Stepwise Two-photon Excited Fluorescence from Higher Excited States of Chlorophylls in Photosynthetic Antenna Complexes

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Stepwise two-photon excited fluorescence (TPEF) spectra of the photosynthetic antenna complexes PCP, CP47, CP29, and light-harvesting complex II (LHC II) were measured. TPEF emitted from higher excited states of chlorophyll (Chl) a and b was elicited via consecutive absorption of two photons in the Chl a/b Qy range induced by tunable 100-fs laser pulses. Global analyses of the TPEF line shapes with a model function for monomeric Chl a in a proteinaceous environment allow distinction between contributions from monomeric Chls a and b, strongly excitonically coupled Chls a, and Chl a/b heterodimers/-oligomers. The analyses indicate that the longest wavelength-absorbing Chl species in the Qx region of LHC II is a Chl a homodimer with additional contributions from adjacent Chl b. Likewise, in CP47 a spectral form at ~680 nm (that is, however, not the red-most species) is also due to strongly coupled Chls a. In contrast to LHC II, the red-most Chl subband of CP29 is due to a monomeric Chl a. The two Chls b in CP29 exhibit marked differences: a Chl b absorbing at ~650 nm is not excitonically coupled to other Chls. Based on this finding, the refractive index of its microenvironment can be determined to be 1.48. The second Chl b in CP29 (absorbing at ~640 nm) is strongly coupled to Chl a. Implications of the findings with respect to excitation energy transfer pathways and rates are discussed. Moreover, the results will be related to most recent structural analyses.

Higher plants possess a complex light-harvesting antenna system for both photosystems, I and II. In photosystem II, the trimeric light-harvesting complex (LHC II),2 harboring almost 50% of the total chlorophyll (Chl) a and b in the thylakoid membranes, comprises the most peripheral antenna. The monomeric minor antenna complexes CP29, CP26, and CP24 (lhb4–6 gene products) are connected to LHC II and are located proximal to the core antenna complexes, CP43 and CP47, which surround the reaction center; for a recent review see Ref. 1.

In contrast to LHC II, for which very recently high resolution x-ray structural analyses have been obtained (2, 3), no such high resolution structures of the minor complexes are available yet. Their structures are assumed to be comparable with that of the monomeric subunits of LHC II but with lower Chl content: According to present knowledge CP29 binds six Chls a and two Chls b (4, 5).

In addition, currently no high resolution structure for CP47 is available. However, x-ray structural analyses of three-dimensional crystals of oxygen evolving PS II core complexes from the thermophilic cyanobacteria Thermosynechococcus elongatus (6–8) and T. vulcanus (9) at medium resolution have been obtained. According to the most recent analysis at 3.0 Å resolution (8), 16 Chl a molecules are bound to cyanobacterial CP47. Purified CP47 from spinach was found to bind 15 ± 1 Chls a and 3–4 β-carotenes (10), which is in good agreement with the recent structural analyses of its cyanobacterial homologues (6–8). The amino acid sequences of the CP47 apoproteins from spinach and T. elongatus are 73% identical. Analysis of putative Chl a ligands of spinach CP47 and comparison with CP47 of T. elongatus reveal that all but Asp-188 (which is swapped for a glutamate residue in plant CP47) are conserved (data not shown). Because the transmembrane topology is predicted to be conserved and the environment of the pigment coordinating amino acids is very similar, or even identical, it is suggested that distances and relative orientation of the chlorin rings as well as the orientation of the Qy and Qx transition dipole moments are analogous in cyanobacterial and plant CP47 (11). Hence, it is highly likely that certain Chls a, with center-to-center distances on the order of 10 Å, in spinach CP47 show strong excitonic interactions.

In plant antenna complexes absorption subbands of individual Chls a and b show strong spectral overlap (a phenomenon also referred to as spectral congestion), in particular in the red-
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most (Q_y) absorption range. This issue strongly hinders the assessment of the extent of excitonic coupling in these pigment–protein complexes and its role in efficient light harvesting. The absorption subbands of the 42 Chls a and b in LHC II are represented in the Q_y spectral range between 620 and 690 nm. Certainly, progress in crystal structure analyses, as achieved most recently for LHC II (2, 3), permits more adequate modeling of the intricate absorption substructure. However, an assignment of individual pigments (at their respective binding sites) to spectral substructures, relevant to understand excitation energy transfer (EET) ultimately ending in the photochemical reaction centers, is still very fragmentary. Moreover, the question as to what extent the available crystal structures reflect the pigment arrangement in native LHCs also has apparently no trivial answer (Ref. 12, cf. also Ref. 3).

