The High Efficiency of Photosystem I in the Green Alga *Chlamydomonas reinhardtii* Is Maintained after the Antenna Size Is Substantially Increased by the Association of Light-harvesting Complexes II*†(S)

Clotilde Le Quiniou, Bart van Oort, Bartlomiej Drop, Ivo H. M. van Stokkum, and Roberta Croce

*From the Department of Physics and Astronomy, Faculty of Sciences, VU University Amsterdam and Institute for Lasers, Life and Biophotonics Amsterdam, LaserLab Amsterdam, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands*

**Background:** Photosystems acclimate to different light conditions modulating their antenna size.

**Results:** In PSI-LHCI-LHCII, energy transfer from LHCII to Photosystem I is relatively slow but still very efficient.

**Conclusion:** The association of LHCII to PSI increases its absorption cross section by ~50%, maintaining ~96% quantum efficiency.

**Significance:** The antenna size of PSI can be increased considerably without sacrificing its efficiency.

Photosystems (PS) I and II activities depend on their light-harvesting capacity and trapping efficiency, which vary in different environmental conditions. For optimal functioning, these activities need to be balanced. This is achieved by redistribution of excitation energy between the two photosystems via the association and disassociation of light-harvesting complexes (LHC) II, in a process known as state transitions. Here we study the effect of LHCII binding to PSI on its absorption properties and trapping efficiency by comparing time-resolved fluorescence kinetics of PSI-LHCl and PSI-LHCl-LHCII complexes of *Chlamydomonas reinhardtii*. PSI-LHCl-LHCII of *C. reinhardtii* is the largest PSI supercomplex isolated so far and contains seven Lhcb's, in addition to the PSI core and the nine Lhca's that compose PSI-LHCII, together binding ~320 chlorophylls. The average decay time for PSI-LHCl-LHCII is ~65 ps upon 400 nm excitation (15 ps slower than PSI-LHCII) and ~78 ps upon 457 nm excitation (27 ps slower). The transfer of excitation energy from LHCII to PSI-LHCII occurs in ~60 ps. This relatively slow transfer, as compared with that from LHCl to the PSI core, suggests loose connectivity between LHCII and PSI-LHCII. Despite the relatively slow transfer, the overall decay time of PSI-LHCl-LHCII remains fast enough to assure a 96% trapping efficiency, which is only 1.4% lower than that of PSI-LHCII, concomitant with an increase of the absorption cross section of 47%. This indicates that, at variance with PSII, the design of PSI allows for a large increase of its light-harvesting capacities.

In eukaryotic organisms, the photosynthetic apparatus contains two main pigment-protein complexes, embedded in the thylakoid membrane, Photosystems (PS) I and II, that harvest light energy and convert it into chemical energy (1). The PS performances are largely determined by their capacities to harvest light and transfer excitation energy to the reaction center (RC) where charge separation occurs (2). To efficiently harvest light, PSI and PSII are equipped with two types of antennae. The inner antenna is composed of chlorophyll (Chl) a-binding complexes that together with the RC form the core complex. The core complexes of PSI and PSII bind 98 and 35 Chls, respectively (3, 4) and are highly conserved in eukaryotic organisms (5). The outer antenna, by contrast, varies in the different organisms as it is optimized for the absorption in their light-growth conditions (2). In plants and green algae, it is composed of members of the light-harvesting complex (Lhc) multigenic family, each coordinating between 10 and 14 Chls (a and b) (6, 7).

Despite the high sequence similarity, the number and properties of the Lhcs vary in plants and algae. LHClII trimers are composed of three major Lhcb's in *Arabidopsis thaliana* (Lhcb1–3 (8)) and of nine major Lhcb's in the green alga *Chlamydomonas reinhardtii*. (LhcbM1–9 (9)). Three monomeric antenna, CP29 (Lhcb4), CP26 (Lhcb5), and CP24 (Lhcb6), located between the core and the LHCII trimers, are present in *A. thaliana* PSI-LHClII (10), whereas CP24 is absent in *C. reinhardtii*, resulting in a different organization of the PSII-LHCII supercomplex in the two organisms (11, 12). Concerning PSI-LHCl, in *A. thaliana*, four Lhca's (Lhca1–4) are associated with the PSI core (13), forming a PSI-LHCl complex that contains 155 Chls (14, 15). In *C. reinhardtii*, nine Lhca's compose the antenna of PSI (16), which is far larger than that of plants (17, 18).

