Two-dimensional Electronic Spectroscopy of Light Harvesting Complex II at Ambient Temperature: A Joint Experimental and Theoretical Study

Hong-Guang Duan^{1,2,3}, Amy L. Stevens², Peter Nalbach^{1,3}, Michael Thorwart^{1,3,*}, Valentyn I. Prokhorenko^{2,†} and R. J. Dwayne Miller^{2,3}

¹*I. Institut für Theoretische Physik, Universität Hamburg,
Jungiusstraße 9, 20355 Hamburg, Germany*

²*Max Planck Institute for the Structure and Dynamics of Matter,
Luruper Chaussee 149, 22761, Hamburg, Germany*

³*The Hamburg Center for Ultrafast Imaging,
Luruper Chaussee 149, 22761 Hamburg, Germany*

(Dated: August 18, 2015)

We have performed broadband two-dimensional (2D) electronic spectroscopy of the Light Harvesting Complex II (LHCII) at ambient temperature. We found that the electronic dephasing occurs within ~ 60 fs and inhomogeneous broadening is approximately 120 cm^{-1} . A three-dimensional global fit analysis allows us to identify several time scales in the dynamics of the 2D spectra ranging from 100 fs up to ~ 10 ps and to uncover the energy transfer pathways in LHCII. In particular, the energy transfer between the chlorophyll *b* and chlorophyll *a* pools occurs within ~ 1.1 ps. Retrieved 2D decay-associated spectra also uncover the spectral positions of corresponding diagonal peaks in the 2D spectra. Residuals in the decay traces exhibit periodic modulations with different oscillation periods. However, only one of them can be associated to the excitonic cross-peaks in the 2D-spectrum, while the remaining ones are presumably of vibrational origin. For the interpretation of the spectroscopic data, we have applied a refined exciton model for LHCII. It reproduces the linear absorption, circular dichroism, and 2D spectra at different waiting times. Several components of the energy transport are revealed from theoretical simulations that agree well with the experimental observations.

I. INTRODUCTION

The light harvesting complex II (LHCII) from Photosystem II of higher plants is one of the most abundant complexes in nature and is responsible for capturing sun light and transferring its energy to the reaction center with a nearly unity efficiency. In the Photosystem II of higher plants, the main photoabsorption occurs in LHCII. It is a pigment-protein trimer of three similar monomeric units.¹ Besides carotenoid molecules which absorb light in the blue-green spectral range, each monomer contains two types of chromophores, chlorophyll *a* (Chla) and chlorophyll *b* (Chlb). They are embedded in the protein matrix and are responsible for absorbing the sunlight in the range of 600-700 nm.

The crystal structure of LHCII was recently solved with a resolution which is high enough to identify 8 Chla and 6 Chlb subunits in each monomer (Fig. 1). Also, their orientation within the protein matrix^{2,3} could be identified. After the capture of a photon and the formation of an exciton, the excitation energy is effectively transferred via different exciton states towards the reaction center. There the energy is converted to chemical energy by splitting of water molecules (see, e.g., Ref. 4 for more detailed review). Yet, for isolated antenna complexes the energy is trapped in the lowest exciton levels from which it is irradiated with a high quantum yield due to fluorescence.

The central importance of the LHCII has been realized and thus, it has long been probed extensively by almost

every available steady-state spectroscopic technique, such as absorption, linear and circular dichroism (CD), fluorescence⁶⁻⁸ and single-molecule spectroscopy,⁹ and also by Stark spectroscopy.¹⁰ The electron-vibrational coupling and the involved intramolecular vibrational modes have been revealed using low-temperature non-photochemical hole burning¹¹ and fluorescence line-narrowing spectroscopies.¹²

Also energy transfer (ET) processes have been extensively studied in the past. For this, ultrafast spectroscopies such as transient absorption and time-resolved fluorescence¹³⁻¹⁵, and three-pulse photon echo peak shift (3PEPS)^{16,17} spectroscopy have been used. More recently, 2D photon echo spectroscopy¹⁸⁻²⁰ has also been applied.

In particular, in the femtosecond transient absorption spectroscopy studies at low temperature (77 K), different time scales of the ET have been investigated.¹³ Various Chla absorption bands have been selectively excited and the induced changes over the entire Q_y region have been studied. A subsequent global fit, together with a target analysis on the basis of a sequential energy transfer model, has revealed different time scales in which the energy transfer dynamics between the pigments in monomeric LHCII occur. Within this excitation range, the population kinetics of the Chla pool involve the time scales of 0.28 ps, 11.5 ps, 160 ps, and a long-lived component with the time scale of 3.4 ns. At room temperature, a transient absorption study of LHCII-trimers has been performed at very low excitations in the Chlb spectral region.¹⁴ The measured kinetics showed lifetimes of 175

FIG. 1. Spatial structure of the LHCII monomer from the stromal side (PDB file 2BHW) with labeling of pigments according to Ref. 3. For clarity of presentation, the carotenoids are not shown. The figure was created using the VMD package.⁵

fs, 625 fs, 5 ps and a long-lived component. The shortest lifetimes were assigned to the energy transfer between the Chlb and Chla pools. Notably, a 300 fs lifetime component was not found in the measured kinetics of the LHCII trimers. However, this 300 fs lifetime was reported in Ref. 16 in the 3PEPS-kinetics in the Chlb absorption band. It was attributed to the energy transfer within the Chlb molecules, whereas a 4-ps component reflects the energy transfer from Chlb to Chla. Together with this, the 150-fs and the 600-fs lifetimes were also found in the 3PEPS-kinetics.

In the subsequent 3PEPS experiment reported in Ref. 17 from the same lab, this 300-fs component was observed in the 3PEPS kinetics and was then attributed to the energy transfer between Chlb and Chla. In a recent 2D spectroscopy study of LHCII trimers, only the Chlb pool was excited with excitation pulses with a relatively narrow spectrum (14 nm), however, with a spectrally broad "reading" pulse.¹⁹ This allowed them to resolve the 300-fs component in a sequence of 2D spectra taken at different waiting times. It was attributed, as well as the 2.4-ps component, to the energy transfer from Chlb to Chla. However, questions remained open. The excitation level was too high for annihilation-free conditions to be presented in view of the relatively large changes in the differential absorbance, up to 50 mOD. Moreover, the interpretation of the Fourier-transformed photon-echo signals in terms of transient absorption changes, which involve changes in populations, is debatable.

In general, there is consensus about basically four timescales for ET in the LHCII (see, e.g., the reviews

4 and 21): a very fast one with the characteristic lifetime of 100-150 fs, a slower one with a 300-600 fs lifetime (whose appearance depends on the excitation wavelength and excitation spectrum), 1.5-2.5 ps which is generally accepted to be the lifetime of the ET between the Chlb and Chla pools, and a 5-10 ps component.