One possibility to disentangle the “spectral congestion” with special regard to Q_y transition dipole coupling between Chls is to search for fingerprints of excitonic coupling outside the Q_y range. We have recently been able to detect weak fluorescence from higher excited (most probably B_n) states, elicited by stepwise two-photon excitation in the Q_y range (TPEF), from Chl a and Chl b in solution as well as from pigment–protein complexes (13). Stepwise absorption of two photons (in contrast to simultaneous two-photon excitation) requires a sufficient excited state absorption in the Q_y range. This condition is warranted for monomeric (uncoupled) Chls (14) as well as strongly excitonically coupled (Bacterio) Chls (15). In the latter case this step is a one-exciton to bi-exciton transition (15, 16). Moreover, in a first attempt to analyze TPEF spectra with regard to excitonic Chl–Chl coupling, it was demonstrated that the red-most Q_y absorption subband of LHC II (at ~680 nm) is dominated by excitonically coupled Chls a with additional contributions from adjacent Chl b, which is in line with previous studies (16–18) and the most recent structural analyses (2, 3). On the other hand, for the structurally related complex, CP29, no indication was found that the red-most absorption band is due to excitonic coupling; in particular, no contribution of Chl b to it was detectable, in accordance with previous reports (5, 12, 19).

We report here an extended TPEF study on the peridinin-Chl a protein (PCP) from the dinoflagellate Amphidinium carterae and the plant complexes LHC II, CP29, and CP47, representing peripheral, proximal, and core antenna complexes. TPEF spectra were obtained at varying excitation wavelengths in the Chl a/b Q_y range. The results of global analyses of TPEF line shapes based on corresponding standard shapes for monomeric Chls in a proteinaceous environment will be discussed with respect to strong excitonic coupling of pigments.

MATERIALS AND METHODS

Sample Preparation and Characterization—PCP was a kind gift from Dr. R. G. Hiller (Macquarie University) and was isolated from A. carterae as described previously (20). PCP was diluted prior to the measurements in 25 mM Tris–HCl, 2 mM KCl, pH 7.5. CP29 and CP47 (co-purifying with CP29) were isolated from spinach as described (5, 10, 13). Crude CP47 obtained after the first and second chromatography were pooled and dialyzed against 50 mM MES–NaOH, pH 6.5, 30 mM NaCl, 0.025 w/v % n-dodecyl β-d-maltoside, 2 mM benzamidine, and 5 mM ε-aminoacaprate, concentrated, and loaded onto a Mono Q HR (5/5) connected to an FPLC system (Amersham Biosciences). CP47 was eluted from the column using a linear gradient of 50–300 mM NaCl in equilibration buffer, dialyzed overnight, and concentrated. LHC II was prepared as described previously (13). Pigment–protein complexes were characterized by Western blotting using peptide-directed and monoclonal antibodies, reverse phase high pressure liquid chromatography, and matrix-assisted laser desorption ionization time-of-flight-mass spectrometry analyses as described previously (10). The following Web-based protein analysis programs were used: BLAST (au.expasy.org/tools/blast/) for similarity searches, ClustalW (www.ebi.ac.uk/clustalw/) for alignment, and Predictprotein (www.predictprotein.org) to analyze transmembrane regions. Absorption spectra were recorded on a Lambda 900 spectrophotometer (PerkinElmer). All samples were adjusted to an optical density of 0.1 cm⁻¹ at the excitation wavelength. Absorption spectra were measured before and after the TPEF spectroscopy experiments to exclude sample degradation.

Fluorescence Measurements—The setup for TPEF spectroscopy has been described in detail previously (13, 21). The 100-fs tuneable laser pulses had ~10 nm spectral width (full width at half maximum); excitation photon flux density was 10^{28} cm⁻² s⁻¹ for measurements of the TPEF band shapes and between 2 × 10^{26} and 6 × 10^{28} cm⁻² s⁻¹ for measurement of intensity dependence of the TPEF signal. The fluorescence band shapes were spectrally corrected for the instrumental response. All measurements were performed at room temperature.