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†This article contains SI1: Materials and Methods, SI2: Results from the target analysis, and SI3: Quantifying the contributions of compartment size, composition and connectivity to the average decay time of PSI-LHCl and PSI-LHCl-LHCII.

‡To whom correspondence should be addressed. Tel: 31-20-5986310; E-mail: r.croce@vu.nl.

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The abbreviations used are: PS, Photosystem; LHC, light-harvesting complex; RC, reaction center; Chl, chlorophyll; DAS, decay-associated spectra; SAS, species-associated spectra.

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Excitation Energy Transfer in PSI-LHCl-LHCII of **C. reinhardtii**

The amount of excitation energy delivered to the RCs by the Lhcs does not only depend on their number but also on their biochemical and spectroscopic properties (19). The different affinity for Chl a and b between Lhcs and Lhcbns and the presence of far-red absorbing forms in Lhcs (20, 21) results in differences in the wavelength-dependent light-harvesting capacities of the two photosystems. This creates an excitation imbalance that can affect electron transport and induce photodamage (22). To avoid this and maintain a maximal photosynthetic efficiency, plants and algae regulate the association of Lhcbns with PSI and PSII in a process known as state transitions (23, 24), in which mobile LHCII is associated with PSII in state 2 transition, only part of it associates with PSI, whereas the rest gets quenched (27, 28, 30). However, due to the complexity of the mechanism and the physiological role of this process are different from plants. It was indeed observed that although a large part of the total LHCII population, and under sunlight, it is mainly associated with PSI i.e. plants in light are in state 2 (25).

The mechanisms of state transitions in the green alga **C. reinhardtii** seem to differ from what is observed in plants (26–28). For a long time, this difference was believed to be the pool size of LHCII involved in the transitions (80% according to Ref. 29); however, recent results suggest that the mechanism and probably also the physiological role of this process are different from plants. It was indeed observed that although a large part of LHCII functionally disconnects from PSII during the state 1 to state 2 transition, only part of it associates with PSI, whereas the rest gets quenched (27, 28, 30). However, due to the complexity of the cells and the presence of different LHCII pools, a conclusion about excitation energy transfer efficiency from Lhcbns to PSI could not be made.

PSI-LHCl-LHCII supercomplexes with different antenna size have been purified (31–34). The antenna size depends on the method used to induce the state transition and/or on the isolation protocol. The largest PSI-LHCII-LHCII isolated so far contains seven Lhcbns (two LHCII trimers and one monomer located on the PsAH/L side of the core complex), in addition to the nine Lhcs located on the other side of the core complex (34). This PSI-LHCII-LHCII is clearly larger than the PSI-LHCl-LHCII from plants that contains only one LHCII trimer (35, 36), although recent results indicate that in the membrane of plants, more than one LHCII trimer can be associated with PSI-LHCl (37).

In this work, we have performed time-resolved fluorescence measurements on the PSI-LHCII-LHCII complex of **C. reinhardtii** with or without preferential excitation of Lhcbns. We have determined the energy transfer efficiency of these Lhcbns and their influence on the trapping yield of PSI. The data indicate a loose connection between the Lhcbns and the PSI core, which is responsible for a relatively slow energy transfer step. These results are interpreted on the bases of the new PSI-LHCII structures (14, 15), and the possible energy transfer pathways in PSI-LHCl-LHCII are discussed.

**Experimental Procedures**

**Sample Preparation**—PSI-LHCl-LHCII was prepared as in Ref. 34 from the PSII-lacking mutant Fl39 of **C. reinhardtii** (34, 38, 39). In short, cells were harvested in mid-logarithmic phase (growth at 25 °C, 20 microeinsteins × m⁻² s⁻¹) and state 2 was induced by incubating them in anaerobic conditions (i.e. shaken in the dark for 20 min in the presence of NaN₃). Thylakoid membranes were solubilized to a final concentration of 0.5 mg of chlorophyll/ml, 0.5% Digitonin (Sigma), and 0.2% dodecyl-α-β-maltoside, and then loaded on a sucrose gradient. PSI-LHCl-LHCII was harvested with a syringe.