In addition to the extensive experimental investigations of the LHCII, great efforts have been made on the side of theoretical simulations of both steady-state and time-resolved spectroscopic data. It should be emphasized that the identification of the energy levels of the excitonic states and the energy transfer pathways from experimental data is not a trivial task and is often limited by typically congested linear spectra, homogeneous broadening, and by the presence of static disorder and inhomogeneous broadening. The most refined exciton model of the LHCII was established by Novoderezhkin *et al.*²² on the basis of the crystal structure with a spatial resolution of 2.72 Å.² They performed a simultaneous fit of the absorption, linear dichroism, steady-state fluorescence, and transient absorption spectra at different delay times and at different excitation wavelengths (notice that the CD spectrum was not included in the fit to the set of data). The excitonic interactions between the pigments (i.e., the off-diagonal terms in the Hamiltonian matrix) were calculated in the dipole-dipole approximation.²³ The model describes reasonably well the linear spectra and the transient absorption data. The time scales and pathways of the ET have been identified upon using modified Redfield theory and an experimental exciton-phonon spectral density.²² According to this advanced model, the initial, fast Chlb→Chla energy transfer is due to a good spatial connection of the Chlb to strongly coupled Chla clusters. Long-lived components of the energy transfer are determined by the population of red-shifted monomeric Chl a604, followed by a very slow (12 ps) flow of excitation energy from this bottleneck site to the remaining low-lying exciton states. The dynamics of the population within the Chla pool was found to be governed by fast (sub 100 fs) excitonic relaxation within the a610-a611-a612 trimer, followed by slower relaxation within a602-a603 and a613-a614 dimers (Fig. 1) on the time scale of 200-300 fs. An even slower migration on a time scale of 300-800 fs between these clusters then sets in, and, finally, a very slow transfer from a604 to the quasi-equilibrated sites terminates the transfer²¹ (we use the nomenclature of Ref. 2). The homogeneous broadening was described within Redfield theory, implying that this approach has no capacity for a proper description of the 2D electronic spectra. The dominating effect is fast dephasing which clearly has a non-Markovian behavior in general. It should also be noted that the dipole approximation for calculating the excitonic interactions is the simplest one and is actually applicable when the distances between the chromophores are large enough as compared to their dimensions. A more accurate approach is the transition density cube (TDC) method²⁴ which includes the Coulomb interac-

tion of the chromophore molecular charge densities. The site energies of the pigments (i.e., the diagonal terms in the Hamiltonian matrix) were obtained by fitting the calculated spectra to the experimental data. They also can be calculated independently by ab-initio, semi-empirical, or other quantum chemistry methods and by accounting for the energy level shifts due to the interaction of the chromophores with the local protein environment. Such an approach has been followed in Ref. 25 and may yield values of the site energies which can be used as very good initial values for a fitting procedure.

In addition to “traditional” transient absorption and time-resolved fluorescence spectroscopies, 2D photon-echo electronic spectroscopy has also been applied to the study of LHCII. **2D spectroscopy provides additional, important information on the dynamics, clearly separable from heterogeneous line broadening, and coupling between chromophores.** To date, only a few investigations using this technique have been performed.^{26–28} Moreover, the only one theoretical analysis devoted to the modeling of 2D-spectra of the LHCII¹⁸ is based on a modified Red-field master equation and uses a simplified technique²⁹ for creating 2D spectra.

Extending spectroscopic measurements to the second frequency dimension allows identifying the locations of excitonic states and mapping the pathways of the energy transfer among them. The first report of 2D spectroscopy of the LHCII at 77 K has been provided in Ref. 18. Several cross peaks between excitonic states were visualized and thus some energy transfer routes were identified. In a subsequent work of the same group, the authors analyzed oscillations arising on the diagonal sections of 2D spectra measured at consecutive waiting times. From the power spectra of the Fourier-transformed oscillations all exciton states in the LHCII have been identified.²⁰ The energies of the excitonic transitions were in an excellent agreement with the values predicted earlier in Ref. 22, however, not with the energies defined later in a newly refined LHCII model from the same authors.³⁰

Furthermore, polarized 2D electronic spectroscopy was applied to reveal the angles between the transition dipole moments of some excitonic states.³¹ The so-called coherence-specific pulse polarization sequence³² isolates cross-peaks and suppresses diagonal peaks. It was also used to investigate dephasing in a 2D study of the LHCII.³³ The coherence of cross-peaks was reported to decay at 77 K on two distinguishable time scales of ~ 50 and 800 fs. However, it remained unclear how these dephasing times relate to electronic dephasing and to the dephasing of exciton states in the LHCII. All 2D spectroscopy studies of the LHCII cited above were performed at low temperature (77 K). Up until present, only one published work applied this tool to the LHCII at room temperature.¹⁹ However, a relatively narrow excitation spectrum, which covers only the Chlb absorption band, was used.

In this work, we present experimental as well as theoretical results for the 2D-electronic spectroscopy of

LHCII at ambient temperature. In particular, to distinguish this work from Ref. 19, we have used a broadband excitation spectrum which covers the whole Q_y absorption band. Most notably, this allows us to trace and resolve the coherent and the ET dynamics within the entire set of exciton states. The experimental 2D spectra indicate fast electronic dephasing on a time scale of ≈ 60 fs. We observe long-lived oscillations in the cross peaks at room temperature. A cross-correlation analysis shows that they are rooted in intramolecular vibrations. In particular, they cannot be attributed to possible beatings between *excitonic* states associated with the Chlb and Chla pools. The flow of excitation energy between these pools is clearly resolved and can be identified in the 2D-spectra as separate peaks starting from waiting times of 100 fs. The 2D spectra calculated at room temperature within the framework of our developed theoretical model are in **very good** agreement with the experimental ones **in relation to the previously published comparisons between theory and experiment** (see, e.g., in Ref. 34). Moreover, a multidimensional global fit approach³⁵ allows uncovering various energy transfer pathways in the two-dimensional decay-associated spectra (2DDAS).

The paper is organized in the following way. In Sec. II, we describe the experimental methods, the measuring conditions, and the data analysis. Sec. III outlines the theoretical model and the method used to calculate the linear and the 2D spectra. In Sec. IV, we provide the experimental results and compare them to the theoretical simulations. Finally, Sec. V summarizes the central conclusions of the present study.

II. MATERIALS AND EXPERIMENTAL METHODS

Experimental setup. All the reported measurements were performed using a 2D coherently-controlled setup described in Ref. 36 with two minor modifications: Aiming to increase the speed of the acquisition of the spectra, the electronics as used in the previous camera were replaced (Tec5, Germany), and, the motorized translation stages were equipped with optically-based actuators. This allowed us to directly measure their absolute positions. The 2D setup was used in a “mono-beam” fashion, i.e., without involving the second beam which has passed through a deformable mirror compressor (see Fig. 3 in Ref. 36). Heterodyne detection of the photon-echo signal (PE) was achieved by placing a thin neutral-density filter with OD 2 into the fourth (reference) beam. This delayed the local oscillator pulse by ~ 400 fs. The spot size of all beams at the sample position was $\sim 60 \mu\text{m}$.

The ultrashort light pulses generated by a home-built noncollinear optical parametric amplifier (NOPA). It was tuned to the red side of the visible spectrum and centered at 670 nm. Since at that position the NOPA spectrum is very broad, and in order to increase the spec-

tral brightness, its spectral width was reduced to ~ 60 nm by placing a 3mm thick fused silica window into the white-light arm of the NOPA. Furthermore, in order to avoid excitation of high-lying vibrational states in the chlorophylls, the wings of the NOPA spectrum were suppressed by using an appropriate amplitude mask applied to an acousto-optical modulator Dazzler (Fastlite, France). This was placed into the first beam of the 2D setup (see Ref. 36). The resulting laser spectrum is shown in Fig. 2 together with the absorption spectrum of the LHCII sample used in the experiments. The light pulses were compressed to the Fourier-transform-limited level (22 fs FWHM) using a corresponding phase mask applied to the Dazzler. The pulses were characterized by a home-built third-order frequency-resolved optical gating setup (FROG) described in detail in Ref. 37. The energy of the excitation pulses was controlled by the Dazzler and limited up to 10-15 nJ per beam at the sample position. All measurements were performed with a repetition rate of 900 Hz.

