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**SOLVENT EFFECTS ON THE SPECTRAL DIFFUSION
DYNAMICS OF CHLOROPHYLLS**

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

2020

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JAMIE SIM HUNG NI

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

A thesis submitted to the Nanyang Technological
University in partial fulfilment of the requirement for the
degree of Master of Science

2020

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

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The contributions of the co-authors are as follows:

- Assoc. Prof. Howe-Siang Tan initiated the project direction and revised the manuscript draft
- Dr. Muhammad Faisal and Dr. Thanh Nhut prepared the manuscript draft
- Dr. Muhammad Faisal, Dr. Pawel J. Nowakowski, Dr Thanh Nhut, Hoang Long Nguyen and I performed 2DES experiments and assisted in the analysis and interpretation of results

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Jamie Sim Hung Ni

Abstract

Chlorophyll *a* (Chl *a*) and Chlorophyll *b* (Chl *b*) are pigment molecules that are vital to the process of photosynthesis in green plants. These molecules bind to the protein matrix through ligation with side groups in the protein scaffold. The manner in which protein side groups bind to the Mg^{2+} ions play an important role in the function of Chls, tuning the energy based on the nature of Chl-ligand interactions which aids in the acceptance of light energy for photosynthesis. In this thesis, we aim to understand the relaxation dynamics of Chl *a* and Chl *b* monomers in solvents to provide a greater insight of its behaviour in the protein matrix. We use ultrafast two-dimensional electronic spectroscopy (2DES) to determine the frequency fluctuation correlation function (FFCF) in a range of solvents, measuring the spectral diffusion process of the Q_y transition using the Centre Line Slope (CLS) method.

In this thesis, we experiment with how the interactions between the surrounding solvent molecules and Chl affect the FFCF in Chl *a* and Chl *b*. In chapter 2, the factor discussed is the polarity and hydrogen bonding ability of the surrounding solvent. We measure the FFCF decay for Chl *a* and Chl *b* in a protic solvent, methanol and aprotic solvents, tetrahydrofuran and diethyl ether, all of which have varying polarity. We find that the polarity of the solvent does not affect the FFCF decay but a significant ~ 40 ps component is only present in the protic solvent, methanol. We also observe that Chl *b* exhibits a higher level of inhomogeneous broadening as compared to Chl *a* despite their similarities in molecular structure.

Next, in chapter 3 we discuss the effect of Lewis basicity of the surrounding solvent. We perform 2DES experiments on Chls in solvents of various Lewis basicity, which is measured by the pK_a and BF_3 affinity. From the decay of the FFCF, we find that certain timescales of the spectral diffusion dynamics are correlated to the Lewis basicity of the solvent. Additionally, control experiments and theoretical calculations are used to try and better understand the molecular mechanism causing this correlation. The sensitivity of Chls to the Lewis basicity of their surrounding solvent is due to the dynamics of the dative bond between the Mg^{2+} of the Chl and the heteroatom of the solvent molecule. We are able to show that the 2DES method can act as a sensitive probe to study the molecular mechanism associated with the Lewis basicity of a solvent.

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1 Introduction

1.1 Chlorophyll

Photosynthesis is a process that is vital to the survival of human life. Green plants absorb sunlight and carbon dioxide to produce glucose and oxygen. The essential pigment-proteins involved in this process are known as Chlorophylls (Chls). Chls are green pigment molecules which exist in the protein complex of green plants. They are the means through which the light harvesting complexes absorb energy, which is crucial to the process of photosynthesis. The two main types of Chls in green plants are Chlorophyll *a* (Chl *a*) and Chlorophyll *b* (Chl *b*). Chls are amazing molecules with the ability to perform multiple functions in the photosynthetic apparatus. In the photosynthetic antenna, they aid in photon absorption and excitation energy transfer and storage [29]. However, in reaction centers their role is electron transfer and primary charge separation. Due to their versatility and their key role in the process of photosynthesis, these molecules have naturally attracted the interest of many research groups all over the world for the century. These two chromophores are also the main focus of this study.

Due to the importance of this pigment, it has been widely studied over the past few decades [3]. It was first discovered in 1818 by French chemists Pelletier and Caventou who gave it the name, Chlorophyll [4]. Then, it was first spectroscopically studied in 1864 [5] by Stokes who suggested the existence of two forms of Chl. There was much debate over a few decades as to whether two forms of Chl existed, until 1913 when Willstatter managed to isolate the two chromophores, Chl *a* and *b* [6]. He also proved for the first time that magnesium (Mg) was present in the molecule and eventually, the basic structure of Chl *a* and *b* was determined by Hans Fischer in 1937 [7]. The first person to successfully synthesise Chl *a* was Woodward in 1960 [8] which he received a Nobel prize for in 1965.

1.1.1 Early Spectroscopic Studies on Chlorophyll

Soon after the successful synthesis of Chls, many spectroscopic studies were carried out on the pigments. Infrared (IR) spectroscopy was used to confirm the structure of Chl by determining the presence of some ambiguous groups [9]. Sauer and coworkers [10] performed UV-Vis spectroscopy on various Chl pigments in CCl_4 , showing the existence of dimeric Chl at high concentrations, with a broadened spectrum and an additional shoulder at ~ 700 nm. Similar studies on the monomers and dimers of Chl *a* were conducted by Brody and coworkers [11] in a more nucleophilic solvent, ethanol but they still yielded similar results. Some early polarised absorption spectroscopic studies were used to study Chls *a* and *b* in lipid membranes to understand the orientation of the Chls, yielding information on the orientation of these Chls in the bilayer [12]. Further site-selected polarisation and fluorescence spectroscopic studies on Chl *a* provided more *in vivo* information, showing the behaviour of Chl *a* in Light Harvest Complex II (LHC II) has similar spectroscopic properties to low temperature monomeric Chl *a* [13].

Extensive Nuclear Magnetic Resonance (NMR) spectroscopy studies on Chl, its derivatives and aggregates helped determine their chemical identities and structure [14]. The association of Chl *a*, Chl *b* and many of its derivatives were also studied using NMR spectroscopy. Katz and coworkers performed many of such studies, playing a major role in the investigation of Chl-Chl and Chl-nucleotide interactions. They concluded that the Chl-Chl aggregation at high Chl concentration resulted mainly from the interaction of the Mg^{2+} of one Chl to the carbonyl group of another. They also showed the NMR spectra of Chl to be extremely solvent dependent between nucleophilic and non-nucleophilic solvents due to interaction between the Mg center of the porphyrin ring and the nucleophilic solvent molecules or ligands. [15]. Chl was already known to be coordinately unsaturated in the absence of ligand-metal coordination, however when dissolved in a nucleophilic solvents, the bare metal atom would bind with heteroatoms in one or both of its axial positions, with the coordination number being highly dependent on the

nature of the ligands [16], with penta-coordinated Chl being the dominant configuration in most nucleophilic solvents.

1.1.2 Recent Spectroscopic Studies on Chlorophyll

In more recent years, the interest in photosynthetic pigments has been more focused on the energy transfer dynamics in such systems. These studies aim to understand and replicate the highly efficient light-to-charge conversion of more than 95% in photosynthetic organisms [17]. Chls in particular have the role of absorbing energy through the light harvesting complex, which then transfers it to the reaction centre where the charge separation takes place. However, charge transfer in this highly complex system is not well understood in steady-state measurements due to the ultrafast rate in which this energy transfer takes place. Studies have shown that the energy transfer takes place on the scale of femtoseconds to picoseconds [18]. Therefore, to try and understand the fast excitation energy transfer in these systems, or solvent reorganization mechanisms and spectral modulation, experimental methods involving ultrafast spectroscopy are now more commonly employed.

Light harvesting complexes have been studied through various time-resolved spectroscopic methods to try and understand the energy transfer between Chls. Earlier ultrafast experiments mainly used transient absorption or, often referred to as pump probe spectroscopy and equilibration time for Chl *a* in LHC II was found to be $\sim 5 - 7$ ps [19,20]. This method was also used to determine the energy transfer from Chl *b* to Chl *a* in LHC II monomers and trimers at 77 K, yielding two transfer times ~ 200 fs and 3 ps [21].

Recently, two dimensional spectroscopy has been used more frequently to study these systems. For example, two dimensional electronic spectroscopy (2DES) has been used to study the transfer in the Chl *a* manifold of LHC II [18], show-

ing the transfer takes place on the scale of hundreds of femtoseconds to a few picoseconds. 2DES was also used to study the spectral diffusion in Chl *a*, making use of the ellipticity method in a range of polar and non polar solvents [22]. Two dimensional electronic vibrational spectroscopy has also been used on both Chl *a* and Chl *b* to distinguish the penta and hexa coordinated states of Chl in various solvents [23]

1.2 Chlorophyll-Environment Interactions

Chl pigments are molecules with a very unique structure. This uniqueness stems mainly from their extensive π -electron system [24] formed by the porphyrin ring which is chelated to a central Mg^{2+} ion. Upon binding to the protein, Chls experience conformational modulation which tunes its optical, electrochemical and electronic properties [25] and in actuality, Chls in proteins adopt a variety of geometries, meaning how they perform in the photosynthesis is not dependent on a unique Chl conformation [26]. To uncover how Chls interact with their environment in the protein matrix, much research has been done to better understand the properties of Chls outside of the protein structure in different solvent environments. Many spectroscopic studies have been conducted on Chls to investigate how hydrogen bonding, polarity and nucleophilicity of its surrounding molecules affect its absorption spectra [27]. It was concluded that there are two main solvation centers in the Chl molecule, a nucleophilic one (the carbonyl group on the porphyrin ring) and an electrophilic one (the electron deficient Mg^{2+} center). Where the former experiences hydrogen bonding with protic surrounding molecules and the latter experiences ligation from surrounding heteroatoms. This leads us to consider how Chls are affected by the polarity, hydrogen bonding ability and nucleophilicity (or basicity) of their surroundings. Understanding Chls in various solvents helps to better identify the Chl-protein interactions in light-harvesting complexes.

1.2.1 Hydrogen bonding and Polarity Effects

In the plant protein matrix, Chls are organised in a complex manner with various Chl-protein interactions such as hydrogen-bonding [28] or non-specific interactions such as hydrophobic forces [27,29]. One way they bind to amino acid side chains [1] is through hydrogen bonds formed between the polypeptide backbone NH and side chains to the C-7 formyl group in Chl *b* and C-13 keto group in both Chls [30].

