Supporting Information

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SI Text

LHCII:PSI:PSII Stoichiometry in the Membranes of *Chlamydomonas reinhardtii*

It has been proposed that in *C. reinhardtii* 80% of the light-harvesting complex II (LHCII) population moves from photosystem II (PSII) to photosystem I (PSI) during the transition from state 1 to state 2. However, to fully understand the extent of the transition, it is important to know the number of LHCII complexes present in the membranes.

We have measured the PSI:PSII ratio (in terms of reaction centers) with a Joliot-type spectrophotometer (Bio-Logic SAS JTS-10) using the carotenoid electrochromic shift as reported previously (1–4). The data indicate that, in our cells, the PSI/PSII ratio is 0.93, in agreement with previous reports indicating that in most conditions this value for *C. reinhardtii* is close to 1 (3, 5).

The Chl *a/b* ratio of the cells was 2.25, also in agreement with previous data (5). The pigment composition of the complexes was analyzed by fitting the spectrum of the 80% acetone extracted pigments with the spectra of the individual pigments in acetone and by HPLC, as described previously (6). Some of us have recently shown that the PSI–LHCI complex of *C. reinhardtii* is composed of a core complex together with nine Lhcas, binding in total around 240 Chls, of which 196 are Chls *a* and 44 are Chls *b* (7).

The PSII core contains 35 Chls *a*, whereas one LHCII trimer binds 42 Chls (24 Chls *a* and 18 Chls *b*) (8). For CP29, we use the stoichiometry obtained from the crystal structure (9) (meaning nine Chls *a* and four Chls *b*), and for CP26 we use the same stoichiometry as for monomeric LHCII (eight Chls *a* and six Chls *b*). We assume that CP29 and CP26 are present in a 1:1 stoichiometry with the PSII core like in plants, meaning that PSII core plus CP29 plus CP26 together bind 52 Chls *a* and 10 Chls *b*.

Using these data, we can calculate the relative number *x* of LHCII trimers according to the following equation:

$$2.25 \pm 0.05 = ((0.93 \pm 0.10) * 196 + 52 + 24\,x)/((0.93 \pm 0.10) * 44 + 10 + 18x),$$

leading to 7.5 ± 1.0 LHCII (estimated uncertainty at most one LHCII) per PSII reaction center (RC) in the membranes of *C. reinhardtii*. Thus, if in state 1 all LHCII trimers (for instance, seven) are associated with PSII, then there are 250 Chls *a* and 155 Chls *b* per PSII RC.

If, as suggested, 80% of the outer antenna moves to PSI in state 2, two-thirds of the Chls *a* of PSII would disappear, leading to a large decrease of the PSII amplitude and a concomitant increase of the PSI amplitude. Using the numbers above, this would mean that in state 1 45% of the Chls *a* would be attached to PSI and in state 2 this percentage would be 83%, an increase of 38%. However, as shown in the main text, the experimentally observed percentage is at most 4%. Even if we assume that one of seven LHCII trimers would move from PSII to PSI, the ratio of the number of Chls *a* in PSI and PSII would change from 196 and 226 to 220 and 202, respectively. This would correspond to an increase of the PSI amplitude of around 6%, more than is experimentally observed.

Global Analysis for *C. reinhardtii* Cells Grown in "Normal" Light Conditions

C. reinhardtii cells were grown under continuous white light illumination in Tris-acetate-phophate medium (10). Cells were shaken in a rotary shaker (100 rpm) at 30 °C and illuminated by a white lamp at normal light intensity (100 μmol·m^2·s^{-1}). All cells were grown in 250-mL flasks with a growing volume of 50 mL, and they were maintained in the logarithmic growth phase. The global analysis results are shown in Table S1.

The 77 K Steady-State Excitation Spectra for State 1-Locked and State 2-Locked Cells.

For steady-state fluorescence excitation spectra, a Jobin Yvon Fluorolog FL3A22 spectrofluorimeter was used. The fluorescence excitation spectra were recorded at 77 K, with excitation and emission bandwidths of 2 and 1 nm, respectively. The emission was collected at 680 and 712 nm. An integration time of 0.4 s was used. Each spectrum was measured six times in a run and then averaged. The state 1 and state 2 spectrum for PSI, detected at 712 nm, are very similar and only show a small difference around 650 nm, indicating a small increase of excitation energy transfer from LHCII to PSI (Fig. S2). Also, the state 1 and state 2 spectrum for detection at 680 nm (PSII and detached LHCII) are very similar and only show a small difference around 475 nm, indicating a small decrease of excitation energy transfer from LHCII to PSI.

