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Measuring the Spectral Diffusion of Chlorophyll *a* Using Two-Dimensional Electronic Spectroscopy

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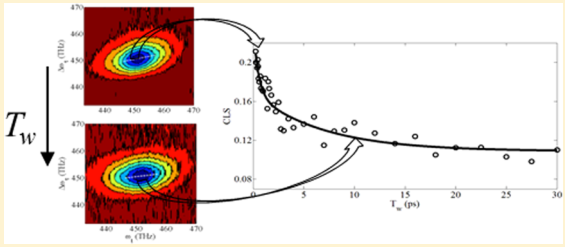
ABSTRACT: Ultrafast two-dimensional electronic spectroscopy has been used to study the spectral diffusion of the Q_y transition of chlorophyll *a* in methanol. The two time frequency-fluctuation correlation function (FFCF) of the transition has been measured using the center line slope method, together with optimized fitting of the linear spectrum. The FFCF was measured to decay over four time scales. The three fastest time constants of which were measured to be ∼65 fs, ∼0.5 ps, and ∼7 ps. These are assigned as the inertial component of solvation and spectrally diffusive solvation processes respectively. The fourth time constant (>1 ns) may be attributed to the chromophore structural inhomogeneity.



INTRODUCTION

Naturally occurring light-harvesting complexes have been extensively investigated for the past few decades.[1−6] Recent studies have observed quantum coherences between different excitonic states in an increasing range of these complexes,[7−9] even at physiologically relevant temperatures.[10] The excitonic states in question are predominantly localized over selections of chlorophyll pigments contained within the protein scaffolding, which has been suggested to play a nontrivial role in the survival of these coherences over extended time scales.[11]

Relevant studies in chlorophyll have been sparse in the literature, given the important role that these pigments play in light capture and the subsequently populated excitonic states in light-harvesting complexes. Becker et al.[12] completed a combined fluorescence quantum yield and transient spectra study of bacteriochlorophyll *a* and bacteriopheophytin *a*. The observed inhomogeneous broadening of the initial Q_y absorption spectra yielded a fluorescence spectra that was shifted to higher than predicted frequencies in a variety of polar solvents. The transient absorption measurements here predominantly showed biexponential dynamics, with time scales of 1.5 and 18 ps in methanol and 1.1 and 82 ps in 1-propanol. These were attributed to solvent rearrangement and/or vibrational relaxation.

Femtosecond pump−probe spectroscopic studies were made on chlorophyll *b* in both acetone and pyridine[13] as well as chlorophyll *a* in pyridine.[14] The transient absorption spectra of these species reveal similar dynamics that consist of two components at ∼100 fs and 1−3 ps. The ∼100 fs component was attributed to transient hole burning while the 1−3 ps component was attributed to the response of the solvent rearrangement. Vibrational relaxation was eliminated as a possible source of this component since no changes were observed in photobleaching or stimulated emission after 200 fs.

More recently, using pump−probe spectroscopy, the vibrational states of the Q_y band of chlorophyll *a* have been mapped in ethanol[15] and a mixed petroleum ether and 2-propanol (100:5) mixture[16] by quantum beat analysis. In the latter study, the electron-vibration coupling constants for several vibrational modes were calculated.

The solvation dynamics of chlorophyll pigments is a simple model to try to understand the role of the local protein environment in light-harvesting complexes. The frequency-fluctuation correlation function (FFCF) measures the ensemble averaged frequency fluctuation of a transition over time and is sensitive to changes in the solvation environment. In this article, we investigate and quantify the spectral diffusion dynamics of the Q_y band of chlorophyll *a* in methanol, by observing the evolution of the time-dependent portion of the FFCF utilizing partially colinear, pump−probe geometry 2D electronic spectroscopy. In order to measure the FFCF, the center-line slope method (CLS) devised by Fayer and co-workers[17] was utilized. This method enables the simplified extraction of the FFCF. With further fits to the experimental linear spectrum, the entire FFCF is recovered. Very recently, using a similar strategy, combining linear and 2D spectroscopies, exciton-coupled porphyrin dimers have been studied.[18] Hitherto, to the best of the author's knowledge, the CLS method has only been applied to vibrational systems.[19] This is the first time that the method has been applied to the study of electronic transitions. Photon echo peak shift (PEPS) measure-

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ments are commonly used to study spectral diffusion in electronic transitions by measuring a function proportional to the FFCF.[20−25] Here, the CLS method is used as it allows for a normalized FFCF of electronic chromophores to be measured directly from the 2D spectra.

