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Supporting Material

Effect of antenna-depletion in Photosystem II on excitation energy transfer in *Arabidopsis thaliana*

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Supplementary material

This Supplementary material contains five sections: (i) details about the experimental conditions, (ii) fluorescence emission spectra, (iii) derivation of $\tau_{trap} = N_{eff}[\tau_{CS} + \tau_{RP}\exp(-\Delta G/kT)]$ and (iv) calculation of the decay associated spectra (DAS), and (v) a schematic overview of the macrostructure of the thylakoid membrane.

(i) Experimental conditions

The sample was kept at 287 K in a flow cuvette and a sample reservoir (25-50 ml). The sample was flowing from the reservoir to the cuvette and back, with a flow speed of ~150 ml/min. The optical path length of the cuvette was 3 mm, the OD at the excitation wavelength was ~0.2/cm, and the size of the excitation spot was 2 mm. The excitation power was 0.5-4 µW. In this way each laser pulse excites 1 out of ~5*10^7 to 4*10^8 Chls. In the time (~7 ms) to travel through the excitation spot ~3*10^4 pulses hit the sample, exciting 1 out of ~2*10^3 to 1.6*10^4 pigments. Supposing equal excitation of PSI and PSII, and a PSII antenna size of ~200 pigments/RC, it follows that while passing through the excitation beam, pigments associated to ~0.6 to 5% of the RCs are excited. After passing through the beam, it takes ~10-20 s until the next passage. Experiments using higher or lower excitation power indicated that under the experimental conditions almost all RCs remain open. Figure S1 shows the WT fluorescence kinetics excited at 475 nm, and detected above 645 nm, at different excitation powers. The final experiment of a measuring series was always a repeat of the first experiment. The resulting decay curves were indistinguishable. Each sample was measured 2-5 times.

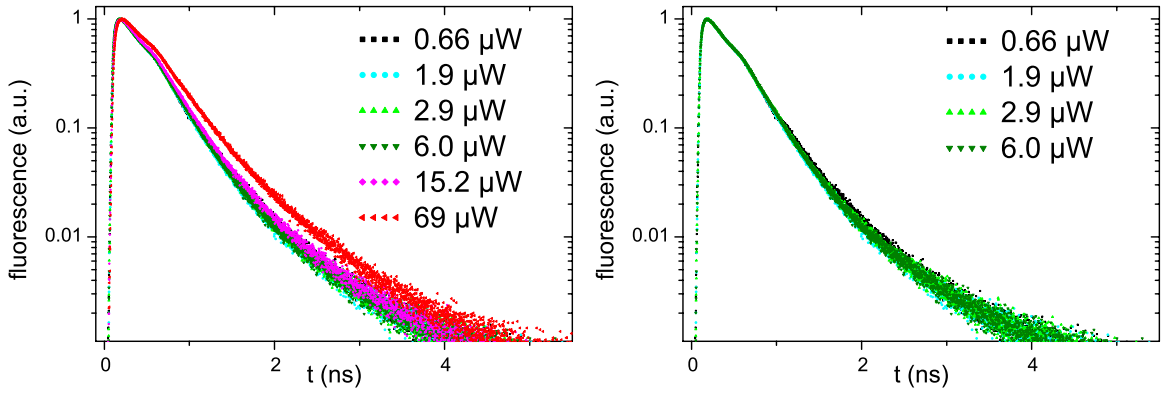

Figure S1. Fluorescence kinetics of thylakoid membranes of WT *Arabidopsis thaliana*, excited at 475 nm, and detected above 645 nm, at different excitation powers. The right figure shows the curves measured around the power range (0.5-4µW) used for the experiments described in the main text.

(ii) Fluorescence emission spectra

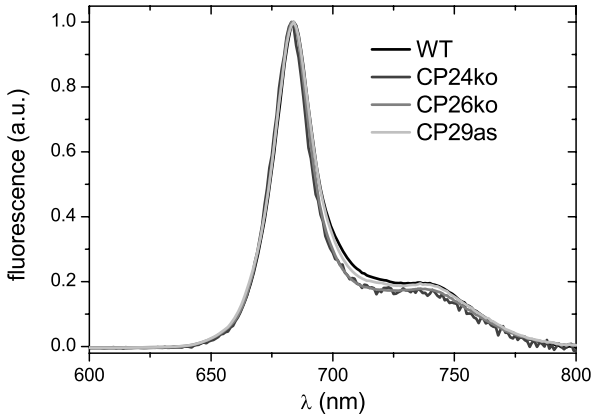

Figure S2. Room temperature fluorescence emission spectra of thylakoid membranes from WT and mutants of *A. thaliana*. The excitation wavelength is 412 nm.