Fluorescence Data Evaluation—TPEF spectra were evaluated using the program LorFit version 3.0.³ The non-linear fitting procedure is based on a simplex algorithm according to Nelder and Mead (22). All TPEF spectra were subjected to a global analysis procedure (i.e. a simultaneous fit of all data sets of the respective antenna complex with the same model functions and set of parameters). Lorentz-shaped profiles were assumed for all subbands. Fit parameters were peak positions, spectral widths, and amplitudes of the subbands. The TPEF line shape of PCP was used as a model function for monomeric Chl a in a proteinaceous environment for all other antenna complexes investigated. Thus, the free parameter space in the respective analyses is drastically reduced, resulting in very stable (against variation of the starting parameters) non-linear fits for CP47, CP29, and LHC II. Additionally, to improve the individual fits a variable amount of a spectrally broad (wavelength-independent) background had to be taken into account. This background most probably results from an onset of white light-like continuum generation.

RESULTS AND DISCUSSION

Peridinin Chlorophyll a Protein—PCP was chosen as a suitable system to obtain the TPEF line shape of monomeric Chl a in a proteinaceous environment. PCP contains only two Chls a per eight peridinins in each monomeric subunit of the trimeric complex (23). The Chls are well separated (by 17 Å) so that excitonic coupling is negligible (23). Furthermore, it is generally

³ J. Ehlert, unpublished results.
assumed that there is no absorption of peridinin in the Chl Qy absorption range.

Fig. 1 shows the TPEF spectra of Chl a in PCP excited by 100-fs laser pulses with peak wavelengths at 660, 670, and 680 nm (full width at half maximum 10 nm). The spectra are all characterized by a pronounced peak and a clearly resolved red-shifted shoulder. Results of a global analysis of the TPEF line shapes are also shown; the experimental spectra can be well reproduced by a superposition of two Lorentzians with maxima at 446.9 nm (assigned to the 0–0 transition) and 473.8 nm, the latter corresponding to a vibronic (0–1) structure. Additionally, a spectrally constant background had to be included. A closer inspection of the spectra reveals a moderate bathochromic shift (~2–3 nm) of the TPEF peak of monomeric Chl a in PCP in comparison to monomeric Chl a in solution (cf. Ref. 13). The shift is obviously due to interaction of the Chl with the protein environment. It appears to be reasonable to assume that a similar shift occurs when Chl b is inserted into a proteinaceous matrix. Hence, applying the same shift to the TPEF line shape of dissolved Chl b provides a model function that can be used as a first approximation for TPEF of monomeric Chl b in pigment-protein complexes. These model functions were used to analyze the TPEF spectra of Chls in the plant antenna complexes CP47, CP29, and LHC II.

CP47—CP47 (and CP43) form the core antenna of photosystem II in all oxygenic photosynthetic organisms. Because CP47 binds only Chl a it is a suitable pigment-protein complex to study excitonic coupling between Chls a by analyses of TPEF spectra.

TPEF spectra of CP47 were measured at excitation wavelengths of 640, 650, 660, 670, and 680 nm. Two typical spectra, obtained at the blue and the red edges of the Qy absorption region, are shown in Fig. 2. A global analysis of all five TPEF spectra using the model function for monomeric Chl a in a proteinaceous environment obtained from PCP (see above) indicates that for a satisfactory fit an additional broad band (peaking at 448.2 nm) is required. The latter band contributes to a varying extent to the overall TPEF line shape at different excitation wavelengths. The broad, rather unstructured additional band is interpreted to represent the contribution of strongly excitonically coupled Chls a to the overall TPEF signals. The band exhibits a minor red-shift with respect to the 0–0 transition of monomeric Chl a (see above).

In each of the five investigated TPEF spectra (excited by pulses with 10 nm full width at half maximum) contributions from both monomeric as well as excitonically coupled Chls a were resolved. A low temperature (77 K) absorption and fluorescence spectroscopic study together with Monte Carlo simulations (24) based on the 3.8 Å structure (6) provided indications for strong excitonic coupling between certain Chls a in CP47. Investigations using nonlinear polarization spectroscopy in the frequency domain in the Qy range are also consistent with

FIGURE 1. Stepwise TPEF spectra of Chl a in PCP. Excitation wavelengths are indicated. Simulation (bold solid line) of the experimental data (○) with a model function (monomeric Chl a, dotted line) and a wavelength-independent background (thin line).