MATERIALS AND METHODS

The experimental setup and theory behind the phase cycling schemes necessary for these pump−probe geometry 2D electronic spectroscopy measurements have been well described[26,27] and demonstrated previously.[28] As such, only a brief description is provided here.

All measurements were conducted at ambient temperatures of approximately 298 K. Chlorophyll *a* (Sigma Aldrich) was dissolved in methanol to provide a sample OD of 0.22 in a 1 mm sample cell (1 mm sapphire windows). A commercially available regenerative amplifier laser system (Legend Elite, Coherent) provided pulses centered at 800 nm (0.8 W, 1 kHz, 40 fs), which were used to pump a home-built, two-stage optical parametric amplifier to generate near-infrared wavelengths, which were subsequently frequency doubled to be resonant with the Q_y band of chlorophyll *a* (herein referred to as Chl *a*) centered at 665 nm. This beam was passed through a commercially available acousto-optic programmable dispersive filter[29] (Dazzler, Fastlite) to generate the first two excitation pulses, with variable delay and relative phase. For all measurements presented here, the shaped pulses were referenced to a carrier frequency of 419 THz to put the signal frequency in the partial rotating frame, taken using a 1 × 2 phase cycling scheme,[27,28] and attenuated to 300 nJ sum energy for the two pulses. The pulse shaper also acted to compress these pulses to 45 fs FWHM (<1.3 times transform limited). The third interaction pulse was a white light continuum (WLC) generated by focusing the 800 nm fundamental through a 2 mm sapphire window, and passing over a motorized delay stage to enable control of the population time (T_w) after the first two interaction pulses. This WLC also acted as a local oscillator field. The shaped 665 nm light and WLC were overlapped on the sample cell, and the WLC was focused into a spectrometer (Acton SP2300, Princeton Instruments) equipped with a CCD detector (PIXIS 100B, Princeton Instruments) and frequency resolved.

For each 2D electronic spectra (2DES) collected, the delay between the first two interaction pulses cover a total time range of 180 fs in 3 fs steps, while the T_w time remained constant. Due to the observation of the coherent artifact which is spectrally overlapped with the desired signal, collection of 2DES was only permissible at a shortest T_w of 250 fs. This is not a significant limitation, as the CLS method used to recover the normalized FFCF only applies for waiting time much longer than the free induction decay (FID) time scale. In this instance, the FID is approximately 70 fs. 2DES were collected at T_w values extending to 400 ps, to ensure that the evolution of the 2DES over time scales pertinent to the time-dependent portion of the FFCF was fully observed.

RESULTS

Figure 1 shows the linear spectrum of Chl *a* in methanol. The main peak is assigned to the Q_y transition, within the plane of the porphyrin ring.[30,31] The peak is centered at 665 nm (451 THz) with a bandwidth of ∼20 nm (14 THz). The other peaks

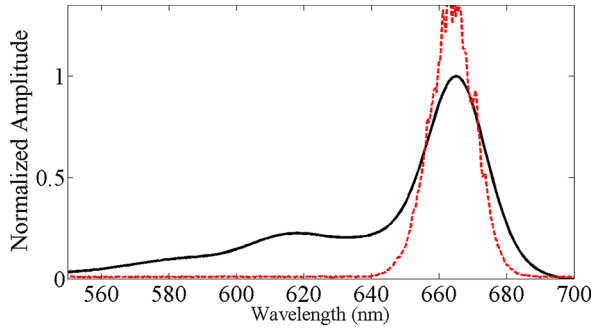

Figure 1. Linear absorption spectra of Chl *a* in methanol (black), with typical spectrum of the frequency doubled OPA output overlaid (red dashed).

blue-shifted from the main peak are generally assigned to a vibronic progression from the Q_y band.[32,33]

Figure 2 shows the evolution of the 2DES line shape of the Q_y band of Chl *a* in methanol as T_w is increased, with the corresponding CLS fits overlaid which are subsequently discussed. These 2DES are typical examples of the observed

