Supporting Material

Functional rearrangement of the light-harvesting antenna upon state transitions in a green alga

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77 K steady-state fluorescence excitation spectra

In the previous studies on ST in plants the fluorescence excitation difference spectrum of PSI revealed a shift of LHCII between PSI and PSII (1, 2). Similarly, in order to characterize the changes that PSI undergoes upon ST in *C.r.*, we measured the excitation spectrum at 715 nm in S1c and S2c and calculated the (S2c - S1c) difference spectrum (Figure S1A). Besides the maxima at ≈430 nm and 670 nm characteristic for Chl *a*, the presence of maxima at 475 nm and 650 nm indicates that the protein(s) involved in the change of excitation energy distribution between PSI and PSII upon ST contain(s) much Chl *b*, which corresponds well with LHCII. This is in line with the recent publication of Drop et al. (3) who isolated and characterized a PSI complex with two attached LHCII trimers from *C.r.* poised in S2. We also measured the excitation spectrum at 695 nm in S1c and in S2c. The (S1c - S2c) difference spectrum is shown in Figure S1B, and primarily reveals changes in PSII emission upon ST. The main maxima in the difference spectrum appear around 650 nm and 675 nm with a shoulder around 665 nm and a broad band towards short wavelengths. These characteristics correspond well with the (S2 - S1) spectrum of PSI reported before for plants, which has been satisfactorily fitted with an excitation spectrum of LHCII (1) and (1-T) spectrum of LHCII (2). Therefore, we assign the change that appears at 695 nm in *C.r.* upon S1→S2 transition to a detachment of LHCII from PSII.

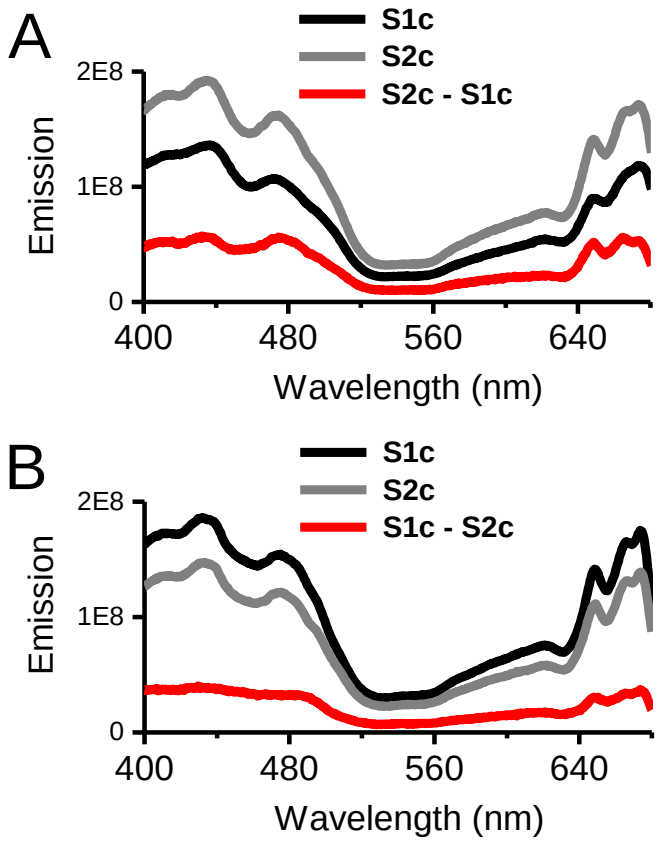

Figure S1 The 77 K steady-state excitation spectra of intact instantly frozen WT *C.r.* cells locked in S1c (kinase inhibitor) or in S2c (phosphatase inhibitor); (A) detected at 715 nm (primarily PSI emission), (B) 695 nm (primarily PSII emission). The presence of a peak at 650 nm in the difference spectra (red) at both detection wavelengths indicates that the pigment-protein complexes responsible for the changes in the absorption cross-sections of PSII and PSI upon ST contain Chl *b*.

6 K steady-state fluorescence

Steady-state fluorescence emission at 6 K was measured on thylakoids isolated from *stt7 C.r.* mutant (S1) and from a WT *C.r.* (S2) according to (4). For these measurements the thylakoids were resuspended in 25 mM HEPES (pH 7.5), 65% glycerol (v/v) 5 mM $MgCl_2$ buffer. The samples were placed in 4K Utreks helium-bath cryostat and the spectra were collected by means of laboratory-built spectrometer equipped with a 0.5 m imaging spectrograph and a CCD camera (Chromex Chromcam I). A broadband excitation was provided by a tungsten lamp (Oriel) and the excitation at 475 nm was further obtained by use of an interference filter (with a 15 nm bandwidth). The spectral resolution was 0.3 nm.

