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Visualization of Excitonic Structure in the Fenna-Matthews-Olson Photosynthetic Complex by Polarization-Dependent Two-Dimensional Electronic Spectroscopy

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Abstract

Photosynthetic light-harvesting proceeds by the collection and highly efficient transfer of energy through a network of pigment-protein complexes. Inter-chromophore electronic couplings and interactions between pigments and the surrounding protein determine energy levels of excitonic states and dictate the mechanism of energy flow. The excitonic structure (orientation of excitonic transition dipoles) of pigment-protein complexes is generally deduced indirectly from x-ray crystallography in combination with predictions of transition energies and couplings in the chromophore site basis. Here, we demonstrate that coarse-grained excitonic structural information in the form of projection angles between transition dipole moments can be obtained from polarization-dependent two-dimensional electronic spectroscopy of an isotropic sample, particularly when the nonrephasing or free polarization decay signal rather than the photon echo signal is considered. The method provides an experimental link between atomic and electronic structure and accesses dynamical information with femtosecond time resolution. In an investigation of the Fenna-Matthews-Olson complex from green sulfur bacteria, energy transfer connecting two particular exciton states in the protein is isolated as being the primary contributor to a cross peak in the nonrephasing 2D spectrum at 400 fs under a specific sequence of polarized excitation pulses. The results suggest the possibility of designing experiments using combinations of tailored polarization sequences to separate and monitor individual relaxation pathways.

Introduction

Photosynthesis begins with the harvesting of sunlight by antenna pigments that rapidly funnel energy to reaction centers. The spectral coverage and energy transfer characteristics of light-harvesting systems are determined by the structural arrangement of pigments and their interactions with the surrounding environment, which is often the interior of a protein. Even among organisms with chlorophyll as the primary light absorber, the architecture of antenna systems varies widely in nature.(1) Investigation of these specialized light-harvesting structures, evolved under different light environments to fuel photosynthesis with optimal efficiency, could have applications in solar energy conversion devices and is increasingly important as we search for clean energy alternatives.

The Fenna-Matthews-Olson (FMO) pigment-protein complex is found in low-light adapted green sulfur bacteria, which harvest light primarily in a large antenna structure called the chlorosome. The FMO complex is tasked with transporting energy collected in the chlorosome to the reaction center, thereby initiating the photochemistry that ultimately leads to the chemical storage of energy. FMO was the first chlorophyll protein structure solved by x-ray crystallography and comprises three identical subunits each containing seven bacteriochlorophyll (BChl) pigments nested within beta sheets.(2; 3) The closest center-to-center distance between neighboring intrasubunit BChls is 11 Å, with the largest coupling energies between them estimated to be on the order of 100 cm⁻¹. The closest approach of intersubunit BChls is approximately 24 Å, with corresponding coupling energies of < 20 cm⁻¹.(4) For this reason it has been assumed (and borne out by spectroscopic studies) that the exciton wavefunction is rapidly localized on individual

subunits upon excitation.(5) Compared to the highly symmetric ring structures of the light-harvesting apparatus in the similarly widely-studied purple photosynthetic bacteria, the asymmetric arrangement of the seven pigments in FMO is more reminiscent of light-harvesting in higher plants. Because of its lack of symmetry and the early availability of x-ray structure information in conjunction with its relatively small size, FMO has been considered a model system for investigation of photosynthetic energy transfer.

A number of spectroscopic experiments and theoretical studies have contributed to a good understanding of the energetic landscape in FMO.(5-7) The lack of symmetry within the FMO subunit has presented a challenge for researchers modeling experimental spectra, because each of the seven BChls experiences a different local environment due to, for example, the proximity of charged residues or bowing of the BChl macrocycle, resulting in variations of Q_y site energies (the $S_0 - S_1$ transition energy of the chromophore) by as much as 600 cm^{-1} .(4) Adolphs, et al. performed calculations of site energies using an electrostatic method that for the first time predicted absorption and linear and circular dichroism spectra.(8) Müh, et al.(9) further incorporated atomic detail of the protein including the backbone into quantum chemical/electrostatic calculations of excitation energies and discovered a pronounced effect of the alpha-helix dipole on BChl site energies. In this way, the protein itself can direct excitation flow through the complex by tuning the site energies.

Several time-resolved spectroscopic studies have shown that energy transfer in FMO proceeds with time constants ranging from ~ 100 fs to several ps, with faster transfer generally occurring on the blue side of the Q_y band.(10) Two-dimensional (2D) electronic spectroscopy is a particularly incisive tool for investigating photosynthetic

energy transfer, yielding maps of coupling and dynamical processes of electronic transitions. Recently, Brixner and coworkers(11) identified two major pathways of energy flow in FMO using 2D electronic spectroscopy. The nature of energy transfer within the protein has also been investigated, and evidence of quantum coherence in fluorescence anisotropy(12) and 2D spectroscopy data(13) implies that quantum mechanical wavelike energy transfer rather than purely dissipative “hopping” may contribute to the efficiency of light-harvesting by FMO.

Excitation energy transfer in photosynthetic complexes is controlled by the orientation and spatial extent of exciton states, referred to here as the excitonic structure, in addition to the energetic landscape of the protein. In a multichromophore system, non-negligible coupling energies give rise to exciton states with transition dipole moments distinct from those of the individual chromophores. The Coulombic couplings redistribute electron transition density among the chromophore sites, shifting transition energies and reorienting transition dipole moments. The excitonic structure of photosynthetic complexes is generally determined indirectly from estimates of site and coupling energies, with the latter often based on the dipole-dipole approximation despite the close packing of chromophores. An experimental method for probing excitonic structure directly in order to further understand energy transfer dynamics is therefore desirable.

In this work, we demonstrate that 2D electronic spectroscopy can be used to obtain excitonic structure information in addition to elucidating energetic relaxation pathways. Based on generation of a three-pulse photon echo, analogous to the spin echo in NMR, 2D spectroscopy is sensitive to the third order optical polarization of the system.

