.4), is plotted together with the experimental spectrum in Figure 3-3.

Table 3-1. Parameters recovered from the fitting for FF CF of Chl *a* in methanol.

Δ_0	τ_{c0}	Δ_1	τ_{c1}	Δ_2	τ_{c2}	Δ_3	τ_{c3}	Δ_4
(cm ⁻¹)	(fs)	(cm ⁻¹)	(ps)	(cm ⁻¹)	(ps)	(cm ⁻¹)	(ps)	(cm ⁻¹)
1006	65	621	0.37	597	3.7	637	44	340

Figure 3-3 Linear spectrum of Chl a (black squares) and the result of the fitting (red line) obtained using parameters from CLS ω 3.

As can be seen from Figure 3-3, modelling the linear spectrum using Kubo lineshape theory give a fairly good fit although some of the experimental data point shows slight deviation from the fitting using parameter from Table 3-1. The reason for this deviation may be due to inaccurate determination of the value for Δ_0 and τ_{c0} . The value of Δ_0 and τ_{c0} was estimated using the least square fit method and not from the experimental data (due to presence of coherence artifact and short time approximation used in CLS method). Furthermore, the used of Kubo line shape, which assume exponential form for $C(T_w)$ neglects the imaginary portion of the line shape function and thus may result in lack of the spectral diffusion process.

Additionally, in order to model the FFCF values obtained using CLS ω_1 and ellipticity methods with different bandwidths of the pump pulses, we utilize the expressions derived by Do *et al.* taking into account finite pulses to model 2D spectra [16]. It is shown that for population times T_w larger than the duration

of the pulses, where the time ordering of the pulses is ensured, the computationally expensive triple integral calculations can be simplified. In cases of T_w -independent response function, or when the response function varies with a time scale slower than the pulse duration, the purely absorptive 2DES spectrum $S_{abs}(\omega_t, T_w, \omega_\tau)$ can be obtained using Eq. 3.6. Eq. 3.6 takes into consideration finite pulse width effects and is obtained by the multiplication of the response function in the frequency domain by the profiles of interacting pulses.

$$\begin{aligned}
S_{abs}(\omega_t, T_w, \omega_\tau) = & R_{NR}(\omega_t, \omega_\tau) e^{-\gamma T_w} \tilde{\epsilon}_1(\omega_\tau - \omega_L) \tilde{\epsilon}_2^*(\omega_\tau - \omega_L) \\
& \times \tilde{\epsilon}_3(\omega_t - \omega_L) \tilde{\epsilon}_3^*(\omega_t - \omega_L) + R_R^*(\omega_t, -\omega_\tau) e^{-\gamma T_w} \\
& \times \tilde{\epsilon}_1(\omega_\tau - \omega_L) \tilde{\epsilon}_2^*(\omega_\tau - \omega_L) \tilde{\epsilon}_3^*(\omega_t - \omega_L) \\
& \times \tilde{\epsilon}_3(\omega_t - \omega_L)
\end{aligned} \tag{3.6}$$

where, R_{NR} and R_R are non-rephasing and rephasing response function, respectively, $\tilde{\epsilon}_i$ is the spectrum at the electric field level of the i -th interaction pulse and ω_L is the laser frequency. In our current case, the pulses used have durations of approximately 27 fs (35 nm bandwidth) and 80 fs (10 nm bandwidth). These durations are short compared to the timescale of the time dependent variation of the 2DES peakshape (after $T_w = 130$ fs) as characterized by the time constants $\tau_{c1} - \tau_{c3}$ (in Table 3-1). The application of Eq. 3.6 is therefore justified in cases reported in this article.

The third order response functions were obtained using Kubo's lineshape theory with parameters from Table 3-1. Following Eq. 3.6 to generate the 2DES spectrum with finite pulse effect, response functions were multiplied twice by the 35 nm or 10 nm bandwidth Gaussian shape function (to include two excitation pulses) in the excitation frequency and twice by 160 nm bandwidth Gaussian

shape function in the detection frequency (to account for the probe and local oscillator). The resulting real part of $S_{abs}(\omega_t, T_w, \omega_\tau)$ at $T_w = 0.2$ ps with normalized correlation function values obtained using CLS ω_1 method are presented together with experimental data in Figure 3-4 a) Experimental (left column) and calculated (right column) 2DES spectra at 0.2 ps population time using 10 nm (top row) and 35 nm (bottom row) pump pulses; b) CLS ω_1 values in population decay time obtained from experimental spectra (symbols) and calculated ones (lines) with 35 nm (black) and 10 nm (blue) pump pulses..

Figure 3-4 a) Experimental (left column) and calculated (right column) 2DES spectra at 0.2 ps population time using 10 nm (top row) and 35 nm (bottom row) pump pulses; b) CLS ω_1 values in population decay time obtained from experimental spectra (symbols) and calculated ones (lines) with 35 nm (black) and 10 nm (blue) pump pulses.

As seen from Figure 3-4, the FFCF values obtained using the CLS ω_1 method from the modelled 2DES spectra match those from the experiment. This indicates that by using Eq. 3.6, the accurate FFCF values can still be recovered using the CLS ω_1 or ellipticity method from 2DES spectra obtained with narrow pump pulses. One way to do this is to perform a fitting of the normalized FFCF

obtained from the modelling of 2DES spectra using finite pulse bandwidth to the one obtained from experimental data. Although the modelling of 2DES spectra using Eq. 3.6 is relatively fast, such fitting can still take a substantial period of time without prior knowledge of approximate values of correlation times and amplitudes as initial fit parameters. Instead, here we show that the accurate FFCF values can be directly recovered during the CLS ω_1 analysis. Assuming a Gaussian shape ($y = A\exp(-\frac{(\omega-\mu)^2}{2\sigma^2})$, where A is the amplitude, μ is centre of the peak and σ is the width) of the pump pulses and slices of the 2DES spectra, and based on the derivation by Do *et al.*, where $y_{finite}(\omega) = y_{infinite}(\omega)y_{pump}(\omega)^2$, the maximum of the 2DES spectrum slice in the case of the finitely wide pump can be found by

$$\mu_{finite} = \frac{\sigma_{pump}^2 \mu_{infinite} + 2\sigma_{infinite}^2 \mu_{pump}}{\sigma_{pump}^2 + 2\sigma_{infinite}^2} \quad 3.7$$

From which, after simple derivation, the maximum of the 2DES spectrum for infinitely wide pump can be found as

$$\mu_{infinite} = \frac{2\sigma_{finite}^2(\mu_{finite} - \mu_{pump})}{\sigma_{pump}^2 - 2\sigma_{finite}^2} + \mu_{finite} \quad 3.8$$

Following the CLS analysis, the slope of the line connecting $\mu_{infinite}$ points gives the normalized FFCF. Figure 3-5 presents results from exercising this method for the measured spectra using 35 nm and 10 nm pump widths compared to FFCF obtained using CLS ω_3 method. Recovered this way, the FFCF values reproduce fairly well those using the CLS ω_3 method, which as shown earlier do not depend on the bandwidth of the pump pulses.

Figure 3-5 Normalized correlation function as a function of decay time recovered using CLS ω 1 and equation 3.6, based on expressions derived by Do *et al.*, for data recorded using pump of 35 nm (red) and 10 nm (blue) bandwidth compared to FFCF obtained using CLS ω 3 method from 10 nm wide pump (black).

The observed finite pulse effects on the recovered FFCF values can be understood using Eq. 3.6 . The equation indicates that the final 2DES spectrum is obtained by multiplication of the response function by the profiles of the interacting pulses. If we take a slice of the 2DES spectrum that is not at the center of the peak (Figure 3-6a) and multiply it by the pulse profile (Figure 3-6b), the center of the resultant peak's profile will shift towards the center of the pulse used (Figure 3-6c). If the same operation is performed for slices at different excitation (ω_1) frequencies, the positions of their maxima will all be 'pulled' towards the center of the pump pulse position. The resultant line that connects the maxima (outlined as a red dashed line in Figure 3-6d. This change in gradient explains the decrease in CLS values for finite pulses.

Figure 3-6 Schematic illustration explaining the origin of the effect of the recovered FFCF from the 2D peak shape due to finite pulse effect based on Eq. 3.6 a) slice of the 2D spectrum with maximum at position -10 (first from left slice from Figure 3-6d); b) interaction pulse with maximum at position 0; c) due to the multiplication of the 2DES spectrum slice by the interaction pulse the peak maximum of the 2DES slice shifted towards the center of the interaction pulse; d) diagram showing change of the CLS due to the multiplication of the series of 2D slices by the pulse profile (each slice was normalized for better visualization).

We now consider the effect of shifting the center frequency of the 2DES slices by the interacting pulse while maintaining the same finite pulse spectral width. This resultant “laser pulling” effect has been considered and discussed by Perlik *et al.* [14]. They showed that it can distort 2D spectra depending on the frequency of the interaction pulses potentially leading to misinterpretation of the probed processes. However, while reducing the pulse bandwidth leads to a decrease in the CLS value, as is shown and explained earlier, the shift in the pulse

center frequency does not further distort the recovered FFCF values. This can be understood by studying Eq. 3.7 , from which the difference between maximum points of two 2DES slices does not depend on the center position of the pump spectrum profile:

$$\Delta\mu_{finite} = \frac{\sigma_{pump}^2(\mu_{infinite1} - \mu_{infinite2})}{\sigma_{pump}^2 + 2\sigma_{infinite}^2} \quad 3.9$$

Thus, no matter what the position of the interaction pulse is, it should always result in the same relative frequency distant between different 2D slices, i.e. CLS. Figure 3-7 shows CLS_{ω_1} of the simulated 2DES spectra from Figure 3-4b using Eq. 3.6, for both narrow (10 nm) and broad (35 nm) pump pulses (Figure 3-7b, blue and black solid lines, respectively), compared with the case when the pump pulses are shifted 242 cm^{-1} off the center of the transition frequency (Figure 3-7b, blue triangles and black squares, respectively). As is clear, for both narrow and broad pump pulses, there is virtually no difference between the pulses with spectrum centered at, and shifted away from, the absorption spectrum peak.