Light-harvesting complexes II (Lhcbns) were prepared as in Ref. 12. Band 2 from the sucrose gradient contained Lhcb monomers such as CP26, CP29 and LhcbMs, and Band 3 contained LHCII trimers.

**Fraction of Excitation in Lhcbns**—To selectively excite Lhcbns, we determined the fraction of excitation in Lhcbns and chose excitation wavelengths for the time-resolved measurements that showed the largest differences. Lhcbns were excited the least upon 400 nm with 27–29% of PSI-LHCl-LHCII excitation present in the Lhcbns, whereas Lhcbns were excited the most upon 475 nm excitation corresponding to 41–46% of the excitation.

To determine the fraction, we used two different methods as described in Ref. 40. For details, see SI1.

**Steady State and Time-resolved Measurements**—The absorption spectra were measured at room temperature with a Varian Cary 4000 UV-visible spectrophotometer. CD spectra were measured at 10 °C on a Chirascan-Plus CD spectrometer (Applied Photophysics, Surrey, UK). The 10 °C steady state fluorescence spectra were measured with a spectrofluorometer (FluoroLog, Horiba Scientific) upon 500 nm excitation. To avoid self-absorption, the sample was diluted to optical density 0.07 at the Qy maximum (1-cm path length) in a buffer containing 20 mM Hepes (pH 7.5) and 0.02% digitonin (Sigma).

Time-resolved fluorescence measurements were performed with a streak camera setup as described in Ref. 40. In short, fs pulses were generated with a repetition rate of 250 kHz using a laser system (Coherent Vitesse Duo and Coherent RegA 9000) and were used to feed the OPA (Coherent OPA 9400) to generate excitation light at 475 and 400 nm. The pulse energy was reduced to 0.3 nJ to avoid singlet-singlet annihilation. The exciting light focused on the sample was 0.151 mol of photons/m²/s at 475 nm and 0.127 mol of photons/m²/s at 400 nm. Fluorescence was detected at the magic angle with respect to the excitation polarization, in the 590–860 nm range from 0 to 400 ps. The sample was measured at 15 °C in its purification buffer (20 mM Hepes, pH 7.8, 0.02% digitonin, 0.7 m sucrose) in a 10 × 10-mm quartz cuvette at optical density 0.35 cm⁻¹ at the Qy maximum. To avoid self-absorption, the laser beam was focused in the sample close to the cuvette wall, and emission was collected at a right angle close to the entry point of the laser beam into the cuvette. The sample was stirred with a magnet bar to avoid singlet-triplet annihilation. A power study confirmed the absence of annihilation (data not shown).

The fluorescence decays measured upon 400 and 475 nm excitation were analyzed with a sequential model (see SI1). The average decay time τav (Equation SI1-1) characterizes the time for the excitation energy to be used for charge separation and is calculated by considering only the components attributed to the PSI-LHCl-LHCII kinetics (excluding ns components attributed to disconnected species). For more details on the acquisition and data analysis, see SI1.

**Target Analysis**—We used the previous kinetic model describing **C. reinhardtii** PSI-LHCl (see Fig. 3a) (40) and extended it with a new compartment representing the seven
Lhcb (called “Lhcb”) in equilibrium with “Bulk” (see Fig. 3b).

The natural decay rate constant of Lhcb was fixed to $k_0 = (3.3 \text{ ns}^{-1})$ as found for isolated LHCII trimers of $C. \text{ reinhardtii}$.

The complete kinetic scheme (Scheme SI2-1) also contains precursors populating the other compartments on the ps time scale, with relative amounts varying depending on excitation wavelength (Table SI1-2), and a disconnected compartment corresponding to species with ns lifetimes. A detailed description of the procedure for the target analysis is given in SI1.

Simulated Kinetic Models—Population dynamics were simulated (MathWorks R2014b 64-bit, MATLAB) for hypothetical particles of: 1) different size (number of Chls $a$); 2) different connectivity; and 3) different composition (energy of Chls) (Scheme SI3-1). This enabled us to independently study the effects of these three factors on trapping time and efficiency. See SI3 for details.