To understand how Chls interact with their environment in the complex, many spectroscopic studies have been conducted on Chls to investigate how hydrogen bonding and polarity of its surrounding molecules affect its spectra. One such study analysed the solvent effects on the visible absorption spectra of Chl *a* in 20 solvents [27] taking into account the electrophilicity and nucleophilicity of the solvents concluding that hydrogen bonding reduces the Soret band significantly. A recent review summarizes the spectral properties of Chl *a* in more than 50 different solvents with varying polarity and hydrogen bonding strength [31]. Further absorption spectroscopy research on BChl *a* correlated the electronic transitions, Q_y and Q_x to the Onsager solvent polarity of the surrounding solvent. However, protic and aprotic solvents formed two groups with different correlation effects [29] where protic solvents resulted in higher sensitivity of the Chl to polarity and was attributed to hydrogen bonding interactions between the solvents and the carbonyl group on the Chl ring.

1.2.2 Ligation Effects

It is well known that Chl molecules bind to the protein matrix in plants through ligation with side groups in the protein scaffold. Chls bind strongly to the protein matrix through axial coordination between the side groups in the protein and the electron deficient Mg^{2+} ion of Chl. The manner in which protein side groups bind to the Mg^{2+} ions play an important role in the function of Chls, tuning the energy based on the coordination number of the Chl [2], altering its absorption characteristics [32] and changing how they contribute to energy transfer in the complex. In particular, the mode of chelation between amino acid residues and the Mg^{2+} ion is vital to the structural function of light harvesting complexes [33]. Hence, the Chl-ligand interaction is of key importance to the function of Chl molecules in the light harvesting complexes.

The different ways in which the coordinatively unsaturated Mg^{2+} center binds to the protein matrix results in many different geometries of the chromophore in the complex. However, the exact role of the Mg^{2+} centre of Chl in the photosynthetic pathway is yet to be well understood but it is well known to be crucial to the process of photosynthesis. Over the years, many studies have been done on Chls to better understand the Chl-ligand interactions. Extensive NMR spectroscopy studies have been conducted on the pigments in various solvents [15, 16]. They concluded that Chl molecules, when dissolved in polar solvents at low concentration, exists in their monomeric form with one or two heteroatoms coordinated to the bare Mg^{2+} center of the porphyrin ring. NMR spectra of Chl *a* was shown to be solvent dependent but when compared to NMR spectra of pheophytin, a chromophore similar to Chls but without the metal ion center, the solvent dependence was absent, concluding the Mg^{2+} center of the ring is crucial to this effect [34].

In non-polar solvents, the unsaturation of the central Mg^{2+} ion is satisfied with the oxygen side groups of another Chl molecule coordinating to the bare metal, forming dimers. Dimers of Chl *a* and Chl *b* are known to show a shoulder in

the UV-Vis absorption spectrum at ~ 700 nm [35]. IR spectroscopy was used to confirm that Chl dimers are formed in non-polar solvents due to coordination of oxygen-containing side groups to the Mg^{2+} center of Chl [34]. When dissolved in a polar solvent, the Mg^{2+} in Chl molecules can either be bonded to one ligand or two ligands, resulting a penta-coordinated or hexa-coordinated complex respectively. Early visible absorption spectroscopy studies on bacteriochlorophyll *a* (BChl *a*) in pyridine at varying concentrations show evidence of both the penta and hexa-coordinated state through the deconvolution of the Q_x band. These show a distinction between the Q_x band of penta and hexa-coordinated BChl *a* at ~ 580 nm and ~ 610 nm respectively [36]. However, this could not be resolved in Chl *a* or Chl *b* due to the overlapping Q_x bands.

In almost all known photosynthetic complexes Chls exist in the penta-coordinated state [37]. Even in various solvents, it is difficult for biligated Chl to be formed due to steric factors. The penta-coordinated Chl can be formed without steric repulsion, but the hexa-coordinated Chl requires the shift of Mg^{2+} ion back into the plane of the porphyrin ring [27]. However, there are some exceptions, such as in Light Harvesting Complex 1 (LHC I), where BChl *a* exists in both conformations [38] or when dissolved in strongly ligating solvents.

The properties of Chls have been studied through many different methods to interpret and assign the features of their absorption spectrum. A redshift of both the Q_y and Q_x band in Chl *a* was observed with increasing solvent polarity. The Q_x redshift is also confirmed to be due to the formation of hexa-coordinated Chl, with this effect being absent in derivatives without the central Mg^{2+} ion like pheophytin [29]. Through studies of the absorption and fluorescence spectra of Chl *a* at ambient temperatures and low temperatures [39] it was found that there is a red-shift and narrowing of the spectra at low temperatures along with an appearance of an additional Q_x band at 639 nm which indicates the presence of hexa-coordinated Chl *a*, confirmed by multiple other studies [40, 41]. In solvents such

as diethyl ether, Chl *a* exists in its penta-coordinated state at ambient temperature, but converts into the hexa-coordinated state at 77 K [42]. In our experiments presented later in this report, the experiments are done on Chl *a* and *b* at ambient temperatures with the aim of uncovering, at the molecular level the interactions between the surrounding ligands and the Chl molecules.

1.3 Two-dimensional electronic spectroscopy (2DES)

Two-dimensional electronic spectroscopy, also referred to as 2DES is a powerful technique to measure electronic coupling, relaxation processes, energy transfer and electron transfer [43]. While more conventional spectroscopy methods such as linear spectroscopy (UV-Vis or IR spectroscopy), Raman spectroscopy and even pump-probe spectroscopy only provide a one-dimensional view of electronic transitions or vibrational motion, 2D spectroscopy provides an additional axis giving us even more insight into the behaviour of a chromophore in its excited state.

1.3.1 Transient absorption spectroscopy

Figure 1: Diagram showing the TA spectroscopy set-up showing the pump-probe beam geometry used. The multi-coloured pulse is the probe pulse while the pink pulse is the pump pulse.

To begin with the explanation of how 2DES works, we often start by explaining a simpler method, transient absorption (TA) spectroscopy which is often referred to as pump-probe spectroscopy. TA spectroscopy is a method that allows one to obtain time-resolved information about the system of interest.

The set up for the TA experiment involves a pump pulse which is a ultrashort laser pulse used to excite the sample. This pump pulse interacts with the sample resulting in the population of the excited state. After some time passes, known as T , the probe pulse interacts with the sample. This probe pulse consists of a broadband pulse that probes the excited population and also acts as a local oscillator. The emitted third order signal is sent to the spectrometer, giving us the heterodyne detected intensity spectrum. This method allows one to track the changes in the

absorption of the system after a period of time, T and can be applied to monitor dynamics in many systems [44]. However, in the TA method only provides frequency resolution in the detection axis. Therefore, this method is limited by the one-dimensionality of the spectra. This led to further develop this method by implementing a pulse train excitation with an interpulse pump delay to resolve an additional axis, leading to the following discussion on 2DES.

1.3.2 Two-dimensional electronic spectroscopy

Two-dimensional spectroscopy is a superior method that manages to gather a significant amount of information within the framework of third-order nonlinear spectroscopy. In comparison to other methods, such as photon echo spectroscopy, transient grating spectroscopy and transient absorption spectroscopy, which was mentioned in the previous section, 2D spectroscopy provides significantly more information from the spectrum. Photon echo spectroscopy is most commonly used to distinguish static and dynamic line broadening and time-scales for energy gap fluctuations and allows for the separation of homogenous and inhomogeneous broadening. As for transient grating spectroscopy, it is a third-order technique often used for characterising relaxation processes but it is suited for looking at optical excitations with a well-defined spatial period with the first two pulses are time-coincident and therefore indistinguishable. It can even be said that, any other third-order experiment could be deduced from a 2D spectrum, which is why we choose to use this method to conduct our studies on Chl.

Figure 2: The pulse shaper assisted pump-probe beam geometry used in 2DES measurements. The multi-coloured pulse is the probe pulse while the pink pulses are the pump pulses after having gone through the pulse shaper

2DES could be viewed as an upgraded method of TA spectroscopy. 2DES involves two pump pulses and one probe pulse in the same pump-probe geometry. In our set-up, the first femtosecond laser pulse is sent through a pulse shaper [45] which shapes the pulse into a phase-locked double pulse train. Unlike the TA spectroscopy method, these pulses have a variable delay time, τ . After the two pump pulses have interacted with the sample and a delay time, T , the third pulse comes, the probe pulse. Once again, this is a broad-band pulse that probes the excited population and doubles as a local oscillator for heterodyne detection [46] of the third order signal. However, since 2DES has an additional delay time, τ , we can scan a range of τ for a specific T and resolve the coherence occurring between two excitations. Next, we Fourier transform along τ to obtain our 2D spectra with two axes, one for excitation and one for detection.

Conventional spectroscopic methods like linear spectroscopy can provide in-

formation on the energy levels of simple molecules, with well separated energy states. However, for complexes, their congested energy levels cannot be fully resolved using such methods. Information would be simply hidden under the featureless lineshapes. 2D spectroscopy also allows us measure the homogeneous and inhomogeneous broadening by measuring the linewidth of peaks, giving us more insight into the spectral diffusion taking place at an ultrafast timescale. Hence using 2D spectroscopy would allow one to dig much further giving us a fuller picture of the processes happening in molecules and complexes.

1.4 Center Line Slope

The Center Line Slope (CLS) analysis is a method commonly used in 2DES to extract frequency-frequency correlation from the spectrum. In this section, the theory of CLS will be discussed as well as why it is used to measure spectral diffusion.

Spectral diffusion is the process by which environmental fluctuations, such as intermolecular or intramolecular fluctuations, result in the frequency of a transition changing over time. In a 2D spectra, this would result in the linewidth of a peak changing or broadening caused by the loss in correlation between the excitation and emission frequencies. The linewidth broadening is a result of both homogeneous broadening and inhomogeneous broadening. The former being due to dynamic evolution caused by solvent-solute interactions and the latter is due to the local environment of each molecule, for example the varying conformations of a chromophore. These two broadening processes occur at extreme timescales, with the intermediary process of spectral diffusion occurring on the sub-picosecond to picosecond timescale [52] and can be quantified by the Frequency Fluctuation Correlation Function (FFCF) [54].

For an ensemble of two level systems, which is the case for this study, the

FFCF is shown by

$$C(t) = \langle \delta\omega(t)\delta\omega(0) \rangle \quad (1)$$

where the angled brackets are the ensemble average and $\delta\omega(t) = \omega(t) - \langle\omega\rangle$, which represents the time dependent fluctuation of the electronic transition from the average frequency, $\langle\omega\rangle$. The FFCF is often described as ‘memory function’ as it is indicative of how much memory the chromophore in the ensemble maintains over a period of time, or in our case a specific time, T_w .