In effect, the technique connects absorption and emission frequencies by interrogating the sample with three time-delayed femtosecond laser pulses and monitoring the emitted signal.(14) The frequency-domain signal, collected as a function of the two scanned time delays called the ‘coherence’ time, or τ (between the first two pulses), and the ‘population’ time, or T (between the second two pulses), is Fourier- transformed over τ to yield 2D frequency-frequency plots as a function of T . The ability to overcome phase fluctuations has played a key role in making 2D spectroscopy feasible at visible wavelengths. To this end, passively-stabilized 2D setups based on diffractive optics(15; 16) or actively-stabilized techniques using feedback-controlled optics are employed.(17- 19) Damrauer, Zanni, and coworkers recently demonstrated phase stable measurement of absorptive 2D electronic spectra using pulse shapers.(20)

Although 2D spectra contain information often unavailable or obscured in other femtosecond laser experiments, broad linewidths can nevertheless complicate interpretation of spectra. Two-dimensional spectra contain signals arising from many different energetic processes, and separation of individual contributions is desirable for better characterization of excited state dynamics. This can be achieved in part by rotating the polarization of the laser pulses to suppress or enhance particular signals based on the relative orientations of transition dipoles in the system. Polarization conditions for 2D IR spectroscopy of an isotropic sample were pioneered by Hochstrasser and coworkers and further explored theoretically by Dreyer et al.(21-23) Sequences have been identified that suppress diagonal peaks in 2D spectra, and it has been shown that disentangling 2D spectra in this way is also feasible in the electronic regime.(24; 25) Using cross-polarized excitation pulses, angles between transition dipole moments have been

determined from 2D IR spectra.(26; 27) These polarization conditions do not require aligning the molecules in the sample cell, but rather take advantage of the set relative orientations of excited transitions within the molecules. Here, we use polarization-dependent 2D femtosecond electronic spectroscopy to probe the excitonic structure and dynamics of FMO from *P. aestuarii*.

Separating rephasing (photon echo) and nonrephasing contributions to the signal is an additional tool for disentangling the complicated interference pattern that makes up a 2D spectrum. So-called phase-twisted lineshapes with mixed absorptive and dispersive character have been identified in 2D NMR and IR spectra when rephasing and nonrephasing signals were measured separately, and addition of the two spectra has been used to obtain absorptive lineshapes.(28; 29) Ge et al. discussed separate measurement of rephasing and nonrephasing signals for enhanced resolution of closely spaced peaks in 2D IR spectra.(30) In rephasing contributions, the system evolves in conjugate frequencies during τ and t (the time delay between the last two pulses). This gives rise to a photon echo signal, which reveals the system's ability to effectively reverse inhomogeneous dephasing. For nonrephasing signals, the phase factors governing evolution of coherence during τ and t have the same sign, resulting in free polarization decay during t . Measurement of rephasing and nonrephasing spectra is achieved experimentally by reversing the order of arrival of the first two pulses at the sample, and the relative amplitude of the two signals is a measure of the degree of inhomogeneous broadening in the system.(27) In this work, we monitor both types of signals separately, and observe distinct spectral features in nonrephasing spectra. Combined with the use of specific polarization schemes, the nonrephasing spectra directly reveal information about

the excitonic structure, or orientation of excitonic transition dipoles, of the protein complex and give a more detailed view of energetic relaxation.

Experimental Methods

FMO from *P. aestuarii* was isolated as described in Li, et al.(31). A Sephacryl S-300 high resolution gel filtration column was employed as a final purification step. The optical density ratio of OD_{267nm}/OD_{371nm} was less than 0.55, indicative of a highly purified complex. The sample was dissolved in a pH 8 buffer solution of 20mM Tris-HCl and 0.1% of LDAO was added to prevent aggregation. The sample was then mixed with glycerol (30:70 by volume) for formation of a glass in a 200 μ m-thick quartz cell for measurements at 77K. The sample OD was 0.22 at 805 nm.

Details of the experimental apparatus have been described elsewhere.(24; 32) Briefly, 45 fs pulses at 805 nm with a spectral bandwidth of 31 nm output from a home-built Ti:Sapphire regenerative amplifier are used to measure 2D spectra. The beam is split into four by a beamsplitter followed by a diffractive optic optimized for transmission of +/-1st orders. The time delay between the first two pulses (coherence time, τ) is scanned by movement of paired glass wedges controlled by a computerized stepper motor, and the time delay between the 2nd and 3rd pulses (population time, T) is controlled by a retroreflector delay stage. The beams are focused to a 70 μ m spot-size on the sample in the boxcar geometry with 4 nJ/pulse in pulses 1,2, and 3. The 4th pulse is attenuated by four orders of magnitude to serve as a local oscillator for heterodyne detection, and passes through the sample before the other pulses. A mask selects signal emitted from the sample in the phase-matched direction $\mathbf{k}_s = -\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3$, collinear with

beam 4. The signal is spectrally resolved with a spectrometer and CCD camera. The polarizations of the pulses are controlled by true zero-order half waveplates inserted in beams 1 and 2, and in this work the combinations $\langle 0^\circ, 0^\circ, 0^\circ, 0^\circ \rangle$, $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$, and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ are used (with the bracket expressions denoting the linear polarizations of pulses $\langle 1, 2, 3, 4 \rangle$, where 4 is the local oscillator). The measurement is performed by scanning the coherence time from -300 to 300 fs, where negative τ corresponds to arrival of pulse 2 before pulse 1, for each population time point. For a given T, Fourier transformation is performed using all coherence time points to yield the total 2D spectra, while for rephasing and nonrephasing spectra only positive and negative time points are Fourier transformed, respectively.

Theoretical Methods

Theoretical calculations of 2D spectra are performed based on Zigmantas, et al.(33) In brief, a Frenkel exciton model of the FMO monomer is adopted in which 1-exciton and 2-exciton manifolds are built from site and coupling energies and Q_y transitions of the seven BChls, with orientations of site transition dipole moments determined from the protein x-ray structure.(34) Couplings between BChls in different monomeric subunits of the protein are neglected, due to their relatively weak strength. An overdamped Brownian oscillator model for nuclear motions resulting from interaction with the protein environment (electron-phonon coupling) is adopted,(35) with no correlation between energetic fluctuations at different sites. Dynamics between exciton states is calculated using modified-Redfield theory in the secular approximation.(36; 37) A Gaussian distribution of site energies represents inhomogeneous broadening due to

slow fluctuations of the protein environment, as determined by the linewidths in the spectra and the amplitude ratio of rephasing to nonrephasing spectra. All model parameters are published in Supporting Information.