Distance Measurements in the Reconstructed PSI-LHCI-LHCII—PSI-LHCI-LHCII EM of higher plants (36) and $C. \text{ reinhardtii}$ (34) were used to position the apoproteins of PSI and Lhcb. The positions were set as close as possible to the EM, but uncertainties remain concerning the exact distance that separates the apoproteins and their relative orientation. The reconstruction was made with the PyMOL Molecular Graphics System (Version 1.3) to have a three-dimensional perspective and a first estimation of distances between the Chls of interest.

**Results**

**Steady State Characterization**—Absorption and fluorescence emission spectra of PSI-LHCI-LHCII are shown in Fig. 1, a and b, together with the spectra of PSI-LHCI. The maximum $Q_y$ absorption of PSI-LHCI-LHCII (at 678 nm) as well as its maximum emission (at 683 nm) are blue-shifted as compared with the PSI-LHCI maxima (at 679.5 and 687.5 nm, respectively). The absorption difference spectrum between PSI-LHCI-LHCII and PSI-LHCI (Fig. 1a, green) overlaps well with the Lhcbs absorption spectrum (Fig. 1a, magenta) in agreement with previous results (34).

CD spectra of PSI-LHCI-LHCII and PSI-LHCI are compared in Fig. 1c. They show similar features in the $Q_y$ absorption region (negative peaks at 648 and 680 nm and a positive peak at 664 nm), as well as in the Soret region (similar shape below 423 nm). Major differences are visible between 423 and 532 nm, as

![FIGURE 1. Absorption and emission and CD spectra.](image-url)
Excitation Energy Transfer in PSI-LHCI-LHCII of C. reinhardtii

Figure 2. Sequential analysis of PSI-LHCI-LHCII fluorescence decays upon 400 nm or 475 nm excitation at 15 °C. a, selected fluorescence decay traces after excitation at 400 nm (gray) and 475 nm (orange). Black and red lines indicate the fit curves. b, DAS of each decay component. The two different experiments were fitted simultaneously to link (I) the fourth lifetime associated to the disconnected species. The amplitude of each decay component (i.e., relative area = \( A_i/\sum A_i \)) is shown in parentheses next to the corresponding lifetime, in %. The DAS are normalized to the initial populations of excited states (i.e., the total area of PSI-LHCI-LHCII-related DAS, \( \sum A_i \)). See SI1 for details. a.u., arbitrary units.

shown in the PSI-LHCI-LHCII minus PSI-LHCI difference spectrum (Fig. 1d, green). This spectrum strongly resembles the spectrum of LHCII trimers in detergent micelles (42) but also has some feature of aggregated LHCII trimers (distinct negative peak at 438 nm and positive peak at 483 nm (43)). This suggests that the Lhcbs are involved in additional (or modified) excitonic interactions and/or that as compared with the detergent, LHCII trimers associated with PSI-LHCI are present in a slightly different conformation similar to LHCII trimers in aggregates.

Excitation Energy Transfer and Trapping Kinetics—In this preparation, Lhcbs have been shown to be functionally associated to PSI-LHCI-LHCII (34). To characterize the excitation energy transfer and trapping kinetics of PSI-LHCI-LHCII and estimate the energy transfer rate between Lhcbs and PSI-LHCI, time-resolved fluorescence of PSI-LHCI-LHCII was measured with a streak camera set-up. In C. reinhardtii, as in higher plants, the PS core antenna contains only Chls \( a \), whereas the peripheral antenna also contains Chls \( b \). Excitation in the Chl \( a \) and in the Chl \( b \) region can then be used to disentangle the contributions of the core and peripheral antennas (44). More specifically, upon 475 nm excitation, 41–46% of the energy was in Lhcbs, whereas this value dropped to 27–29% upon 400 nm excitation. These two excitation wavelengths were chosen for the time-resolved measurements.