In Kubo’s stochastic theory of lineshapes, [48] he describes the FFCF by a multi-exponential decay function, shown by

$$C(t) = \sum_i \Delta_i^2 e^{(-t/\tau_{c_i})} \quad (2)$$

where Δ_i^2 is the fluctuation amplitude and τ_{c_i} is the correlation lifetime of the i^{th} component.

Many methods have been developed to measure the FFCF, such as three-pulse photon echo peak shift spectroscopy [49]. In more recent years, multi-dimensional spectroscopy has been employed to study spectral diffusion by using the nodal line slope (NLS) [50], ellipticity [22] and CLS [51] method. Such methods were first used in the field of two dimensional infrared (2DIR) spectroscopy [52] to measure vibrational transitions and later using 2DES to measure electronic transitions [53].

A recent review was published comparing the above mentioned methods of extracting the FFCF from 2DES data [54]. The CLS method was mentioned to be a relatively simple method to extract the FFCF by fitting slices of the 2D spectrum either parallel to the pump axis or probe axis, resulting in CLS ω_1 and CLS ω_3 respectively. The ellipticity method was also discussed, where the diagonal and antidiagonal slices of the diagonal peak are extracted from the 2D spectrum and fit

with a Gaussian function. Lastly, the NLS method was mentioned to be more suitable for systems that have overlapping spectral features by measuring the slope of the nodal line formed between two spectral peaks with opposing signs [50].

In our studies, we find the CLS method the most suitable to extract the normalised FFCF from our 2D spectra. Kwak and group first proposed the CLS method [52, 55] to measure the normalised FFCF by tracking the changes of the frequency dependent peak maxima in slices of 2DIR spectra. They first proved that the CLS is equal to the normalised FFCF under certain approximations.

The CLS ω_3 is the inverse slope of the center line, which is determined by the maximum point of slices of the 2D spectra. We refer to Fig 3 to explain how the CLS ω_3 value is obtained. For spectra at different T_w , we select the window in which we want to extract the CLS ω_3 value, in this case it would be the bleach peak plus stimulated emission peak, as seen in Fig 3 (a). Then, 1D slices of the spectra are extracted within a certain window as seen in Fig 3 (b). As in Fig 3 (c), the actual data (black line) is then fit (orange line) to obtain the minimum point (white circle). All of the minimum points of the individual 1D slices are determined in Fig 3 (d) and finally, in Fig 3 (e) this line is fit with by a linear function to obtain a value of the slope. This value is the CLS ω_3 value for that specific delay time. The CLS ω_3 is essentially the gradient of the minima points which varies from 0 to 1 where all the memory of an initial frequency has been lost, resulting in a rounded lineshape. For our measurements, we take CLS ω_3 which means our slices are parallel to the emission or probe axis. We choose the CLS ω_3 method over others mentioned previously such as ellipticity, nodal line slope and CLS ω_1 (where the slices are parallel to the pump or excitation axis) as the CLS ω_3 method has shown to be the most robust method to analyse experimental data where there is the presence of noise, Stokes shift and other effects causing deviation in the spectra [53, 56].

Figure 3: Diagram describing the steps taken to determine the CLS ω_3 value of a purely absorptive 2DES spectrum. The different portions of the diagram are described as follows: (a) - the CLS ω selection window, (b) - the 1D slices of the spectra, (c) - the actual fit to the slice of data, (d) - the minimum point of each slice and (e) - the linear fit the to minimum points on the spectra.

The CLS ω_3 method is a robust one, which has been used to measure spectra diffusion properties of the Q_y band of Chl *a* [51] in methanol, measuring the FFCF to decay on three timescales with residual inhomogeneity. It was also used to measure the correlation between the Q_x and Q_y transitions in Chl molecules [57] by determining the frequency fluctuation cross-correlation function showing minimum correlation between the transitions.

For our study, we selectively excite the Q_y transition of both Chl *a* and Chl *b* in different solvents. This is with the assumption that the stochastic fluctuations

of this transition follow Gaussian statistics. The Q_y transitions are thought of as two-level systems, allowing us to use the CLS ω_3 method to study the spectral diffusion for waiting times, T_w between 100 fs and 800 ps. 1D slices of the main diagonal peak on the 2D spectra are taken parallel to the emission axis, ω_3 and the minimum points are extracted and fit with a linear line which gives us the final CLS ω_3 used for further analysis.

1.5 Experimental Methods

1.5.1 Sample Preparation

Chlorophyll molecules were purchased from Sigma-Aldrich and Chlorin e6 (Chl e6) was purchased from Fisher Scientific and used without any further purification. The solvents (2,3-dihydrofuran, tetrahydropyran, tetrahydrofuran, diethyl ether, 3-fluoropyridine, pyridine, 3-methoxypyridine and 4-methoxypyridine) were all of HPLC or analytical grade. Before use, they were soaked in $3\text{\AA}$ molecular sieves (Sigma-Aldrich) and bubbled with nitrogen before using them to dissolve the molecules to ~ 0.2 optical density (OD) for Chl a and Chl e6 and ~ 0.3 OD for Chl b. Due to the low oscillator strength of the Q_y band in Chl b, a higher OD was required to obtain a clean 2DES spectrum. We ensure the Chls are in their monomeric form by keeping the concentration of our samples low and ensuring the ~ 700 nm dimer peak is absent from the linear spectrum. Once the sample is prepared, it is put in a 1 mm quartz cuvette and immediately used for the 2D measurement. Before and after the 2D measurement, the linear spectrum and thin layer chromatography are checked to ensure minimal degradation (less than 5%) has taken place.

1.5.2 2DES set-up

We use a commercial Ti:Sapphire Amplifier (Legend Amplifier, Coherence, 800 nm at 1 kHz repetition rate) to generate an 800 nm output that is focused with a concave mirror into a hollow tube containing pressurised argon gas at a pressure of ~ 2.2 bar. The output of the tube is a supercontinuum pulse [58] that is then split by a beam splitter (90:10) to produce the pump and probe respectively.

The pump was compressed with an AOPDF (Dazzler, Fastlite) pulse shaper and measured using an autocorrelator to be sub-50 fs while the probe is compressed using a pair of chirped mirrors (DCM 9, Laser Quantum). The probe is set to be polarised at the magic angle (54.7°) with respect to the pump to remove isotropic effects. Finally, the 2DES experiment is carried out using the pump-probe geometry [59].

The 2DES experiment uses a 1×2 phase cycling scheme [?, 60, 61, 63]. The AOPDF is used to generate a two-pulse sequence with a coherence time, τ and delays the two excitation pulses from 0 fs to 120 fs with a step size of 5 fs. The two pulses gave a sum energy of 40 nJ per pulse pair. The experiment was conducted in the partially rotating frame with a reference wavelength of 710 nm ($1.41 \times 10^4 \text{ cm}^{-1}$) for all experiments. The pump and probe beams were spatially overlapped on the sample and the population time (T_ω) from -0.3 ps to 800 ps. However, we were only able to analyse data from ≥ 100 fs due to the presence of the coherent artifact which spectrally overlaps with our signal.

Figure 4: 2DES experimental set-up beginning with the white light generated from a pressured Argon tube [58]. The abbreviations are, ND - neutral density filter, BS - beamsplitter, P - polarizer, AOPDF - acousto-optic programmable dispersive filter, short-pass - short pass filter, flip - flip mirror, I/2 - half-wave plate, T - delay time, ref- reference beam, pr - probe beam, pu - pump beam, CCD - charge-coupled device.

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2 Solvent-Dependent Spectral Diffusion Dynamics of Chlorophylls

2.1 Introduction

Figure 5: Molecular structure of Chl *a* (left) and Chl *b* (right), where the -Phytyl chain represents a hydrocarbon chain $-\text{C}_{20}\text{H}_{39}$

Chls *a* and *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, the Chls in LHCs capture 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 within 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. A recent review summarizes the Chl *a* absorption and emission spectra from more than 50 solvents belonging to different groups (protic, non-polar, aromatic, etc.) [16]. It describes the mechanisms of solvatochromism and differentiation between the specific and non-specific interactions in Chl *a*. However, studies on steady-state measurement do not explain the details on time-dependent processes such as the solvent reorganization mechanism and spectral modulation which commonly occurs on the sub-picosecond to hundreds of picosecond timescales. To uncover such information, ultrafast nonlinear spectroscopic techniques such as time-resolved fluorescence (TRF) [17] and three-pulse photon echo peak shift (3PEPS) [18] have been applied. 2DES is a nonlinear optical spectroscopic tool that has become a powerful technique to elucidate the dynamics in condensed matter [19–22]. In recent studies, 2DES was employed to characterize the spectral diffusion dynamics of Chl *a* in a whole broad range of solvent environments [23–25]. The results indicate the amplitudes and time scales of frequency fluctuation and were attributed to the solvent reorganization effects occurring after the laser excitation. However, little has been reported on Chl *b*, specifically, the spectral diffusion dynamics of Chl *b* monomer.

Despite their close resemblance in structure, shown in Fig 5, the spectral properties of Chl *a* and Chl *b* molecules are quite distinct. For instance, in the linear spectrum shown in Fig 6, the 1st excited state (Q_y band) of Chl *b* can be seen to be blue shifted with respect to the Chl *a* [26]. Apart from that, studies on the ultrafast relaxation dynamics, such as transient absorption and multidimensional spectroscopy, demonstrate distinct kinetics in 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* [27]. This was attributed to the stronger mixing between the Q_x and Q_y bands in Chl *a* [28]. More recently, a new technique called the two-dimensional electronic-vibrational spectroscopy (2DEV) developed by Fleming et

al. has revealed the specific molecular vibrational spectra assigned to each type of the Chl allowing further distinction of these molecules [29].

In this chapter, we present results from the 2DES measurements performed on the monomer of Chl *a* and Chl *b* molecules in different solvents and investigate the nature of inhomogeneous broadening from the effect of specific interaction occurred in Chl-solvent systems. We applied the center line slope method (CLS ω_3) devised by Fayer et al. [30] to retrieve the normalized 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. Here, we measure and discuss the spectral diffusion dynamics as measured from the obtained CLS values for Chl *a* and Chl *b* in various solvents. Despite their similarities in terms of the structure, we observed distinct CLS ω_{3t} 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.

2.2 Results and Discussion

The linear absorption spectrum of Chl *a* and Chl *b* are shown in the diagram below with the broadband pump spectrum covering the Q_y transition of both Chls.