Two-dimensional spectra are calculated using the third order nonlinear response function, $R^{(3)}$, described in detail in Mukamel.(35) The response function describes a sum of Liouville energy level pathways contributing to the signal in the phase-matched direction assuming the impulsive excitation limit and the rotating wave approximation. The 2D spectrum, S_{2D} is then proportional to the response function:

$$S_{2D}(\omega_\tau, T, \omega_t) \propto \int_{-\infty}^{\infty} R^{(3)}(\tau, T, \omega_t) \exp(-i\omega_\tau \tau) d\tau. \quad (1)$$

The rephasing and nonrephasing spectra are calculated using response pathways

$$R^{(3)}_{RP} = R_2 + R_3 - R_1^* \quad (2)$$

$$\text{and } R^{(3)}_{NR} = R_1 + R_4 - R_2^*, \quad (3)$$

respectively, in the conventional nomenclature. Each of these time-domain response pathways describes a sequence of promotions and demotions of electrons between states of the system stimulated by the laser pulse fields and includes the evolution of the system during intervening time periods. The full form of the response functions can be found elsewhere,(23; 35) but here it is useful to note that the contribution to the 2D spectrum by each pathway is controlled by a prefactor of the form $\langle i_\alpha j_\beta k_\gamma l_\delta \rangle \mu_\alpha \mu_\beta \mu_\gamma \mu_\delta$, where $\alpha, \beta, \gamma,$ and δ represent transitions connecting pairs of states, $\langle i_\alpha j_\beta k_\gamma l_\delta \rangle$ is the orientationally averaged projection of the lab-frame linear polarizations of the laser pulses onto the molecular frame transition dipole moments, and μ_α denotes the dipole moment magnitude of molecular transition α .

Results and Discussion

Figure 1 shows the real total, rephasing, and nonrephasing 2D spectra of FMO from *P. aestuarii* at 77K, denoted as $S(\lambda_{\tau}, T, \lambda_t)$, $S(\lambda_{+\tau}, T, \lambda_t)$, and $S(\lambda_{-\tau}, T, \lambda_t)$, respectively. Features with positive sign are shown in green to red and correspond to stimulated emission or bleaching, while blue features are negative, indicating excited state absorption. Peaks in the rephasing and nonrephasing parts tilt along and perpendicular to the diagonal, respectively, as is characteristic in 2D spectra from NMR through the optical regime. The total spectrum, which is equivalent to the summation of the two signals, is dominated by the rephasing part. The ratio of rephasing to nonrephasing signal amplitudes (all normalized in Fig. 1 for clarity) is 4.7 at $T=0$ and 3.3 at $T=400\text{fs}$. The rephasing signal decays more quickly than the nonrephasing signal due to rapid homogeneous dephasing, or loss of memory, which diminishes the system's ability to emit a photon echo. As discussed previously by Ernst and Bodenhausen,(28) phase-twisted lineshapes in separated rephasing and nonrephasing signals give rise to distinctive interference effects in the spectra. Nonrephasing spectra have the advantage that closely spaced peaks are easier to distinguish than in rephasing spectra due to their anti-diagonal tilt; that is, the diagonal linewidth of an individual transition is narrower in the nonrephasing spectrum than in the rephasing spectrum. In both types of spectra, interference can give rise to shifts in the center positions of peaks, obscuring the true transition energies. Adding the two types of signals to obtain the total spectrum serves to cancel the dispersive character of peaks in favor of purely absorptive lines.

In Figure 1, the $T=0$ total spectrum is characterized by a strong positive signal along the diagonal comprising several overlapping bands, weaker positive off-diagonal

features that merge into the diagonal peaks, and excited state absorption above the diagonal. The rephasing and nonrephasing signals are strikingly different. The $T=0$ rephasing spectrum contains almost no features aside from one wide, inhomogeneously broadened diagonal band, while the nonrephasing spectrum contains several distinct peaks along the diagonal, as well as strong off-diagonal features. The total spectrum, the sum of both signals, retains some of this structure. In particular, the two most prominent diagonal bands of the nonrephasing spectrum give rise to the strong double peak along the diagonal in the total spectrum (peaked at 814 nm and 804 nm). In the second row of Figure 1, the $T=400$ fs spectra show that the nonrephasing spectrum gives a clearer view of dynamics. The rephasing spectrum shows little evolution on this timescale, whereas excitation energy migration out of the upper bands (most notably the disappearance of the ($\lambda_r=804$ nm, $\lambda_i=804$ nm) diagonal peak) is clearly visible in the nonrephasing spectrum.

The rephasing and nonrephasing parts of the third-order polarization are sensitive to the same excitation energy transfer dynamics; however, energy transfer is more apparent in the nonrephasing spectrum. This results from the narrower diagonal linewidths of nonrephasing signals and from the photon echo effect that gives rise to dominant diagonal peaks in rephasing spectra, obscuring off-diagonal peaks which may reveal information about energy transfer. In addition, as discussed in Cheng, et al.,(38) Liouville pathways that cause excitonic quantum beating appear off and on the diagonal in rephasing and nonrephasing spectra, respectively. Thus a clearer view of energetic relaxation is obtained from the off-diagonal region of nonrephasing spectra. From Figure 1 it is clear that splitting the total spectrum into its constituent $+\tau$ and $-\tau$ parts aids

analysis because, although the total spectrum has the advantage of absorptive lineshapes, the nonrephasing part shows more clearly the individual excitonic features.

Additional separation of signals can be achieved in 2D spectra by the use of specific polarization combinations of the excitation pulses. The polarization conditions used in this experiment were chosen because it is theoretically possible to derive ϕ , the projection angle between a pair of exciton states, directly from the ratio of the $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ spectra (with the exception that it is always impossible to distinguish between ϕ and $180^\circ - \phi$). This scheme is similar to the cross-polarized $\langle 90^\circ, 90^\circ, 0^\circ, 0^\circ \rangle / \langle 0^\circ, 0^\circ, 0^\circ, 0^\circ \rangle$ method used previously in 2D IR spectroscopy.(26; 27) The advantage of the sequences used here is that the diagonal peak amplitudes are more suppressed compared to those in the cross-polarized experiment, which is necessary for gleaning information from 2D electronic spectra with broad linewidths and closely spaced states. Despite the fact that it is still difficult to separate individual transitions, which prevents direct acquisition of ϕ , comparison of the spectra measured under these polarization conditions enables deduction of excitonic structural information.