The emission bands measured at 6 K are narrower and can be more easily resolved when compared to bands in the 77 K spectra. For these measurements thylakoids isolated from WT cells poised in S2 and cells of the *stt7* mutant (unable to perform ST and thus locked in S1) were used. The resulting emission spectra were *simultaneously* fitted using a superposition of skewed Gaussian bands, where the spectral shapes of the bands between S1 and S2 were linked but the amplitudes of the bands were allowed to vary. The result of the fit is shown in Figure S2 and the estimated relative areas under the used bands are summarized in

Table S1. The main PSI emission at about 707 nm is clearly more pronounced in S2, whereas the main PSII emission bands around 687-690 nm are more pronounced in S1. The maxima of PSII are blue-shifted at 6 K compared to 77 K, as reported earlier (5). A narrow emission band at 681 nm with about equal intensity in S1 and S2 can be attributed to trimeric LHCII (6). Interestingly, a narrow emission band peaking at 684.5 nm with considerable amplitude is observed in S2 only. According to the fit, the much higher shoulder around 682 nm in the 6 K S2 emission spectrum is entirely caused by this band, which we denote X-685. The implications of this observation are discussed in the target analysis of time-resolved emission at 77 K in the main text.

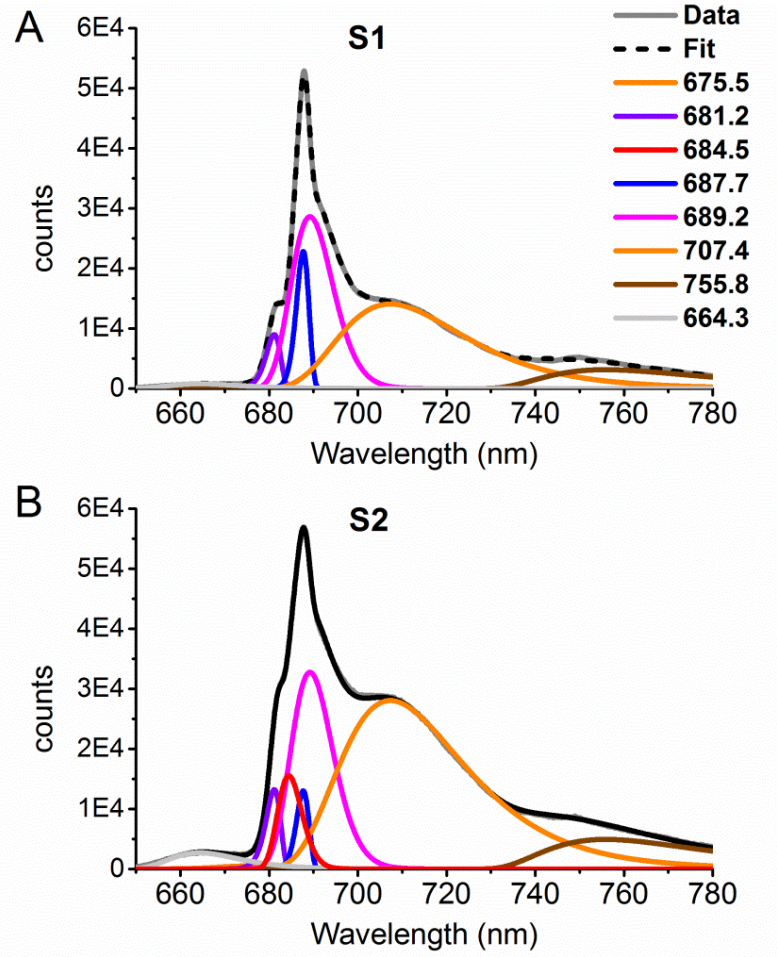

Figure S2 The 6 K steady-state fluorescence emission spectra of thylakoids isolated from *C.r.* in S1 (*stt7*) or in S2nc (WT) fitted simultaneously with a set of skewed Gaussian bands with spectral shapes linked between the two states and with variable amplitudes. Note that the 685-nm band (red) is necessary to satisfactorily fit the spectrum of S2.

Table S1 Areas of the Gaussian bands used in the fit of the 6 K steady-state fluorescence emission spectra of thylakoids isolated from *C.r.* in S1 (*stt7*) or in S2nc (WT).