Global Sequential Analysis—The fluorescence decays measured upon 400 and 475 nm excitation were analyzed with a sequential model. The data can be well described by a minimum of four components (no structure in the residuals, Fig. 2). A clear difference between both excitation wavelengths is observed in the decay of PSI-LHCI-LHCII at an early time where fast Chl \( b \) decay is clearly visible after their preferential excitation at 475 nm (see in particular 656 nm in Fig. 2a). The decay-associated spectra (DAS) for both excitation wavelengths are shown in Fig. 2b. The first component is a pure energy transfer component (conservative positive-negative shape of the DAS) from blue Chls \( a \) and \( b \) to red Chls \( a \) for both excitation wavelengths. This transfer is faster at 400 nm than at 475 nm (1.8 ps as compared with 3.5 ps, respectively), possibly because of an additional transfer step occurring from Chls \( b \) to blue Chls \( a \) upon 475 nm where Chls \( b \) are preferentially excited. Indeed, the Chls \( b \) emission at \( \sim 650 \) nm appears in the DAS of this fast component upon 475 nm excitation. The next two components are mainly decay components for both excitation wavelengths, with faster lifetimes upon 400 nm excitation as compared with 475 nm (\( ~20/90 \) ps as compared with \( \sim 30/100 \) ps, respectively). The fourth component has very small amplitude (\( ~3–4\% \)) and represents disconnected Lhcbs/Lhcbs/Chls with ns lifetime (41, 45). Fit parameters are summarized in Fig. 2b.

The average decay time \( \tau_{2b} \) of PSI-LHCI-LHCII is \( \sim 65 \) ps upon 400 nm and \( \sim 78 \) ps upon 475 nm. The association of Lhcbs increases the average decay time by \( \sim 15 \) ps at 400 nm and by \( \sim 27 \) ps at 475 nm as compared with the average decay times of PSI-LHCI (49.7 and 51.4 ps, respectively (40)).

Target Analysis—To estimate the energy transfer rate between PSI-LHCI and LHCII within PSI-LHCI-LHCII, a target analysis was performed on the two datasets (400 and 475 nm excitation) simultaneously. The previous kinetic scheme reported in Ref. 40 (Fig. 3a) was used to model PSI-LHCI-related kinetics, and the Lhcbs were modeled by an extra compartment (called “Lhcb”) transferring energy to the “Bulk” compartment (Fig. 3b). Several constraints were imposed on the fit parameters (rate constants, species-associated spectra (SAS), and initial populations) as described in SI1-iii. An additional compartment (Scheme SI2-1) accounts for a small population of disconnected species (\( \sim 3\% \)) whose lifetime (3.2 ns) is very close to that of the Lhcbs in C. reinhardtii (41).

The kinetic model (Fig. 3b) fits the data well (Fig. SI2-1). Rate constants fitted from the target are given in Fig. 3b (in red), and the SAS are given in Fig. 4. The Lhcb SAS has a significantly lower red emission and a narrower bandwidth as compared with Bulk as expected (Fig. 4 and Fig. SI2-2b). The red shift visible in the Lhcb SAS as compared with the isolated Lhcb steady state emission spectrum (Fig. 4) suggests that Lhcbs...
Excitation Energy Transfer in PSI-LHCI-LHCII of C. reinhardtii

It corresponds to an enthalpy difference $\Delta H$ of $-21\,\text{meV}$ ($\Delta H = H_{\text{Bulk}} - H_{\text{Lhcb}} = -k_B T \ln (N_{\text{Lhcb}}/N_{\text{Bulk}} \times k5/k6)$) with 147 Chls $a$ estimated in Bulk, see Table SI2-iv, and 55 Chls $a$ in Lhcb, Table S11-1). This enthalpy difference corresponds to an $\sim 9$-nm blue shift of Lhcb as compared with Bulk.