Figure 2 shows the absolute magnitude 2D spectra of FMO from *P. aestuarii* measured under the two polarization schemes, $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$, at $T=400\text{fs}$. The spectra on the top row are absolute magnitude rephasing, $|S(\lambda_{+T}, T, \lambda_t)|$, and those on the bottom absolute magnitude nonrephasing, $|S(\lambda_{-T}, T, \lambda_t)|$. In magnitude spectra, the diagonal and anti-diagonal tilts of rephasing and nonrephasing peaks become difficult to distinguish. The striking aspect of the spectra is that large differences between these two different polarization experiments are only readily visible in the nonrephasing

spectra. This again points to the utility of isolating the nonrephasing component in order to decongest spectra and shows that polarization conditions become particularly powerful when combined with separation of the $+\tau$ and $-\tau$ parts. The disadvantage to the polarization schemes used here is the inability to phase the data using a separate pump-probe experiment, as is done for the real spectra shown in Figure 1. Under experimental conditions of extremely good phase stability, it is possible to collect 2D spectra with reliable phase without the need for an auxiliary experiment, as discussed in Read and Engel, et al.(24) However, as shown by these data, in the absence of sufficient phase stability, the absolute magnitude spectra are still valuable.

To simulate the spectra, we begin with the full Hamiltonian of the FMO monomer published by Müh, et al.,(9) and perform simultaneous fitting of the linear and 2D rephasing, nonrephasing, and polarized spectra. The parameters arrived at after fitting are similar to those from Müh, et al. Because the peaks overlap less, the parameters required to model polarized and nonrephasing spectra satisfactorily are more constrained, thus the similarity of our fit parameters to those arrived at by pure theory in Müh, et al. underscores the robustness of their method. Theoretical nonrephasing spectra are shown on the top row in Figure 3. The general features of the experimental spectra at 400 fs are captured, and the model shows excellent agreement with the experimental rephasing spectra as well (not shown). The upper right panel of Figure 3 shows experimental and theoretical linear absorption spectra, along with the spectrum of the laser pulses used to measure the 2D spectra. The pulse spectrum is incorporated in 2D simulations by weighting each calculated dipole strength by the experimental laser spectrum, which has

the small but noticeable effect on the calculated spectra of enhancing the higher-energy peaks.

The T=400 fs $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ spectra in Figure 3 simulated using the full FMO monomer Hamiltonian reproduce the major aspect of the corresponding experimental spectra (bottom row): in the $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ case at 400 fs the spectrum is dominated by a diagonal peak at ($\lambda_r=814$ nm, $\lambda_t=814$ nm) while in the $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ spectrum a pronounced cross-peak at ($\lambda_r=804$ nm, $\lambda_t=814$ nm) is also visible. This cross peak is due primarily to excitonic energy transfer during the population time, T. The simulated $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ nonrephasing spectrum in Figure 3 shows complete disappearance of the diagonal peak at 804 nm by 400 fs, whereas the experimental spectrum shows that the upper diagonal peak persists on this timescale. This may result from an overestimation of the excitation energy transfer rate or from an incomplete description of coherence dynamics (expected to contribute strongly to the diagonal in nonrephasing spectra) afforded by modified Redfield theory. However, the generally similar experimental and theoretical spectra suggest that the model accurately accounts for the energetic and excitonic structure of the FMO complex and the major pathways of energy flow. The striking difference between the $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ spectra can be explained by the orientational factors $\langle i_\alpha j_\beta k_\gamma l_\delta \rangle$ controlling peak amplitudes for Liouville pathways corresponding to energy transfer between 1-exciton states, as discussed above. Figure 4a shows the dependence of these orientational factors on the projection angle ϕ between a pair of exciton states for the two polarization schemes. Note that the orientational factors can be positive or negative, which we cannot distinguish in the current work due to the incompatibility of the

polarization schemes with the auxiliary phasing experiment. As seen in Figure 4a, the amplitude of an energy transfer cross peak in a $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ spectrum is maximum at a projection angle of 0° , and vanishes at 52° , while in a $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ spectrum it increases (in absolute value) monotonically from 0° to 90° .

The manifestation of the orientational factor dependence on φ is illustrated in Fig4 b and c by simulated nonrephasing 2D spectra of an excitonically coupled dimer with coupling energy 40 cm^{-1} , site energies 810 and 802 nm, and an energy transfer time constant from the upper to lower exciton states calculated from modified Redfield theory to be 350 fs. The dimer Hamiltonian parameters were chosen to roughly reproduce the two most prominent peaks in the FMO spectrum at 814 and 804 nm, in order to illustrate the effect of transition dipole orientation on polarization-dependent 2D nonrephasing spectra. Figure 4b shows the $T=400$ fs spectra calculated for the dimer with $\varphi = 5^\circ$, while Figure 4c shows the spectra calculated for the same dimer system, but with $\varphi = 40^\circ$ (the dipole strengths are adjusted to give the same relative amplitudes of diagonal peaks). The $\varphi = 5^\circ$ spectra under the $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ polarization schemes are nearly indistinguishable, whereas the $\varphi = 40^\circ$ spectra approximate the behavior we observe in the FMO experiment, with a cross peak due to energy transfer appearing strongly in the $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ spectrum and only weakly in the $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ spectrum. Characterization of the observed energetic states and dynamics of FMO requires use of the full Hamiltonian taking into account all seven of the BChls, as shown in Figure 3. However, the simplified dimer model of Figure 4 clearly illustrates that the origin of the contrast between the $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ nonrephasing 2D spectra lies in a relative orientation between prominent transitions near $\varphi = 40^\circ$.