In Fig. S3, the 77 K emission spectra are shown for state 1 and state 2 after excitation at 440 nm (absorption peak of Chl *a*) and 475 nm (Chl *b*). For state 1, the relative height of the PSI peak around 710 nm is somewhat lower for 475-nm excitation than for 440-nm excitation. This indicates that the amount of Chl *b* that is present in the outer antenna complexes is somewhat lower for PSI than for PSII. Upon going to state 2, the spectra are completely different with a substantial relative increase of the PSI peak for both excitation wavelengths. Nevertheless, the PSI peak is still somewhat (although less) smaller for 475-nm excitation and also these results do not indicate massive movement of outer antenna complexes from PSII to PSI.

The Average Lifetime Distribution for FLIM Image—Fig. 3.

The average lifetimes per pixel were analyzed with the SCP image software and plotted as a histogram to show the average lifetime distribution in the image (Fig. S4). The overall average lifetime in the image was around 400 ps.

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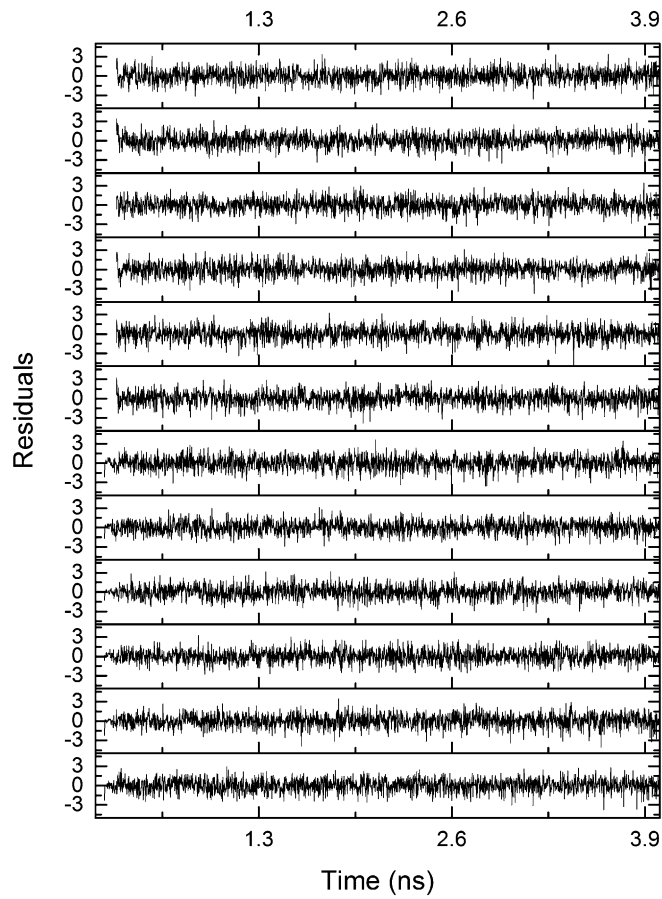
Fig. S1. Weighted residual plots of fluorescence decay curves in Fig. 2. The residuals show that five decay components are sufficient for a successful fit.
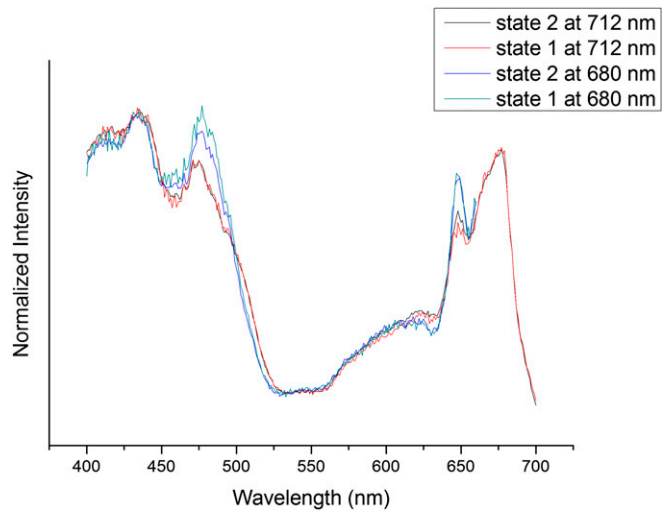