Sample preparation and measuring conditions.

The LHCII trimers were isolated from spinach according to the procedure described in Ref. 38, dissolved in a 50-mM HEPES buffer, and stored at -40°C . Directly before the measurements, the samples were filtered using a $0.2\ \mu\text{m}$ micropore filter in order to reduce unwanted light scattering which significantly affects the 2D spectra in particular around zero waiting time. The absorption and CD spectra were measured using a Jasco J-810 spectropolarimeter (USA) in a 1-mm path length cell. The 2D measurements were performed at room temperature using a 0.4 mm thick closed cell with inner volume of 1.5 mL, mounted onto a precise home-built X-Y motorized translator with a moving speed of ≈ 6 mm/sec. The actual spectrum of the sample is shown in the left panel of Fig. 2. The OD at the maximum of the absorbance of the Q_y band was 0.33. To minimize unwanted contributions of the nonresonant response to the PE signal, a 0.2 mm fused silica cover slip was used as an input window in the cell. In order to avoid the so-called annihilation of excitons in LHCII trimers, the excitation energy was kept below 5 nJ. At these excitation conditions the magnitude of the homodyne PE was proportional to the third power of the incoming pulse energy (see the dependence of the PE on the injected energy in the right panel of Fig. 2). This ensures the excitation is in the linear regime. Deviations from the linear regime start to occur at energies > 6 nJ per beam. We have verified that under these illumination conditions, the maximal degradation of the absorbance of the LHCII sample in the cell was $\sim 5\%$ within 12 hours of operation.

The PE spectra were collected at each fixed “waiting time” T by scanning the delay time τ in the range of $[-100, +150]$ fs with a step size of 1 fs. We collected 280 spectra that were averaged at each delay point. The waiting time T was linearly scanned within 0-2 ps in steps of 25 fs, and with logarithmically spaced steps up to 50 ps. Since the accumulation of a large number of PE scans

FIG. 2. Left: Absorption spectrum of the LHCII sample and the overlap with the spectrum of the excitation laser. Right: Dependence of the homodyne PE signal (symbols) on the excitation energy measured at waiting time $T = 0$. To illustrate the deviation from the linear regime (solid line), we plot the cubic root of the magnitude of the PE signal on the ordinate.

takes a couple of hours, the NOPA spectrum was actively stabilized using an appropriate feedback.

Data treatment. The 2D spectra were created from the measured PE signals according to the procedure described in detail in Ref. 36. Briefly, the PE traces extracted from the heterodyne signals were Fourier transformed along the delay axis τ in the whole measured range. This results in a dependence of the PE signal on the frequency ω_τ . The spectra along the observation axis corresponding to the wavelength scale in the spectrometer were interpolated to equally-spaced frequencies ω_t . The phasing of the measured 2D spectra, i.e. finding the correct delay of the local oscillator filter, was performed using an “invariant theorem”.³⁶ It states that the real parts of both the heterodyne-detected PE and the transient grating (TG) spectra at $\tau = 0$ are equal to the pump-probe (PP) spectrum measured at the same waiting time T . We used both these methods for the phasing of the heterodyne signals. While both methods give almost equal LO-delays (≈ 400 fs), the phasing of TG to PP spectra is more accurate, since for waiting times $T > 20 - 30$ fs, the TG spectra are not affected by non-resonant contributions from the solvent and from the front window. Thus, they are free of the artifacts caused by interference of scattered light with intrinsic PE/TG signals.

Multidimensional global fits of both experimental and measured arrays of 2D spectra was performed in accordance with the previously developed algorithm.³⁵ A detailed description can also be found in the SI to Ref. 39. In this method, a sequence of 2D spectra taken at different T is collected to form a three-dimensional array $S(\omega_\tau, \omega_t, T)$. This 3D array is then decomposed into a sum of two dimensional decay-associated spectra (2DDAS) $A_i(\omega_\tau, \omega_t)$ with individual exponential decays with associated lifetimes τ_i , according to

$$S(\omega_\tau, \omega_t, T) = \sum_i A_i(\omega_\tau, \omega_t) \exp(-T/\tau_i). \quad (1)$$

The number of exponentials used to describe the experimental data was limited to 4 due to the measuring noise. In order to avoid the possible influence of a nonlinear response of the solvent on the PE, the fit of the experimental data was performed starting at $T = 50$ fs.

III. THEORETICAL MODELING

We consider a molecular model of the LHCII monomer described by the Hamiltonian H_{mol} consisting from the set of molecules in two-level approximation. For spectroscopy, the total transition dipole operator $\boldsymbol{\mu}$ of the molecule is coupled to the semiclassical electric field $\mathbf{E}(t)$ of the laser pulse, which results in the time-dependent Hamiltonian

$$H(t) = H_{mol} - \boldsymbol{\mu} \cdot \mathbf{E}(t). \quad (2)$$

We note that our model is restricted to the LHCII monomer due to limitations in the available computer power. The total dipole operator is expressed via the transition dipole moments of individual molecules $\boldsymbol{\mu}_m$ according to

$$\boldsymbol{\mu} = \sum_{m=1}^N \boldsymbol{\mu}_m (\alpha_m + \alpha_m^\dagger), \quad (3)$$

with α_m and $\alpha_m^\dagger$ being the creation and annihilation operators of the electronic excitation on the m th molecule. The molecular Hamiltonian H_{mol} is given as

$$H_{mol} = H_e + H_{ph} + H_{e-ph}. \quad (4)$$

Here, the Frenkel Hamiltonian H_e describes the electronic degrees of freedom, and is given by

$$H_e = \sum_{m=1}^N \epsilon_m \alpha_m^\dagger \alpha_m + \sum_{m=1}^N \sum_{n < m} J_{n,m} (\alpha_m^\dagger \alpha_n + \alpha_n^\dagger \alpha_m),$$

where ϵ_m are the site transition energies of the molecules, and $J_{n,m}$ is the inter-molecular excitonic coupling. H_{ph} describes the nuclear (phonon) degrees of freedom. We consider a continuous distribution of phonon modes which can be modeled as a bath of harmonic oscillators. In our model, we assume that the electronic excitation on the m th molecule couples independently to its own harmonic bath given as

$$H_{ph} = \sum_{m=1}^N \sum_{j=1}^{N_b^m} \left(\frac{P_{mj}^2}{2} + \frac{1}{2} \omega_{mj} x_{mj}^2 \right). \quad (5)$$

Here, N_b^m is the number of bath modes which couple to the molecule m , x_{mj} and p_{mj} are the mass-weighted position and momentum of j th harmonic oscillator mode with the frequency ω_{mj} . The system-bath spectral density has the form

$$J_m(\omega) = \frac{\pi}{2} \sum_{j=1}^{N_b^m} \frac{c_{mj}^2}{\omega_{mj}} \delta(\omega - \omega_{mj}). \quad (6)$$

As usual, we assume a continuous distribution of the frequencies of the bath modes for $N_b^m \rightarrow \infty$ and, in particular, we assume an Ohmic spectral density. The electron-phonon coupling H_{e-ph} is assumed to cause only fluctuations of the electronic energy and is independent for each molecule, such that

$$H_{e-ph} = \sum_{m=1}^N \sum_{j=1}^{N_b^m} c_{mj} x_{mj} \alpha_m^\dagger \alpha_m. \quad (7)$$

For the parametrization of the model, the site transition energies ϵ_m of the chromophores were pre-calculated in an ab-initio approach for the molecules in the gas phase. We found higher transition energies ($S_0 \rightarrow S_1$) for Chlb than for Chla due to the additional carbonyl group in Chlb. This is known and consistent with the linear spectroscopic data. **In a subsequent step, the pre-calculated site energies have been then optimized by fitting of calculated absorption and CD spectra to the experimental ones.** The intermolecular excitonic couplings $J_{n,m}$ have been taken from Ref. 40 without any further modifications. In particular, they were calculated by the transition density cube method.²⁴ The final form of the model Hamiltonian after the optimal fitting to the measured spectra is given in Eq. (1) in the SI. For the system-bath interaction, the parameters of the Ohmic spectral density $J(\omega) = \gamma \omega e^{-\omega/\omega_c}$ were fitted by assuming three artificial spectral densities, thereby using Eq. (15) of Ref. 41. This approach significantly speeds up the numerical calculations.