Figure 6: Absorption spectrum of Chl *a* (green line) and Chl *b* (red line) in Pyridine (Pyr) overlaid with the excitation spectra.

Figure 7: Chl *a* purely absorptive 2DES in methanol (MeOH) (first row), tetrahydrofuran (THF) (second row) and diethyl ether (DEE) (third row)

Figure 8: Chl *b* purely absorptive 2DES in MeOH (first row), THF (second row) and DEE (third row)

The purely absorptive 2DES spectra of Chls *a* and *b* at two different delay times in various solvents are shown in Fig 7 and 8 above. The response after pumping the Q_y bands of these Chls is a strong negative signal, due to ground state bleaching and stimulated emission of the first excited state. However, the signal in Chl *b* is always significantly lower than that of Chl *a* even though the same pump power is used. This is attributed to the low oscillator strength of the Q_y transition in Chl *b* [31]. Since the 2DES signal scales as fourth order with respect to the transition dipole moments, the signal for Chl *b* is significantly lower as compared to Chl *a*. This explains the rationale behind the preparation of Chl *b* samples of higher concentration, to try and minimise this difference but for all Chl *b* measurements, a longer data acquisition time is required to obtain a clean spectrum. Additionally, the larger excited-state absorption (ESA) in Chl *b* measurements is noticeable. Assuming the ESA is similar in magnitude for both Chls, the higher oscillator strength in Chl *a* would cause the ESA to appear to be weaker as compared to Chl *b* or even cause a larger Stokes shift in the peak position of the Q_y Chl *b* from the diagonal.

In terms of the peak shape, a typical 2D spectra shows a bleach lobe that is initially elongated along the diagonal and gradually becomes symmetrical as the T_w increases as can be seen in Fig 7 and 8. This evolution is ascribed to the spectral diffusion process which causes an initially inhomogeneously broadened spectrum to become homogeneous at later T_w [32]. Quantification of these dynamics using CLS ω_1 for Chl *a* dissolved in MeOH has been reported by Wells et al. [23]. More recently, Moca et al. reported the spectral diffusion dynamics of Chl *a* in various solvents using the ellipticity method. They concluded that the inhomogeneous broadening was promoted the most by viscosity and hydrogen bonding solvents and to a lesser degree by polarity [25]. Here, we use 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 [24, 33, 34]. CLS ω_3 was carried out by using a Gaussian function fitted to a slice parallel to the detection axis ω_3 in a 2D

spectra to determine the maximum signal position for the slice, which is so-called the center points. This was repeated for a series of slices at different excitation axis, ω_3 . In our analysis, we have analysed ω_3 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 center points were then fitted by linear regression to determine the center line, from which the gradient with respect to the vertical axis of the retrieved center line is defined as CLS ω_3 . The CLS ω_3 data is fit with a tri-exponential decay, $\sum_i A_i \exp(-\tau_i/T_w)$ with timescales, τ_i and amplitudes, A_i . We carefully choose a tri-exponential decay as it provides the best fit to the data. The fitted CLS ω_3 from the 2D spectra is denoted by cyan line superimposed onto the plot as shown in Fig 7 and 8.

Figure 9: CLS values (circles) plotted against their delay time, T_w with their resulting tri-exponential decay fit (solid line) in MeOH (blue), DEE (yellow) and THF (green) for Chl *a* (left) and Chl *b* (right) plotted on the logarithmic scale

Table 1: Fit results of the CLS values for Chl *a* in various solvents with their corresponding polarity [35], where E_T^N (water) = 1.0

Solvent	Polarity E_T^N	τ_1 (ps) [A ₁]	τ_2 (ps) [A ₂]	τ_3 (ps) [A ₃]	τ_4 [A ₄]
MeOH	0.76	0.27 [0.19]	4.63 [0.16]	42 [0.17]	[0.03]
THF	0.21	0.46 [0.28]	2.44 [0.16]	32 [0.04]	[0.01]
DEE	0.12	0.57 [0.24]	4.51 [0.10]	59 [0.02]	[0.02]

Table 2: Fit results of the CLS values for Chl *b* in various solvents with their corresponding polarity [35], where E_T^N (water) = 1.0

Solvent	Polarity E_T^N	τ_1 (ps) [A ₁]	τ_2 (ps) [A ₂]	τ_3 (ps) [A ₃]	τ_4 [A ₄]
MeOH	0.76	0.67 [0.12]	7.10 [0.18]	47 [0.22]	[0.06]
THF	0.21	0.33 [0.24]	2.10 [0.23]	45 [0.04]	[0.05]
DEE	0.12	0.41 [0.35]	2.40 [0.21]	32 [0.06]	[0.07]

We present the 2DES results for Chl *a* and *b* in protic solvent methanol (MeOH) and aprotic solvents, tetrahydrofuran (THF) and diethyl-ether (DEE) with their corresponding CLS ω_3 in Fig 7 and 8 above. The CLS results are then fit with an exponential decay function as explained previously and the results of the tri-exponential decay fits are shown in Fig 9 and the fit timescales are presented in Table 1 and 2. For both Chls, the results show a significant difference in the slope of the CLS ω_3 decay in polar solvent MeOH as compared to the less polar solvents. However, there is not a noticeable difference in the decay slope for solvents THF and DEE despite the difference in polarity, which agrees with previous spectral diffusion measurements using the ellipticity method [25]. This suggests that the difference in the decay is most likely due to the hydrogen-bonding that exists in MeOH but not in THF or DEE.

The first CLS ω_3 decay lifetime, τ_1 for both Chl *a* and *b* in range from ~ 200 fs to ~ 700 fs. We did not resolve this lifetime with further precision as

we did not have sufficient spectra for $T_w < 1$ ps. We assigned the first spectral diffusion timescale, τ_1 to the inertial component of solvation resulting from the libration motion of surrounding solvent molecules. These values can be compared to related values using time-resolved fluorescence (TRF) and three-pulse photon echo peak shift spectroscopy (3PEPS) solvation dynamics measurements. In TRF experiments using coumarin 153 molecule as a probe, the sub-picosecond fast timescale of the solvation dynamics in solvents MeOH and THF were measured to be in the range of 200 to 300 fs [17]. In a 3PEPS experiment using dye molecule IR144 as a probe molecule, a 50 to 70 fs timescale is observed for the hydrogen bonding protic solvents as well as other aprotic solvents [18] while a similar 3PEPS experiment with DTTCl as the probe molecule measures a fast component for 260 fs [36]. Our measured values are consistent with these timescales in general, which are assigned to the inertial component of solvation that results from the libration motion of the solvent molecules.

The next two decay lifetimes, τ_2 and τ_3 for both Chls show comparable A_2 amplitudes. However, there is a noticeable difference in the A_3 amplitudes where the A_3 value for MeOH is significantly greater than the other two aprotic solvents, where the A_3 is small. This becomes clearer when we compare the A_3/A_2 ratios, with a A_3/A_2 ratio of Chl *a* and Chl *b* in MeOH giving values of 1.06 and 1.22 respectively while the ratios in THF and DEE are only between 0.17 and 0.28. This is in agreement with TRF experiments [37] where the reported solvation dynamics of MeOH have similar relative amplitudes for the 3.2 ps and 15 ps timescale, while THF only has amplitude in the 1.5 ps timescale with no amplitude in the > 10 ps timescale. These picosecond timescales are attributed to the diffusion dynamics of solvent molecules [38] through the translational or rotational motion of the molecules. The ~ 40 ps timescale is observed in both Chl *a* and Chl *b* in MeOH. This timescale is likely to be due to the hydrogen-bond network which hinders the rearrangement of the solvent molecules.

The slowest component τ_4 in the exponential decay corresponds to a timescale that is larger than our maximum measured population time of $T_w \sim 1$ ns, therefore there are no timescale values for τ_4 , only the amplitude. The amplitude for this timescale, A_4 is consistently larger for Chl *b* in all three solvents as compared to Chl *a*. This component is a measure of the inhomogeneity of the system. 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, seen in Fig 5. 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, 39–42]. In the structure of Chls, the carbonylic oxygen in the porphyrin ring is the most nucleophilic center serving as the hydrogen-bond recipient [11]. In protic solvents such as MeOH, Chls form a hydrogen bond between the –OH group of the solvent and the carbonyl groups on the porphyrin ring. Therefore, two of the potential binding sites 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 [39]. This extra hydrogen bond with MeOH will limit the faster fluctuation of the porphyrin ring and thus the frequency fluctuation of Chl *b* electronic transition and explain the higher inhomogeneity observed in Chl *b* than Chl *a*.

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^{2+} 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, 43]. For the case of Chl *b* in THF and DEE, even though hydrogen bond interaction is not formed between the formyl groups and the solvent molecules, the 7-formyl group in Chl *b* is reported to have an effect to the strength of interaction between ligand and the Mg^{2+} center of the porphyrin. As proposed by Hooper and Eggink, the Mg^{2+}

center in Chl *b* expresses a stronger positive charge than Chl *a* [16,42]. The reason is that the additional formyl group in Chl *b* pulls the electron density from the pyrrole nitrogen towards the substituent group and thus makes the Mg^{2+} 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 the 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. [1]. 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 molecules and central Mg^{2+} ion as speculated earlier by Moca et al. [25]. For Chl *b*, this can increase the probability of the solvent- Mg^{2+} ion interaction to persist beyond 1 ns. There may be a distribution in the configurations of this solvent- Mg^{2+} ion interaction thus giving rise to higher A_4 values of the fit to the CLS ω_3 values as observed for Chl *b*.

2.3 Conclusion

The spectral diffusion process of the Q_y transition of Chl *a* and Chl *b* has been studied using 2DES. Although there have been several 2DES spectral diffusion studies performed on Chl *a* [23, 25], this is the first report of similar studies on Chl *b*. By utilizing the CLS ω_3 method to extract the normalized FFCF from the 2D spectra, we have shown two main points about the spectral diffusion dynamics of Chl *a* and Chl *b*.

Firstly, the spectral diffusion dynamics of Chls are sensitive to different solvent parameters, being highest for the highly polar solvents or those forming hydrogen bond interaction with the Chl's formyl group, shown in the case of MeOH. Secondly, the spectral diffusion dynamics respond to the interaction formed with the different types of substituent groups in Chls. The same trend was found in the previous study, implying that the method used here is also suitable to study Chl-environment interactions. We have made a comparison between the CLS ω_3 for Chl *a* and Chl *b* in protic solvent MeOH and nonpolar aprotic solvents THF and DEE, observing distinct values of CLS ω_3 of both Chls despite their close resemblance in terms of structure. Higher contributions to inhomogeneous broadening as observed for Chl *b* were attributed to the effect of the hydrogen bond interaction formed with the additional formyl group and 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.