The exciton model from the full FMO monomer Hamiltonian determined by fitting 2D spectra is consistent with analysis of the $\phi = 40^\circ$ dimer. According to the present FMO model, excitons 2 and 4 have the highest dipole strength and are primarily responsible for the two strongest bands in the linear and 2D spectra at 814 and 804 nm, respectively. These states are characterized by a projection angle of 38° and a 4-2 energy transfer time constant of 440 fs. Therefore, the $\phi = 40^\circ$ dimer spectra at $T=400$ fs generally reproduce the dominant features, resulting from excitons 2 and 4, of the corresponding FMO experimental spectra, and the ($\lambda_r=804$ nm, $\lambda_r=814$ nm) cross-peak is mainly due to relaxation from excitons 4 to 2. As discussed above, the exciton transition dipoles contribute to the cross-peak through $\mu_2^2 \mu_4^2$, and the peak is enhanced in the $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ spectrum by a favorable orientational factor. The positioning of excitonic transition dipoles is strongly dependent on the model Hamiltonian parameters, so that subtle changes in site and coupling energies have a pronounced effect on ϕ . In fitting the spectra, input parameters can be identified that produce good fits to rephasing and all-parallel spectra, but overestimate the amplitude of the cross-peak in the $T=400$ fs $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ nonrephasing spectrum, due to a too-large (too close to 90°) projection angle between the dominant transitions.

The simulations underscore the fact that the nonrephasing polarized spectra are uniquely sensitive to excitonic structure, and can furthermore be used to isolate individual energy transfer pathways. The polarization conditions serve to identify particular relative orientations of coupled transitions, and considering the nonrephasing spectra, in which fewer Liouville pathways contribute to the off-diagonal region, is useful for focusing on energy transfer processes. Relative amplitudes of energy transfer cross

peaks under different polarization conditions yield excitonic structural information directly. This point is demonstrated here, in that an estimate of the relative orientation of two transition dipoles determined from the orientational factor dependence on ϕ (as illustrated by the dimer model) is borne out by the calculation based on the full FMO Hamiltonian. In other words, even without previously obtained crystallographic or spectroscopic data, a coarse-grained view of excitonic structure can be obtained using combined polarization sequences in 2D electronic spectroscopy. This is however only feasible when cross peaks can be sufficiently resolved, and therefore the use of nonrephasing spectra is particularly powerful in conjunction with polarization-dependent 2D experiments.

The site and exciton basis transition dipole moments predicted by the full FMO model are shown in Figure 5. In the absence of interchromophore interaction, that is when the J coupling is 0, the transitions correspond to the Q_y transition dipoles localized on each BChl, resulting from π - π^* excitation within the conjugated plane of the molecule parallel to the axis connecting the NB and ND nitrogens (in the Protein Databank notation). The lengths of the transition dipole moments shown in figure 5 are proportional to their magnitudes, which are all assumed equal in the site basis (Figure 5a). In the absence of coupling, the site and exciton basis pictures are equivalent. When interchromophore electronic coupling is introduced (Figure 5b), linear combinations of the sites give rise to reoriented transition dipole moments with redistributed dipole strength. The excitons are numbered according to their energies, the site numbering used is in accordance with the Protein Databank crystal structure,⁽³⁴⁾ and the color-coding in Figure 5 matches the sites on the left with the exciton states on the right to which they

contribute predominantly. However, the correspondence is only approximate, as some of the excitons are delocalized over multiple sites. Transition dipole moments are positioned in Figure 5b according to the site basis coefficients making up the exciton states, thereby taking into account the delocalization length of the excitons. In particular, exciton 5 is highly delocalized over sites 4,5,6, and 7, reflected by the pronounced shift in position of the green arrow from site 6 to a position spanning sites 4,5,6, and 7 in the exciton basis. In contrast, excitons 1 and 7 are largely localized on sites 3 and 5, respectively, as evidenced by the similar positioning of the dipole moments in the site and exciton bases. The energetic ordering of states changes upon moving from the site to the exciton basis, reflecting the change in energetic landscape effected by the Coulombic coupling. The dominant transitions corresponding to excitons 2 and 4 are shown in red and cyan in Figure 5b, respectively, in the geometry supported by spectral fits as discussed above.

The orientation of the FMO complex between the chlorosome and reaction center has not yet been verified by crystallography, however as discussed by Adolphs et al.,(8) the funneling of energy by FMO suggests that the lower energy states are linked to the reaction center. Thus, the most probable conformation for the complex is with BChl sites 1 and 6 interfacing the chlorosome and sites 3 and 4 close to the reaction center. Like the result of Brixner, et al.(11) on FMO from *C. tepidum* (a structurally similar but spectroscopically distinct complex from a different green sulfur bacterium) and of Adolphs, et al.,(8) the present model supports two major energetic pathways through the *P. aestuarii* FMO complex. In the present model, these pathways correspond generally to migration from exciton 7 to 5 to 4 to 2 to 1 and from 6 to 3 to 2 to 1. As discussed in

Brixner et al., the spatial extent of the excitons determines the energy migration pathway, rather than the energy levels alone, so that relaxation can skip energetically intermediate states. When sites 1 and 6 are linked to the chlorosome, energy can couple into both of the dominant pathways, corresponding generally to the left- and right-hand sides of the protein in Figure 5. The model suggests that at 77K energy equilibrates among the six highest exciton states within ~ 1 ps, and further relaxation occurs only after 3 ps. That energy transfer among the higher states is so much more rapid than transfer to the lowest state is apparent in the short-time experimental spectra presented in this work, where pronounced relaxation into, but not out of, the 814 nm band (corresponding to excitons 2 and 3) is visible. The discrepancy in energy transfer rates is a manifestation of the localization of exciton 1 on site 3 due to the pronounced redshift of its site energy relative to others, so that little spatial overlap occurs with other exciton states. The evolutionary advantage is most likely the resultant unidirectionality of energy flow, such that once the excitation migrates to site 3 it is essentially trapped and directed to the reaction center.