The quantum dynamical calculations were performed using time nonlocal quantum master equation method (TNL).^{41,42} Further details about the numerical calculation procedures are provided in the SI. Inhomogeneous broadening is generated by low frequency nuclear motion and static disorder. In this work, we have accounted for inhomogeneous broadening ($\text{FWHM} \sim 106 \text{ cm}^{-1}$) by convoluting the theoretically calculated homogeneous spectra with a Gaussian-shaped distribution having a width of $\sigma = 90 \text{ cm}^{-1}$ ($\text{FWHM} = \sqrt{2 \ln 2} \sigma$). The dipole strengths and the orientations of the chromophores were also taken from Ref. 40. In particular, the relative strengths of the transition dipole moments of the Chla and Chlb molecules were set to $|\mu_a|=1.0$ and $|\mu_b|=0.85$, respectively.

To gauge the parameters of our model, we calculated the linear absorption and the CD spectra as defined in Eq. (2) in the SI, and compare them to the experimental ones. To achieve the optimal fit of the CD spectrum, it was necessary to rotate the transition dipole moments of the Chla molecules by 8° . The directions of the corresponding dipole moments of the Chlb molecules were unchanged.⁴³ In Fig. 3, we show the absorption and CD spectra calculated at room temperature, together with the experimentally measured spectra. The calculated stick spectrum is represented by the bars. **The 2D spectra were created using the equation of motion-phase matching approach⁴⁴ including the double excited states. The**

FIG. 3. Top: Linear absorption spectrum at room temperature calculated by the TNL method (solid line) and measured (symbols). In addition, the stick spectrum of the molecular Hamiltonian is shown. The dashed line indicates the spectrum of the excitation. Bottom: Circular dichroism spectrum both from theoretical calculation (solid line) and from experimental measurement (symbols) at room temperature. The optimal fit yields the parameters $\gamma = 0.6$ and $\omega_c = 400 \text{ cm}^{-1}$ for the Ohmic spectral density. The calculated results were convoluted with a Gaussian-shaped distribution with the width $\sigma = 90 \text{ cm}^{-1}$ both for the fit of the absorption and the CD spectrum simultaneously.

spatial averaging of 2D spectra was performed according to prescription given in Ref. 45 (Chapter III). Detailed description of the calculations is provided in the SI.

IV. RESULTS AND DISCUSSION

Dephasing and inhomogeneous broadening. Figure 4 shows a collection of representative experimental 2D spectra (left column) collected at different waiting times as indicated. The right column shows the corresponding 2D spectra calculated on the basis of our theoretical model. Both are in very good agreement, which supports the refined exciton model of the LHCII. At an initial waiting time $T = 0$, the 2D spectrum is significantly stretched along the diagonal which manifests rather strong inhomogeneous broadening in the LHCII. The off-diagonal section of the spectrum is related to homogeneous broadening. It has a FWHM of 170 cm^{-1}

which corresponds to an electronic dephasing time of 62 fs, assuming a Lorentzian line shape. As expected, dephasing at room temperature is much faster than at low temperature. For 77 K, the dephasing time has been estimated for LHCII also from 2D spectroscopy.¹⁸ Nevertheless, it is still slightly longer than the typical electronic dephasing time for, e.g., organic dyes (40-50 fs). The degree of inhomogeneous broadening can be estimated as the ratio of the diagonal and the anti-diagonal widths. We find ≈ 3.1 (the FWHM of the diagonal section is 530 cm^{-1}). However, this estimation is not strictly valid for an excitonic system having several transitions distributed over a wide spectral range (cf. stick spectrum on Fig. 3). Even without inhomogeneous broadening the 2D spectrum of such system will display some diagonal stretch due to spectral distribution of the excitonic transitions. We can deduce the degree of inhomogeneous broadening by comparison of diagonal widths for the measured (inhomogeneous) and calculated homogeneous 2D-spectrum (FWHM 410 cm^{-1} ; not shown). From this comparison follows that the degree of inhomogeneous broadening at $T = 0$ is approximately 120 cm^{-1} which is actually closed to the inhomogeneous parameter of 106 cm^{-1} used in the calculations of all spectra. Some features, which are visible in the left-top sector of the 2D spectrum (label C), can be associated with the cross-peak arising from the interference of the exciton states associated with the two manifolds of the Chla and Chlb molecules. However, at this waiting time, the corresponding diagonal peaks are not yet well resolved since they are “blurred” by fast dephasing processes. They become much better resolved at waiting times beyond $\sim 100 \text{ fs}$. Then, dephasing is over, and the magnitude of the diagonal peak, which can be associated with the Chlb pool (label B), decays with increasing waiting times due to energy transfer to the manifold of the exciton states associated with the Chla pool (label A). This energy transfer manifests itself as a growing cross-peak C, which becomes clearly visible at waiting times between 100 fs and up to a few tens of ps, see Fig. 4.

The time evolution of the diagonal (A,B) and the cross peaks (C,D) is shown in Fig. 5. The different characteristics in the decay traces are caused by the energy transfer basically between the Chlb and Chla pools (see below).

Energy transfer pathways in LHCII. The energy transfer pathways and the associated lifetimes can be resolved by applying a multidimensional global fit approach to the series of consecutive 2D spectra acquired at different waiting times. The procedure for a global fitting of frequency- and time-resolved data is applicable only when the dynamics are not dispersive, i.e., when the lifetimes do not significantly depend on the wavelengths and frequencies. To validate the applicability of the global fitting to our experimental LHCII 2D data, we independently fitted each decay trace (a single fit) in the set of the 2D spectra which contains about 26000 traces. We found that the retrieved lifetimes form three clearly distinguishable groups with quite narrow distributions of

FIG. 4. Real part of the experimental (left) and the theoretical (right) 2D photon echo spectra of the LHCII at different waiting times. The theoretical result is calculated with the model parameters obtained by fitting the linear absorption and CD spectra (see text). The diagonal and cross peaks are labeled by capital letters in the frame $T = 0$ fs. The curves at the top show the respective linear absorption spectra.

lifetimes. For the probability of occurrence of lifetimes, retrieved from the single fits, we refer to Fig. 3S in the SI. The quality of the 3D global fits can be inspected by comparing the experimental and the fitted decay traces shown in Fig. 5. The global fitting provides a 2DDAS, which is shown in the left panel of Fig. 6.