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3 Direct accessing the molecular mechanism of solvent Lewis basicity using 2DES

3.1 Introduction

The acidity or basicity of an organic compound is one of the most fundamental chemical properties that it possesses. The concept of acids and bases was first proposed by Bronsted to be proton donors and acceptors respectively [1,2]. Later, the definitions were revised by Lewis who proposed a more general approach. He pointed out that many substances do not have protons or accept protons, falling outside the rather narrow definition of Bronsted acids and bases. He then defined acids to be electron pair acceptors and bases to be electron pair donors resulting in what is commonly known as the Lewis definition of acids and bases [3]. In the scope of this chapter, we mainly follow Lewis' definition of acids and bases.

The measurement and understanding of the molecular mechanism of acid-base behavior is of much importance in various fields of Chemistry. In coordination chemistry, it is used to understand the interaction between the ligands and metal centers of new catalysts by predicting the strength of the Lewis acid-base interaction. In medicinal chemistry, it is used to determine the binding sites of drug molecules in complicated enzymes. Lastly, in physical chemistry, it allows one to gain greater insights into various solvation dynamics in solutions.

The International Union of Pure and Applied Chemistry (IUPAC) definition of Lewis acidity/basicity is defined as the thermal tendency of a substance to act as a Lewis acid/base [4]. For aprotic organic compounds, the Lewis basicity is estimated by measuring the pK_a of the conjugate acid, or protonated form of the base. For such a compound, a higher pK_a would indicate a stronger basicity, or electron pair donating ability. The pK_a of an organic compound is a crucial parameter to estimate its Lewis acidity or basicity. Being such a useful parameter,

the pK_a values for various compounds have been well researched and presented in literature [2, 5]. However, there is one main drawback of the pK_a scale, which is the fact that it can only be used to measure compounds dissolved in aqueous solutions. This poses a huge problem as many chemical reactions are carried out in the absence of water due to their sensitivity to such an environment.

In light of this problem, another parameter can be used to determine Lewis acidity and basicity or the ligation strength of a Lewis base. One such parameter is the BF_3 affinity, which is defined as the change of enthalpy of the reaction between BF_3 and the investigated compound in an inert organic solvent, usually CH_2Cl_2 [6]. Due to the electron deficient Boron center and the relatively small size of the molecule, BF_3 is a good reference Lewis acid to measure the Lewis basicity in non-aqueous conditions and the BF_3 affinity has become a popular scale to estimate the Lewis basicity of organic compounds [7]. Various other scales to determine Lewis basicity have been proposed over the years. One such scale for a solute scale of Lewis affinity is the $SbCl_5$ affinity scale and $CH_3NH_3^+$ affinity scale which are similar to the above mentioned BF_3 scale, based on calorimetric measurements of Lewis acid-base reactions with the mentioned compound acting as the Lewis acid [8]. However, the BF_3 is the best reference Lewis acid due to its simple electronic structure, allowing for cleaner chemical reactions and straight forward quantum chemical calculations. Other methods such as solvatomagnetic quantification of hydrogen-bond basicity based on ^{19}F NMR chemical shifts [9], or solvatochromic scales measuring the Lewis basicity based on the shift of infrared absorption bands of ICN , ICl or I_2 [10–12] have also been developed.

Despite the diversity of established methods, the currently available basicity scales are purely phenomenological, based on steady-state observables. The physical process linking a detailed molecular mechanism to the macroscopic property of Lewis basicity is still lacking as it requires a time-resolved technique. In this study, we aim to provide a clearer physical picture using 2DES to study the spec-

tral diffusion of Chls *a* and *b* in various solvents.

Chl is the main pigment responsible for the light-harvesting and energy regulation of most photosynthetic organisms on Earth. Chls have been extensively studied in a wide range of scientific research fields. The two main types of Chls existing in green plants are Chl *a* and Chl *b*. As they usually function under a flexible and dynamical protein scaffold, solvent-dependent properties of Chls *a* and *b* have been investigated under various studies using both steady-state and time-resolved techniques. The solvation equilibrium constants of Chl *a* in various solvents were calculated from absorption spectra by Seely [13]. The study of Seely and Jensen analyzed the linear absorption spectra of Chl *a* dissolved in 40 different solvents and recovered a correlation between the absorption width and the solvents' refractive indexes [14]. Renger and Avarmaa investigated the absorption spectra of Chl *a* in terms of empirical parameters of Lewis basicity and steric constant to determine the two predominant solvation centers – an electrophilic Mg^{2+} center and a nucleophilic carbonylic oxygen. They also suggested that it is highly probable for the Mg^{2+} center to be attached to two axial ligands if the ligands have negligible steric effect [15].

Various time-resolved techniques have been implemented to study the solvation dynamics of Chls, especially the ultrafast dynamics, in different environments. Martinsson et al. reported the 3-ps solvation timescale of Chl *a* in pyridine using transient absorption spectroscopy [16]. A similar study was done on Chl *b* in pyridine and the solvent relaxation timescale was determined to be 1-3 ps [17]. Broadband pump-probe vibrational spectroscopy was implemented to investigate both electronic and vibrational dynamics of the Q_y band of Chl *a* by Du et al. They observed more than 25 vibrational modes, in which 11 and 8 of them were mainly contributed from the excited and ground state, respectively. Through vibronic coupling, the vibrational relaxation in Chl *a* was estimated to occur with the timescale below 1 ps [18].

With the recent development of multidimensional spectroscopic techniques, many studies have been conducted to further investigate the solvation dynamics in Chls. These techniques, coupled with data analysis methods such as ellipticity [19,20] and CLS [21,22] allow for better time resolution on the ultrafast solvation dynamics in a range of solvents. Moca et al conducted 2DES experiments on Chl *a* in a various polar and non-polar solvents, measuring the spectral evolution with the ellipticity method [23]. More recently, 2DES was also used, coupled with the CLS method to study the correlation between the Q_x and Q_y transitions in Chl *a*, showing only minimal correlation up to 2 ps [24].

In chapter 2 of this thesis, we used 2DES together with the CLS ω_3 method to measure the time-evolution of the diagonal 2D peakshape in both protic and non-protic solvents [25]. By measuring the CLS ω_3 with respect to the delay time, we resolved several dynamic timescales of Chls *a* and *b*, a sub-picosecond, few to tens of picosecond and tens to hundreds of picoseconds. The sub-picosecond timescale is usually attributed to the inertial solvation component originated from the libration motion of solvent molecules. The few to few tens of picosecond dynamics are assigned to the solvation relaxation of Chls [23,25]. Although the detailed mechanism is still elusive, the picosecond dynamics were observed to be promoted by hydrogen bonding and solvent polarity [23]. Other 2D electronic-vibrational (2DEV) spectroscopic experiments could distinguish between solvation states of Chls *a* and *b* and reaffirmed that in polar nucleophilic solvents, Chls *a* and *b* exist predominantly in the hexacoordinated state [26].

Despite the wealth of literature, the molecular mechanisms of Chl solvation dynamics, that cause the spectral diffusions at picosecond timescales, are not clear. The results in chapter 2 showed how 2DES together with CLS ω_3 can be used to distinguish Chl-environment interactions, exploring the Lewis basicity of the surrounding solvent. For this reason, we conduct 2DES experiments of Chls in a

broader range of different solvents to explore the solvent effect on the Chl diffusion dynamics. We are able to present the determination of spectral diffusion timescales of Chls *a* and *b* in 9 different solvents. From the correlation between the spectral diffusion timescale and the corresponding solvent's Lewis basicity, we can for the first time, determine at the molecular level, the spectral diffusion mechanism of Chl molecules after the optical excitation. This knowledge is valuable for chemists as the spectral diffusion lifetime can be used to estimate the basicity, i.e., ligation ability of a synthetic ligands. In addition, the insights about the ligation between Chls and nucleophilic molecules can be useful to understand the interaction between amino acid residues and the Chl pigments in the biological photosynthetic complexes.

3.2 Results and Discussion

We conduct 2DES experiments on Chl *a* and Chl *b* in various solvents. These solvents were selected based on a few criteria, first being their pK_a values, giving a wide range of Lewis basicity. The second would be the solvation ability of Chl in the solvents and the absence of dimer formation, which we see in non-polar solvents like hexane resulting in a ~ 700 nm peak in the absorption spectrum. We also chose these solvents to avoid the hydrogen bonding interactions between the solvent molecules and Chls that have been mentioned in chapter 2 to contribute to the picosecond dynamics, therefore none of the solvents studied can form hydrogen bonds. We were unfortunately unable to find solvents that could dissolve the Chl molecules with pK_a values ~ 0 . With these considerations in mind, the following solvents, 2,3-dihydrofuran (DHF), tetrahydropyran (THP), diethylether (DEE), tetrahydrofuran (THF), 3-fluoropyridine (3F-Pyr), 3,5-difluoropyridine (3,5F-Pyr), 3-methoxypyridine (3MeO-Pyr), pyridine (Pyr) and 4-methoxypyridine (4MeO-Pyr) were selected for these experiments. The molecular structures of the solvents are shown in Fig 10 below divided into two groups (ethers and pyridines) based on their heteroatom (O and N).

Group of Ethers

Group of Pyridines

Figure 10: Molecular structures of solvents used divided into two groups based in their heteroatom.

Figure 11 shows the linear spectrum of Chl *a* and Chl *b* overlaid with their corresponding pump spectra. Despite the similarities in structure between Chl *a* and Chl *b*, it can be seen from the absorption spectrum that the Q_y band in Chl *a* is redshifted as compared to Chl *b*.

Since the absorption spectrum of Chls differ based on the their solvent environment, the Q_y transition of both Chls are also more redshifted in more nucleophilic solvents like Pyr as compared to THF or DEE. For all 2DES of Chls in various solvents, we tune our excitation pulse to cover the Q_y transition depending on the solvent being used.

Figure 11: Absorption spectrum of Chl *a* (a) and Chl *b* (b) in Pyr (red), 3F-Pyr (green) and THF (blue) overlaid with their corresponding pump spectra covering the Q_y bands

Figure 12: Molecular structure of Chl *a* (left) and Chl *b* (right)

The structures of Chl *a* and *b* are shown above in Fig 12, it can be seen that the structures of both molecules are almost identical, with the exception of the formyl group present on the 7th position of the ring of Chl *b*.