The amplitude matrices (Table I upon 475 nm and Table SI2-1 upon 400 nm excitation) detail the extent of (de)population of each compartment for each lifetime and give the time scale at which Lhcbs transfer energy to the rest of the supercomplex. The three shorter lifetimes do not have large amplitudes in the trapping (overall amplitude $\sim 0$) and correspond to times where precursors transfer energy to the other four compartments. The $\sim 23$-, $\sim 64$-, and $\sim 97$-ps lifetimes represent trapping components with most of the trapping occurring with the longest lifetime. With an $\sim 64$-ps lifetime, Lhcb equilibrates (large positive amplitude) with all the other compartments. Similar observations can be made when exciting PSI-LHCI-LHCII at 400 nm (Table SI2-1) except that the $\sim 23$-ps lifetime contributes more to the trapping than upon 475 nm. The average decay times obtained by target analysis of PSI-LHCI-LHCII are 70 ps upon 400 nm and 80 ps upon 475 nm excitation (see S11-iii), consistent with the average decay times calculated from the sequential analysis (see above). Contributions of Antenna Size, Connectivity, and Composition to the Average Decay Time of PSI-LHCI and PSI-LHCHII—The equilibration time between two compartments is influenced by their size (number of Chls in each compartment), by their composition (energy of the Chls), and by the connectivity between them. To disentangle these different contributions, the kinetics of hypothetical PSI-LHCI and PSI-LHCLHII complexes with different compartment size, composition, and/or connectivity were simulated (Scheme SI3-1), and their average decay time was compared (Table SI3-1). In the simulation, we have compared the effect of the presence of Lhca2 and Lhca9, the most loosely bound antenna complexes of PSI (18), with that of the seven Lhcbfs. The simulations show that: 1) The addition of 16 Chls $a$ (as many as initially contained in Lhca2 and Lhca9, see Table SI1-1 and SI2-iv) when isoenenergetic with the bulk slows down the average decay time by $\sim 2.5$ ps, while the addition of 55 Chls $a$ (as many as initially contained in the seven Lhcbfs, see Table SI1-1) slows it down by 4–6 ps. 2) The connectivity of PSI with Lhca2 and Lhca9 is very good and does not influence the average decay time of PSI-LHCLHII, whereas the connectivity between PSI-LHCI and Lhcbfs is not optimal and is responsible for an increase of the decay time of 16–25 ps as compared with the best possible case. 3) The red forms contained in Lhca2 and Lhca9 (46) slow down the kinetics of PSI-LHCLHII by $\sim 5$ ps, whereas the bluer Chls in Lhcbs (as compared with bulk Chls of PSI-LHCLHII) speed up the kinetics by 2–3 ps. A detailed description of the simulations is presented in SI3.

Discussion

Time-resolved measurements of the PSI-LHCLHII complex of C. reinhardtii, the largest PSI supercomplex isolated so

| Lifetimes | Red | Bulk | Lhca2/a9 | Lhcb | Overall amplitude of the trapping |
|-----------|-----|------|---------|------|-------------------------------|
| $\sim 23$- | $0.1$ | $0.000$ | $0.008$ | $0.000$ | $-0.047$ | $0.000$ |
| $\sim 64$- | $1.1$ | $-0.054$ | $-0.491$ | $0.016$ | $0.001$ | $-0.023$ |
| $\sim 97$- | $1.4$ | $0.000$ | $0.011$ | $-0.065$ | $0.000$ | $0.001$ |
| $\sim 23$- | $6.5$ | $-0.007$ | $0.106$ | $-0.067$ | $-0.002$ | $0.029$ |
| $\sim 64$- | $22.9$ | $-0.047$ | $0.152$ | $0.059$ | $-0.013$ | $0.151$ |
| $\sim 97$- | $63.8$ | $-0.308$ | $0.064$ | $0.018$ | $0.397$ | $0.171$ |
| $\sim 23$- | $97.3$ | $0.416$ | $0.151$ | $0.040$ | $0.063$ | $0.670$ |

Amplitude matrices of PSI-LHCLHII upon 475 nm excitation and the overall amplitude of the trapping at each lifetime.

A detailed description of the simulations is presented in SI3.
far (34), show that the trapping time of this supercomplex is \( \sim 65 \) ps upon 400 nm excitation and \( \sim 78 \) ps upon 475 nm excitation (Table 2). This large difference between the two excitation wavelengths is attributed to the differential excitation of Lhcb (more Lhcb excitation at 475 nm, see Table S1-2). This implies that the transfer from Lhcb to PSI-LHCI is slow relative to the trapping within PSI-LHCI. The presence of seven Lhcb slows down the overall trapping by 15 ps at 400 nm and by 27 ps at 475 nm excitation (Table 2). This result helps in interpreting the time-resolved data on \( C. \) reinhardtii \( \textit{in vivo} \) (27). If a large population of PSI-LHCI-LHCII particles should be expected in the cells in state 2 as compared with state 1, not only a large difference in the amplitude of the PSI component should be observed but also a significant difference in its lifetime. The fact that none of these effects were observed in the time-resolved measurements in the cells indicates that the difference in the amount of LHCII associated with PSI in state 1 and 2 is relatively small as concluded by Ünlü \textit{et al.} (27).