Figure 3-7 a) Calculated 2DES spectra at 0.2 ps population time using 10 nm (top row) and 35 nm (bottom row) pump pulses shifted 242 cm^{-1} off the center of the Q_y transition frequency. Panels on the left show the linear spectrum of Chl *a* (black line) with overlaid used pump spectra (red line). b) CLS₀₁ values obtained with pump positioned at the center of Q_y transition (symbols) and off the center (lines) for 10 nm (blue) and 35 nm (black) pump bandwidths.

Previous studies have shown that distortions in 2D peak shape can be caused by the chirp present in the used interaction pulses what potentially could affect results obtained. As discussed by Ogilvie *et al*, the chirp in either pump or probe pulse can affect the peak shape of a 2D spectra by the broadening of cross peak width of the lower frequency or higher frequency part of the 2D peak. This distortion is due to the asynchronous waiting time, T_w for the lower or higher frequencies part of the pulse spectrum. For example, positively chirped pump will result in longer T_w for lower frequencies compared to higher frequencies. Thus, the lower frequencies will undergo longer variation in the transition frequency (spectral diffusion) compared to the higher frequencies part (and opposite case for negatively pump chirp) [30,31]. However, the amount of chirp

in either of the interaction pulses over the bandwidth of the Q_y transition in the performed experiments compared to the timescale of the measured processes as well as minimum analysed value of T_w (130 fs) suggest that no significant distortions should be observable here. In cases of the chirp present in used pulses comparable with timescale of the measured processes the 2D spectra can be corrected according to procedure showed by Tekavec *et al.* [32].

In this study, we do not consider the contributions of the excited state absorption (ESA) to the 2DES peak due to its small contribution to the 2DES spectrum. The ESA overlaps with the fundamental peak [33] and extends beyond the peak towards the blue end of the spectrum. It manifest as a constant small T_w independent offset, estimated in our 2DES spectra to be about 0.08 of the 2DES peak amplitude of the Q_y transition under study.

3.4 Conclusion

We presented an experimental comparison of three methods (ellipticity, CLS_{ω_1} , CLS_{ω_3}) of extracting FFCF parameters from peakshape of 2DES spectra while using different spectral bandwidths of the pump pulses. The obtained results show that for broader pulses covering the absorption band of the transition under study, all of the tested methods recover similar values of normalized FFCF. However, in cases when the spectral bandwidth of the pump pulses is narrower than the studied band, the methods of ellipticity and CLS_{ω_1} give underestimated values of FFCF. Therefore, according to study conducted here, which also concurs to the previous related tests [11,13], it is noted here that CLS_{ω_3} method is the most robust in the conditions typically encountered during experiments. Furthermore, using expressions derived by Do *et al.* to model 2DOS spectra

including effects of finite bandwidth [16], we see that the accurate FFCF values should still be possible to be recovered using any of these methods with narrow pump and probe pulses used. Here we proposed and utilized a fast method of achieving this based on the CLS method and expressions by Do *et al.* (Figure 3-5).

Comparing the FFCF values for Chl *a* in methanol measured here with those previously measured, we see that they resemble values measured by Moca *et al.*[20] using ellipticity method, where a very broad pump was used. In accordance with the study presented here, the FFCF values recovered by Wells *et al.*[19] using the CLS ω_1 method with narrow pump pulses is underestimated.

3.5 References

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Chapter 4

Studies on Spectral Diffusion of Chlorophyll *a* and Chlorophyll *b* using Two-Dimensional Electronic Spectroscopy (2DES)

4.1 Introduction

Chlorophyll *a* (Chl *a*) and Chlorophyll *b* (Chl *b*) are the basic building blocks of natural light-harvesting complexes (LHC) which occur in plants and green algae. They have been extensively studied due to their widespread occurrence and important functional roles in the LHCs. Essentially, both of the Chlorophylls (Chls) capture the light energy and deliver it to the reaction center through a highly optimized energy transfer network on a femtosecond to picosecond timescales [1–5]. The optimization of this process is governed by the organization of Chls within the protein matrix [6–9]. It is well established that Chls are organized in a complex and orderly manner with the lipoprotein through a specific (hydrogen bond, donor-acceptor complex formation) and non-specific interaction (hydrophobic and dispersive forces) [10–15]. Spectroscopic studies on the isolated systems of Chls in various solvents have been widely studied to identify this interaction and ultimately understand the role of local protein environment in light-harvesting complexes.

Recent review on this, summarize the Chl *a* absorption and emission spectrum from more than 50 solvents belong to different groups (Protic, apolar,

Despite their close resemblance in structure (Figure 4-1), the spectral properties of Chl *a* and Chl *b* molecules are quite distinct. For instance, in the linear spectrum (Figure 4-2), the 1st excited state (Q_y band) of Chl *b* can be seen to have blue shifted spectrum with respect to the Chl *a* [19,20]. Apart from that, studies on the ultrafast relaxation dynamics, such as transient absorption and multidimensional spectroscopy, demonstrate distinct kinetics between the two molecules. It was shown that the internal conversion between the Q_x (2nd excited state) and Q_y bands is faster in Chl *a* compared to Chl *b* [21]. This was attributed to the stronger mixing between the Q_x and Q_y bands in Chl *a* [22]. More recently, a new technique called the two-dimensional electronic-vibrational spectroscopy (2DEV) was developed by Fleming *et al.* and has revealed the specific molecular vibrational spectra assigned to each type of the Chl allowing further distinction of these molecules [23].

In this chapter, we present results from the performed 2DES of Chl *a* and Chl *b* and investigate the nature of inhomogeneous broadening from the effect of specific interaction occurred in Chl-solvent systems. Different from the previous studies [17,18], we applied the center line slope method ($CLS\omega_3$) devised by Fayer and co-worker [24] to retrieve the frequency fluctuation correlation function (FFCF) from the 2D spectra. This method has been mostly used to study spectral diffusion in the 2DIR regime, and it is sensitive towards the solute-solvent interaction. Recently, CLS has been implemented by Tahara *et al.* to study the noncovalent bond interaction (hydrogen bonding interaction) inside the

interfacial water by comparing the CLS_{ω_3} values for DPPG and DPTAP[25]. Here, we will be studying Chl *a* and Chl *b* in various solvent using CLS_{ω_3} and discussed the comparison between the extent of inhomogeneous broadening and spectral diffusion as measured from the obtained CLS values. Despite their similarities in terms of the structure, we observed distinct CLS_{ω_3} values for each of Chls in different solvents. We discuss these findings in relation to the modification at the periphery of the Chl molecules which influences the structural fluctuation and thus the frequency modulation of the excited state transition.

4.2 Experimental method

The experimental setup for 2DES measurement using pump-probe geometry has been described and demonstrated previously [26,27]. This experiment was based on a commercial Ti: Sapphire Amplifier (Legend Amplifier, Coherent) which was used to generate 800 nm laser pulses with 120 fs in pulse duration. A major part of it was used to pump the Non-Collinear Optical Parametric Amplifier (TOPAS White) to obtain excitation pulses centered between 14900 cm^{-1} (671 nm) and 15380 cm^{-1} (650nm). All of the excitation pulses (Pump) were compressed to < 1.3 transform limited pulses using the AOPDF (Dazzler, Fastlite). The laser center frequency, $\lambda_{\text{Excitation}}$ and bandwidth (in bracket) for each experiment are summarized in the Table 4-1 below.

Table 4-1 Parameters of the used excitation pulses corresponding to performed experiments.

Sample	Solvent	Normalized empirical parameter of solvent polarity, E_T^N at 25°C^{a,b}	λ_{Max} /nm and (Pump Bandwidth/nm)
Chl <i>a</i>	MeOH	0.76	665 (35)
	Py	0.30	671 (35)
	THF	0.21	665 (35)
	Diethyl ether	0.12	662 (32)
Chl <i>b</i>	MeOH		650 (30)
	Py		655 (30)

^aObtained from ref [28]

^bWater $E_T^N=1.00$

The minor portion of 800 nm light was focused into a 2 mm Sapphire crystal to generate a white light continuum serving as a probe. The white light pulses were compressed using two pairs of negative group velocity dispersion mirrors (DCM 11 Laser Quantum Novanta Inc.). Before overlapping onto the sample, the probe light passed through a half-waveplate and a polarizer to plane polarize it at the magic angle (54.7 °) with respect to the pump polarization.

The ultrafast two-dimensional electronic spectroscopy (2DES) measurements were carried out using a partially collinear (pump-probe) geometry implementing a 1 x 2 phase cycling scheme [29]. The first coherence time, τ , was scanned by using the AOPDF to delay the first two excitation pulses from 0 fs to 141 fs in an interval of 3 fs and attenuated to 20 nJ per pulse.

Additionally, the experiment was done in a partially rotating frame by setting λ_{ref} at 13990 cm^{-1} (715 nm) and satisfying the Nyquist sampling limit. Pump and probe beams were spatially overlapped into a 1 mm flow cell after which a signal was directed to the spectrometer (HORIBA Jobin Yvon, TRIAX 190) equipped with a CCD detector (Princeton Instruments, Pixis 100) to record the 2D spectra. The population time (T_w) was recorded in a logarithmic scale to monitor the dynamics of spectral diffusion from -0.3 ps to 400 ps.

The samples were purchased from Sigma-Aldrich without further purification. All of the samples were dissolved in various HPLC and spectroscopic grade solvents to match optical density of 0.2 at the Q_y band. No formation of aggregation can be observed at this concentration [18]. After the sample was prepared, it was used immediately to collect 2D spectra at room temperature (23°C). Each experiment was then repeated for at least three times using a new sample to ensure repeatability. Additionally, we measured the linear absorption spectrum before and after the experiment to ensure that no photo-degradation had occurred.

4.3 Results and discussion

4.3.1 Linear Spectra.

Figure 4-2 shows the linear absorption spectrum of Chl *a* and Chl *b* dissolved in methanol superimposed with the spectrum of corresponding pump excitation used for 2DES experiment. In all experiments, the laser spectrum was set to cover the Q_y excitation band.

Figure 4-2 Linear Absorption of Chl *a* and Chl *b* normalized to the B band with the excitation pulses overlaid. The inset shows the same data for higher range of wavenumber.