We find the lifetimes of 100 fs, 1.1 ps, 9.8 ps, and a long lived component with a lifetime >100 ps, which is not resolved in the delay time window used (marked as “Inf” in Fig. 6). This long-lived 2DDAS can be associated to the trapping of excitation energy in low-lying exciton states after the fast energy transfer between excited states has been completed. A corresponding DAS

FIG. 5. Representative traces of $S(\omega_\tau, \omega_t, T)$ taken at (ω_τ, ω_t) as indicated. The blue lines mark the experimental data and the red lines show the fits.

with long lifetimes of a few ns was previously observed in numerous transient absorption and time-resolved fluorescence studies. The fastest resolved 2DDAS with the lifetime of 100 fs can be associated to the fast electronic dephasing. It appears due to the decay of the coherence of the excited exciton states. We note that the excitation spectrum used here covers the whole Q_y band, so that all exciton states are excited. This 2DDAS shows a smaller degree of inhomogeneity as compared to the 2D spectrum collected at $T = 0$, see Fig. 4. A red-shifted cross peak is also present there. The section of the 100-fs 2DDAS along $\omega_\tau = 15340$ cm^{-1} clearly illustrates this feature. Some energy transfer is also observed in the 100-fs 2DDAS along $\omega_\tau = 14900$ cm^{-1} as indicated by the negative shoulder around $\omega_t = 14600$ cm^{-1} . This ET is associated with the main diagonal peak of Chla, but it can be attributed to the ET from the exciton states located at 14750 cm^{-1} to low-lying state(s) located around 14600 cm^{-1} (see in Fig. 2). As opposed to the experimental data, the corresponding 2DDAS with the comparable lifetime of 70 fs retrieved from calculated 2D spectra has a rather different shape. It displays a negative peak around 15000 cm^{-1} which can be associated either with an efficient ET between Chlb-Chla pools or with the contribution of the excited state absorption.

We did not find a spectral component with a lifetime of 300 fs, either in the single or in the global fit analysis of the experimental data. The existence of this component was discussed in several experimental papers (see in Introduction). We note that we have performed a separate conventional pump-probe experiment in which this com-

ponent was also not detected in the measured kinetics of the same LHCII sample. However, interestingly enough, a spectral component with a lifetime of 400 fs is resolved in the 2DDAS of our theoretically calculated 2D spectra, see Fig. 2S in the SI. Nonetheless, it should be realized that there is no direct link between the transient spectroscopy and the electronic 2D spectroscopy in terms of the observed spectra due to the difference in the measured signals and to the different dimensionality. Therefore, a direct comparison of the one-dimensional DAS derived from the transient absorption spectrum and the 2DDAS retrieved from the 2D photon-echo spectroscopy experiments is not straightforward, especially taking into account the different excitation conditions. Usually, a narrow excitation spectrum located at the blue side of the absorption spectrum is used in the pump-probe experiments, while a broad excitation spectrum covering the whole absorption Q_y band in the photon echo experiment reported here is used. However, when 2DPE spectroscopy is performed under similar conditions with respect to the excitation spectrum as in the pump-probe experiment, i.e., with a narrow and blue-located excitation spectrum, the degree of similarity in the retrieved DAS is much higher. Yet, such conditions lead to a substantial loss of useful information in the 2D spectroscopy (e.g., excitonic interaction as visualized via cross peaks). Such excitation conditions were realized in the recently reported experiment in Ref. 19. In particular, this significant difference in the excitation conditions can explain the absence of the 300fs component in our experimental data.

The overall energy transfer from the Chlb to the Chla pool is clearly resolved in the 2DDAS with a lifetime of 1.1 ps, see Fig. 6. Also, the corresponding Chla and Chlb diagonal peaks are clearly separated and much better resolved in this 2D decay-associated spectrum as compared to the 2D spectra at different waiting times T as shown in Fig. 4. To further illustrate this, we show in Fig. 7 cuts along the horizontal axis through the 2DDAS. In particular, the viewgraph for the waiting time of 1.1 ps shows a section along $\omega_\tau = 15340 \text{ cm}^{-1}$ which belongs to the Chlb pool and whose “butterfly” shape is similar to the decay-associated spectra in conventional time-resolved pump-probe spectroscopy. It reveals the “downhill” Chlb $\rightarrow$ Chla energy transfer, whereas the “uphill” Chla $\rightarrow$ Chlb transfer is observed in the cut along $\omega_\tau = 14700 \text{ cm}^{-1}$. It crosses the diagonal peak associated with the Chla pool. For the given spectral split between the positive and the negative shoulder ($\sim 400 \text{ cm}^{-1}$), the ratio of their amplitudes could be estimated from a Boltzmann distribution and yields 0.14. On the other hand, the observed ratio is 0.4. However, taking into account that in 2D spectroscopy the measured signal is proportional to the polarization rather than to the population as in the transient spectroscopy, this ratio has a reasonable magnitude ($\sqrt{0.14} = 0.38$). Hence, we can conclude that the overall shapes of the spectra are similar and the same energy transfer pathways are re-

FIG. 6. The experimental (left) and theoretical (right) 2DDAS denoted by $A_i(\omega_\tau, \omega_t)$ in Eq. (1) which result from the global fitting analysis. They clearly reveal the four components of the energy transport in LHCII.

solved. Moreover, the lifetimes in the 2DDAS retrieved from the experimental and from the calculated 2D spectra are similar, although the uphill ET is not present in the calculated spectra.

The 2DDAS associated to the lifetime of 9.8 ps displays a significant cross peak C with a less populated diagonal peak B. The latter is associated with the exciton states corresponding to the Chlb pool. This cross peak is well resolved in the section of the 2DDAS at $\omega_\tau = 15340 \text{ cm}^{-1}$. The shape of this 2DDAS does not show a signature of inhomogeneous broadening, and we thus can conclude that the inhomogeneous broadening in LHCII has diffusive character. Clearly, it has died out after ~ 10 ps. The calculated 2DDAS with a similar lifetime (~ 7 ps) shows a strong elongation along the diagonal di-

FIG. 7. Horizontal cuts through the 2DDAS at the fixed frequency ω_T as indicated. This yields the lineshape of the global fitting components for the 2DDAS.

rection. This is associated with a strong inhomogeneous broadening even after ~ 10 ps. This discrepancy between the measured and the calculated spectra is due to drawbacks in accounting for the inhomogeneous broadening while calculating the 2D spectra (see discussion in Theoretical Modeling). In particular, the effective inclusion of the inhomogeneous broadening via a convolution with a Gaussian distribution seems insufficient here. Therefore, the measured and the calculated 2DDAS cannot be directly compared for lifetimes longer than a few ps. This also holds for the shapes of 2D spectra. In addition to the inhomogeneous broadening, the ET between the exciton states located around $\omega_t = 14900 \text{ cm}^{-1}$ and the low-lying state(s) at 14500 cm^{-1} is also resolved in the 2DDAS associated to the lifetime of 9.8 ps, see the negative shoulder in the section at $\omega_t = 14840 \text{ cm}^{-1}$ in Fig. 6. This allows us to conclude that there are two energy transfer pathways from the exciton states located around 14900 cm^{-1} to the low-lying “energy trap” states at the red shoulder of the absorption spectrum ($14500\text{--}14600 \text{ cm}^{-1}$) with very different lifetimes of 100 fs and ~ 10 ps.