Band [nm]	State 1	State 2
664.3	1.4%	2.9%
675.5	0.3%	0.4%
681.2	3.3%	2.9%
684.5	0.0%	5.5%
687.7	7.1%	2.4%
689.2	29.7%	20.1%
707.4	46.2%	54.5%
755.8	12.0%	11.2%

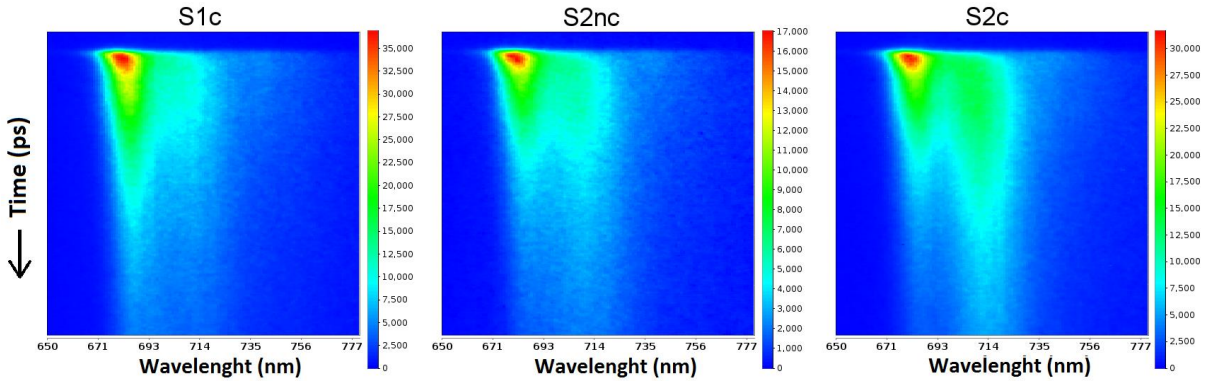

Figure S3 The streak camera images measured at 77 K on intact instantly frozen WT *C.r.* cells in S1c (locked with kinase inhibitor), S2nc (not locked chemically), S2c (locked with phosphatase inhibitor). The excitation was at 475 nm. Note the increased emission above 700 nm in S2c (and to lesser extent in S2nc) vs S1.