FIGURE 2. TPEF spectra of Chl a in CP 47. Excitation wavelengths are indicated. Simulation (bold solid line) of the experimental data (○) with contributions from monomeric Chl a (dotted line), excitonically coupled Chls a (dashed line), and background (thin line).
the existence of strong excitonic Chl $a/a$ coupling in CP47. A particularly remarkable feature is the dominant contribution of monomeric Chl $a$ to the TPEF spectrum excited at 680 nm. Our results suggest that the 680-nm centered band in CP47 has to be assigned to an uncoupled Chl $a$. This band, however, does not appear to be the terminal state of the intra-complex EET in CP47 (24). A schematic representation of the strongly excitonically coupled Chls $a$ in the 3.0 Å structural model of cyanobacterial CP47 (8) is given in Fig. 6a: Chls $a_{11}, 12, \text{and } 13$ (according to the designation in Ref. 8) form a trimer; Chls $a_{25}$ and $26$ form a dimer. Most probably, the strongly coupled trimer is responsible for the red-most shifted subband (peaking at 683 nm) in the Q$_y$ spectral region.

CP29—CP29 is one of the minor, monomeric LHCs of photosystem II in higher plants. The TPEF spectra of CP29, measured at five different excitation wavelengths between 640 and 680 nm, as well as the results of a global analysis of the data, are shown in Fig. 3.

The following results were obtained by the global analysis. i) Two-step excitation at 640 and 650 nm (i.e. in the absorption region of the two Chls $b$) gives rise to strikingly different TPEF spectra. ii) Two-step excitation of CP29 at 660, 670, and 680 nm (in the Chl $a$ absorption region) results in TPEF line shapes that can be simulated using components that reflect monomeric Chl $a$ and excitonically coupled Chls $a/b$, with decreasing weight of the latter toward the red edge.

The different TPEF line shapes observed upon excitation in the Chl $b$ region can be explained by different excitonic coupling strengths of the two Chl $b$ molecules in CP29 to neighboring Chl(s) $a$. Obviously, the Chl $b$ excited at 640 nm is strongly coupled to Chl $a$, giving rise to the dominant broad TPEF band centered at 483.8 nm (note the red shift as compared with TPEF of monomeric Chl $b$; compare also the legend to Fig. 3). Excitonic coupling of the 640-nm absorbing Chl $b$ with Chl $a$ in CP29 has been demonstrated also by nonlinear polarization spectroscopy in the frequency domain experiments (19). In the TPEF line shape obtained upon 640-nm excitation also a certain contribution from monomeric Chl $a$ is resolved. The latter observation is most probably due to ultrafast EET from the first excited singlet state ($S_1$) of the initially excited Chl $b$ to $S_1$ of a neighboring Chl $a$ and subsequent absorption of a second photon by that Chl $a$.

In marked contrast to the results obtained with 640-nm excitation, two-photon excitation at 650 nm (in the absorption region of the other Chl $b$) results in a TPEF spectrum that can be predominantly assigned to monomeric Chl $b$. No indication for excitonic coupling of this Chl $b$ was obtained. A certain contribution of TPEF attributable to monomeric Chl $a$ can be explained as the result of ultrafast EET as in the case of 640-nm excitation (see above).

The results obtained upon excitation in the Chl $a$ region are in line with previous studies suggesting that a monomeric Chl $a$ is the origin of the red-most Q$_y$ subband of CP29 (see Ref. 19 and references therein). This interpretation is further corroborated by the intensity dependence of 680-nm-excited TPEF of CP29 (see below).

LHC II—TPEF spectra of trimeric LHC II have been measured at various excitation wavelengths in the range of Chl $a/b$ Q$_y$ absorption. Fig. 4 shows TPEF spectra obtained upon excitation at 640, 650, 660, and 680 nm. In addition, the results of a global analysis of the data are depicted. The following results are particularly remarkable.

i) Stepwise two-photon excitation at 640 nm results in a TPEF line shape that is dominated by a response due to excitonically coupled Chls $a$ and $b$. Therefore, the absorption in the range ~640 nm is mainly due to Chl $b$ molecule(s) that is (are) a component of a heterodimer or an oligomeric cluster. There is also indication of a minor contribution from monomeric Chl $b$ (in contrast to the TPEF spectra obtained upon 640-nm excitation of CP29, see above) as well as from monomeric Chl $a$ (which can be explained by ultrafast EET to the latter as in the case of CP29; see above).

ii) Excitation at 650 nm results in TPEF emission that can be assigned to originate from both, monomeric Chl $b$ as well as monomeric Chl $a$. Obviously, the observation of TPEF emanating from Chl $a$ is again (as for CP29) the result of ultrafast EET from $S_1$ of Chl $b$ to Chl $a$ and subsequent absorption of a second photon by this Chl $a$. Most interestingly, there is no unequivocal indication of a TPEF contribution from excitonically cou-

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4 B. Voigt, K.-D. Irgang, G. Renger, D. Leupold, and H. Lokstein, unpublished results.
pled Chls. This differs somewhat from the explanation of the 650-nm-excited TPEF of LHC II that was presented previously (13). The previous interpretation, however, was based on a much smaller data set without employing global analysis of the data.