Fig. S2. Excitation spectra of state 1-locked and state 2-locked *C. reinhardtii* cells. The spectra are recorded at 77 K at two different emission wavelengths (680 and 712 nm).
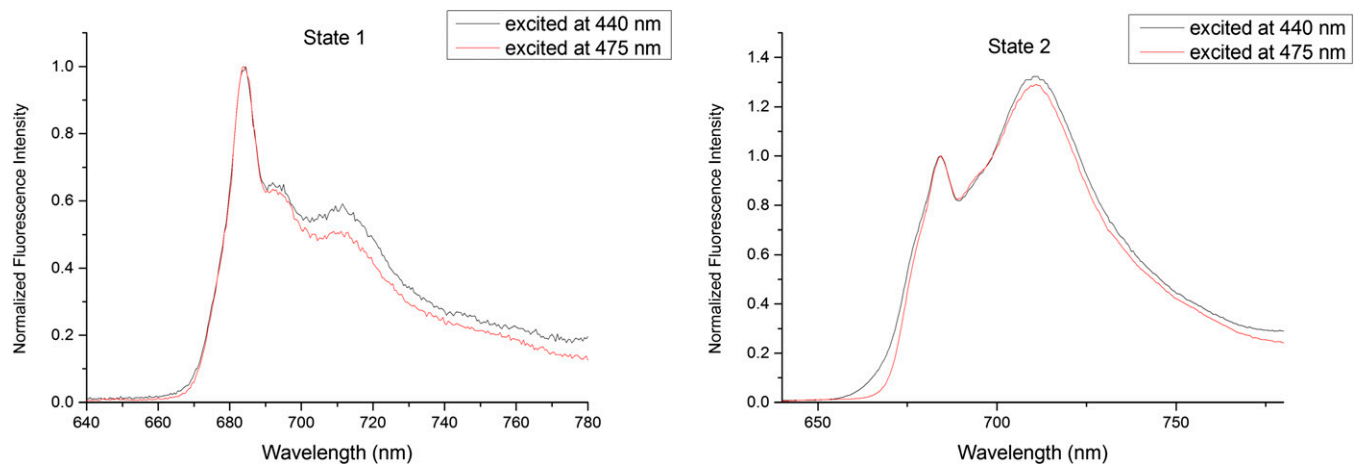
Fig. S3. Excitation spectra of state 1-locked and state 2-locked *C. reinhardtii* cells. The spectra are recorded at 77 K for two different excitation wavelengths (440 and 475 nm).
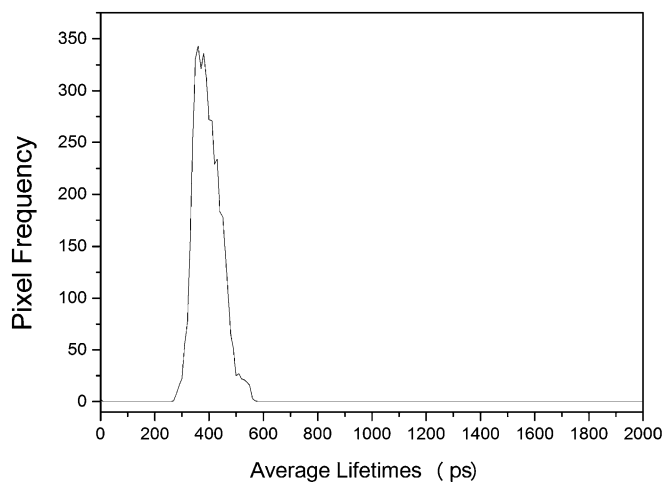

Fig. S4. The average lifetime distribution for Fig. 3.

Table S1. Global analysis results for TCSPC data obtained upon excitation at 400 nm at room temperature for state-locked *C. reinhardtii* grown in normal light conditions

State	Detection τ, ps	680 nm p	707 nm p
		Condition: normal light	
State 1			
	73	0.48	0.64
	258	0.19	0.21
	612	0.33	0.15
	1,553	0.004	0.004
	τ_{avg}	289.1 ps	198.0 ps
State 2			
	61	0.44	0.54
	173	0.26	0.26
	520	0.28	0.18
	1,066	0.02	0.02
	τ_{avg}	234.5 ps	191.4 ps

Confidence intervals of fluorescence lifetimes (τ) as calculated by exhaustive search were <5%; lifetimes were calculated with two to four repeats; p represents relative amplitudes. The fit results were interpreted in terms of the overall average fluorescence lifetime (τ_{avg}) for τ_1, τ_2, τ_3, and τ_4 according to $\tau_{avg} = \sum_{i=1}^{4} p_i \tau_i$, where $\sum_{i=1}^{4} p_i = 1$.