4.3.2 Two-dimensional electronic spectroscopy (2DES).

Representative purely absorptive 2D spectra of Chl *a* and Chl *b* at two different delay times were plotted in Figure 4-3 and Figure 4-4 respectively. The horizontal axis in the 2D spectra represents the detection frequency, ω_{probe} , whereas the vertical axis represent the excitation frequency, ω_{pump} . The white

dotted line indicates the diagonal line where $\omega_{\text{pump}} = \omega_{\text{probe}}$.

Figure 4-3 Normalized 2DES of the Q_y band of Chl *a* in MeOH, Py, THF, and Et₂O at T_w values of 0.15 ps (left) and 10 ps (right) with corresponding CLS ω_3 fits overlaid (red line).

Figure 4-4 Normalized 2DES of the Q_y band of Chl b in methanol (top) and pyridine (bottom) at T_w values of 0.15 ps (left) and 10 ps (right) with corresponding $CLS\omega_3$ overlaid (red lines).

The response from pumping the Q_y band of Chl a and Chl b shows a strong negative signal which arises from ground state bleaching (GSB) and stimulated emission (SE) of the 1st excited state. Figure 4-5 shows examples of Feynman diagram accounting for these two processes. We noted that the signal strength in the 2D spectra for Chl b is lower than Chl a despite the same excitation energy was being used in the experiment. This is due to the lower dipole strength of Q_y band in Chl b ($D(Q_y) = 18.94 \text{ debye}^2$ in methanol) compared to Chl a ($D(Q_y)$

= 27.98 debye² in methanol) obtained from the index mappings of dipole strengths of the Chl *a* and Chl *b* as reported by Knox *et al.* [30].

Figure 4-5 Examples of double-sided Feynman diagram accounting for the stimulated emission (left) and ground state bleaching process (right)

In term of the peak shape, a typical 2D spectra shows a bleach lobe that is initially elongated along the diagonal and gradually becomes symmetrical as T_w increases [17,18]. This evolution is ascribed to the spectral diffusion process which causes an initially inhomogeneously broadened frequency to become homogeneous at later T_w [31]. Quantifying these dynamics using CLS ω_1 on Chl *a* dissolved in methanol has been reported by Wells *et al.* [17]. More recently, Moca *et al.* have reported the spectral diffusion dynamics of Chl *a* in various solvents using ellipticity method, concluding that the inhomogeneous broadening was promoted the most by viscosity and hydrogen bonding solvents and to a certain degree, polarity [18]. Differing from the previous studies, we chose the CLS ω_3 method to study the spectral diffusion dynamics of Chl *a* and Chl *b*. This method was chosen due to its robustness in retrieving the FFCF as recently reported by Cheatum *et al.* and Hauer *et al.* [32,33]. Despite the common experimental problems that can arise from the 2D experiment such as low signal to noise ratio, Stokes shift, and finite pulse duration, CLS show minimal

perturbation and give a better analysis to measure the dynamics of spectral diffusion. CLS was carried out by using a Gaussian function fitted to a slice parallel to the probe wavelength in a 2D spectra, and the center frequency was then determined. This was repeated for a series of slices at different ω_{pump} . In our analysis, we have analyzed ω_{probe} in a range of 300 cm^{-1} around the center of the signal to reduce the effect of noise at the sides of the peak. Collected in such way, series of peak positions were then fitted by linear regression, from which the gradient is defined as $\text{CLS}\omega_3$. The fitted $\text{CLS}\omega_3$ from the 2D spectra is denoted by the red line superimposed onto the plot as shown in Figure 4-3 and Figure 4-4.

4.4 Solvent dependence studies on Chl *a* and Chl *b*.

In order to understand the effect of local environment on the Chl *a* and Chl *b*, it is first important to investigate how different types of solvents affect the retrieved values of FFCF. We will start this section by discussing the $\text{CLS}\omega_3$ results of Chl *a* and Chl *b* in protic solvent methanol (MeOH), and solvents with decreasing polarity: Pyridine (Py) $\rightarrow$ Tetrahydrofuran (THF) $\rightarrow$ Diethyl Ether (Et₂O). Figure 4-6 shows the comparison of $\text{CLS}\omega_3$ among different solvents for the first 15 ps for Chl *a* molecule. The results show a significant difference in term of the slope values between the highly polar hydrogen bonding solvent (methanol) and other aprotic nonpolar solvents. When the solvent polarity was varied from Py to Et₂O, it does not result in a significant change in the values of $\text{CLS}\omega_3$. This illustrates that the FFCF is most affected by the high polarity or hydrogen bond solvent like MeOH and give only a minimal effect to the change of polarity in nonpolar solvents, which is in close agreement with existing results using the

ellipticity method [18]. We revise the solvent dependent study to clarify whether the method used shows sensitivity to the different type of environment which interacts with Chl *a* monomer. In addition, similar study was applied to Chl *b* molecules using methanol and pyridine solvents. The obtained results are plotted in Figure 4-7. As expected, the CLS_{ω_3} for Chl *b* also shows a similar trend as Chl *a* when two of the solvents were compared qualitatively ($CLS_{MeOH} > CLS_{Py}$). However, an analysis on the CLS_{ω_3} through quantitative comparison shows that the initial values of the slope (<500 fs) for Chl *b* in methanol started at a much higher value than that for Chl *a*. On contrary, in pyridine, the Chl *a* and Chl *b* started at an almost same slope value. This difference suggested that there are additional features that promote the inhomogeneous broadening in case of Chl *b*. It is likely that the additional formyl group in Chl *b* which participates in the hydrogen bond formation with the methanol largely limit the fast fluctuation of the porphyrin ring and thus the frequency fluctuation of Chl *b* electronic transition. We will further elaborate on this in the next section, where the CLS_{ω_3} of Chl *a* and Chl *b* will be compared in more details. We note here that the timescales obtained from the exponential decay fitted to the curve do not imply any direct correlation between the parameters of the solvent used. The decay time scales (τ_1) between 0.21 ps and 0.72 ps is attributed to the fast change of excitation frequency of Chl *a* and Chl *b* due to thermal fluctuation of the solvent molecules. The refilling of the hole in the ground state absorption is caused by this thermal fluctuation which is described as the fluctuation caused by the non-equilibrium distribution of thermal bath in excited state upon impulsive excitation to the system. Meanwhile, from from 1.9 ps to 63 ps decay times, it is assigned as the dielectric relaxation of solvents and intramolecular vibrational

redistribution (IVR) and/or vibrational relaxation. The long decay times from <1 ps to about 100 ps have been previously assigned as dielectric relaxation of solvent and intramolecular vibrational redistribution (IVR) and/or vibrational relaxation by several other groups [34,35]. Studies by Han *et al.* for example, have reported transient absorption of Chl *a* in methanol and found time constant range from 1.7-20 ps. They attributed this to the dielectric relaxation of solvents [36]. In other studies, time constant of 0.8 and 20 ps [37], 17 and 100 ps [38] and <30 ps and 60 ps [39] have been observed in BChl *a* and other dyes molecules dissolved in methanol and other solvents. Therefore, our result resembles well with the time constant measure in these studies. The range of time scales and assignment for τ_1 , τ_2 and τ_4 resemble with the previous results reported by Wells *et al.* except for the τ_3 decay time constant [17]. Recent studies by Moca *et al.* using the ellipticity method and 2DES modelling utilizing Brownian oscillator on Chl *a* molecules, have reported values of 30 ps and 60 ps in methanol and other solvents and thus resemble with our τ_3 decay time range [18]. The different between Wells *et al.*, Moca *et al.* and our studies may be due to narrow excitation bandwidth of pump and different methods used to extract FFCF. This issue has been thoroughly discussed in Chapter 3 of this thesis.

Although the spectral diffusion process occurs with almost similar time constants, the decay amplitudes (values showed in bracket in Table 4-2) for τ_1 (τ_3) in methanol are much lower (higher) compared to the other solvents. This suggest that the presence of hydrogen bond interaction between formyl and hydroxy group in protic solvent such as methanol largely limit the fast fluctuation of Chl *a*. Recent work by Tahara *et al.* studying DPPG and DPTAP using two dimensional heterodyne-detected vibrational sum frequency (2D HD-VSFG) and

CLS method have found similar trend, where the DPPG shows slow frequency modulation compared to DPTAP due to hydrogen bond interaction that formed between interfacial water and DPPG but not in DPTAP [25]. Therefore, it is possible that hydrogen bond interaction between formyl group in Chl *a* and hydroxyl group in methanol conformationally restrict the fluctuation of electronic transition and thus lower down the amplitude of fast fluctuation, τ_1 . The lack of amplitude for τ_1 in methanol is then compensated by the amplitude of τ_3 where in this time constant, the amplitude for methanol is significantly higher compared to the other solvents. Therefore, we suggested that this timescale is corresponds to hydrogen bond rearrangement of Chl molecule in methanol. Meanwhile, the relative amplitude for other aprotic solvent such as pyridine, tetrahydrofuran and diethyl ether show only a slight decrease in amplitude (τ_2 and τ_3) as the polarity of the solvents is decreases. This may be due to minimal effect of solvent polarity to the correlation function as reported by Moca *et al.* [18].

Figure 4-6 CLS ω_3 against T_w for Chl *a* (points) with fitted triexponential decays (lines) in methanol (Black), pyridine (Red), tetrahydrofuran (Blue), diethyl ether (Purple)

Table 4-2 Fit parameters of tri-exponential decays of CLS ω_3 values for Chl

***a.* The amplitudes are denoted in the bracket**

Solvent (Chl <i>a</i>)	τ_1(ps)	τ_2(ps)	τ_3(ps)	τ_4
Methanol	0.62 ± 0.15 (0.04 ± 0.03)	2.25 ± 0.45 (0.16 ± 0.01)	32 ± 4 (0.16 ± 0.01)	>1 ns (0.04 ± 0.01)
Pyridine	0.55 ± 0.19 (0.16 ± 0.06)	2.56 ± 0.74 (0.19 ± 0.06)	40 ± 8 (0.06 ± 0.01)	>1 ns (0.02 ± 0.01)
Tetrahydrofuran	0.21 ± 0.15	1.90 ± 0.50	48 ± 6	>1 ns

	(0.15 ± 0.02)	(0.18 ± 0.01)	(0.05 ± 0.01)	(0.02 ± 0.01)
Diethyl ether	0.60 ± 0.13	2.83 ± 1.38	34 ± 10	$>1 \text{ ns}$
	(0.19 ± 0.04)	(0.10 ± 0.03)	(0.03 ± 0.01)	(0.03 ± 0.01)

Figure 4-7 CLS ω_3 against T_w for Chl *b* (dots) with fitted triexponential decays (lines) dissolved in methanol (Black) and pyridine (Red).