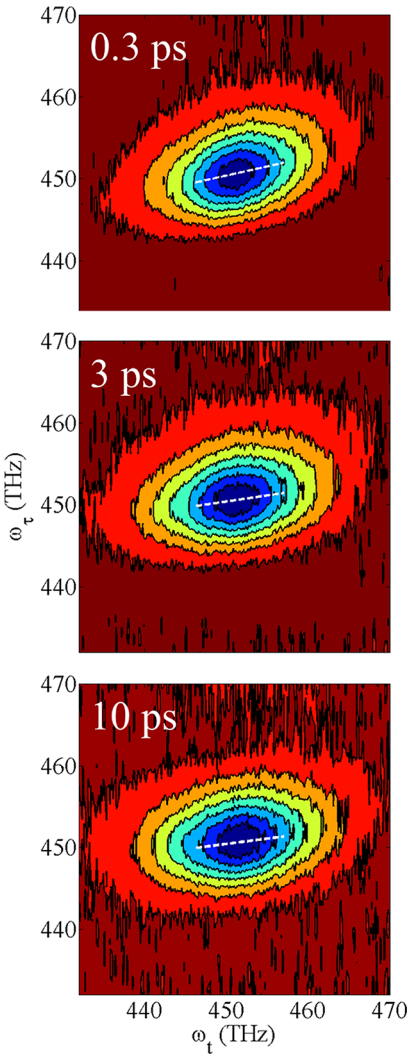

Figure 2. 2DES of the Q_y band of Chl *a* in methanol shown at T_w values of 0.3, 3, and 10 ps (top to bottom) with corresponding CLS fits overlaid (white dashed lines).

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2DES at these T_w values. The use of a continuum probe has been demonstrated to cause frequency dependent distortions to the antidiagonal 2DES line width.[34,35] The limited minimum T_w value of 250 fs, combined with the long time scales of the processes measured here compared to the chirp of the probe over the bandwidth of the Q_y transition means no such distortions are easily discernible here. It is however anticipated that at short T_w values, and in cases where the time scales of the measured processes and probe chirp are comparable, corrective procedures such as those suggested by Tekavec et al.[36] will become necessary.

Retrieving the FFCF from experimental 2D spectroscopy data generally requires a complicated model response function[37−39] to be fitted to experimental data. In recent years, efforts have been made to simplify the extraction of the FFCF from experimental 2D data, with the ellipticity method suggested by Roberts et al.[40] and CLS method of Kwak et al.[17] being the most straightforward to implement. In the CLS method, one fits a line through the maxima of the slice of the 2D spectrum at each detection frequency. Kwak et al. have shown analytically that, under the short time approximation where the coherence time is short compared to T_w, the gradient of this line equates the normalized FFCF as a function of T_w.

$$S(T_w) = \bar{C}(T_w) \equiv \frac{\langle \delta\omega(T_w)\delta\omega(0)\rangle}{\langle \delta\omega(0)\delta\omega(0)\rangle} \quad (1)$$

In this work, a band of about 10 THz corresponding to the highest signal intensity (to reduce the influence of noise at the edges of the peak) was fitted with a Gaussian function at each value of ω_t and linear regression of the determined maxima were performed through a least-squared method to yield the CLS values. Figure 3 depicts the CLS progression as T_w

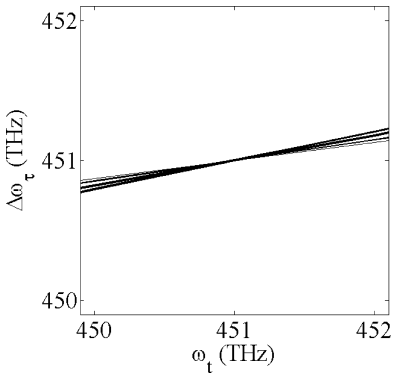

Figure 3. CLS fits of 2DES at T_w values of 0.5, 1.4, 6, and 10 ps (progressing from thickest line to thinnest respectively).

increases. Figure 4 depicts the measured CLS versus T_w. These data points were fitted with a biexponential decay with amplitude A_1 and A_2 and decay constant of τ_1 and τ_2 as well as a baseline offset (A_3) corresponding to a process with a time scale larger than the observation window of these measurements (≫ 500 ps). The determined fit parameters are displayed in Table 1. The optimal values of the two amplitudes are similar, with $A_1 \approx 0.07$ and $A_2 \approx 0.06$, and have time constants of $\tau_1 \approx 0.5$ ps and $\tau_2 \approx 7$ ps, respectively. Supplementary to these 2DES, pump−probe scans of the transient spectra of Chl *a* in methanol were also measured (not presented here) and the Q_y excited-state lifetime was found to be 110 ± 15 ps. High