The detailed modeling of the measurements (Fig. 3b) shows that the excitation energy transfer between Lhcb and the rest of the supercomplex occurs in \( \sim 60 \) ps (Table 1). Several factors can influence the transfer rates: the antenna size, the energy of the pigments associated with the complexes, and the connectivity between Lhcb and PSI-LHCI. To discriminate between

**TABLE 2**

Average decay times and trapping efficiencies of PSI-LHCI-LHCII compared to PSI-LHCI

\[
\begin{array}{|c|c|c|c|c|c|}
\hline
& \text{PSI-LHCI (40)} & \text{PSI-LHCI-LHCII} \\
\hline
\text{Excitation wavelength (in nm)} & 400 & 475 & 400 & 475 \\
\text{Average decay time } \tau_{av} \text{ (in ps)} & 49.7 & 51.4 & 64.8 & 78.4 \\
\text{Trapping efficiency } (\Phi_{CS} = 1 - \tau_{av}/\tau_{CS} ) & 97.5\% & 97.4\% & 96.7\% & 96.0\% \\
\hline
\end{array}
\]

**FIGURE 5.**

(a), EM images of PSI-LHCI-LHCII from \( C. \) reinhardtii (34) viewed from the stromal side superimposed with apoproteins of PSI-LHCI of higher plants in cyan (Protein Data Bank (PDB) 4XK8 (14)) and LHCII trimer in brown (PDB 1RWT (20)) assembled as PSI-LHCI-LHCII of higher plants (black outline of EM of \( A. \) thaliana (36)) (left); or superimposed with LHCII assembled as PSI-LHCI-LHCII of \( C. \) reinhardtii by slightly rotating the LHCII trimer in brown into the LHCII trimer in blue and by adding a new LHCII trimer in green, CP29 in yellow (PDB 3PL9 (6)), and the five additional Lhcas present in \( C. \) reinhardtii in magenta (duplicates of Lha1 from Ref. 14) (right). The scale bar is 10 nm. The reconstruction was made with PyMOL. b, three-dimensional views of porphyrin rings from Chls bound to the apoproteins presented in a (right) with the same color: view from the stromal side (left) rotated 45° (upper right) or 90° (lower right) along the black axis. c, selection of the Chls present at the interface of Lhcb and PSI core (1.8 \( \times \) magnification of the red frame in b). The shortest distances between the closest atoms of the porphyrin rings of PSI Chls and Lhcb Chls are indicated in \( \AA \) for the different sides of PSI core. d, Chls a trimer (red) binding PsaA (apoprotein in gray) and Chl a1401 (pink) newly found in Ref. 14, viewed from the side indicated with a red arrow in a (right). These Chls could be involved in energy transfer pathways with LHCII (apoprotein in light blue).
Excitation Energy Transfer in PSI-LHCI-LHCII of C. reinhardtii

the contributions of these factors to the trapping time of PSI-LHCI and PSI-LHCl-LHCII, we have simulated excited state population dynamics of hypothetical particles where each factor was controlled. The connectivity of the Lhcb monomer was compared with that of the most loosely bound Lhcas (Lhca2 and Lhca9) (18), which also show a relatively slow equilibration (~7 ps) with the rest of the complex (40). The results indicate that the functional connectivity between Lhca2/a9 and the PSI complex is very high and that the observed relatively slow migration is due to the presence of low energy forms (46) in these Lhcas. This conclusion is in agreement with the observation that the presence of red forms slows down the trapping kinetics in different organisms (40, 47–49). On the contrary, the results show that in PSI-LHCl-LHCII the favorable downhill energy transfer profile of Lhcb cannot compensate their low connectivity with PSI core. This low connectivity largely explains the slow transfer from Lhcb to the PSI core and can be due to a low number of transfer pathways.

To determine the possible transfer pathways in the PSI-LHCl-LHCII, we reconstructed its three-dimensional structure by mapping the structures of LHClII trimer (20), CP29 (6), and PSI-LHCl (14, 15) of higher plant onto the EM projection map of PSI-LHCl-LHCII of C. reinhardtii (34). The structure of all subunits of C. reinhardtii is expected to be similar to that of higher plants given the sequence similarity (50, 51). Different views of the structural model are presented in Fig. 5.