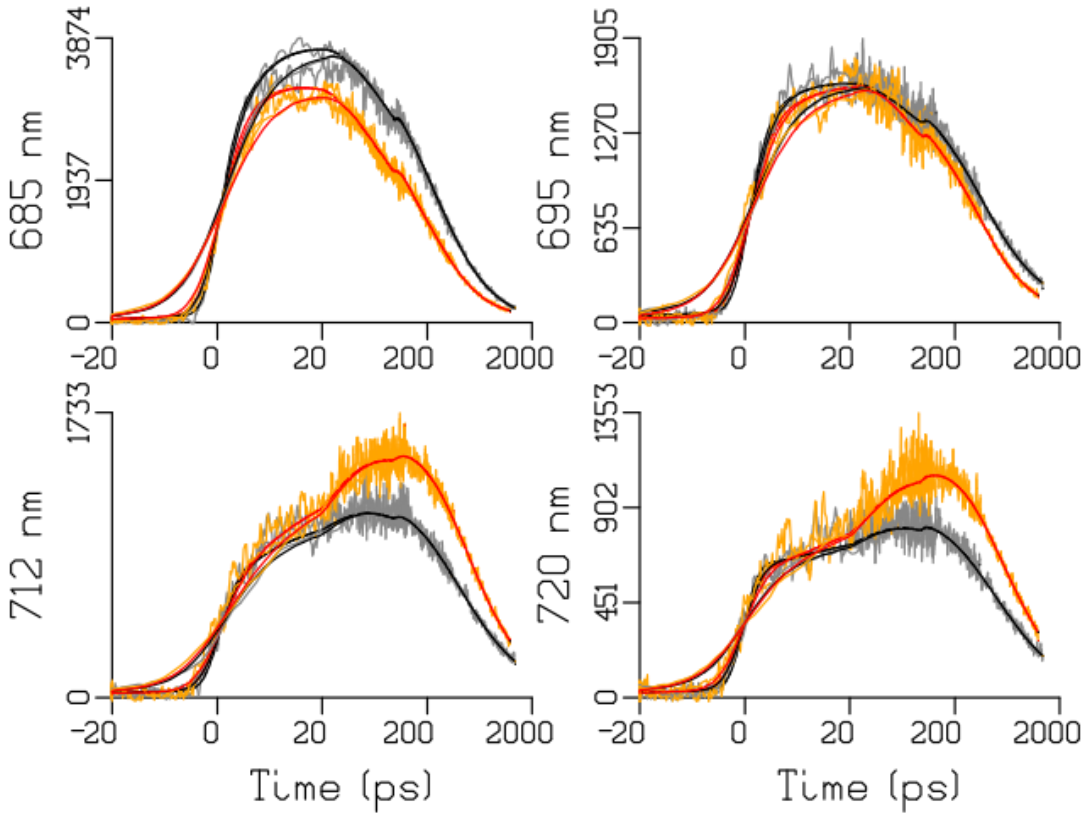

Figure S4 Selected traces (and fits) at wavelengths (written at the ordinate label) relevant for state transitions. Color key: S1c grey (black), S2c orange (red). The 712 and 720 nm traces are dominated by PS I emission and show a clear increase upon S1c→S2c. Concomitantly, the emission at late times decreases at 685 nm and 695 nm. Note that after 101 ps a small (4%) second response is visible as a hump in the black and red fitted traces.

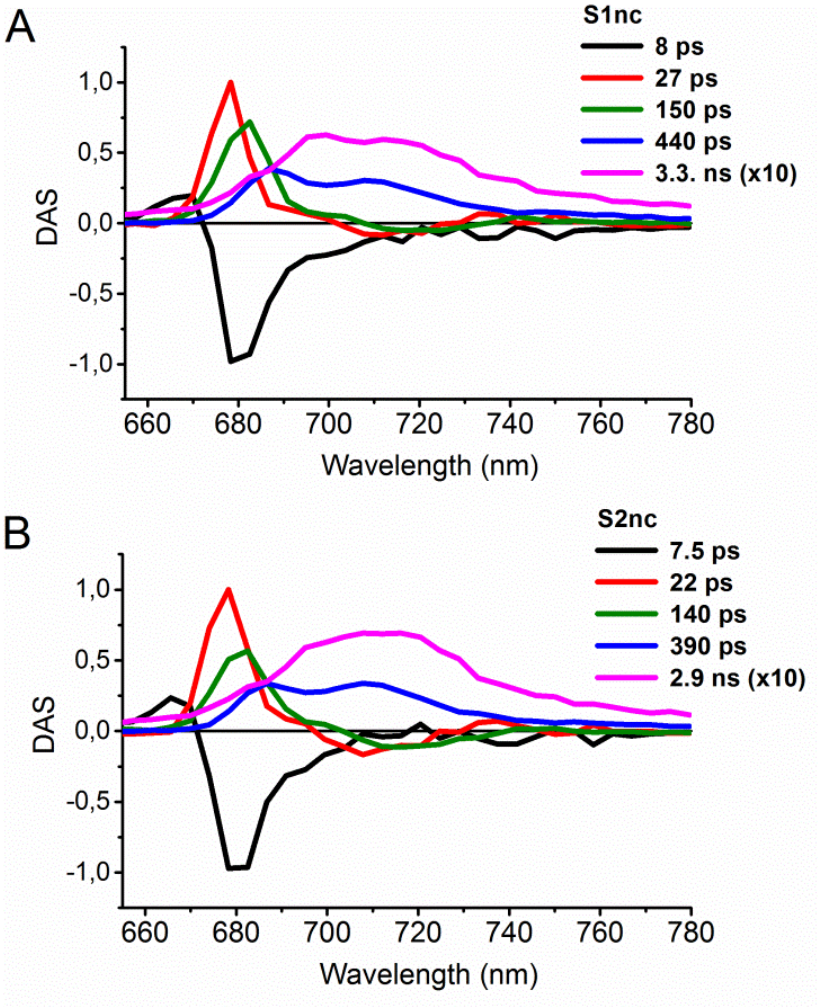

Figure S5 Decay associated spectra (DAS) estimated from global analysis of 77 K time-resolved fluorescence emission spectra of intact instantly frozen WT *C.r.* cells in S1nc and S2nc where no inhibitors were used to lock the respective states.