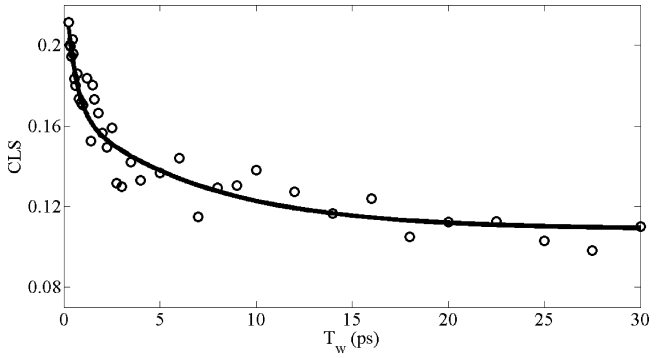

Figure 4. CLS data as T_w progresses (circles) with the corresponding fitted biexponential decay described in Table 1 overlaid (line).

temporal resolution pump−probe scans at pump−probe delay times <1 ps do not show any discernible oscillations.

■ ANALYSIS AND DISCUSSION

2D spectra are heterodyne-detected photon echo signals and can be modeled using nonlinear optical response theory.[37] The connection between FFCF, $C(t)$, and the 2D spectrum is well documented[41] and will not be repeated here. The FFCF can be phenomenologically modeled with a generalized Kubo line shape, using a series of exponential decays, where each term may represent a certain dynamical process

$$C(t) = \sum_i \Delta_i^2 \exp(-t/\tau_i) \quad (2)$$

where Δ_i and τ_i are the amplitude and the correlation time of the frequency fluctuation of the ith component, respectively. No discernible oscillation is observed in the CLS data presented in Figure 4, as well as in the pump−probe traces. This leads us to conclude that there is no underdamped oscillating component in the FFCF of eq 2 in this instance. $C(t)$ is related to the line shape function $g(t)$

$$g(t) = \int_0^t \mathrm{d}t_2 \int_0^{t_1} C(t)\, \mathrm{d}t_1 \quad (3)$$

which is in turn related to the linear optical response, $R^{(1)}(t)$, which gives rise to the linear spectrum and the third-order nonlinear optical response, $R^{(3)}(t_1,t_2,t_3)$, that produces the 2D spectra.[37]

In the case where $\Delta_i\tau_i \ll 1$, the line-shape function can be expressed as $g(t) = \delta(t)/T_2$ and is said to be motionally narrowed (where T_2 is the homogeneous dephasing constant), and the observed linear spectrum line shape is Lorentzian in nature. Conversely, when $\Delta_i\tau_i \gg 1$, it simplifies to yield $g(t) = (\Delta_i/2)t^2$ and can be approximated as a constant, and we are said to be in the inhomogeneous limit and a Gaussian line shape is observed. In many condensed phase systems, $\Delta_i\tau_i \approx 1$ and are classified as being in the spectral diffusion regime.

From a purely absorptive 2D spectrum, the CLS gives the normalized FFCF as shown in eq 1. Comparing eqs 1 and 2, the experimental amplitudes of the exponential decay used to fit the CLS can therefore be recognized as $A_i = \Delta_i^2/\sum\Delta_i^2$. By definition, $\bar{C}(T_w{=}0) = 1$. The three amplitudes in Table 1 sum to only 0.239. The experimental data points only begin at T_w = 250 fs. It is therefore clear that at least one fast process is occurring within the first 250 fs that is not directly observed in these measurements. We therefore assign an additional exponential decay with quantities Δ_0 and τ_0 to account for

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Table 1. Fit Parameters to Measured Biexponential Decays of CLS Values

sample	A_1	τ_1 (fs)	A_2	τ_2 (ps)	A_3
Chl *a* in methanol	0.070 ± 0.022	500 ± 299	0.060 ± 0.012	6.9 ± 3.5	0.109 ± 0.007

this short process which makes up for the shortfall of 0.761. From Table 1, we know the values of τ_1 and τ_2, while the time scale for the baseline component, τ_3 (corresponding to A_3), is assigned as >1 ns. This leaves only the value of τ_0 and the total sum of the square of the fluctuation amplitude, $\sum\Delta_i^2$, to be determined. In order to obtain these values, we make use of the linear spectrum. The linear spectrum is related to the FFCF via the line-shape function $g(t)$, as shown in eq 4.