Stepwise two-photon excitation at wavelengths ≥660 nm (Fig. 4 shows two typical spectra obtained upon excitation at 660 and 680 nm) results in TPEF line shapes that predominantly arise from the Chl \textsubscript{b} region (representing excitonically coupled as well as monomeric Chls \textsubscript{b}; the mechanism underlying the latter emission, however, is not yet understood). This is clearly different from the behavior of CP29 (compare Fig. 3). Particularly remarkable is the significant contribution of heterodimer/-oligomer-derived TPEF upon 680-nm excitation. This is in line with the intensity dependence of the TPEF signal of LHC II (see below) as well as with several studies indicating strong excitonic coupling between Chls in the longest wavelength range of LHC II (16, 18). Meanwhile, high resolution three-dimensional structural analyses of LHC II have become available (2, 3) allowing an unambiguous assignment of all eight Chls \textsubscript{a} and six Chls \textsubscript{b} to their respective binding sites. Moreover, their mutual distances have been determined with unprecedented accuracy as well as the orientations of their transition dipole moments, thus allowing more precise estimations of excitonic interaction strength between neighboring pigments (2). Indeed, strong excitonic Chl a-a (Chls a611 and a612, in their notation) as well as Chl a-b (a604 and b606, a603 and b609) interactions have been inferred from the structural data (2). A schematic representation of the strongly excitonically coupled Chls a in the 2.72 Å structural model of LHC II (2) is given in Fig. 6b. The strongly excitonically coupled Chls a611/a612, located at the surface of the monomeric LHC II subunit (as well as of the LHC II-trimer), has been inferred to be of paramount importance for efficient EET in the photosystem II peripheral antenna system (13, 16, 17).

**Intensity Dependence of the TPEF Signal Amplitude**—TPEF emission from monomeric Chls \textsubscript{a} and \textsubscript{b} (in organic solvents) exhibits a quadratic dependence of signal amplitude on excitation intensity. This behavior can be modeled with a reasonable parameter set as outlined in Ref. 13. A similar behavior has been observed for certain excitation wavelengths (corresponding to absorption of Chls that are not excitonically coupled) in PCP, CP47, and CP29. The dependence of the TPEF signal amplitude on excitation intensity for CP47 and CP29 (with intensity variation over two orders of magnitude, in both cases for the excitation wavelength 680 nm) is shown to fit a straight line in the log/log plot in Fig. 5. This observation provides additional evidence that the red-most spectral form in the Q\textsubscript{y} absorption region of CP29 is due to a monomeric Chl \textsubscript{a}, being in line with the well established fact of non-interacting Chls \textsubscript{a} in PCP. LHC II shows a markedly different behavior: in the given excitation intensity range a deviation from the quadratic response is observed (Fig. 5). This is consistent with an 2-fold-enhanced Chl \textsubscript{a} absorption cross-section (as compared with monomeric Chl \textsubscript{a}) at ~680 nm (16). The experimentally determined intensity dependence of the TPEF of LHC II can be readily simulated by taking into account this increased absorption cross-section. Moreover, any significant contribution of exciton-exciton annihilation to the measured intensity dependence can be excluded.\(^3\)

**Implications for Excitation Energy Transfer**—Although two-photon-excited fluorescence from higher excited (B\textsubscript{x}) states...
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(TPEF) of Chls a and b has a rather low quantum yield on the order of $10^{-4}$ (13) it is nevertheless possible to detect this emission, even from isolated photosynthetic pigment-protein complexes. Excitation via consecutive two-step absorption of photons elicited by tunable 100-fs pulses, is based on the existence of Chl a and b $S_0 \rightarrow S_1$ (Q_y) absorption bands with significant $S_1 \rightarrow S_0$ excited state absorption in the same spectral range. It is very important to note that the selective Soret region excitation of Chls accomplished via this route circumvents any excitation of fluorescence-emitting components of these complexes other than Chl a/b and/or of sample impurities that might confound the extremely weak B_y fluorescence. This problem would arise in case of direct one-photon excitation into transition(s) of the Chl a/b Soret range.