3.2.1 2DES results

Figure 13: Representative 2D spectra of Chl *a* in THF (first row), 3F-Pyr (second row) and Pyr (third row) at $T_w = 0.2, 10, 47.5$ and 100 ps overlaid with the corresponding central points (white circles) and the linear fit (blue, green and red line respectively) to obtain the CLS.

Figure 13 shows the 2D spectra of Chl *a* in three different solvents. These measurements were chosen to display the spectral evolution of Chl *a* in solvents with a wide range of basicity. As seen from the 2DES spectra, the 2D peakshape at early T_w is much more diagonally elongated in all solvents as compared to at later T_w where the peakshape is much more rounded. This results in a lower CLS

ω_3 value at later times (seen by the larger slope of the line), in fact it is almost zero. Since the CLS ω_3 is proportional to the normalized FFCF, as stated in previous sections, we can say that spectral diffusion has taken place, resulting in the loss of memory of the molecule.

Figure 14: 2D spectra of Chl *b* dissolved in 4-methoxy pyridine at $T_w = 0.2, 10, 47.5$ and 100 ps. The white circles indicate the center points ω_t^{max} where the amplitudes of the horizontal slices peak. The cyan lines represent the linear fit to obtain the CLS values at each T_w .

As mentioned in introduction, the CLS method to extract FFCF is only applicable for the 2D peakshape of an ensemble of two-level systems. The peakshape

must be well-resolved, with no overlap with other spectral features and the fluctuation of the energy level must follow Gaussian statistics. In the case of Chl *b* dissolved in 4MeO-Pyr presented in Fig 14, we can clearly see that beside the negative ground-state bleach (GSB) signal, which is the main focus of CLS analysis, there are significant positive features due to the excited-state absorption (ESA). As shown in Fig 14, at early time of 0.2 ps, the GSB peak is slightly distorted, probably due to the ESA signal.

Furthermore, at later T_w , spectral diffusion has occurred and the amplitude of GSB signal decreases, resulting in a stronger distortion of the negative peakshape. This effect is clearly shown in the 2D spectrum at $T_w = 100$ ps, where the center points ω_t^{max} cannot form a straight line to determine the CLS. Therefore, the CLS values and spectral diffusion timescale of Chl *b* in 4MeO-Pyr cannot be determined accurately, reflected by the big error bar in Fig 16 seen later in this chapter.

3.2.2 BF₃ affinity calculations

In this study, we aim to observe how the spectral diffusion dynamics of Chls changes with the Lewis basicity of its surrounding solvent molecules. To do this, we chose the BF₃ affinity as one of the quantitative measurements of the basicity of the solvents used. However, some of the BF₃ affinity values were not readily available in current literature. For these solvents, we carried out our own BF₃ affinity calculations as shown for the following solvents.

We used the Gaussian 09 Rev D.01 [27] software to perform the DFT-RwB9TXD calculation of the BF₃ affinity values for the following solvents DHF, 3,5F-Pyr and 3F-Pyr. First, the molecular structures of BF₃, DHF, 3,5F-Pyr and 3F-Pyr are optimized both individually and with their Lewis coordinated complexes, using the DFT-RwB9TXD calculation [28] and DGDZVP basis set [29, 30] with the standard solvation model of CH₂Cl₂. Next, the enthalpy change of complexation, ΔH

is calculated using the following equation:

$$\Delta H = E_{BF_3-solvent} - E_{BF_3} - E_{solvent} \quad (3)$$

From the definition stated in chapter 1 of this thesis, the BF_3 affinity of a compound is the negative value of the corresponding ΔH and E denotes the energy after enthalpy correction of the optimized molecular structure. [7].

Table 3: Energy of the optimized molecular structures of BF_3 , solvent molecules, and the corresponding Lewis complexes

Molecule	Energy (Hartree/molecule)	Energy (kJ/mol)
BF_3	-324.515639	-852015.7284
DHF	-231.075041	-606687.4619
3,5F-Pyr	-446.594659	-1172534.165
3F-Pyr	-347.358417	-911989.4363
BF_3 -DHF complex	-555.615392	-1458768.072
BF_3 -3,5F-Pyr complex	-771.154049	-2024664.761
BF_3 -3F-Pyr complex	-671.922288	-1764131.798

Table 4: BF_3 affinities of three solvents calculated from the enthalpy change of the complexation processes (Eqn. 3)

Molecule	ΔH of complexation (kJ/mol)	BF_3 affinity (kJ/mol)
DHF	-64.881350	64.881350
3,5F-Pyr	-27.454168	27.454168
3F-Pyr	-30.266038	30.266038

3.2.3 CLS ω_3 results

The CLS ω_3 values for delay times, T_w between 100 fs and 800 ps is obtained and fit with a tri-exponential decay, $\sum_i A_i \exp(-\tau_i/T_w)$ with timescales, τ_i and amplitudes, A_i . We carefully choose a tri-exponential decay as it provides the best

fit to the data. The plot of the CLS ω_3 decay for three solvents is shown in Fig 15 and the fit result is obtained in Tables 5 and 6 below. It should be noted that since the solvents used are Lewis bases, the pK_a shown in the table represents the pK_a of the protonated species, or the conjugated acid.

Figure 15: The CLS ω_3 values in different solvents plotted against T_w (circles) with their corresponding multi-exponential fits (line) up till $T_w = 100$ ps, where most of the decay has finished

For these experiments, we were able to resolve three exponential decay lifetimes with an addition baseline which is due to the inhomogeneous contribution resulting in a long lifetime of > 1 ns.

Table 5: Fit results of CLS ω_3 values for Chl *a* in different solvents using tri-exponential functions presented with the corresponding pK_a of the solvents' conjugate acids and BF₃ affinities. Normalized FFCF amplitudes are denoted in squared brackets. The error bars are the standard deviations obtained from all independent measurements. *pK_a values estimated using ChemAxon. †BF₃ affinities calculated with Gaussian 09 Rev. D.01 as stated in the previous section.

Solvent	pK _a	BF ₃ affinity [kJ/mol]	τ_1 (ps) [A ₁]	τ_2 (ps) [A ₂]	τ_3 (ps) [A ₃]	τ_4 [A ₄]
DHF	-4.7*	64.881†	0.27 ± 0.04 [0.22 ± 0.01]	2.09 ± 0.25 [0.20 ± 0.02]	33.6 ± 7.3 [0.047 ± 0.005]	[0.020 ± 0.001]
THP	-4.1*	85.36±0.46	0.26 [6] ± 0.02 [0.23 ± 0.02]	2.45 ± 0.16 [0.227 ± 0.005]	24.85 ± 0.66 [0.067 ± 0.005]	[0.017 ± 0.005]
DEE	-3.59 [31]	78.77±0.38 [32]	0.39 ± 0.08 [0.17 ± 0.01]	2.08 ± 0.33 [0.16 ± 0.02]	31 ± 10 [0.05 ± 0.01]	[0.025 ± 0.005]
THF	-2 [5]	90.40±0.28 [32]	0.24 ± 0.10 [0.26 ± 0.02]	1.86 ± 0.23 [0.21 ± 0.02]	34.0 ± 5.3 [0.045 ± 0.005]	[0.010 ± 0.001]
3F-Pyr	2.8 [33]	126.633†	0.39 ± 0.16 [0.21 ± 0.02]	3.8 ± 1.1 [0.23 ± 0.03]	56 ± 15 [0.07 ± 0.01]	[0.018 ± 0.008]
3MeO-Pyr	4.81 [34]	127.67±0.48 [7]	0.90 ± 0.21 [0.17 ± 0.02]	10.7 ± 1.4 [0.23 ± 0.01]	203 ± 62 [0.0074 ± 0.005]	[0.026 ± 0.008]
Pyr	5.23 [35]	128.08±0.50 [32]	0.49 ± 0.30 [0.18 ± 0.02]	5.5 ± 2.3 [0.20 ± 0.02]	118 ± 45 [0.11 ± 0.01]	[0.026 ± 0.008]
4MeO-Pyr	6.58 [34]	135.27±0.45 [7]	0.76 ± 0.06 [0.18 ± 0.01]	10.96 ± 0.61 [0.21 ± 0.02]	120 ± 25 [0.097 ± 0.005]	[0.077 ± 0.012]

Table 6: Fit results of the CLS values for Chl *b*, presented in a similar manner

Solvent	pKa	BF ₃ affinity [kJ/mol]	τ_1 (ps) [A ₁]	τ_2 (ps) [A ₂]	τ_3 (ps) [A ₃]	τ_4 [A ₄]
DHF	-4.7*	64.881†	0.40 ± 0.13 [0.21 ± 0.01]	2.45 ± 0.23 [0.20 ± 0.02]	33 ± 7 [0.048 ± 0.008]	[0.048 ± 0.004]
THP	-4.1*	85.36±0.46 [6]	0.42 ± 0.09 [0.193 ± 0.004]	3.43 ± 0.54 [0.24 ± 0.02]	38 ± 11 [0.057 ± 0.005]	[0.063 ± 0.005]
DEE	-3.59 [31]	78.77±0.38 [32]	0.49 ± 0.09 [0.28 ± 0.04]	2.66 ± 0.15 [0.24 ± 0.01]	32.7 ± 1.5 [0.0047 ± 0.005]	[0.053 ± 0.005]
THF	-2 [5]	90.40±0.28 [32]	0.28 ± 0.04 [0.24 ± 0.02]	2.00 ± 0.19 [0.24 ± 0.01]	31.8 ± 3.0 [0.047 ± 0.005]	[0.053 ± 0.005]
3,5F-Pyr	0.18*	114.868†	0.46 ± 0.14 [0.20 ± 0.02]	4.77 ± 0.60 [0.19 ± 0.02]	67.0 ± 1.7 [0.118 ± 0.08]	[0.065 ± 0.005]
3F-Pyr	2.8 [33]	126.633†	0.53 ± 0.09 [0.22 ± 0.02]	5.2 ± 1.7 [0.20 ± 0.01]	102 ± 59 [0.09 ± 0.02]	[0.067 ± 0.005]
3MeO-Pyr	4.81 [34]	127.67±0.48 [7]	1.0 ± 0.5 [0.19 ± 0.02]	11.5 ± 1.3 [0.21 ± 0.02]	228 ± 41 [0.100 ± 0.001]	[0.060 ± 0.008]
Pyr	5.23 [35]	128.08±0.50 [32]	0.45 ± 0.15 [0.16 ± 0.04]	5.45 ± 0.81 [0.17 ± 0.01]	148.9 ± 6.8 [0.23 ± 0.01]	[0.093 ± 0.004]
4MeO-Pyr	6.58 [34]	135.27±0.45 [7]	0.29 ± 0.22 [0.28 ± 0.18]	3.25 ± 0.69 [0.20 ± 0.01]	116 ± 85 [0.14 ± 0.03]	[0.14 ± 0.03]

Upon further observation of the peak shape in Fig 13 the different rates of spectral diffusion are already noticeable in THF, 3F-Pyr and Pyr with $\tau_3 = 34.0 \pm 5.3$, 56 ± 15 and 134 ± 18 ps respectively. It can be seen that in THF even at $T_w = 10$ ps, the peakshape is very rounded and the spectral diffusion is almost complete which is supported by results in Fig 15, showing the CLS decay in THF is almost negligible after 10 ps. However, in 3F-Pyr the 2D peakshapes at $T_w = 10$ ps and 47.5 ps show very different central lines, where the CLS at 10 ps is more slanted. Referring to Fig 15, we can see an obvious decay in the CLS values in 3F-Pyr from 10 ps to 47.5 ps. Lastly, in Pyr the spectral diffusion rate is the slowest of the three, where the central line at $T_w = 100$ ps is still significantly tilted as compared to the central lines in 3F-Pyr and THF. This indicates the spectral diffusion process in Pyr at 100 ps is not yet complete which corresponds to the largest τ_3 value for this solvent.