Oscillations in 2D spectra and their origin. The residuals of the experimental decay traces shown in Fig. 5 show several periodic oscillations within the first 2 ps of the delay time. A Fourier analysis of all residuals provides the 3D spectrum where the axes are ω_t , ω_τ and ω_T . Several 2D slices from this 3D spectrum taken at different frequencies ω_T overlap the positions of the main diagonal peaks or the cross-peaks in the 2D-spectra (see Fig. 4SI in the SI), but the overlap does not occur simultaneously. In addition, the spectral positions of the resolved

“cross-peaks” with respect to the diagonal do not match the corresponding frequencies ω_T at which they are measured. Thus, we can conclude that the origin of these well-resolved peaks in the 3D spectrum has rather intramolecular character and involve vibrational states in the chromophores. This is in contrast to the interference beatings between excitonic states. In an additional analysis of the oscillations along the diagonal, we also were not able to attribute the resolved frequencies to particular excitonic states, as has been done in the 2D experiment at 77 K.¹⁸ In fact, this is not surprising since almost all peaks associated with the oscillations of notable magnitudes are located away from our diagonal region (see Fig. 5S in SI).

FIG. 8. Correlation 2D spectrum of the residuals obtained after the 3D global fitting of the 2D spectra. The color bar shows the magnitudes of the correlation coefficients.

To verify the origin of the oscillations observed in the 2D spectra, we have performed a cross-correlation analysis of the residuals across the diagonal $\omega_\tau = \omega_t$. In particular, we have calculated the correlation coefficients C between the residuals R for each pair of conjugated spectral positions in the delay time window up to 2 ps. The delay time steps were equally distributed and the correlation coefficients are given as

$$C(\omega_t, \omega_\tau) = \text{corr}(R(\omega_t, \omega_\tau, T), R(\omega_\tau, \omega_t, T)), \quad (8)$$

where corr evaluates the correlation with respect to T . This yields a correlation 2D spectrum which is plotted in Fig. 8. Negative values correspond to anticorrelated residuals, whereas positive values correspond to correlated residuals. We find strong correlations and anticorrelations between different areas of the 2D spectrum. Many cross-peaks can be resolved in this correlation spectrum and the positions of some of them are correlated

with the Fourier-components in the 3D spectrum. In particular, peaks in the 630 cm^{-1} slice of the 3D spectrum overlap with the main correlated areas and the 252 cm^{-1} slice overlaps with the main anticorrelated areas (see Fig. 5SI in the SI). The anticorrelated cross peaks in the correlation spectrum show that the oscillations contained in corresponding residuals have opposite phases. According to the theoretical investigations in Ref. 46, this confirms the intramolecular or vibronic origin of these oscillations. There is only one area of positively correlated residuals around $\omega_t = 14700\text{ cm}^{-1}$, $\omega_r = 15200\text{ cm}^{-1}$. It can be associated with the beatings between excitonic states located within the Chlb and the Chla pools, especially when taking into account the 630-cm^{-1} oscillations in the residuals at these spectral positions. This is consistent with the Chlb-Chla spectral splitting.

FIG. 9. Evolution of the cross peak signals (real part) located at $\omega_r=15370\text{ cm}^{-1}$, $\omega_t=14810\text{ cm}^{-1}$ (label C) and $\omega_r=14810\text{ cm}^{-1}$, $\omega_t=15370\text{ cm}^{-1}$ (label D) for increasing waiting times. The oscillation period of $\sim 50\text{ fs}$ ($\omega=667\text{ cm}^{-1}$) can be extracted from the time window up to $\sim 200\text{ fs}$.

In our theoretical model, localized vibrational states of the chlorophylls were not included due to the exponential growth of the associated Hilbert space and the demanding size of the required computer hardware equipment. Therefore, if oscillations appear in the sequence of the 2D spectra calculated within our model, they safely can be attributed only to beatings between exciton states. This clearly allows us to uniquely determine the origin of the oscillations observed in the experimental spectra. In the calculated 2D spectra, we find only strongly damped oscillations of small amplitudes within the initial waiting time window up to $\sim 200\text{ fs}$ for the spectral positions of the cross-peaks C and D. The corresponding signals are shown in Fig. 10. Their underlying period can be estimated to $\sim 50\text{ fs}$ ($\omega_T=667\text{ cm}^{-1}$). This frequency

matches well the energy gap between the exciton transitions 14666 cm^{-1} and 15333 cm^{-1} . They belong to the Chla and the Chlb pools, respectively (see the stick spectrum in Fig. 3). Therefore, we can conclude that the 630-cm^{-1} oscillation observed in the experimental 2D spectra is associated with the beatings between exciton states, while all other resolved oscillations originate from the involved vibrational states of the chlorophylls. We cannot exclude that some of them could belong to vibronic states as well which would require a strong coupling between the electronic and the vibrational degrees of freedom. This plausible line of reasoning should be tested theoretically by explicitly including vibrational states to our model Hamiltonian and a subsequent dynamical description of the vibrational degrees of freedom in the corresponding calculations.

V. CONCLUSIONS

In this paper, we report the results of a broadband 2D electronic spectroscopic study of LHCII trimers at room temperature. Dephasing of the electronic excitation at room temperature is much faster as compared to low temperatures and occurs within a 60-fs range. Our data directly visualize the significant impact of strong inhomogeneous broadening in the LHCII. Equally important is the loss of inhomogeneity observed at longer waiting times. This allows us to conclude that the inhomogeneous broadening in the LHCII at room temperature has diffusive character and dies out after a few tens of ps. An analysis of the experimental data is given in terms of an exciton model of the LHCII-monomer and an explicit dynamics treatment.

The numerically-calculated 2D spectra coincide with the experimentally-measured ones in the range of waiting times of up to several ps. Together with the advanced global fit analysis, this relatively new spectroscopic technique allows us to reveal and literally visualize the energy transfer pathways of one of the most important light harvesting complexes of higher plants. Our analysis for ambient temperature conditions allows us to identify which of the channels, active at low temperatures, do contribute predominantly in the living species. We have safely resolved the energy transfer channels from the Chlb to the Chla pools in both the experimental and the modeled spectra. These transfer dynamics occur within 1.1 - 1.2 ps as retrieved from the 2D spectra. Moreover, the fastest 2DDAS with a lifetime of $\sim 100\text{ fs}$ can be attributed to the loss of coherence among excited exciton states. It also resolves the energy transfer pathway from the exciton states associated with the main absorption peak to the exciton states lying at its low-energy edge. The main diagonal peaks associated with the Chla and Chlb pools are resolved in the 2D spectra and even a cross peak between them is visible within a waiting time up to 1 ps. Yet, these peaks and cross-peaks are much better visualized in the 2DDAS obtained after applying the

multidimensional global fit to the sequence of 2D-spectra collected at different waiting times.

Besides resolving the energy transfer pathways and visualizing the diagonal as well as the cross-peaks in the 2D spectra, also long-lasting oscillations in the kinetics of the coherence appear. The analysis of these oscillations and the cross-correlations applied for the first time to 2D spectroscopy, together with the comparison to the results of the numerical simulations shows that almost all of them are associated with vibrational transitions of individual chlorophylls or corresponding vibronic states. Only one component of the oscillations with a frequency of $\sim 630 \text{ cm}^{-1}$ can be attributed to the beatings between excitonic states located in the Chla and Chlb pools. The damping of these oscillations can be estimated to be $\sim 100 \text{ fs}$ (Fig. 8) which is consistent with the electronic dephasing in LHCII.