The potential of the information contained in TPEF profiles is mainly due to its reflection of excitonic coupling of Chls in the Q_y absorption range. The representation of the putative strongly coupled Chls in the recent structural models of CP47 (8) and LHC II (2) is shown in Fig. 6, a and b. Investigations in the Q_y region directly are less informative because of the issue of severe spectral congestion.

From the TPEF profiles of CP47 and CP29 Chl a excitonic coupling and Chl a/b heterodimeric/-oligomeric coupling, respectively, can be deduced. However, in both cases a subband centered at about 680 was found to be due to monomeric Chl a. This band is also the red-most Q_y absorption subband in the case of CP29. In contrast, for LHC II the TPEF spectra suggest that the corresponding subband at 680 nm reflects strong excitonic coupling of two Chls a with inclusion of adjacent Chl(s) b. TPEF also indicates that heterodimeric/-oligomeric coupling is dominant in the absorption region around 640 nm in CP29 and LHC II, whereas monomeric Chl b absorbs around 650 nm. These findings are consistent with fs pump-probe data obtained with CP29 (25, 26). Whereas the ultrafast absorption kinetics upon 653-nm excitation could be readily simulated assuming Förster-type EET, similar simulations fail for 640-nm excitation in CP29. The measured EET kinetics are $\sim 7$ times faster than values calculated for Förster-type EET (27). According to the results presented in the current study, the transient absorption changes upon 640-nm excitation of CP29 mainly reflect exciton relaxation, consistent with previous studies (19).

The finding that one of the two Chls b in CP29 (which has an absorption maximum of 652 nm at room temperature) is monomeric (not coupled excitonically to other Chls) allows the determination of the refractive index $n$ of its microenvironment. Comparison with a plot of the Chl b Q_y absorption maxima versus solvent polarizability (Lorentz-Lorenz factor) for a wide range of solvents $^5$ (compare also Ref. 28) resulted in $n = 1.48$ for the environment of this particular Chl b. A value of $n = 1.5$ has been obtained previously as a result of EET simulations in CP29 (25). Using an analogous approach as employed by us (but based on xanthophyll absorption) in LHC II a $n = 1.54$ was deduced (29). Precise knowledge of $n$ is crucial to calculate reliable rate constants for Förster-type EET kinetics due to inverse fourth-power dependence on $n$ (for a detailed discussion see, for instance, Ref. 30).

Concluding Remarks—The present study shows that stepwise TPEF of Chls a and b is a suitable experimental tool to gain information on excitonic coupling of pigments in photosynthetic pigment-protein complexes, in particular under conditions of severe "spectral congestion", cf. also Ref. 31). The TPEF line shapes, measured as a function of excitation wavelength in the Q_y range, allow distinction between monomeric pigments, homodimers/-oligomers, and heterodimers/-oligomers in that particular spectral region.

Future studies of TPEF may profit from a variation of excitation pulse duration: Laser pulses in the ps range can be used to

\[ \text{FIGURE 6.} \text{ a, strongly excitonically interacting Chls a in CP47. Protein backbone and carotenoids are omitted for clarity. Chls a11, 12, and 13 (according to the designation of Loll et al. (8, 11) forming a trimer are shown in red, and Chls a25 and 26 forming a dimer are shown in orange. The picture was generated from the Protein Data Base accession code 2AXT (3.0 Å resolution) using the program PYMOL (www.pymol.org).} \]

\[ \text{b, strongly excitonically interacting Chls a and b in a monomeric subunit of (trimeric) LHC II. Protein backbone and xanthophylloid cofactors are omitted for clarity. Strongly excitonically coupled Chls a and b are shown in dark blue and red, respectively. Chls a611 and a612 (according to the designation of Liu et al. (2) form a homodimer; Chls a604/b606 and Chls a603/b609 form heterodimers. Not excitonically coupled Chls a and b are shown in light blue and light green, respectively. The picture was generated from the Protein Data Base accession code 1RWT (2.72 Å resolution).} \]

\[ ^{5} \text{I. Eichwurzel, H. Stiel, A. Scherz, H. Scheer, and D. Leupold, unpublished results.} \]
improve spectral resolution (due to their much narrower pulse widths). On the other hand, pulses shorter than 100 fs would be of advantage to minimize the competing EET processes, in particular among Chl S₁ states.

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