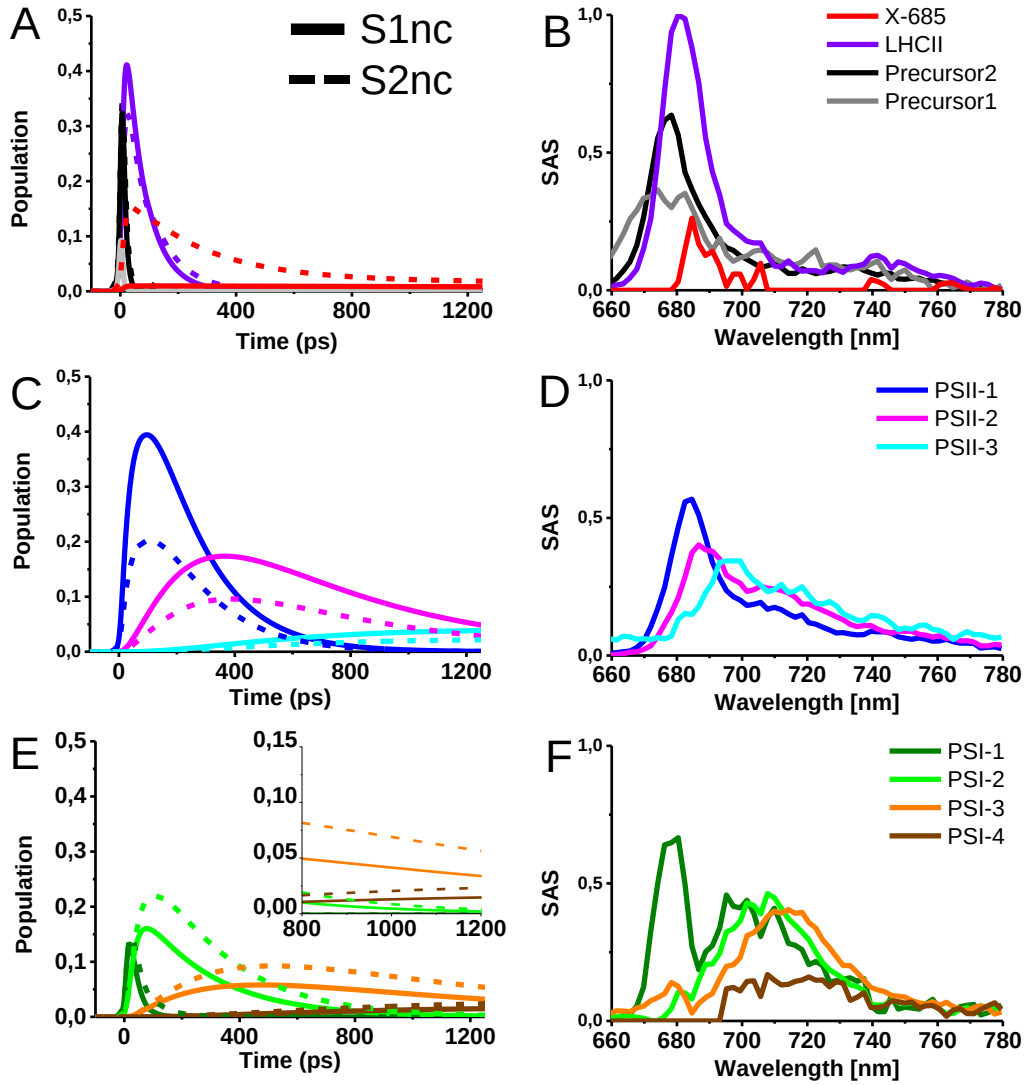

Figure S6 Population profiles (A, C, E) and corresponding species associated spectra (SAS) (B, D, F) estimated from the target model (Figure 3) of the 77 K time-resolved fluorescence emission spectra of intact instantly frozen WT *C.r.* cells in S1nc or in S2nc where no inhibitors were used to lock the respective states.

Reconstructed 77 K steady-state emission spectra

This reconstruction allows us to resolve the contributions from LHCII, X-685, PSI and PSII emission to the steady-state spectra (only chemically induced S1 and S2 are presented). One of the observations is that in S1c the spectrum at 680 nm and at 695 nm is indeed dominated by PSII (blue in Figure S7A). However, at 712 and 720 nm, wavelengths typically associated with PSI, only ≈50% of the emission originates from PSI due to the large contributions of the PSII vibrational bands at these wavelengths (cf. dark green and blue in Figure S7A). This is an important aspect to take into account when estimating the relative increase/decrease of PSI/PSII emission measured in S1 and S2 (cf. (7)). In S2 the emission at 712 and 720 nm is indeed dominated by PSI emission. However, the emission at 685 nm now largely originate from the X-685 nm component (red in Figure S7B) and PSI, therefore changes in amplitude around this wavelength should not be purely assigned to PSII.