$$I(\omega) = Re[FT\{\exp[-g(t)]\}] \quad (4)$$

Using this relationship, we can obtain values for $\sum\Delta_i^2$ and τ_0 by fitting to the experimental linear spectrum. In the fit optimization performed here, the relative amplitudes between Δ_i^2 are constrained according to values in Table 1. This means that the relative normalized amplitude, Δ_0, of the extremely fast component, is set to be 0.761. The total of the square of the fluctuation amplitudes $\sum\Delta_i^2$ and τ_0 were allowed to vary to obtain the best fit to the experimental linear spectra, while the transition frequency was allowed to vary over tight bounds around the experimentally observed transition maxima. For the experimental linear spectrum to be fitted, it is imperative that only the band of the Q_y transition of Chl *a* is used. However, the experimental linear spectrum consists of vibronic progressions on the blue side of the main Q_y transition peak. In light of this, only the red side of the Q_y transition band is fitted. It has also been determined that the vibronic progression does not extend and contribute to the red side of the band. Figure 5 depicts the fit to the experimental linear spectrum.

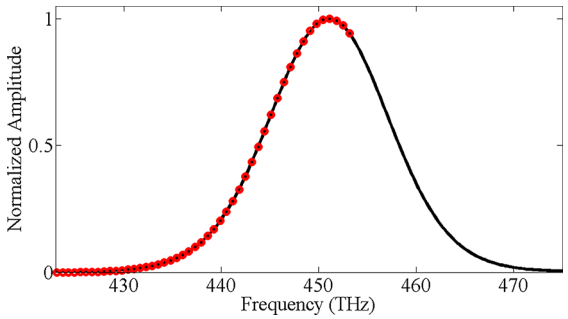

Figure 5. Fitted region of the experimental linear spectrum of Chl *a* in methanol (red circles) with corresponding fit (black line).

The solid black line depicts the numerical optimized fit while the red circles represent the range of experimental linear spectrum that the fit was optimized over.

An excellent fit to the linear spectrum is obtained. The optimized fit value for the short time constant τ_0 = 65 ± 10 fs while that for the total of the square of the fluctuation amplitudes $\sum\Delta_i^2$ = 42.4 THz. Despite the large errors associated with the fit to the CLS, the recovered τ_0 and $\sum\Delta_i^2$ values are extremely robust since the relative amplitude of the unknown component is large (0.761) compared to the sum of the other components (0.239). In cases where the relative amplitude of the unknown component is small, the propagation of error will result in greater uncertainty in the recovered values for the unknown component. Table 2 summarizes the values recovered from both the fit and experiment.

Numerous experiments such as 3PEPS have been performed on dye molecules. One common feature is the presence of a fast component that is attributed to the inertial component of solvation. In the present study, the time constant τ_0 of 65 ± 10 fs is consistent with values recorded for other species elsewhere in the literature.[21−24] This fast component with $\Delta_0\tau_0$ = 2.36 is not in the motionally narrowed regime that gives rise to homogeneous line-width contributions to the spectrum. This is also obvious from observing the linear line shape, as there is no discernible Lorentzian component, typified by a gentle slope at the spectral wings of the Voigt profile making up the spectrum. The relative proportion of the fast component is also consistent with these other studies. Sub-10 fs time scale components have been observed in studies of IR144 and attributed to the rapid dephasing of the initially prepared vibronic wavepacket.[25] In the measurements presented here, these extremely fast components are not observed. If there is indeed a very fast sub-10 fs component, it does not significantly contribute to the line shape, as it may well be in the motionally narrowed regime ($\Delta\tau \ll 1$), and the resultant Lorentzian line shape would be too narrow to contribute to the final line shape, as discussed above.