Table 4-3 Fit parameters of fitted triexponential decays of $CLS\omega_3$ values for Chl *b*. The amplitudes are denoted in the bracket

Solvent (Chl <i>b</i>)	τ_1 (ps)	τ_2 (ps)	τ_3 (ps)	τ_4
Methanol	0.42 ± 0.20 (0.07 ± 0.02)	4.47 ± 1.77 (0.16 ± 0.02)	38 ± 7 (0.22 ± 0.02)	>1 ns (0.09 ± 0.01)
Pyridine	0.72 ± 0.23 (0.14 ± 0.04)	4.08 ± 1.36 (0.16 ± 0.03)	63 ± 15 (0.05 ± 0.01)	>1 ns (0.06 ± 0.01)

4.5 Comparison of spectral diffusion process between Chl *a* and Chl *b*.

In this section, we highlight a more important part of this study where we compare the results of $CLS\omega_3$ between Chl *a* and Chl *b*. As mentioned earlier, Chl *a* and Chl *b* have a very close resemblance in terms of the structure. The distinction comes from the side group substituent in which the methyl group at position 7 in Chl *a* is replaced with a formyl group (Figure 4-1). There have been numerous experiments performed, trying to identify the role of this formyl group in the hydrogen bond interaction with the protein residual in the photosynthetic membrane[10,13,40–42]. In the structure of Chls, the carbonylic oxygen in the porphyrin ring is the most nucleophilic center serving as the hydrogen-bond recipient [43]. In protic solvents such as methanol, it forms a hydrogen bond with the –OH group of the solvent ($C=O \cdots H-O$). Therefore, two of the potential

binding sites (13^1 and 13^3) that can form this type of hydrogen bond interaction can be found in Chl *a* tetrapyrrole ring[11], whereas an extra hydrogen bond site exists in case of Chl *b* with the formyl group (C=O) at position 7 [13]. In another study, Hooper *et al.* have proposed that modification at the periphery of the Chl molecules influences the coordination chemistry of the central Mg ion and this effect plays an essential role in the interaction of Chl *b* with LHC apoprotein and thus in the assembly of LHCs [16,42,44].

Our result shows that the CLS ω_3 analysis carried out on the Chl *a* and Chl *b* does not just respond to different types of solvent, but also to the interaction formed with the other type of substituent group in Chls. Figure 4-8 and Figure 4-9 show the comparison between Chl *a* and Chl *b* in methanol and pyridine respectively. As depicted in the graph, the CLS ω_3 for Chl *b* exhibit higher values than Chl *a*. This trend is apparent for both methanol and pyridine solvents at longer T_w points (> 0.5 ps). However, only a slight difference was found in pyridine at an initial time ($T_w < 0.5$ ps), where it shows that the CLS ω_3 for both Chls, started at almost the same values. This indicates that the absence of hydrogen bond interaction in pyridine solvent, may not affect significantly to the spectral diffusion dynamics at the short time scales.

The E_T^N value shown in Table 4-1 indicates that pyridine exhibits only 30% of the solvent polarity of water, thus classifying pyridine as a less polar, aprotic solvent [28]. Even though hydrogen bond interaction does not form between the formyl groups and pyridine molecules, the 7-formyl group in Chl *b* is reported to have an effect to the strength of interaction between ligand and the metal center of the porphyrin (Mg ion). As proposed by Hooper and Eggink, the central Mg ion in Chl *b* expressed more strongly positive charge than Chl *a*

[16,42]. The reason is that the additional formyl group in Chl *b* pull the electron density from the pyrrole nitrogen towards the substituent group and thus makes the Mg ion less shielded by the electron cloud than in Chl *a*. As a result, the negative end of a fixed dipole (solvent) will have a stronger interaction with the positively charged group of Mg^{2+} ion. This argument has been supported experimentally by measurement of the equilibrium constant of various tetrapyrrole derivatives with specific ligands done by Tamiaki *et al* [45].

Although it is not straightforward to relate this measurement with quantitative values of the FFCF, it is likely that the measured FFCF can be associated with the strength of ligation between solvent and central Mg^{2+} ion as speculated earlier by Moca *et al.* [18]. Interestingly, our results in Figure 4-9 indicate that the values of $\text{CLS}\omega_3$ for Chl *b* are higher than Chl *a* even after several hundred picoseconds of solvent relaxation (residual inhomogeneity).

Whereas, for Chl *b* dissolved in methanol (Figure 4-8), the same argument used for Chls in pyridine may also explained the slower spectral diffusion dynamics when compared to Chl *a*. However, the presence of hydrogen bond between methanol and 7-formyl group in this case will further enhance the strength of the electrostatic bond between the ligand and Mg ion [42]. This is because the hydrogen bond interaction will cause the pulling of the electron density outward of the tetrapyrrole ring. Thus, the Mg^{2+} ion is less shielded by the electron density of the ring and forms stronger electrostatic interaction with the ligands compared to an aprotic solvent. We proposed that this additional strong interaction is one of the contributing factors in the pronounced increase of $\text{CLS}\omega_3$ values as observed for Chl *b* in methanol. Therefore, to recapitulate our argument, we proposed that an additional presence of hydrogen bond due to the

7-formyl group in Chl *b*, is not the only reason affecting the inhomogeneity of Chl *b*, but it is also associated with the additional interaction between methanol and central Mg^{2+} caused by the effect of electron pulling from the hydrogen bond. Our results complement with the previous studies by Hooper *et al.* explaining that interaction between the protein in LHC and Chl *b* forms stronger coordination bonds than Chl *a* [16]. In another relevant studies, it has been reported that a stable reconstitution of LHCII in vitro can be formed with Chl *b*, but not Chl *a* [46]. This is due to the ability of Chl *b* to bind with the sites normally occupied by Chl *a* through multiple strong interactions but not vice versa [44,47].

Figure 4-8 CLS ω_3 as T_w progresses for Chl *a* (Red) and Chl *b* (blue) in methanol with the fitted triexponential decay, inset presents a CLS ω_3 decay at short T_w in range 100fs - 1ps.

Figure 4-9 CLS ω_3 as T_w progresses for Chl *a* (Red) and Chl *b* (blue) in pyridine with the fitted tri-exponential decay, inset presents a CLS ω_3 decay at short T_w in range 100fs - 1ps.

Table 4-4 Fit parameters to fitted triexponential decays of CLS ω_3 values for Chl *a* and Chl *b* in methanol

Sample	τ_1 (ps)	τ_2 (ps)	τ_3 (ps)	τ_4
Chl <i>a</i>	0.62 ± 0.15 (0.04 ± 0.03)	2.25 ± 0.45 (0.16 ± 0.01)	32 ± 4 (0.16 ± 0.01)	>1 ns (0.04 ± 0.01)
Chl <i>b</i>	0.42 ± 0.20 (0.07 ± 0.02)	4.47 ± 1.77 (0.16 ± 0.02)	38 ± 7 (0.22 ± 0.02)	>1 ns (0.09 ± 0.01)

4.6 Conclusion

The spectral diffusion process of Q_y transition of Chl *a* and Chl *b* has been studied using 2DES. By utilizing the CLS ω_3 method to extract the FFCF from 2D spectra, we have shown that the FFCF: (1) is sensitive to different solvent parameters, being highest to the solvent that is highly polar or form hydrogen bond interaction with the Chl's formyl group, (2) respond to the interaction formed with the different type of substituent group in Chls. In the first part, the same trend was found for Chl *a* using CLS ω_3 method as compared to the previous study, implying that the method used is also suitable to study Chl-environment interaction. In the second part of this chapter, we have made a comparison between the CLS ω_3 for Chl *a* and Chl *b* in both methanol and pyridine and observed distinct values of CLS ω_3 despite their close resemblance in term of structure. Slower decays of the FFCF (compared to FFCF of Chl *a*) was attributed to the effect of the hydrogen bond interaction formed with the additional formyl group present in Chl *b*. In addition, it is attributed to the stronger electrostatic interaction between the center metal of porphyrin (Mg^{2+}) and ligand (solvent) resulting from the electron withdrawing effect from the formyl group. Whereas, the environment surrounding methyl group at position 7 in Chl *a* is mostly non-polar, the hydrophobic repulsion or the steric hindrance may be one of the factors for fast decay of FFCF as observed in Chl *a*.

4.7 Reference

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Chapter 5

Measuring the Ultrafast Correlation Dynamics between the Q_x and Q_y bands in Chlorophyll Molecules

5.1 Introduction

Chlorophylls (Chls) are fundamental building blocks in plants and play a very significant role in photosynthesis. The primary function of Chls in plant is to absorb energy from the Sun and direct it to the reaction centre where the charge separation occurs. Due to its importance in understanding the energy capture and transfer processes in photosynthetic systems, there has been much experimental effort to measure the internal conversion rate in Chl. Han *et al.* studied Chl *a* using fluorescence up conversion techniques and reported an extremely fast internal conversion transfer from Q_x to Q_y band occurring in the range of 100 fs to 226 fs depending on the solvent [1]. This was then further corroborated by the transient absorption spectroscopy [2] and the non-adiabatic excited-state molecular dynamics simulation (NA-ESMD) studies [3]. In addition, studies on the Bacteriochlorophyll *a* monomer by Kosumi *et al.* using time-resolved fluorescence shows a relaxation time scale for the Q_x to Q_y band in around 50 fs. [4].