Conclusion

Because it has been so widely studied, FMO is a model photosynthetic light-harvesting protein. In this work, we demonstrate the use of polarization sequences for 2D electronic spectroscopy that, through comparison of nonrephasing spectra, generally corroborate the orientation of exciton transitions predicted for FMO previously and allow further refinement of Hamiltonian parameters. In addition, the experiment allows identification of a cross-peak dominated by energy transfer coupling two particular states in a crowded spectrum. Rapid energy transfer from exciton 4 to exciton 2 is observed

within 400 fs, and the projection angle between the excitons is estimated to be approximately 40° by comparison to theoretical spectra. The technique can be used to obtain a coarse-grained view of excitonic structure directly without prior knowledge of a system's structure or energetics, and without alignment of the sample. When prior knowledge is available, specific polarization sequences can be chosen to isolate particular energetic pathways. For example, identifying a polarization scheme under which relaxation between states with a known ϕ disappears allows that process to be essentially subtracted out of the spectrum, enabling clearer visualization of other processes. More complex experiments using combinations of polarization sequences, similar to the work presented here, could be designed by choosing two polarization schemes with the maximum difference between orientational factors for a predicted cross peak. In this way, the subtraction of one type of spectrum from the other would highlight peaks arising only from the process of interest. Extending these techniques using oriented samples, one could further suppress unwanted signals and probe individual relaxation pathways. The method promises to be especially useful for studies in photosynthesis, allowing researchers to tease out underlying states and their dynamics disguised by the spectral overlap typical of large multi-chromophore aggregates. On the complex energetic landscape of the photosynthetic light-harvesting apparatus, existing dynamical theories are often less than satisfactory owing to high disorder, wide range of coupling energies, and poorly understood interactions with the protein environment. It is therefore necessary to employ experimental tools that can probe specific relaxation processes and thereby obtain rate constants directly from experiment to be used for calibration of dynamical theories.

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Figures

Figure 1.

Real 2D spectra at 77K of FMO from *P. aestuarii*. Spectra shown from left to right are total, rephasing, and nonrephasing signals at T=0 (top row) and T=400 fs (bottom row). All spectra are normalized to unity. The T=0 nonrephasing spectrum clearly shows distinctive diagonal and cross peaks not apparent in the rephasing spectrum, and energetic relaxation out of the upper band is visible in the nonrephasing spectrum at T=400 fs.

Figure 2.

Rephasing (top) and nonrephasing (bottom) absolute magnitude 2D spectra of FMO from *P. aestuarii* at T=400 fs measured with polarizations $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ (left) and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ (right) of laser pulses 1,2,3, and 4, respectively. The nonrephasing spectra show distinct spectral features under the two polarization conditions that can be used to obtain excitonic structural information. The strong cross peak at $(\lambda_\tau=804 \text{ nm}, \lambda_r=814 \text{ nm})$ in the $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ nonrephasing spectrum is indicative of excitation energy transfer.

Figure 3.

Theoretical and experimental nonrephasing spectra of FMO from *P. aestuarii* at T=400 fs and 77K. Top row, left to right: theoretical $\langle 0^\circ, 0^\circ, 0^\circ, 0^\circ \rangle$, $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$, and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ 2D spectra. The top right panel shows experimental and theoretical linear absorption spectra in black and red, respectively, and the dotted line is the laser spectrum of the pulses used to measure 2D spectra. Bottom row, left to right: experimental $\langle 0^\circ, 0^\circ, 0^\circ, 0^\circ \rangle$, $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$, and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ 2D spectra.

Figure 4.

Energy transfer pathway orientational factor dependence on projection angle between exciton states for $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ polarization sequences (a). Below, calculated 2D nonrephasing dimer spectra at 400 fs (top to bottom: all parallel, $\langle 45^\circ, -45^\circ, 0^\circ, 0^\circ \rangle$ and $\langle 75^\circ, -75^\circ, 0^\circ, 0^\circ \rangle$ polarizations) for a dimer with projection angle $\phi=5^\circ$ (b) and $\phi=40^\circ$ (c). The spectra in (c) reproduce more closely the behavior seen in the FMO polarization-dependent nonrephasing experiment due to the $\sim 40^\circ$ relative orientation of the dominant transition dipoles.

Figure 5.

Q_y transition dipole moments in the site basis (a) and exciton basis (b). The site basis is the case in the limit of negligible inter-chromophore couplings, and the transitions are localized on individual BCHs. Coupling between BCHs redistributes dipole strength among sites, resulting in different positions and orientations of the excitonic transition dipoles. The dominant peaks in the short-time 2D spectra arise from excitons 2 and 4 in red and cyan, respectively.

Figure 1.