From the results in the Tables 5 and 6 above, there are three main timescales. The first being the subpicosecond timescale, τ_1 ranging from $\sim 0.2 - 1$ ps which can be attributed to inertial solvation dynamics [36] through 3PEPS experiments. The picosecond timescales, τ_2 and τ_3 respectively are attributed to solvent relaxation processes. However, there are few studies that give further insight to the molecular mechanisms of these relaxation processes. Lastly, the nanosecond timescale, τ_4 is outside of our T_w range but is often assigned to a measure of the structural inhomogeneity of the chromophore [23].

The following discussion focuses on the third lifetime, τ_3 and its dependence on the nature of the solvent, in particular the basicity. As discussed previously, the pK_a of an organic compound is well known as one of the most commonly used methods to quantitatively measure its acid strength. It can be calculated by $pK_a = -\log_{10}(K_a)$ where K_a is the acid dissociation constant. A low pK_a indicates a stronger acid while a high pK_a would indicate a strong base. In our case we use the pK_a as a measure of the basicity of the conjugated acid. We also use the

BF₃ affinity as it is a broader description of Lewis basicity through the donation of a lone pair from a ligand (Lewis base) to BF₃ (Lewis acid) and measuring the strength of the BF₃-ligand bond. BF₃ is the perfect example of a Lewis acid due to its electron deficient boron atom, which would then easily accept a lone pair from heteroatoms. This method would better describe the interaction between the surrounding solvent molecules and their chelation to the Mg²⁺ ion of the Chl. The BF₃ values are listed in Table 5 and 6 with a higher BF₃ affinity indicating a higher Lewis basicity.

The τ_3 , third spectral diffusion lifetimes for Chl *a* and Chl *b* are plotted against the pK_a and BF₃ affinity values in Fig 16. We can see clearly from the graphs that the third diffusion lifetime of these Chls are correlated to the Lewis basicity of the solvent it is surrounded by, with a larger Lewis basicity resulting in a longer τ_3 . This correlation is particularly interesting as it could provide further insights into the relaxation mechanism happening at this timescale and will be discussed further.

esis that the third spectral diffusion component observed from the CLS dynamics is directly related to the fluctuation of the dative bond between the heteroatom of the nucleophilic solvent and the electrophilic Mg center of the Chl molecule.

We can then propose the spectral diffusion mechanism as such, after excitation, the coordinating solvent molecule would undergo some fluctuations which could change the bond length between the Mg^{2+} ion and the solvent molecule. This change in bond length alters the electron cloud of the porphyrin ring, resulting in the change in the frequency of the Q_y transition of the Chl. When the Chls are dissolved in solvents with lower Lewis basicity, the ligand- Mg^{2+} bond length would be longer due to the lower coordination ability of the solvent molecule. This longer bond would be more flexible increasing the degree of fluctuations that take place after excitation resulting in the faster decay of the FFCF, or we could describe this as the chromophore losing its ‘memory’ at a faster rate. This mechanism could explain the fast τ_3 lifetimes for solvent molecules with lower Lewis basicity and vice versa. However, the actual details of the fluctuation motions that cause the differences in FFCF decay cannot be pinpointed in this study. Instead, some possible fluctuation motions could be proposed such as the random wobbling motion of the solvent molecules [37] as suggested in the study of the structural and rotational dynamics CO_2 in an ionic liquid. Another possibility could be the structural reorganization of the entire Chl-solvent complex, seen in the study of molecular-organic frameworks in the presence and absence of dimethylformaldehyde [38]. This reorganization which would alter the dative bond length in our Chl-ligand system and affect the ligand dissociation-association process [38].

It can be seen in Fig 16 that the correlation to pK_a is seen more strongly in the Chl *b* measurements which is evident by the higher τ_3 values of the pyridine group in Fig 16. We attribute this to the difference in molecular structure of the Chls. As seen in Fig 12 and as mentioned previously, the only difference between the

structure of the Chls is the presence of the 7-formyl group in Chl *b* which is not present in Chl *a*. This 7-formyl group on the periphery of the ring pulls the electron cloud from the ring towards itself resulting in the metal center being more deshielded [39]. With a more electron poor metal center, the Mg^{2+} ion is more sensitive to the ligands around it. Hence, the heteroatom of the solvent molecules with higher Lewis basicity would bind more strongly to Chl *b* with a shorter bond length. With a less flexible bond and by the mechanism explained previously, this would lead to slower FFCE decay and higher τ_3 values. Chl *b* is hence more sensitive to the changes in the pK_a of surrounding molecules.

In a recent study, Okuda and group used 2DIR spectroscopy and theoretical calculations to investigate the vibrational modes of some ionic and non ionic molecules in water [40]. Using 2DIR and the CLS method, they obtained three timescales for the multi-exponential decay of the CLS which support the ones found in our experiments. For the anionic molecules, SCN^- and N_3^- , three ranges of lifetimes were found. These lifetimes are in the range of sub picoseconds, several picoseconds and tens of picoseconds. Where all of these timescales were caused by the change in the hydrogen bond network between the ions and the surrounding solvent molecules. In particular, the timescale in the range of several picoseconds was assigned to the structural reorganisation of the first solvation shell around the ions which is similar to our lifetime, τ_2 . The time scale in the range of tens of picoseconds was attributed to the vibration of the hydrogen bonds directly on the ion. This is similar to our explanation of τ_3 being caused by the coordination of the solvent molecules to the metal center of Chl. However, in the 2DIR study, the last lifetime was less than 10 ps causing difficulties in accurately determining this value due to noise. Comparing this to our experiments where the electronic excitation in Chl is up to nanoseconds, making this a good alternative method to probe slower dynamics.

3.2.4 Chlorin e6 control experiments

To support our hypothesis explaining the correlation between τ_3 and Lewis basicity being caused by the interaction between the ligand and the Mg^{2+} center, we carried out 2DES experiments on Chlorin e6 (Chl e6). The molecular structure of Chl e6 is shown below in Fig 17. This molecule was specifically selected as it has a structure very similar to that of Chls with its porphyrin ring. The main differences being the absence of the Mg^{2+} in the center of the ring and the hydrophobic tail. Studies on various derivatives of Chl have shown that the presence or absence of this hydrophobic tail has minimal effect on the solvation of the pigment and does not impact the first solvation shell of the chromophore [?]. Since the correlation of basicity of solvent and τ_3 is hypothesised to be due to ligand- Mg^{2+} bond strength, the following measurements act as controlled experiments.

Figure 17: Chlorin e6 molecular structure

The linear absorption of Chl e6 differs quite drastically from that of Chl, with its Q_y (~ 665 nm) and Q_x (~ 610 nm) band well separated as compared to Chl where they overlap. Also, the Q_y band of Chl e6 in different solvents shows mi-

nor changes in different solvents. The pump is adjusted to ensure the Q_y band is covered. All experiments on Chl e6 were conducted in the same manner as for the experiments on Chls mentioned previously.

Figure 18: Absorption spectra of Chl e6 in THF (blue), 3F-Pyr (green) and Pyr (red) overlaid with the pump spectrum covering the Q_y band

Figure 19 below shows the representative 2DES spectra of Chl e6 in Pyr. The CLS ω_3 was extra from the data in the same manner as in previous experiments. We fit the CLS ω_3 results with a tri-exponential decay, $\sum_i A_i \exp(-\tau_i/T_w)$ with timescales, τ_i and amplitudes, A_i .

Figure 19: Representative 2D spectra of Chl e6 in Pyr at $T_w = 0.2, 2.0, 800$ ps overlaid with the corresponding central points (white circles) and the linear fit (green line) to obtain the CLS.

Table 7: Fit results of the CLS values for Chl e6

Solvent	pK_a	τ_1 (ps) [A ₁]	τ_2 (ps) [A ₂]	τ_3 (ps) [A ₃]	τ_4 [A ₄]
THF	-2	0.39 [0.25]	2.29 [0.16]	26 [0.05]	[0.02]
3F-Pyr	2.8	0.35 [0.19]	2.83 [0.22]	25 [0.08]	[0.03]
Pyr	5.23	0.40 [0.24]	3.59 [0.19]	28 [0.70]	[0.08]

The results for the tri-exponential decay fits for the CLS of Chl e6 are presented in Table 7 above. The three solvents (THF, 3F-Pyr and Pyr) were selected as they span a broad range of basicity. On analysis of Table 7 the stark difference is that the exponential fit results for Chl e6 are essentially solvent independent, with all τ_3 values being similar, despite the difference in the solvent environment. We attribute this observation to the absence of the Mg^{2+} center in Chl e6. Therefore, Chl e6 does not experience the coordination of the surrounding ligands to a metal center, resulting in a relatively constant τ_3 even with a change in the solvent Lewis basicity. IR and NMR spectroscopy experiments conducted on Chl *a* and pheophytin, a molecule identical to Chl *a* with the absence of the central Mg^{2+}

show a solvent dependence in Chl *a* that is not present in pheophytin [41], showing the Mg^{2+} is crucial to this effect. These results further support our hypothesis that the third correlation lifetime at the tens to hundreds of picosecond timescale is due to the dative bond formed between the nucleophilic solvent molecule and the electrophilic Mg^{2+} center of the Chls. The τ_3 values in Chl *e6* could be attributed to intra-chromophore relaxation, which would explain the solvent independence.