It is known that the $Q_x \rightarrow Q_y$ relaxation rate is highly complicated and depend on the energy gap ΔE between the two states. There is also evidence that

coherences between Q_x and vibronic states of Q_y can affect the transfer rate [5]. However, due to line broadening effects of the Q_x and Q_y transitions, there will also be a distribution of ΔE in an ensemble which will affect the transfer rate and the dephasing process of any coherences involving Q_x and Q_y transitions. Therefore, it is necessary to better characterize the distribution of ΔE . The extent of the distribution of ΔE depends on several factors. These factors include the linewidth of Q_x and Q_y transitions and more importantly, on whether the Q_x and Q_y transitions are correlated. There have not been many studies on the correlation between the Q_x and Q_y transitions. Friedrich *et al.* reported the frequency correlation of the Q bands in Mg-mesoporphyrin horseradish peroxidase (MgMP-HRP) using spectral hole burning spectroscopy [6]. Their results point to a conclusion that there is a lack of correlation between the two bands based on a broad non-resonant satellite hole in the Q_x band observed when a selective subpopulation of the Q_y band in an inhomogeneously broadened spectra was burnt. (We note that the Q_x and Q_y bands were assigned conversely from our experiment). These experiments are steady-state experiments and do not give the indication of the time-dependent correlation. The question of whether the Q_x and Q_y transitions are correlated at ultrashort timescales remain unresolved. Previous studies by Sundström and co-worker using transient hole-burning experiment on Chl *b* in pyridine showed that there is the transient hole-burning effect (spectral equilibration after early-time bleaching spectrum) when Q_y sideband was excited [7]. However, in a nonselective burning of the Q_x band, a similar effect was found to be diminished. In this thesis, we report on the ultrafast time-resolved correlation between the Q_x and Q_y transitions of Chl *a* using 2DES.

An absorption peak in the condensed phase, for example in the case of the Q_y transition of Chl *a* solution, is always broadened due to homogeneous, inhomogeneous and spectral diffusion contributions. There have been various studies using modern nonlinear optical techniques such as photon echoes and 2D electronic spectroscopy to characterize these contributions [8–12]. Under the Kubo lineshape theoretical framework, the frequency fluctuation correlation function (FFCF) of the Q_y electronic transition is given by $C_{yy}(T_w) = \langle \delta\omega_y(T_w) \delta\omega_y(0) \rangle$ and contains the relevant details to describe the linewidth [13]. Analogous to this function, we can also define a function $C_{xx}(T_w) = \langle \delta\omega_x(T_w) \delta\omega_x(0) \rangle$ and $C_{xy}(T_w) = \langle \delta\omega_y(T_w) \delta\omega_x(0) \rangle$ that measure the FFCF for the Q_x band and correlation between the transition of the Q_x and Q_y bands respectively. However, due to the fast internal conversion from Q_x to Q_y band (less than ~ 200 fs) and weak oscillator strength of the Q_x band compared to Q_y band, the term $C_{xx}(T_w)$ is omitted from this study. Therefore, only the term $C_{yy}(T_w)$ and $C_{xy}(T_w)$ will be the main focus of this study.

Recent analysis has shown that apart from the Q_y 0-0 band, the peaks that were originally assigned as Q_x and Q_y 0-1 bands (first vibronic band of Q_y) in Chl *a* are possibly overlapping [14]. However, in pyridine, Freiberg *et al.* have shown that the maximum peak of Q_y 0-1 band were distinct from Q_x by about 557 cm^{-1} in room temperature [15]. Due to a possible interference of the overlapping peaks, we have also performed similar studies on one of the molecules analogue to Chl *a* called Chlorin-e6 (Chl-e6). Chl-e6 differs from Chl *a* in that it has a greater separation between the Q_x and Q_y bands and thus provides a better model to study the correlation dynamics between the two transition states [14]. The chemical structure and linear absorption spectrum overlapped with the

spectrum of the excitation pulses uses for Chl *a* in pyridine and Chl-e6 in methanol are summarized in Figure 5-1 and Figure 5-2 respectively.

Figure 5-1 Chemical structures of chlorophyll *a* (left) and Chlorin-e6 (right).

Figure 5-2 Linear absorption of chlorophyll *a* (left) and Chlorin-e6 (right) overlaid with the spectrum of the excitation pulses used for 2DES measurement of Q_{yy} (red) and Q_{xy} (blue) experiment.

In this chapter, we use 2DES to characterize the correlation function $C_{xy}(T) = \langle \delta\omega_y(T) \delta\omega_x(0) \rangle$ which gives details to the correlation dynamics between Q_x and Q_y bands of the Chl *a* in pyridine and in a Chl-e6 in methanol.

In the next section, we describe the experimental technique as well as the CLS technique applied to both $Q_y \rightarrow Q_y$ and $Q_x \rightarrow Q_y$ experiment.

5.2 Theory

Based on the derivation used to obtain a diagonal peak based on FFCF [16], we can derive an expression for a 2DES cross peak function based on $C_{xy}(T) = \langle \delta\omega_y(T) \delta\omega_x(0) \rangle$. The rephasing and nonrephasing signal accounting for population transfer process from Q_x to Q_y can be pictured by the double-sided Feynman diagrams shown in Figure 5-3

Figure 5-3 Double-sided Feynman diagram, nonrephasing (left) and rephasing (right) accounting for population transfer of Q_x to Q_y band.

The third order response function for cross peak can be expressed as

$$R_{xy}^{R/NR}(t_1, t_2, t_3) \equiv i\mu_x^2\mu_y^2 (e^{\pm i\omega_x t_1} e^{-i\omega_y(t_3-t_2)}) F_{xy}^{R/NR}(t_1, t_2, t_3) \quad 5.1$$

where μ_x, μ_y is the transition dipole moment for the Q_x and Q_y band respectively.

$F_{xy}^{R/NR}(t_1, t_2, t_3)$ is a function that accounts for dephasing and therefore describe the shape of crosspeak:

$$F_{xy}^{R/NR(+/-)}(t_1, t_2, t_3) \equiv \left\langle \exp \left[\pm i \int_0^{t_1} d\tau \delta\omega_x(\tau) - \int_{t_2}^{t_3} dt \delta\omega_y(t) \right] \right\rangle \quad 5.2$$

We can expand the exponential function in Eq. 5.2 using 2nd order cumulant expansion and obtain the peak shape function as the following expression

$$\begin{aligned} F_{xy}^{R/NR}(t_1, t_2, t_3) &\equiv \\ &\equiv e^{i\omega_x t_1} e^{-i\omega_y(t_3-t_2)} \exp \left[-[g_x(t_1) + g_y(t_2) + g_y(t_3) \right. \\ &\quad \left. \pm h_{xy}(t_1, t_2) \mp h_{xy}(t_1, t_3) - h_{yy}(t_2, t_3)] \right] \end{aligned} \quad 5.3$$

Where $g_i(t)$ and $h_{ij}(t_1, t_2)$ are defined as

$$g_i(t) \equiv \int_0^t d\tau_2 \int_0^{\tau_1} d\tau_1 \langle \delta\omega_i(\tau_1) \delta\omega_i(\tau_2) \rangle \quad 5.4$$

$$h_{ij}(t_1, t_2) \equiv \int_0^{t_1} d\tau_2 \int_0^{t_2} d\tau_1 \langle \delta\omega_i(\tau_1) \delta\omega_j(\tau_2) \rangle \quad 5.5$$

$g_i(t)$ and $h_{ij}(t_1, t_2)$ are the lineshape functions which account for the spectral broadening of the signal. After some simplification Eq 5.3 becomes

$$F_{xy}^{R/NR}(\tau, T, t) \equiv \exp \left[- \left[g_x(\tau) + g_y(t) \mp \langle \delta\omega_y(T) \delta\omega_x(0) \rangle t\tau \right] \right] \quad 5.6$$

Utilizing the Kubo's stochastic theory of lineshapes, eq. 5.6 which account for rephasing and nonrephasing signal can be further expressed as

$$F_{xy}(\tau, T, t) \equiv \exp \left[- \frac{\Delta_x^2}{2} \tau^2 - \frac{\Delta_y^2}{2} t^2 - C_{xy}(T) t\tau \right] \quad 5.7$$

Where $C_{xy}(T)$ is defined as

$$C_{xy}(T) = \langle \delta\omega_y(T) \delta\omega_x(0) \rangle \quad 5.8$$

Upon Fourier transformation along τ and t and after some simplification, the peak shape function gives the frequency expression of

$$F_{xy}(\omega_\tau, T, \omega_t) \equiv \exp \left[\frac{-\omega_t^2 \Delta_x^2 - \Delta_y^2 \omega_t^2 + 2C_{xy} \omega_t \omega_\tau}{2(\Delta_x^2 \Delta_y^2 - C_{xy}^2)} \right] \quad 5.9$$

Using similar approach to the centre line slope (CLS) method devise by Kwak *et al.* [17], we can then determine the frequency fluctuation cross correlation function (FXCF) by taking the partial derivatives of the response function to zero, $d\bar{F}/d\omega_\tau = 0$. The normalized FXCF is readily shown to become proportional to the CLS by the following expression

$$\frac{d\omega_\tau^{max}}{d\omega_t} = \frac{\Delta_x}{\Delta_y} \bar{C}_{xy}(T) \quad 5.10$$

$$\text{Where } \bar{C}_{xy}(T) = \frac{C_{xy}(T)}{\Delta_x \Delta_y} \quad 5.11$$

In addition, we take into account of the effect of lifetime coherence broadening to the 2D peak shape by including the $1/2T_1$ term into Eq. 5.3 and Eq. 5.7, where T_1 is internal conversion time of Q_x to Q_y .

$$\begin{aligned} F_{xy}^{R/NR(+/-)}(t_1, t_2, t_3) \\ \equiv e^{i\omega_x t_1} e^{-i\omega_y(t_3-t_2)} \exp \left[-[g_x(t_1) + g_y(t_2) + g_y(t_3) \right. \\ \left. \pm h_{xy}(t_1, t_2) \mp h_{xy}(t_1, t_3) - h_{yy}(t_2, t_3)] \right] \exp \left(-\frac{t_1}{2T_1} \right. \\ \left. - \frac{t_3}{2T_1} \right) \end{aligned} \quad 5.12$$

Or after using Kubo's stochastic theory of lineshapes equation 5.12 become

$$F_{xy}(\tau, T, t) \approx \exp \left[-\frac{\Delta_x^2}{2} \tau^2 - \frac{\Delta_y^2}{2} t^2 - C_{xy}(T) t \tau - \frac{\tau}{2T_1} - \frac{t}{2T_1} \right] \quad 5.13$$

5.3 Centre line slope method

In recent years, there have been several methods developed to simplify the extraction of the FFCF from the 2D peak shape. These methods include nodal line slope [18], ellipticity [19], eccentricity[20] and center line slope (CLS). CLS method can be performed in two ways either taking slices parallel to pump (CLS_{ω₁})[16] or slices parallel to probe (CLS_{ω₃})[17]. Here, we employed the CLS_{ω₃} method to study both spectral diffusions in the Q_{yy} (FFCF) and Q_{xy} (FXCF) experiment. To retrieve FFCF we analyzed the diagonal peak (ω_τ, ω_t) = (ω_y, ω_y), whereas, the FXCF was obtained by analyzing the cross peak (ω_τ, ω_t) = (ω_y, ω_x) of Chl *a* and Chl-e6. Using similar protocol as obtaining FFCF, we imposed CLS_{ω₃} to the cross peak and retrieved the FXCF. The result of fitted linear regression of the determined maxima is denoted as the red line superimposed into Figure 5-4.