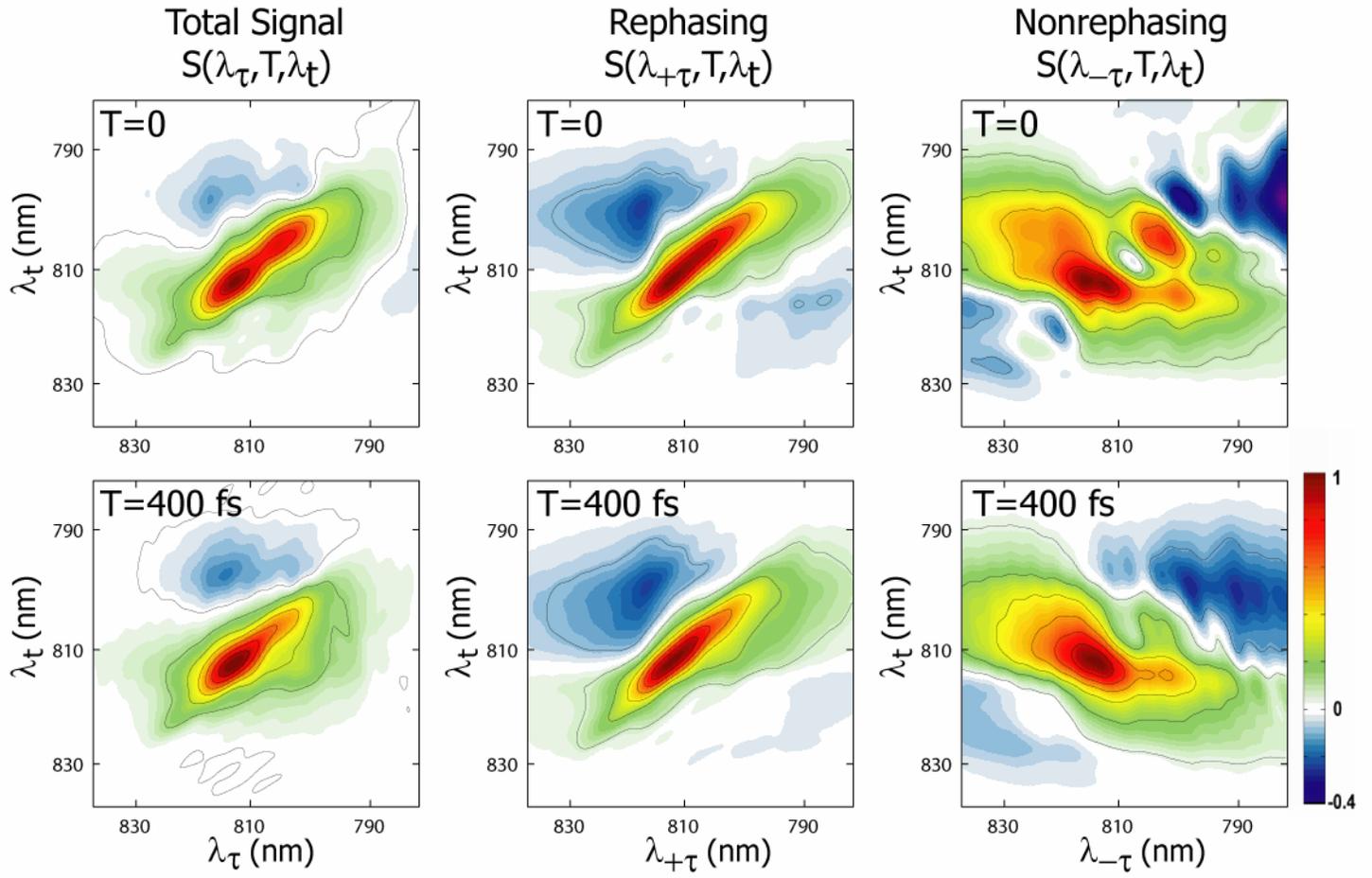


Figure 2.

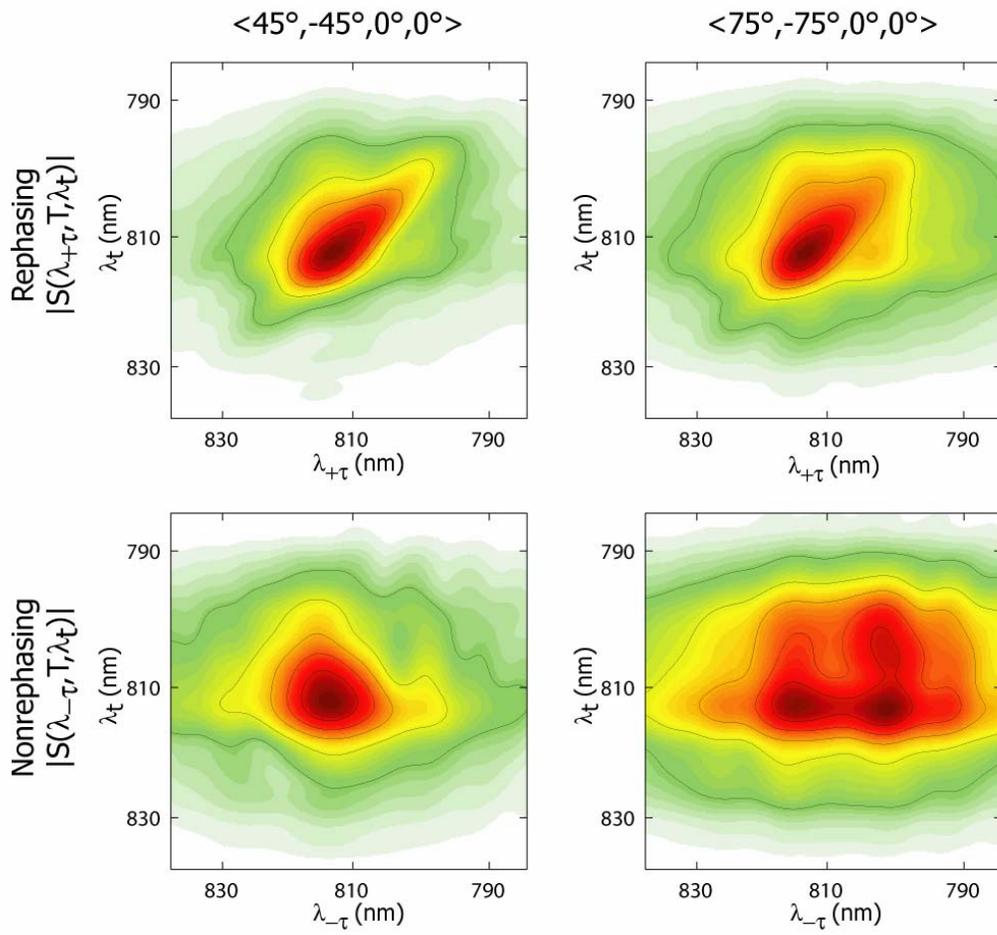


Figure 3.