Using the reconstructed model of the supercomplex, we looked for the shortest distances between the Chls of Lhcbs and those of the PSI core (Fig. 5, b and c), which should represent possible energy transfer pathways. We could not identify pairs of Chls that are separated by less than 18 Å (nearest edge-to-edge distance), in line with the slow energy transfer between LhcbS and PSI core observed in the measurements. The shortest distances are observed between Chl a612 of one LHClII trimer (which is in a similar position as in PSI-LHCl-LHCII of higher plant, and is depicted in blue in Fig. 5, nomenclature for LHClII Chls from (20)) and two PSI core Chls: Chl a1501 (nomenclature for the core Chls from Ref. 14, distance ~21 Å) bound to Psal and the additional Chl (distance ~18 Å) associated with Psah, which is only present in the structure of Mazor et al. (15) (PDB 4Y28, Chl designated as H1 in this structure). The next shortest distance is ~25 Å and involves Chl a611 of the same LHClII trimer and Chl a1801, belonging to a PsAA Chl trimer that was proposed to connect PSI and LHClII trimer in plants in Ref. 15. Other pathways suggested in Refs. 14 and 15 involve Chl a1401 (coordinated to Psaa on the luminal side, Fig. 5, c and d) and Chl a1403 (coordinated to Psak, Fig. 5, c and d) but are not expected to play a major role given the large distances that separate them from the Chls of LHClII (>30 Å). Similarly, PSI Chls are very far from the second LHClII trimer (depicted in green in Fig. 5, with Chl a612 at a distance of ~45 Å from Chl a1501 in Ref. 14 or ~37 Å from H1 in Ref. 15) as well as from the Lhcb monomer (Fig. 5c).

The three-dimensional reconstruction should be considered with some caution as the distances between the Chls can only be approximately estimated due to the low resolution of the EM maps, and the presence of one or two additional Chls in between the complexes cannot be completely excluded. However, it is clear that the gap between LHClII and the PSI core is rather large, and only a few energy transfer pathways seem to be available in agreement with the experimental results. The large distance between the Chls of PSI and LHClII also indicates that the observed differences in the CD signal between the sum of PSI-LHClI and LHClII spectra and the spectrum of the PSI-LHClI-LHClII complex are likely due to a different conformation assumed by LHClII when associated with PSI and not to the presence of new excitonic interactions between Chls of LHClII and PSI core.

It should be noticed that the observed loose connectivity between LHClII and PSI core has a small influence on the trapping efficiency of PSI-LHClI-LHClII. Indeed, even with a 43% increase in the number of Chls (Table SI1-1), the trapping yield of PSI-LHClI-LHClII is as high as 96% (Table 2) (52), only 1.4% lower than in the absence of Lhcb. The 43% increase in Chls corresponds to an increase of the absorption cross section by 47% (averaged over the spectral range), which can make a significant difference in terms of light-harvesting capacities, especially under low light conditions. In this respect, it is interesting to notice the very large difference in efficiency and flexibility between PSI and PSII. We have previously estimated that 240 Chls a per RC represent a maximal antenna size for PSII because above this value the increase in light harvesting would be compensated by the slow migration toward the RC, resulting in an effective loss of efficiency (53). This is clearly not the case for PSI, which is able to accommodate a very large antenna, maintaining a high efficiency even in the presence of slow transfer. Indeed, the results presented here show that in PSI-LHClI-LHClII of C. reinhardtii, which contains 240 Chls a per RC, 96 out of 100 photons absorbed lead to charge separation in the RC. The design of PSI seems then to represent the way to go for efficient light harvesting.

**Author Contributions**—R. C. conceived the research; C. L. Q., B. v. O., and R. C. designed the research, B. D. prepared the samples; C. L. Q. performed all the spectroscopic experiments, the global analysis, and the modelling; and C. L. Q. and I. H. M. v. S. performed the target analysis. B. v. O. and R. C. supervised the research, and C. L. Q. and R. C. wrote the paper with contributions from all the authors. All authors approved the final version of the manuscript.

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DECEMBER 18, 2015•VOLUME 290•NUMBER 51

JOURNAL OF BIOLOGICAL CHEMISTRY 30595

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