5.4 Experimental setup

Chl *a* (Chl-e6) was dissolved in Pyridine (methanol) to match 0.2 OD at the maximum of either Q_x or Q_y excited transition in the 1 mm quartz cuvette. The Chl *a* and Chl-e6 samples, purchased from Sigma Aldrich and Fisher Scientific respectively, were used without further purification. Spectral positions of the generated by the Noncollinear Parametric Amplifier (TOPAS White) pulses exciting Q_x and Q_y bands for Chl *a* and Chl-e6 are summarized in Table 5-1

Table 5-1 Summarized of the excitation wavelength for Chl-e6 and Chl *a*

Sample	Q_y-Q_y (cm^{-1})	Q_x-Q_y (cm^{-1})
Chl-e6 in MeOH	15150	18800
Chl <i>a</i> in Pyridine	14900	15630

Excitation pulses in all of the experiments were compressed to less than 1.3 times transform limited pulses using acousto-optic programmable dispersive filter pulse shaper (AOPDF) and the pulse duration was measured using a homemade autocorrelator ($\sim 29\text{-}35$ fs). To perform 2DES, AOPDF was further used to generate two pulse sequence with an interpulse delay τ (1st coherence time) varying from 0 fs to 153 fs in an interval of 3 fs. Experiments were performed in a partial rotating frame with a reference frequency (λ_{ref}) set at 13990 cm^{-1} for all experiment except for Q_{xy} in Chl-e6 experiment which was set at 17240 cm^{-1} . Phase cycling scheme of 1 X 2 was used in all of the experiments to eliminate strong background pump-probe signal as previously reported by Zhang *et al.* [23]. In all experiments, pump pulses were set to 40 nJ per pulse pair. The third interaction pulse (probe pulse) was a white light continuum pulse generated by $\sim 1 \text{ }\mu\text{J}$ of 12500 cm^{-1} (800nm) light focused onto a 2 mm thick sapphire window and collimated using a curved mirror afterwards. As the procedure of generating white light will introduce a positive group velocity dispersion from the nonlinear self-phase modulation effect (SPM), two pairs of the chirp mirrors were used to compensate the temporal chirp. Afterward, the probe light was passed through a half-wave plate and a polarizer, set at a magic angle (54.7°)

with respect to pump polarization, in order to eliminate rotational contributions to measured spectral diffusion. The spectral diffusion and the energy transfer of Chl *a* were recorded by collecting a series of waiting times, T_w , defined as the time delay between the second and third excitation pulses and was controlled by a high precision motorized delay stage with a translational motion resolution of one femtosecond. After the pump and the probe beams were spatially overlapped in the 1 mm flow cell, the pump beam was blocked while the probe beam sent to the spectrometer equipped with a CCD detector (Pixis 100B, Princeton Instrument) cooled at -75°C . The experiment was then repeated three times to ensure the reproducibility of our results. Finally, the absorption was recorded before and after the experiment to check for sample integrity.

5.5 Results

The representative results of purely absorptive 2DES for Q_{yy} and Q_{xy} at selected population time, T_w ($T = T_w$ from herein) for Chl *a* in pyridine, are shown in Figure 5-4.

Figure 5-4 Normalized 2DES of the Q_{xy} (top) and Q_{yy} (bottom) experiment for Chl *a* in pyridine at T_w values of 0.17, 1 and 10 ps (left to right) with corresponding overlaid CLS fits (red line). White dashed line indicated diagonal.

A common feature between the Q_{yy} and Q_{xy} is the formation of the negative peak at $\omega_3 \sim 14600 \text{ cm}^{-1}$ to 15300 cm^{-1} . This was attributed to the bleaching and stimulated emission signal of Q_y band for the Q_{yy} case and stimulated emission signal after internal conversion of Q_x to Q_y band for the Q_{xy} case. As can be seen in Figure 5-4, the peaks show an evolution from an elliptical peak shape at early population time to circular-like shape at longer T_w . This process is attributed to the spectral diffusion, where an inhomogeneous distribution of frequencies becomes homogeneous. It is noted that the bleaching signal at the diagonal peak resulting from excitation of Q_x band (15630 cm^{-1}) was not clearly seen at the initial T_w recorded in the experiment (data not shown). This is due to the instantaneous internal conversion from Q_x to Q_y in the range of 100 fs and low oscillator strength of the Q_x band[5,14]. Analyzing the cross peak, which dominantly results from the internal relaxation between Q_x to Q_y band,

shows an almost circular-like shape at the initial T_w in the Q_{xy} experiment as seen in Figure 5-4 (top). We can further analyze the 2D peak shape using CLS method on the cross and diagonal peaks for the Q_{xy} and Q_{yy} experiment respectively. The extracted CLS data plotted against T_w together with fitted exponential decay function for both experiments is shown in Figure 5-5. Comparing between FFCF and FXCF, the results show dramatically reduced values in the case of FXCF compared to the FFCF. Although FFCF and FXCF processes result from different origins, FXCF would be expected to have similar values if the Q_x and Q_y bands are highly correlated. Hence, FFCF provide a reference values on the relative correlation between the Q_x and Q_y band.

Figure 5-5 CLS ω_3 data as T_w progresses for Chl a in Pyridine with the corresponding fitted exponential decay described in Table 5-2 for FFCF (red) and FXCF (blue)

In the second part of the experiment, a similar study was conducted on Chl-e6 molecules. The absorption spectrum of Chl-e6 in methanol shows a large separation between the Q_x and Q_y bands, therefore, making Chl-e6 a suitable model to study the correlation between Q_x and Q_y bands independently. The representative results of 2DES measurements for Chl-e6 at selected T_w is shown in Figure 5-6.

Figure 5-6 Normalized purely absorptive 2DES spectra for the Q_{xy} (top) and Q_{yy} (bottom) experiment for Chl-e6 in methanol at T_w values of 0.17, 1 and 10 ps (from left to right) with corresponding CLS fits (red line)

The spectra for Q_{xy} experiment in Figure 5-6 (top) show a symmetrical circular shape for the cross peak. Whereas, in the Q_{yy} experiment the diagonal peak clearly undergoes spectral diffusion similarly as observed in the case of Chl *a*. Our experimental results on Chl-e6 are consistent with the previous observation on Chl *a*, which clearly show the lack of correlation between Q_x and Q_y bands. To put it into quantitative form, we have plotted the extracted CLS

data points against T_w with fitted exponential decay functions for Chl-e6 in Figure 5-7. Similar as in the Chl *a* case, our results show tremendous difference between extracted values of the normalized FXCF and FFCF, indicating the lack of correlation between Q_x and Q_y transitions. The time scales and amplitudes of the components of the fitted exponential decays for the FXCF and FFCF, for both Chl *a* and Chl-e6, are summarized in Table 1. We also note that no noticeable signal is observed on the diagonal peak in Q_{xy} case even for earliest recorded time delays (data not shown). This indicates that the internal conversion time from Q_x to Q_y band is instantaneous, faster than approximately 170 fs. Such process cannot be measured with the present experimental time resolution (IRF = ~150 fs). This is in contrast with the previous calculation results from Reimer *et al.* [14] on Chl-e6 dissolved in TME dioxane, which showed the Q_x to Q_y relaxation time of about 477 fs $\pm$ 10 fs. We note however that different solvent was used in their studies which may affect the relaxation time from Q_x to Q_y [1].

Figure 5-7 CLS data as T_w progresses for Chl-e6 in methanol with the corresponding fitted exponential decay described in Table 5-2 for FFCF (red) and FXCF (Blue)

Table 5-2 Fit parameter to measured exponential decays of CLS₀₃ values for Q_{yy} and Q_{xy} experiment

Experiment	A_1	τ_1 (ps)	A_2	τ_2 (ps)	A_3	τ_3 (ps)	$A_4 > 1$ ns
Chl <i>a</i>							
Q_{yy}	0.16	0.54	0.19	2.45	0.06	35	0.02
Q_{xy}	0.08	1.81	-	-	-	-	-
Chl-e6							
Q_{yy}	0.13	0.53	0.14	4.15	0.10	32	0.03
Q_{xy}	0.05	2.45	-	-	-	-	-

5.6 Analysis and Discussion

The FFXF reveals the time-dependent correlation between two transition states. In the present article it describes correlation between the Q_y and Q_x transition states of Chl *a* and Chl-e6. In general, as the value of $C(T_w)$ varies between -1 and 1, we can classify correlations into three main types: fully correlated with $C(T_w)$ equal to +1, fully anti-correlated for -1, and non-correlated for 0 [21]. These scenarios are depicted in Figure 5-8.

Figure 5-8 Three possible scenarios of correlation between two coupled electronic states when comparing the frequency fluctuation of the diagonal peak and the off-diagonal peak: correlated (left), anti-correlated (center) and non-correlated (right).

Under the assumption that Q_x and Q_y band have the same amount of inhomogeneous broadening, the Q_x and Q_y bands are correlated when the spectral motion for the Q_x band shifts in the same direction as the spectral motion for the Q_y band. The resulting values of $C_{xy}(T_w)$ for the fully correlated scenario will become positive and the cross peak will be elongated in the same fashion as the diagonal peak (Figure 5-8 (left)). Two transitions are anti-correlated if the spectral motion of the Q_x band shifts the transition frequency one way (e.g.

towards high frequency), while the spectral motion of Q_y band shifts the frequency the opposite way (e.g. towards low frequency). In this case the value of $C_{xy}(T_w)$ will be negative and the cross peak will be oriented perpendicular to the diagonal peak as shown in Figure 5-8 (center). In the last scenario, the Q_x and Q_y transitions are non-correlated if the spectral motion between Q_x and Q_y are randomized. The value of $C_{xy}(T_w)$ in this case will be equal to zero and the cross peak will form symmetrical circular shape as shown in Figure 5-8 (right).