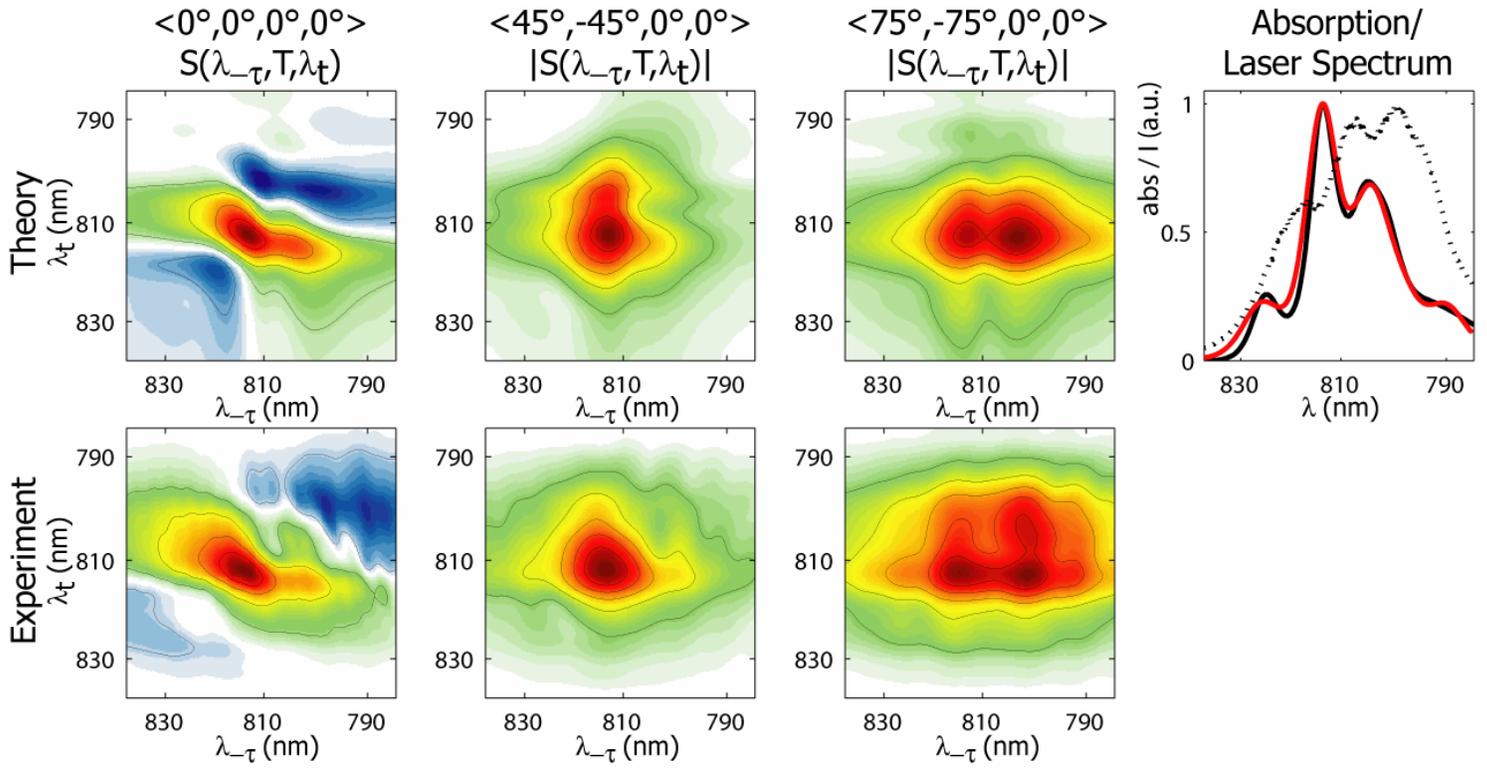


Figure 4.

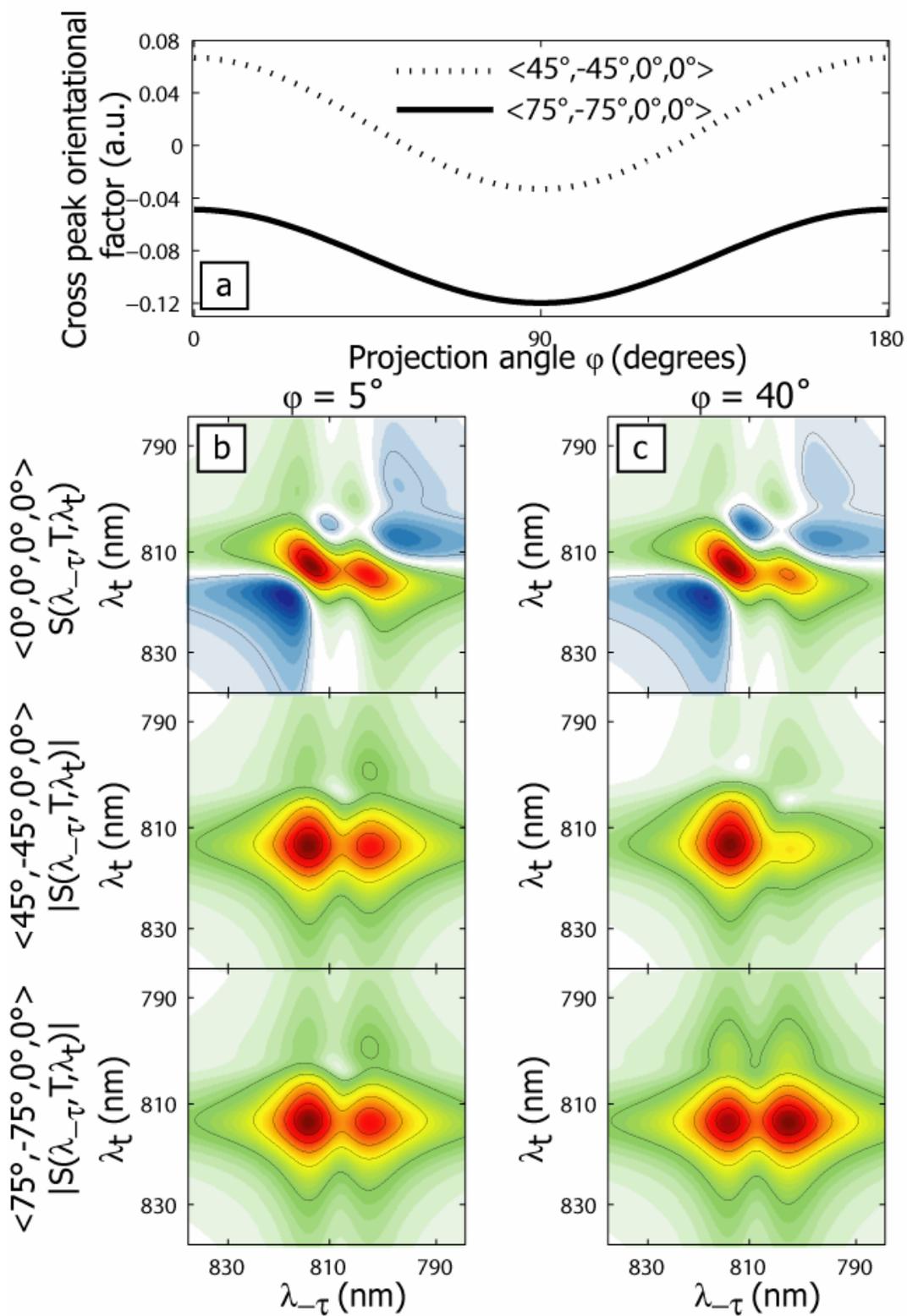


Figure 5.