(iii) Derivation of $\tau_{trap} = N_{eff}[\tau_{CS} + \tau_{RP}\exp(-\Delta G/kT)]$

Derivation of this equation: The system of linear kinetic equations for the antenna complex excitation probabilities (as presented in the Appendix of (1)) is mapped by Laplace transformation into a system of algebraic equations that can easily be solved. For the zero Laplace variable this system reduces into an algebraic system for the vector of partial lifetimes corresponding to the complexes of the coarse-grained model of PSII. The sum of the vector components gives the analytical expression for the average fluorescence lifetime depending on the model parameters like the hopping, CS and radical pair relaxation times as well as the free energy gap. Taking the zero hopping time limit we arrive at the trapping time expression.

(iv) Calculation of decay associated spectra

The calculations above require decay associated spectra (DAS). DAS are calculated from the steady-state spectra and the time-resolved experiments as follows: The relative amplitudes (p_i^{λ}) at detection wavelengths λ were scaled with factor α^{λ}, such that for each detection wavelength, the area under the theoretical fluorescence decay curve equals the intensity of the steady-state spectrum at that wavelength (F(λ)): $F(\lambda) = \alpha^{\lambda}\int_0^{\infty}\sum_{i=1}^{3} p_i * e^{-t/\tau_i} = \alpha^{\lambda}\sum_{i=1}^{3} p_i^{\lambda} * \tau_i^{\lambda}$.

The resulting values $\alpha^{\lambda} * p_i^{\lambda}$ are the values of the DAS of decay component i at detection wavelength λ. F(λ) was calculated from the convolution of the steady-stated fluorescence emission spectrum and the transmission spectrum of the interference filters (measured on a Cary 5E UV-Vis-NIR spectrophotometer).

The longest fluorescence lifetime (> 1.7 ns, with very small amplitude, ≤1%) probably originated from free Chl, from closed RCs of PSII, or from antennae that are disconnected from the RCs. These are not the topic of interest, so the fit results were rescaled omitting this component.

(v) Macrostructure of the thylakoid membrane

The main components of the thylakoid membrane and their relative positions are schematically drawn in Figure S3. PSII and LHCII are present mainly in the stacked regions of the membranes, whereas PSI is mainly present in the unstacked regions and on the topmembranes of the granum stacks. Other complexes are cytochrome b_6f and ATP synthase.

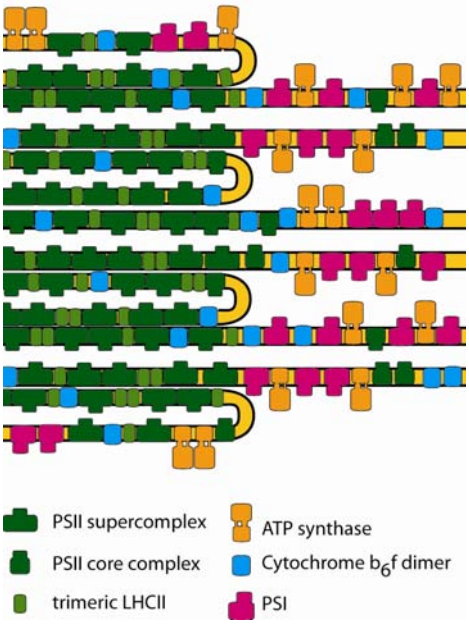

Figure S3 Schematic overview of the macrostructure of the thylakoid membrane, based on refs (2, 3).

References

1. Broess, K., G. Trinkunas, C. D. van der Weij-de Wit, J. P. Dekker, A. van Hoek, and H. van Amerongen. 2006. Excitation energy transfer and charge separation in photosystem II membranes revisited. Biophys. J. 91:3776-3786.
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