Despite some simplifications, our developed and carefully fitted exciton model matches well the linear spectra of the LHCII (both absorption and CD spectra). It properly simulates the 2D-spectra on the basis of one set of model parameters. It is limited by a simplified accounting of the effects of the inhomogeneous broadening. By this, the calculated kinetics of the coherence deviates from the experimentally observed data for times greater than 2 ps where spectral diffusion begins to sig-

nificantly affect the spectra. This leads to differences between the shapes and the lifetimes of some 2DDAS. Finally it should be mentioned that in additional computer simulations of simpler model systems, we have found that the proper accounting of the inhomogeneous broadening via an ensemble averaging of 2D spectra calculated with disordered site energies can lead to significant changes in retrieved lifetimes (up to a factor of 1.5 - 3) and in the shapes of the 2DDAS.⁴⁷

ACKNOWLEDGMENTS

This work was supported from the Max Planck Society and the excellence cluster "The Hamburg Center for Ultrafast Imaging - Structure, Dynamics and Control of Matter at the Atomic Scale" of the Deutsche Forschungsgemeinschaft. H.-G.D. acknowledges financial support provided by the Joachim-Herz-Stiftung (Hamburg) within the PIER Fellowship program. A. S. acknowledges L. Chen for assisting with the preparation of LHCII samples. We furthermore acknowledge R. van Grondelle for providing the LHCII samples used in initial experiments and for sharing the absorption spectrum of LHCII measured at 77 K.

* Corresponding author: michael.thorwart@physik.uni-hamburg.de

† Corresponding author: valentyn.prokhorenko@mpsd.mpg.de

¹ Scholes, G. D.; Fleming, G. R.; Olaya-Castro A.; Grondelle, R. v. Lessons from Nature about Solar Light Harvesting. *Nature Chem.* **2011**, 3, 763-774.

² Liu Z. F.; Yan, H. C.; Wang, K. B.; Kuang, T. Y.; Zhang, J. P.; Gui, L. L.; An, X. M.; Chang, W. R. Crystal Structure of Spinach Major Light-Harvesting Complex at 2.72 Å Resolution. *Nature* **2004**, 428, 287-292.

³ Standfuss, R.; Scheltinga, A. C. T. v.; Lamborghini, M.; Kuhlbrandt, W. Mechanism of Photoprotein and Nonphotochemical Quenching in Pea Light-Harvesting Complex at 2.5 Å Resolution. *EMBO J.* **2005**, 24, 919-928.

⁴ Amerongen, H. v.; Croce, R. in *Primary Processes of Photosynthesis: Basic Principles and Apparatus*, RSC Publ. Cambridge, UK Vol. I, p. 329, 2008.

⁵ W. Humphrey, A. Dalke, K. Schulten, *J. Molec. Graph.* **14**, 33 (1996).

⁶ Hemelrijk, P. W.; Kwa, S. L. S.; Grondelle, R. v.; Dekker, J. P. Spectroscopic Properties of LHCII, the Main Light-Harvesting Chlorophyll a/b Protein Complex from Chloroplast Membranes. *Biochim. Biophys. Acta* **1992**, 1098, 159-166.

⁷ Nussberger, S.; Dekker, J. P.; Kuhlbrandt, W.; Bolhuis, B. M. v.; Grondelle, R. v.; Amerongen, H. v. Spectroscopic Characterization of Three Different Monomeric Forms of the Main Chlorophyll a/b Binding Protein from Chloroplast Membranes. *Biochemistry* **1994**, 33, 14775-14783.

⁸ Peterman, E. J. G.; Hobe, S.; Calkoen, F.; Grondelle, R. v.; Paulsen, H.; Amerongen, H. v. Low-temperature Spectroscopy of Monomeric and Trimeric Forms of Reconsti-

tuted Light-Harvesting Chlorophyll ab Complex. *Biochim. Biophys. Acta* **1994**, 1273, 171-174.

⁹ Tietz, C.; Jelezko, F.; Gerken, U.; Schuler, S.; Schubert, A.; Rogl, H.; Wrachtrup, J. Single Molecule Spectroscopy on the Light-Harvesting Complex II of Higher Plants. *Biophys. J.* **2001**, 81, 556-562.

¹⁰ Olszowska, D.; Krawczyk, S.; Maksymiee, W. A study of Molecular Interactions in Light-Harvesting Complexes LHCIIb, CP29, CP26 and CP24 by Stark effect spectroscopy. *Biochim. Biophys. Acta*, **2004**, 1657, 61-70.

¹¹ Piper, J.; Ratsep, M.; Jankowiak, R.; Irrgang, K. D.; Voigt, J.; Renger, T.; Small, G. J. Q_y -level Structure and Dynamics of Solubilized Light-Harvesting Complex II of Green Plants: Pressure and Hole Burning Studies. *J. Phys. Chem. A* **1999**, 103, 2412-2421.

¹² Piper, J.; Ratsep, M.; Irrgang, K. D.; Freiberg, A. J. Chromophore-Chromophore and Chromophore-Protein Interactions in Monomeric Light-Harvesting Complex II of Green Plants Studied by Spectral Hole Burning and Fluorescence Line Narrowing. *J. Phys. Chem. B* **2009**, 113, 10870-10880.

¹³ Gradinaru, C. C.; Ozdemir, S.; Gulen, D.; I. Stokkum, H. M. v.; Grondelle, R. v.; Amerongen, H. v. The Flow of Excitation Energy in LHCII Monomers: Implications for the Structural Model of the Major Plant Antenna. *Biophys. J.* **1998**, 75, 3064-3077.

¹⁴ Connelly, J. P.; Müller, M. G.; Hucke, M.; Gatzert, G.; Mullineaux, C. W.; Ruban, A. V.; Horton, P.; Holzwarth, A. R. Ultrafast Spectroscopy of Trimeric Light-Harvesting Complex II from Higher Plants. *J. Phys. Chem. B* **1997**, 101, 1902-1909.