Sundström and co-workers performed pump−probe spectroscopic studies on chlorophyll *b* in both acetone and pyridine[13] and Chl *a* in ethanol.[14] In those studies, the pump pulse bandwidth was less than the absorption bandwidth of the Q_y transitions. The rate and extent of the refilling of the spectral hole is measured in their transient absorption spectra. The dynamics measured were fitted with a biexponential decay with time constants of ∼100 fs and ∼3 ps. Sundström and co-workers attribute the faster component to the transition frequency fluctuation of the chromophore caused by the thermal fluctuation of the solvent molecules. The slower component was attributed to the energy level shifts arising from the solvent molecules reconfiguration in response to the excited state of the chromophore. It is not straightforward to relate this dynamical spectral hole refilling to the quantitative values of the FFCF, but the spectral hole refilling is certainly correlated with the processes that leads to the decay in the FFCF of the molecules. As such, the process with the time constant $\tau_1 \sim 500$ fs measured here is assigned to the fast changes in instantaneous absorption frequency of the chromophore due to the thermal fluctuation of the solvent molecules. The fluctuation amplitude Δ_1 = 11.21 THz indicates the spectral fluctuation bandwidth of the chromophore. For the process measured with the time constant $\tau_1 \sim 7$ ps, this is assigned to the methanol solvent molecules rearrangement due to the

Table 2. Recovered Real Valued Parameters for the FFCF from the Fitted Linear Spectra of Chl *a* in Methanol

sample	Δ_0 (THz)	τ_0 (fs)	Δ_1 (THz)	τ_1 (fs)	Δ_2 (THz)	τ_2 (ps)	Δ_3 (THz)	τ_3 (ns)
Chl *a* in methanol	36.96	65 ± 10	11.21	500	10.40	6.9	13.97	≥1

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charge redistribution of the Chl *a* molecules after excitation. This rearrangement perturbs the environment around the Chl *a* chromophore and affects the transition frequency. Processes of this time scale (~1 to 10 ps) are also often observed in other studies of solvent dynamics using PEPS and spectral hole burning spectroscopy and have been attributed to solvent rearrangement in bacteriochorophyll *a* in methanol;[12] elsewhere similar processes have been observed in a variety of dye and other relevant systems.[12−14,21,22,42] The fluctuation amplitude squared Δ_2^2 gives a direct measure of the polar solvent rearrangement energy.

A more exact value of the long time component of τ_3 cannot be determined due to the limited scanning range of this experimental setup, as well as limitations in achieving reasonable signal-to-noise ratios as the signal decays. It is therefore assigned as $\tau_3 > 1$ ns. This long time component is not due to the lifetime of the excited state. This is because the FFCF measured here is not the intensity of the signal as a function of T_w but that of the slope of the peak. This long time component is a measure of the inhomogeneity of the chromophore and has a value of 13.97 THz. In a 3PEPS experiment performed to measure FFCF of the cyanine dye DTCCI by Joo and co-workers, a nanosecond component is also measured.[20] The authors hypothesize that this may be due to the molecule's conformational inhomogeneity due to its large size and flexibility. As Chl *a* is a larger molecule than DTCCI, it is also plausible that the inhomogeneity that is measured here has similar origins.

■ CONCLUSIONS

The two time frequency-fluctuation correlation function of the Q_y transition of Chl *a* in methanol has been quantified by 2D electronic spectroscopy, the CLS method, and optimized fitting to the linear spectrum. To the best of the authors' knowledge, this is the first use of the CLS method to study electronic transitions. The full FFCF occurs over four time scales, with each represented by an exponential decay. The shortest time scale whose amplitude accounts for ~75% of the FFCF decay has a time constant of 65 fs and is attributed to the inertial component of solvation. The second and third components are attributed to spectral diffusion components, τ_1, with a time scale of ≈500 fs and a picosecond component, τ_2, of ~7 ps, assigned to solvent rearrangement. The long time (>1 ns) is attributed to inhomogeneity and could be intramolecular in origin.

The absolute amplitudes of these processes are also obtained and give a measure of the strength of the processes involved.

It is hoped that this measurement of the FFCF of Chl *a* will lead to more theoretical and computational studies toward understanding how an important molecule such as Chl *a* behaves in different biologically relevant environments. It is envisaged that this work will be extended to monitor spectral diffusion dynamics in protein-bound chromophores. The basic understanding of solvated chlorophyll's spectral diffusion dynamics will also be useful as a building block to further understand the dynamics of chlorophyll dimers and further extended structures, such as those in light-harvesting complexes.

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Notes

The authors declare no competing financial interest.

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