Results presented here indicate a lack of correlation between Q_x and Q_y transitions for both Chl *a* and Chl-e6. The normalized FFCF and FXCF at the earliest measured population time show values of 0.43 (0.4) and 0.08 (0.05) for Chl *a* (Chl-e6), respectively. The FXCF decays from slight correlation to become non-correlated in 2.45 ± 0.44 ps for Chl *a* and 1.81 ± 0.266 ps for Chl-e6. FFCF for both samples do not decay to zero in the probed delay window of up to 400 ps and is left with the residual inhomogeneity which has been reported to decay in nanosecond timescale [8,9]. As shown in Eq 5.10, the amount of correlation can be affected by the ratio of frequency distribution of Q_x and Q_y bands. Therefore, we analyzed the effect of Q_x and Q_y distribution by taking the results reported by Reimer *et al.* using the vibronic coupling model for Chl *a* in pyridine and Chl-e6 in TME dioxane [14]. Table 5-3 shows the results of relative bandwidth of the Q_x and Q_y peaks.

Table 5-3 Half-width at half maximum (HWHM) of Q_x and Q_y band and their ratio.

Sample	$\Delta Q_x \text{ cm}^{-1}$ (1000)	$\Delta Q_y \text{ cm}^{-1}$ (1000)	$\Delta Q_x/\Delta Q_y$
Chl <i>a</i>	0.31	0.20	1.55
(Pyridine)^a			
Chl-e6 (TME dioxane)^a	0.33	0.20	1.65
Chl-e6 (MeOH)^b	0.32	0.27	1.19

^aObtained from [14]

^bCalculation using Gaussian fitting

For Chl *a* dissolved in pyridine, the distribution ratio of Q_x and Q_y is 1.55 and for Chl-e6 in TME dioxane 1.65. However, since the solvent used here for Chl-e6 is different from the one reported by Reimers *et al.*, we also include results from fitting Gaussian functions to the linear absorption spectrum of Chl-e6 in methanol. As can be seen in Table 5-3, the ratio for all the cases have values greater than unity ($\Delta Q_x > \Delta Q_y$). The same can be noticed for most of Chl analogues reported [14]. Therefore, the distribution ratio of Q_x and Q_y bands in Eq. 5.10 will not be the leading factor causing the decrease in FXCF values, but rather its increase, making the transitions seem more correlated than they are. Figure 5-9 shows schematic illustration explaining the effect of Q_x and Q_y distribution ratio to the peak shape of 2D spectra under the assumption that Q_x and Q_y bands are fully correlated.

Figure 5-9 Schematic illustration showing the effect of a different Q_x and Q_y frequency distribution on the peak shape of the cross peak. The diagram on the left depicts situation when $\Delta Q_x > \Delta Q_y$. The cross peak here will result in overestimated slope values. Whereas, for the diagram on the right, when $\Delta Q_x < \Delta Q_y$, the slope values of the cross peak become underestimated. Both cases are under the assumption that Q_x and Q_y are totally correlated.

Additionally, because of a very short lifetime of the Q_x band (< 170 fs), we analysed the effect of the lifetime coherence broadening on the 2D peak shape by modeling the 2D spectra. Simulated 2D spectra for different T_1 lifetimes (infinity, 100 fs, 50 fs and 5 fs) are shown in Figure 5-10. The simulation results show broadening of the 2D peak along ω_1 and ω_3 with decreasing of the Q_x lifetime. However, the CLS analysis of the simulated 2D spectra for multiple T_w delay times (Figure 5-11) shows that this effect is negligible for T_1 at 50 fs or longer. In the case of Chl *a* in pyridine, where the Q_x relaxation lifetime is around 107 fs [14], the effect of the lifetime broadening on the analysis can be assumed to be diminished. As for Chl-e6, although the internal conversion lifetime from the Q_x state in methanol is not yet known to the author, we hypothesize that it is between the ranges of ~ 50 -100 fs as reported in most of Chl analogue systems

[1,3,14,22]. Therefore, it is assumed here that this effect is also negligible to the FXCF measurement for Chl-e6 in methanol.

Figure 5-10 Lifetime coherence broadening effect to the 2D spectra at $T_w = 100$ fs and T_2 values equal to infinity, 100 fs, 50 fs and 5 fs.

Figure 5-11 CLS data from the simulated 2D spectra with different population lifetimes: infinity(Red), 100 fs (Orange), 50 fs (Green), 25 fs (Blue) and 5 fs (Magenta) (compared with the experimental value for Q_{xy} (Black Square) experiment

2DES has been shown as a powerful technique to resolve hidden couplings, increasing the resolution of states, etc. In addition, we have been able to demonstrate that it can also provide information on correlation in terms of frequency fluctuation of two electronic excited frequency states with a high temporal and frequency resolution. It is important to note here, that these processes are population free and the observed decay dynamics are based on the system-bath interaction induced by the instantaneous electric field of the excitation pulses. This process is governed by the memory function $C(T_w)$ which measures how well is the initial excited frequency being preserved under the influence of the surrounding solvent. Studies by Fidy and co-workers using the

fluorescence excitation spectra and hole burning experiment on MgMP-HRP, have shown lack of correlation between the two Q bands in the steady-state measurement [6] whereas our time-dependent studies using 2DES have shown that the Q_x and Q_y bands are slightly correlated at initial time of a few ps which then becomes non-correlated afterwards.

5.7 Conclusion

In summary, we have carried out experiments on Chl *a* in pyridine to study the relationship of frequency fluctuation between the Q_x and Q_y transition implementing 2D electronic spectroscopy. To the best knowledge of the author, this is the first use of the CLS method to determine the type of correlation between two coupled excited state in Chl *a*. Our results for Chl *a* dissolved in pyridine and Chl-e6 in methanol show a small amount of correlation at the initial time, and it becomes non-correlated after few picosecond timescales.

The spectral motion is randomized between Q_x and Q_y possibly due to a different environment “feels” by the perpendicular transition dipole moment of Q_x and Q_y band which causes it to fluctuate differently. It is hoped that the observed 2DES spectra here can be further studied using time-dependent computational methods such as ab-initio quantum mechanics calculation in order to determine the correlation between two coupled electronic state such as in chlorophyll *a* and Chl-e6 systems. The basic understanding of the porphyrin electronic system will be useful to further understand mechanisms of non-radiative relaxation process of the higher excited state of isolated chlorophylls or in a protein-bound system such as those in light-harvesting complexes (LHC).

5.8 Reference

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Chapter 6

Conclusion and Future Work

6.1 Conclusion

In the last two decades, studies in ultrafast spectroscopy have been focused on the core scientific questions in biology and material science. With the recent advancement in spectroscopic techniques, such as two-dimensional optical spectroscopy (2DOS), complicated information underlying congested spectra can be studied with high frequency and time resolution. In the early days of 2DOS, most of the studies were focused on the mid-infrared region (2DIR) which concerns mainly the vibrational motion of the molecular systems. Although 2DIR is still an active area of research, it is also important to investigate the energy landscape and dynamics of the electronic states of a system. 2DES is a powerful technique utilizing the ultrafast visible pulses to unravel hidden couplings between electronic and vibrational states, quantum coherence processes, energy relaxation pathways and solvation dynamics in a variety of systems. The aim of this thesis is to use 2DES to study spectral diffusion dynamics of Chl *a* and Chl *b* monomers in various solvents. In addition, we also investigate the type and amount of correlation between the two lowest electronic states of Chl *a*, labeled Q_x and Q_y .

2DES can be implemented either in non-collinear or partially collinear geometry. The advantage of using partially collinear geometry is overcoming of the “phasing” problem, which commonly occurs in the non-collinear (boxcar)

geometry. Inaccurate phasing of the pulses may distort the measured peak shape, thus giving inaccurate results of the performed experiment. We have used Chl *a* molecules to test the quality of the 2D spectra using pulse shaper assisted 2DES method. The technical details of our experimental setup have been explained in chapter two of this thesis. We provided a step-by-step approach for those who wish to convert their conventional pump-probe setup into a 2DES setup with minimal amount of disruption to the optical alignment. Data processing and analysis procedures were included to provide a description of how to retrieve frequency fluctuation correlation function (FFCF) from a purely absorptive 2D spectra.

FFCF is a physical quantity sensitive to changes in the solvation environment. However, correct analysis techniques must be first established in order to avoid ambiguous data interpretation. In the work of this thesis, we have utilized the 2DES experiment to study the effect of laser pulse bandwidth on the FFCF. Several methods for extraction of the FFCF from the 2D spectra such as ellipticity, center line slope for slices along pump CLS_{ω_1} and probe CLS_{ω_3} were tested to determine their robustness when using laser pulses with finite bandwidth. We have concluded that narrower excitation pulse can significantly distort the 2D spectra giving incorrect values obtained from the line shape analysis. However, for the most common case with narrow pump pulses and white light continuum used as the probe, we observed that CLS_{ω_3} method gives robust results not dependent on the bandwidth of excitation pulses. Furthermore, using simplified expression derived by Do *et al.*, we can recover the “full” FFCF using any of the mentioned methods regardless of the broadness of the pulses used.

Frequency evolution of the molecular oscillator described by FFCF can reveal dynamics and their timescales of the spectral diffusion processes. Using the CLS ω_3 method, we elucidate the spectral diffusion process occurring for the 1st excited state of Chl *a* and chlorophyll *b* (Chl *b*) molecules in various solvents. In a polar hydrogen-bonded solvent and non-polar solvent, our results showed four component decays. The first three timescales were attributed to the solvent relaxation and reorganization process (<100 ps). The last component which decays in nanosecond timescales was attributed to the residual inhomogeneity. Although the time scales of solvent relaxation for Chl show no direct correlation with any of the solvent parameters, the extent of inhomogeneous broadening, which manifests itself through the CLS amplitude, suggests a relationship with the solvent polarity. The extent of inhomogeneous broadening, being largest in polar protic solvent and lowest in the non-polar solvent, arise mostly due to dipole-dipole interaction and hydrogen bond interaction. Additionally, by comparing the FFCF between Chl *a* and Chl *b* in the same type of solvent we observed an effect resulting from the different substituent group. We observed that the replacement of methyl group in Chl *a* to formyl group in Chl *b* at position 7 cause a significant increase of the FFCF values. We associated this increase with the change in the ligation strength of the central metal, Mg²⁺, and increased number of available sites for hydrogen bond interaction.