The Chl *e6* experiments act as control experiments and support our hypothesis that the correlation between the third lifetime, τ_3 and the pK_a is due to the ligation of the solvent molecules to the metal center. Although τ_3 and the pK_a are shown to be well correlated, this spectral diffusion lifetime cannot be used as an analytical tool to determine the pK_a of a solvent. This is due to the following reasons - firstly, the CLS analysis that we use to measure the FFCF is based on the assumption that the system of interest is an ensemble of two-level systems. However, in actuality, molecules like Chls have systems with more than two electronic levels which results in an additional signal known as excited state absorption (ESA). This ESA is positive and can alter the main bleaching signal that we use to determine the CLS value. Furthermore, if the ESA is very strong it could even alter the peakshape of interest. In our experiments, the ESA signal is minimal in comparison to the main bleach signal and our CLS analysis is not significantly affected.

Another point to note is that if the ligand's Lewis basicity is not high enough, the dative bond formed between the metal center of Chl and the ligand will not be significant enough to result in a detectable spectral diffusion lifetime. We can see in tables 5 and 6 that the τ_3 values of the group of ethers do not differ greatly. However, the group of pyridines show quite a large difference in their third diffusion lifetimes. We attribute this to the fact that the group of pyridines form much stronger coordinate bonds to the electron deficient Mg^{2+} center. If one were to take a closer look at Fig 16, it is also clear that the τ_3 measured for 3MeO-Pyr is consistently longer in both Chl *a* and Chl *b* than a solvent with significantly

larger pK_a , 4MeO-Pyr. This is odd as according to our hypothesis, molecules with stronger pK_a would form stronger bonds with the Mg^{2+} center, resulting in a longer diffusion lifetime. However, in actuality there could be other factors that should be considered. In this case, we think that the molecular structure of the complex results in this anomaly. We assume that the bond formed between the N of the solvent molecule and the Mg^{2+} of the Chl is perpendicular to the porphyrin ring. In the case of 4MeO-Pyr, the methoxy group is on the opposite side N-Mg bond and would therefore not affect the interaction with the ring. However, in 3MeO-Pyr, the methoxy group would be nearer to the ring, in fact it would form a 120° angle with the lone-pair of the N atom. We think that when 3MeO-Pyr forms a dative bond to the Mg^{2+} metal, the methoxy group would have some secondary interactions with the ring which causes a stronger ligand metal bond, resulting in this high τ_3 value. Many other factors could contribute to the τ_3 values such as steric factors or even secondary interactions with groups on the porphyrin ring. However, we cannot expound further on this from the results collected in this study.

3.3 Conclusion

In this study, we demonstrate for the first time the application of 2DES to investigate the spectral diffusion of 2D absorptive peakshape of abundant photosynthetic molecules (Chls *a* and *b*) in various nucleophilic solvents to propose the spectral diffusion mechanism at the molecular levels. From the strong correlation between the longest spectral diffusion lifetime and the solvents' Lewis basicity, we can attribute the corresponding diffusion process to the fluctuation of the dative bond formed between the nucleophilic solvent molecule and the electrophilic Mg^{2+} center of the Chls. The proposed picture about the spectral diffusion mechanism through the dative bond between solvent and Mg^{2+} center is in good agreement with another similar study about hydrogen bond network in H_2O . It has been confirmed through 2D-IR measurements and molecular dynamics simulations that the direct interactions between the probe species and the solvent molecules in the first solvation shell undergo much more restricted fluctuation and the diffusion timescales are in tens of ps range. The several ps timescale dynamics are majorly induced by the molecules in the outer solvation shell with more flexibility and faster fluctuation. Compared to the vibrational excitation used in 2D-IR, our electronic excitation is more long-lived, providing an advantage for measuring the dynamics in the tens to hundreds of ps window.

The proposed mechanism was also verified with the controlled experiments on Chl *e*6, a molecule similar to Chl but lacking the Mg^{2+} center. Our results suggest that 2DES measurements on Chls can be used to estimate the Lewis basicity of an aprotic organic compound subjected to the solubility of Chl in that compound. Unfortunately, with the current experimental conditions, our 2D peakshape analysis cannot be used as an analytical tool to measure the Lewis basicity yet. But the insights about spectral diffusion mechanism are still useful to understand the dynamics of Chls in more complicated but important systems such as biological photosynthetic complexes.

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4 Summary and Outlook

4.1 Summary

In this thesis, we measure the spectral diffusion processes of pigment-proteins, Chl *a* and Chl *b* in various solvents. We obtain the 2DES spectrum and utilize the CLS ω_3 method to obtain the normalised FFCF of the Q_y ground state bleach peak. The CLS ω_3 data is measured and fit with a tri-exponential decay function to determine the timescales of processes causing the spectral diffusion at the ultra-fast timescale. These results demonstrate how information extracted from 2DES spectra are related to properties of the solvent environment including hydrogen bonding ability, polarity and Lewis basicity.

In Chapter 2, the CLS ω_3 values for Chl *a* and Chl *b* in three solvents, THF, DEE and MeOH were obtained with the aim of understanding Chl-environment interactions better. These three solvents had varying polarity, with DEE and THF being aprotic solvents and MeOH being the only protic solvent. The tri-exponential decay fit of the CLS ω_3 data yielded three timescales. It was found that although the polarity of DEE and THF differed quite significantly, there was minimal differences in their FFCF decay lifetimes. However, what was noticeable was the significant ~ 40 ps component in the only protic solvent, MeOH in both Chls. Additionally, when comparing the results between Chl *a* and Chl *b*, we observe that Chl *b* exhibits a higher level of inhomogeneous broadening as compared to Chl *a* despite their similarities in molecular structure. This was concluded to be due to the 7-formyl group that is present on the of the Chl *b* porphyrin ring, pulling electron density away from the Mg^{2+} center and strengthening the level of ligand-metal interaction as well as the hydrogen bonding interactions between the MeOH molecules and the formyl group

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In Chapter 3, we investigate the effects of Lewis basicity of the solvent on the normalised FFCF of Chl *a* and Chl *b* using the CLS ω_3 . For this study, we

measure the Lewis basicity of solvent molecules using two methods. The first is the more conventional method, the pK_a of the protonated molecule. The second, is the BF_3 affinity, measured by the negative enthalpy change of the coordination process between BF_3 and the solvent molecule. From the decay of the FFCF in 9 different solvents, we can clearly see a correlation between the third timescale (tens to hundreds of picoseconds) of the spectral diffusion dynamics and the Lewis basicity of the solvent environment. This correlation was attributed to the strength of the dative bond formed between the nucleophilic solvent molecules and electrophilic Mg^{2+} center of the Chls. This correlation is seen to be more significant in Chl *b* and is attributed to the stronger ligand-metal interaction due to presence of the 7-formyl group. A mechanism is proposed which is supported by controlled experiments on Chl *e*6, a similar molecule without the Mg^{2+} center. These studies showed minimal change in lifetimes despite significant change in the Lewis basicity of the solvent environment, showing that the Mg^{2+} center is the main factor in the correlation of Chls and Lewis basicity. We are able to show that the 2DES method can act as a sensitive probe, allowing us to study the molecular mechanism associated with the Lewis basicity of a solvent. These results open the door to the possibility that 2DES measurements may even be used to estimate the Lewis basicity of an organic solvent.

Overall, this thesis demonstrates how 2DES is a robust method that can be applied to help us understand the dynamics of Chl-environment interactions. Through the use of the CLS ω_3 method, we can extract the normalised FFCF and track the solvent dynamics. In Chl *a*, the effects of changes in the solvent environment seem to be less significant as compared to in Chl *b* due to the minor difference in molecular structure. In light harvesting complexes, the manner in which Chls interact with the protein matrix are crucial to their functionality in the process of photosynthesis. These studies give us more information as to how Chls interact with their environment with the aim of understanding how they interact with the protein matrix *in vivo*.

4.2 Outlook

The above mentioned experiments using 2DES have provided many insights into the molecular dynamics of Chl-ligand interactions and even presented the possibility of using 2DES and the $\text{CLS}\omega_3$ method as a measure of Lewis basicity of organic solvents. However, there are some limitations and possible improvements that will be presented below.

Firstly, our results are limited by the number of solvents that we used. For the study in Chapter 2 comparing the effects of hydrogen bonding on the FFCF decay of Chls, further studies could be done with more protic and aprotic solvents to confirm that the presence of hydrogen bonding results in a significant tens of picoseconds lifetime. Additionally, we could do 2DES measurements on Chls in various alcohols with different hydrogen bonding strength which has been measured using atomic force microscopy [1, 2], computational calculations based on ab initio and atoms in molecules methods [3] and cyclic voltammetry [4] to measure the effect of hydrogen bonding ability on the amplitude of the long timescale. For the study in Chapter 3 our measurements suggest that 2DES can be used with Chls as molecular probes to estimate the Lewis basicity. However, we cannot use it as analytical tool yet. The solvents chosen had to meet certain criteria - where the Chls were soluble and existed in their monomeric form. As a result, we only used two groups of solvents, the group of ethers and the group of pyridines resulting in the lack of data points in the region where $\text{pK}_a = 0$. In future studies, a wider range of solvents could be used to give us a clearer correlation.

Secondly, all of our $\text{CLS}\omega_3$ decays are fit with a tri-exponential function as these give the best fit results. However, we lack a way to measure the accuracy of the fit and there could be more timescales than indicated from our results. One of the possible ways to overcome this problem would be through low tempera-

ture measurements of Chls in various solvents. At low temperatures, the ultrafast dynamics would slow down and spectral diffusion would occur at a slower rate, allowing us to identify the number of timescales and fluctuations occurring at these timescales, more clearly. Additionally, further mathematical analysis could be done on the exponential decays to better identify the number of lifetimes present though deconvolution of the multi-exponential decay.

Lastly, to better understand the nature of interactions between the solvent molecules and Chls that cause these fluctuation lifetimes, more complex simulations and calculations could be conducted. Quantum mechanical and molecular mechanical (QM/MM) calculations have been conducted on Chl *a* and Chl *b* to calculate the absorption energies in various solvents [5] and similar QM/MM calculations were conducted on Chls in the reaction center to determine the effect of axial ligands bound to the Mg^{2+} , concluding that a hydrogen bond network exists in photosystem II [6]. Such calculations would provide a clearer picture of how ligands interact with the Mg^{2+} center of Chls dissolved in solvents and how other factors such as steric effects play into the strength of the metal-ligand bond. Such calculations could give insight into other interactions between solvent molecules such as steric factors, secondary interactions and hydrogen bonding and how it directly affects the measured FFCF.

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