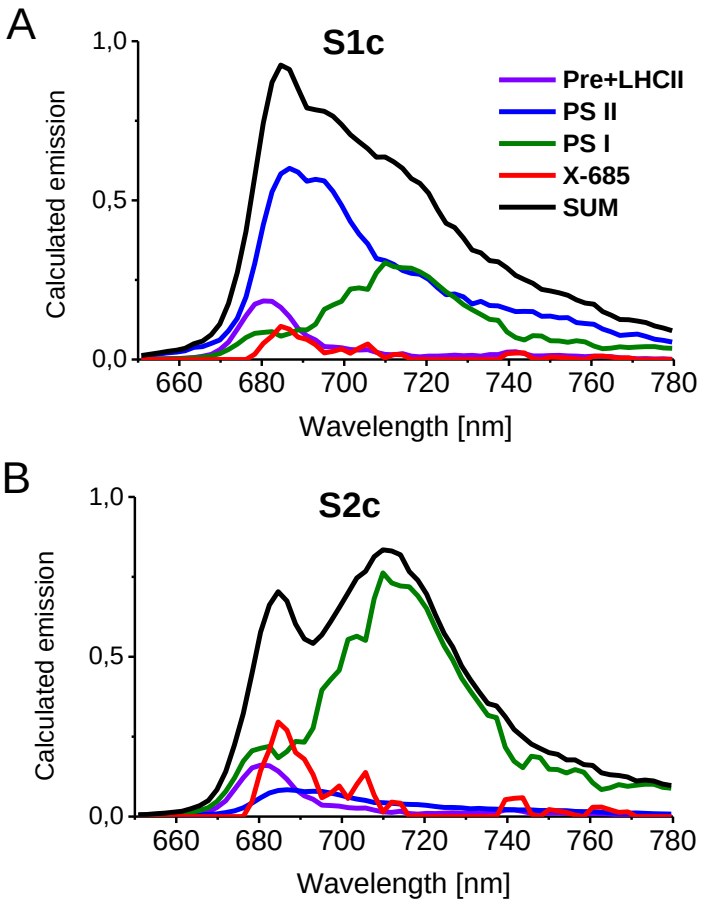

Figure S7 Reconstruction (black) of the steady-state fluorescence emission spectra, based on the target analysis of the 77 K time-resolved fluorescence emission spectra of intact instantly frozen WT *C.r.* cells locked in S1c (kinase inhibitor; A) or in S2c (phosphatase inhibitor; B).

Supporting References

1. Kouril, R., A. Zygadlo, A. A. Arteni, C. D. de Wit, J. P. Dekker, P. E. Jensen, H. V. Scheller, and E. J. Boekema. 2005. Structural characterization of a complex of photosystem I and light-harvesting complex II of Arabidopsis thaliana. Biochemistry 44:10935-10940.
2. Ruban, A. V., and M. P. Johnson. 2009. Dynamics of higher plant photosystem cross-section associated with state transitions. Photosynth. Res. 99:173-183.
3. Drop, B., K. N. S. Yadav, E. J. Boekema, and R. Croce. 2014. Consequences of state transitions on the structural and functional organization of photosystem I in the green alga Chlamydomonas reinhardtii. Plant J. 78:181-191.
4. Germano, M., A. E. Yakushevska, W. Keegstra, H. J. van Gorkom, J. P. Dekker, and E. J. Boekema. 2002. Supramolecular organization of photosystem I and light-harvesting complex I in Chlamydomonas reinhardtii. FEBS Lett. 525:121-125.
5. Andrizhiyevskaya, E. G., A. Chojnicka, J. a. Bautista, B. a. Diner, R. van Grondelle, and J. P. Dekker. 2005. Origin of the F685 and F695 fluorescence in photosystem II. Photosynth. Res. 84:173-180.
6. Hemelrijk, P. W., S. L. S. Kwa, R. van Grondelle, and J. P. Dekker. 1992. Spectroscopic properties of LHC-II, the main light-harvesting chlorophyll a/b protein complex from chloroplast membranes. Biochim. Biophys. Acta. 1098:159-166.

7. Unlü, C., B. Drop, R. Croce, and H. V. Amerongen. 2014. State transitions in Chlamydomonas reinhardtii strongly modulate the functional size of photosystem II but not of photosystem I. Proc. Natl. Acad. Sci. USA. 111:3460-3465.