Apart from measuring spectral diffusion, 2DES has the capability to identify the correlation between the two states of the molecule. A variation to the CLS method was derived in order to measure the frequency fluctuation cross-correlation function (FXCF) or $C_{xy}(T_w)$. The FXCF is related to the energy gap fluctuation process which determines the type and amount of present correlation.

This was used to investigate the correlation between the two lowest states (Q_x and Q_y) of Chl *a*. Using the frequency fluctuation correlation function (FFCF) of the Q_y transition as the reference, it was found that only a minimal correlation lasting few hundreds of femtoseconds exist between Q_x and Q_y transitions. This is likely due to the perpendicular orientation of Q_x and Q_y transition dipole moments which causes these two bands to fluctuate independently of each other.

Whilst much more experiments can be performed using 2DES, such as higher order 3-Dimensional electronic spectroscopy or two quantum two dimensional electronic spectroscopy (2Q2D), here we focused on the development of 2DES in terms of establishing a robust analytical and theoretical method to extract the FFCF and FXCF and their use to study the dynamics of spectral diffusion of Chl *a* and Chl *b* and establish correlation between Q_x and Q_y bands in ultrafast timescales.

6.2 Future work

Due to the small oscillator strength of the Q_x band of Chl, compared to Q_y band, as well as its strong overlap with the vibronic progression of the Q_y state (Q_{y1}) and Q_y state itself, determining its properties can be a challenging task. In order to overcome this, one of the directions for future work is to study system analogous to Chl where Q_x state is easily distinguishable. An interesting example of this is metal-free phthalocyanine (H_2Pc). Phthalocyanines have found wide applications as pigments and dyes but also, in organic electronics, solar cells, information recording media, photodynamic therapy or as nonlinear optical materials. Despite that, their electronic properties are yet to be fully determined. In future studies, we plan to focus on understanding the photophysics of the two

lowest excited states (Q_x and Q_y) of a metal-free phthalocyanine (H_2Pc) (Figure 6-1) using 2DES. As opposed to metal phthalocyanines or porphyrins, H_2Pc has non-degenerate Q_x and Q_y states allowing it to be independently resolved on a 2D spectrum.

Figure 6-1 Linear spectrum of H_2Pc overlapped with excitation pulse (left) and structure of H_2Pc (right)

Figure 6-2 shows an example of preliminary data on phthalocyanine taken by separately exciting Q_x and Q_y band.

Figure 6-2 Preliminary 2DES data of Phthalocyanine excite at Q_x band (top left) and Q_y band (bottom left) and plot of CLS against T_w for four visible 2D peaks of Q_{yy} (red), Q_{yx}(black), Q_{xx} (blue), Q_{xy} (pink).

Interestingly, our preliminary results show the evolution of the FXCF to progress from being correlated (positive CLS) to anti-correlated (negative CLS) during the first few picoseconds before becoming non-correlated in 50-60 ps (Figure 6-2 (right)). Furthermore, using H₂Pc, we can further study the polarization dependent response and the spectral anisotropy properties. Such an experiment is currently being performed in our laboratory. Besides exploring the dynamics of metal-free phthalocyanine, it is also promising to study phthalocyanine molecules with various metal centers exploring the role of central metal atom on spectral diffusion. Such molecules are commercially available with the central metal atom such as Vanadium, Iron, Osmium, and Aluminium.

Appendix

Autocorrelation setup

Figure A1. Autocorrelation setup for pulse width characterization

Figure A2. Autocorrelation measurement for the laser pulse (Black square) with fitted Gaussian function (red). The pulse duration is ~ 29 fs for $\lambda = 670$ nm and FWHM = 35 nm

Mathlab files

Program to process raw 2D data into a 2D purely absorptive spectra

```
%close all
clearvars -except LastFolder;
if exist('LastFolder','var')
    GetFileName = sprintf('%s/*real.txt',LastFolder);
else
    GetFileName = '*real.txt';
end

[FileName, PathName] = uigetfile(GetFileName,'Select the
sig2D.real.txt file for 2D data');

LastFolder = PathName;
FullFileName = sprintf('%s%s',PathName,FileName);

% FileNameCmplx = strrep(FileName, 'real', 'cplx');
% FullFileNameCmplx = sprintf('%s%s',PathName,FileNameCmplx);

% SigRawCmplx = load(FullFileNameCmplx);
```



```

SigRawReal = load(FullFileName);

FileHead = FileName(1:end-4);

PositionComma = strfind(FileName, ',');
FileName(PositionComma) = ' ';

Position = strfind(FileName, 'lambda=');
CenWave = sscanf(FileName(Position+7:end), '%d');

Position = strfind(FileName, 'tau=');
StepSize = sscanf(FileName(Position+4:end), '%d');

Position = strfind(FileName, 'tw=');
Tw = sscanf(FileName(Position+3:end), '%d');

Position = strfind(FileName, 'seq=');
Seq = sscanf(FileName(Position+4:end), '%d');

Position = strfind(FileName, 'phasecycle=');
PhaseCycle = sscanf(FileName(Position+11:end), '%d');

%-----
SigRawData = SigRawReal(2:end, 2:end);
XOffset = 8;
beta=-1;
if PhaseCycle==2;
    file_length2=size(SigRawData,2);
    file_length=file_length2/2;
    SigRaw1=SigRawData(:, (1+XOffset):(file_length-1));
    SigRaw2=SigRawData(:, (file_length+1+XOffset):(file_length2-1));

    SigData=(1/2)*((SigRaw1*exp(-1i*beta*0))+(SigRaw2*exp(-1i*beta*((2*pi)*(1/2)))));
elseif PhaseCycle==3;
    file_length2=size(SigRawData,2);
    file_length=file_length2/3;
    SigRaw1=SigRawData(:, (1+XOffset):(file_length-1));

    SigRaw2=SigRawData(:, (file_length+1+XOffset):((file_length*2)-1));

    SigRaw3=SigRawData(:, ((file_length*2)+1+XOffset):(file_length2-1));

    SigData=(1/3)*((SigRaw1*exp(-1i*beta*0))+(SigRaw2*exp(-1i*beta*((2*pi)*(1/3))))...
        +(SigRaw3*exp(-1i*beta*((2*pi)*(2/3)))));
elseif PhaseCycle==4;
    file_length2=size(SigRawData,2);
    file_length=file_length2/4;
    SigRaw1=SigRawData(:, (1+XOffset):(file_length-1));

    SigRaw2=SigRawData(:, (file_length+1+XOffset):((file_length*2)-1));

```

```

SigRaw3=SigRawData(:, ((file_length*2)+1+XOffset):((file_length*3)-1));

SigRaw4=SigRawData(:, ((file_length*3)+1+XOffset):(file_length2-1));

    SigData=(1/4)*((SigRaw1*exp(-1i*beta*0))+(SigRaw2*exp(-1i*beta*((2*pi)*(1/4))))...
    +(SigRaw3*exp(-1i*beta*((2*pi)*(2/4))))+(SigRaw4*exp(-1i*beta*((2*pi)*(3/4)))));

elseif PhaseCycle==5;
    file_length2=size(SigRawData,2);
    file_length=file_length2/5;
    SigRaw1=SigRawData(:, (1+XOffset):(file_length-1));

SigRaw2=SigRawData(:, (file_length+1+XOffset):((file_length*2)-1));

SigRaw3=SigRawData(:, ((file_length*2)+1+XOffset):((file_length*3)-1));

SigRaw4=SigRawData(:, ((file_length*3)+1+XOffset):((file_length*4)-1));

SigRaw5=SigRawData(:, ((file_length*4)+1+XOffset):(file_length2-1));

    SigData=(1/5)*((SigRaw1*exp(-1i*beta*0))+(SigRaw2*exp(-1i*beta*((2*pi)*(1/5))))...
    +(SigRaw3*exp(-1i*beta*((2*pi)*(2/5))))+(SigRaw4*exp(-1i*beta*((2*pi)*(3/5))))...
    +(SigRaw5*exp(-1i*beta*((2*pi)*(4/5)))));
end

%-----

StepSize = StepSize*1e-15;
CenWave = CenWave*1e-9;
CenFreq = 3e8/CenWave;
NStep = size(SigRawData,1);
Pad = 513;

TauAxis = 0:StepSize:StepSize*(NStep-1);
TauAxis = TauAxis*1e15;
YAxis = 3e17./linspace(CenFreq-(0.5/(StepSize)),CenFreq+(0.5/(StepSize)),Pad);
% YAxis = (linspace(-(0.5/(StepSize)), (0.5/(StepSize)),Pad))*1e-12;

XAxis = SigRawReal(1, (1+XOffset):(file_length-1));

% SigData(1,:) = 0.5*SigData(1,:);

SigPC = SigData;

Sig2D = fftshift(fft(SigPC,Pad),1);

```

```

c = 3e8;

YAxis=(c./YAxis)*(10^(-3))
XAxis=(c./XAxis)*(10^(-3))

data.X=YAxis
data.Y=XAxis
data.T=Tw/1000
data.Abs=Sig2D

figure (1)
contourf(XAxis,TauAxis,real(SigPC),10)
colorbar
xlabel('Probe (THz)');ylabel('Tau (fs)');
% axis equal
% ylim([0 100])
% xlim([426 484])
figure;
contourf(XAxis,YAxis,real(Sig2D),11)
xlabel('Probe (THz)');ylabel('Pump (THz)');
title(['Tw = ', num2str(Tw)])
axis equal
hline = reffline(1,0);
set(hline,'LineStyle','--','color','w','linewidth',2)
% axis equal
colorbar
% caxis([-30 1]);
% axis square
% ylim([540 600])
% xlim([480 600])
colormap parula(60)
% colormap jet
% xlim([50 725])

```

White light continuum (Probe) spectrum

Figure A3. White light continuum generation using 800 nm laser pulse (pulse duration = 120 fs) focused into a 2 mm sapphire crystal.