- ¹⁵ Croce, R.; Müller, M. G.; Bassi, R.; Holzwarth, A. R. Carotenoid-to-Chlorophyll Energy Transfer in Recombinant Major Light-Harvesting Complex (LHCII) of Higher Plants. I. Femtosecond Transient Absorption Measurements. *Biophys. J.* **2001**, 80, 901-915.
- ¹⁶ Agarmal, R.; Krueger, B. P.; Scholes, G. D.; Yang, M.; Yom, J.; Mets, L.; Fleming, G. R. Ultrafast Energy Transfer in LHC-II Revealed by Three-Pulse Photon Echo Peak Shift Measurements. *J. Phys. Chem. B* **2000**, 104, 2908-2918.
- ¹⁷ Salverda, J. M.; Vengris, M.; Krueger, B. P.; Scholaes, G. D.; Czamoleski, A. R.; Novoderezhkin, V.; Amerongen, H. v.; Grondelle, R. v. Energy Transfer in Light-Harvesting Complexes LHCII and CP29 of Spinach Studied with Three Pulse Echo Peak Shift and Transient Grating. *Biophys. J.* **2003**, 84, 450-465.
- ¹⁸ Schlau-Cohen, G. S.; Calhoun, T. R.; Ginsberg, N. S.; Read, E. L.; Ballottari, M.; Bassi, R.; Grondelle, R. v.; Fleming, G. R. Pathways of Energy Flow in LHCII from Two-Dimensional Electronic Spectroscopy. *J. Phys. Chem. B* **2009**, 113, 15352-15363.
- ¹⁹ Wells, K. L.; Lambrev, P. H.; Zhang, Z. -Y.; Garab, G.; Tan, H. -S. Pathways of energy transfer in LHCII revealed by room-temperature 2D electronic spectroscopy. *Phys. Chem. Chem. Phys.* **2014**, 16, 11640-11646.
- ²⁰ Calhoun, T. R.; Ginsberg, N. S.; Schlau-Cohen, G. S.; Cheng, Y. -C.; Ballottari, M.; Bassi, R.; Fleming, G. R. Quantum Coherence Enabled Determination of the Energy Landscape in Light-Harvesting Complex II. *J. Phys. Chem. B* **2009**, 113, 16291-16295.
- ²¹ Grondelle, R. v.; Novoderezhkin, V. I. Energy Transfer in Photosynthesis: Experimental Insights and Quantitative Models. *Phys. Chem. Chem. Phys.* **2006**, 8, 793-807.
- ²² Novoderezhkin, V. I.; Palacios, M. A.; Amerongen, H. v.; Grondelle, R. v. Excitation Dynamics in the LHCII Complex of Higher Plants: Modeling Based on the 2.72 Å Crystal Structure. *J. Phys. Chem. B* **2005**, 109, 10493-10504.
- ²³ Novoderezhkin, V. I.; Salverda, J. M.; Amerongen, H. v.; Grondelle, R. v. Exciton Modeling of Energy-Transfer Dynamics in the LHCII Complex of Higher Plants: A Redfield Theory Approach. *J. Phys. Chem. B* **2003**, 107, 1893-1912.
- ²⁴ Krueger, B. P.; Scholes, G. D.; Fleming, G. R. Calculation of Couplings and Energy-Transfer Pathways between the Pigments of LH2 by the ab Initio Transition Density Cube Method. *J. Phys. Chem. B* **1998**, 102, 5378-5386.
- ²⁵ Adolphs, J.; Renger, T. How Proteins Trigger Excitation Energy Transfer in the FMO Complex of Green Sulfur Bacteria. *Biophys. J.* **2006**, 91, 2778-2797.
- ²⁶ Hybl, J. D.; Albrecht, A. W.; Gallagher Faeder, S. M.; Jonas, D. M. Two-dimensional Electronic Spectroscopy. *Chem. Phys. Lett.* **1998**, 297, 307-313.
- ²⁷ Brixner, T.; Stenger, J.; Vaswani, H. M.; Cho, M.; Blankenship, R. E.; Fleming, G. R. Two-dimensional Spectroscopy of Electronic Couplings in Photosynthesis. *Nature* **2005**, 434, 625-628.
- ²⁸ Jonas, D. M. Two-dimensional Femtosecond Spectroscopy. *Annu. Rev. Phys. Chem.* **2003**, 54, 425-463.
- ²⁹ Zigmantas, D.; Read, E. L.; Mančal, T.; Brixner, T.; Gardiner, A. T.; Cogdell, R. J.; Fleming, G. R. Two-dimensional Electronic Spectroscopy of the B800-B820 Light-Harvesting Complex. *Proc. Natl. Acad. Sci. (USA)* **2006**, 103, 12672-12677.
- ³⁰ Novoderezhkin, V. I.; Marin, A.; Grondelle, R. v. Intra- and inter-monomeric Transfers in the Light Harvesting LHCII Complex: the Redfield-Förster Picture. *Phys. Chem. Chem. Phys.* **2011**, 13, 17093-17103.
- ³¹ Schlau-Cohen, G. S.; Calhoun, T. R.; Ginsberg, N. S.; Ballottari, M.; Bassi, R.; Fleming, G. R. Spectroscopic Elucidation of Uncoupled Transition Energies in the Major Photosynthetic Light-Harvesting Complex, LHCII. *Proc. Natl. Acad. Sci. (USA)* **2010**, 107, 13276-13281.
- ³² Zanni, M. T.; Ge, N. H.; Kim, Y. S.; Hochstrasser, R. M. Two-dimensional IR Spectroscopy Can Be Designed to Eliminate the Diagonal Peaks and Expose Only the Cross-peaks Needed for Structure Determination. *Proc. Natl. Acad. Sci. (USA)* **2001**, 98, 11265-11270.
- ³³ Schlau-Cohen, G. S.; Ishizaki, A.; Calhoun, T. R.; Ginsberg, N. S.; Ballottari, M.; Bassi, R.; Fleming, G. R. Elucidation of the Timescales and Origins of Quantum Electronic Coherence in LHCII. *Nature Chem.* **2012**, 4, 389-395.
- ³⁴ Gelzinis, A.; Valkunas, L.; Fuller, F. D.; Ogilvie, J. P.; Mukamel, S.; Abramavicius, D. Tight-binding Model of the Photosystem II Reaction Center: Application to two-dimensional Electronic Spectroscopy. *New J. Phys.* **2013**, 15, 075013.
- ³⁵ Prokhorenko, V. I. *European Photochemistry Association Newsletter*, issue June 2012, 21.
- ³⁶ Prokhorenko, V. I.; Halpin, A.; Miller, R. J. D. Coherently-controlled Two-dimensional Photon Echo Electronic Spectroscopy. *Opt. Express* **2009**, 17, 9764-9779.
- ³⁷ Prokhorenko, V. I.; Nagy, A. M.; Miller, R. J. D. Coherent Control of the Population Transfer in Complex Solvated Molecules at Weak Excitation. An Experimental Study. *J. Chem. Phys.* **2005**, 122, 184502.
- ³⁸ Liu Z.; Chang, W. in *Biophysical Techniques in Photosynthesis II*, eds. T.J. Aartsma and J. Matysik, pp. 77-96 (Berlin, Springer 2008).
- ³⁹ Minlota, F.; Prokhorenko, V. I.; Mančal, T.; Berlepsch, H. v.; Bixner, O.; Kauffmann, H. F.; Hauer, J. Vibronic and Vibrational Coherences in Two-Dimensional Electronic Spectra of Supramolecular J-Aggregates. *J. Phys. Chem. A* **2013**, 117, 6007-6014.
- ⁴⁰ Frähmcke, J. S.; Walla, P. J. Coulombic Couplings Between Pigments in the Major Light-Harvesting Complex LHC II Calculated by the Transition Density Cube Method. *Chem. Phys. Lett.* **2006**, 430, 397-403.
- ⁴¹ Meier, C.; Tannor, D. J. Non-Markovian Evolution of the Density Operator in the Presence of Strong Laser Fields. *J. Chem. Phys.* **1999**, 111, 3365.
- ⁴² Kleinekathöfer, U. Non-Markovian Theories Based on a Decomposition of the Spectral Density. *J. Chem. Phys.* **2004**, 121, 2505.
- ⁴³ Oviedo, M. B.; Sánchez, C. G. Transition Dipole Moments of the Qy Band in Photosynthetic Pigments. *J. Phys. Chem. A* **2011**, 115, 12280-12285.
- ⁴⁴ Gelin, M. F.; Egorova, D.; Domcke, W. Efficient Method for the Calculation of Time- and Frequency-Resolved Four-wave Mixing Signals and Its Application to Photon-echo Spectroscopy. *J. Chem. Phys.* **2005**, 123, 164112.
- ⁴⁵ M. Cho, *Two-Dimensional Optical Spectroscopy*, (CRC Press, Boca Raton, 2009).
- ⁴⁶ Pislakov, A. V.; Mančal, T.; Fleming, G. R. Two-dimensional Optical Three-Pulse Photon Echo Spectroscopy. II. Signatures of Coherent Electronic Motion and Exciton Population Transfer in Dimer Two-dimensional Spectra. *J. Chem. Phys.* **2006**, 124, 234505.
- ⁴⁷ We have calculated the 2D spectra for simple models of

a dimer and a trimer and either have included an exponential broadening or have performed an explicit ensemble average. The lifetimes of the components of the 2DDAS calculated by exponential broadening are ~ 1.5 to 3 times shorter than those from the ensemble averaged results.

FIG. 10. Graphical abstract