

Supplementary Information

The quantitative contribution of different photosystem II compartments to non-photochemical quenching in *Arabidopsis*

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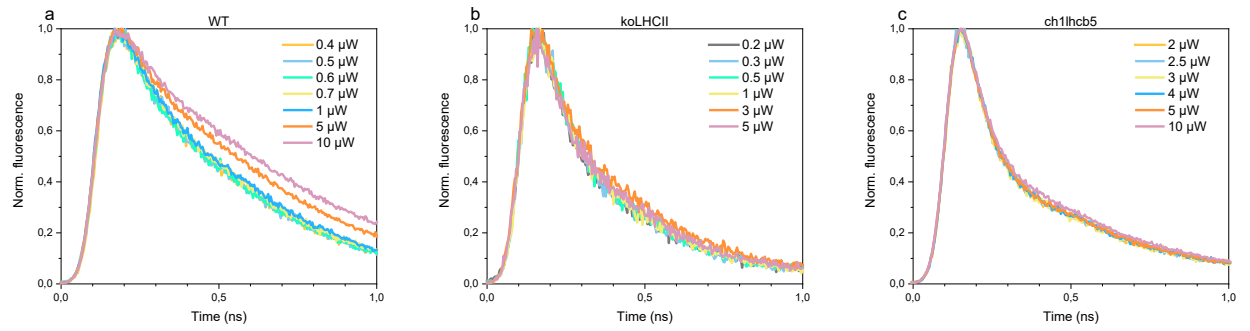
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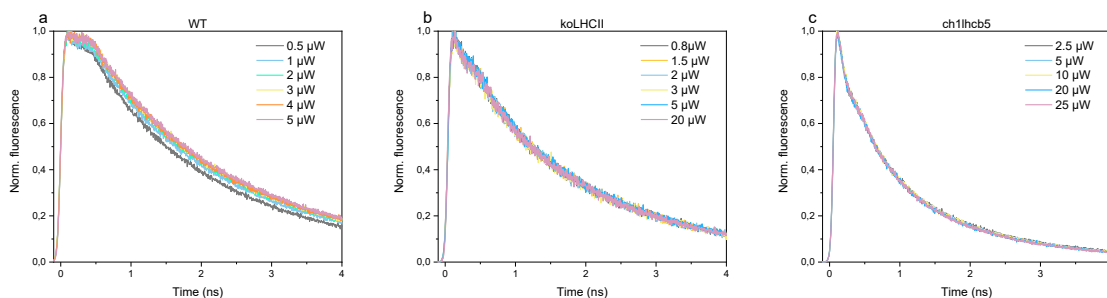
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Supplementary Fig. 1. Power study to determine which excitation powers keep thylakoid membranes in the open state. Samples were excited at 468 nm using different excitation powers and fluorescence was detected at 680 nm. Traces are representative of three biologically independent replicas with similar results. Powers of 0.5, 0.5, 3.0 μW were selected for further experiments on WT, kolHClI, and Ch1lhcb5, respectively.



Supplementary Fig. 2. Power study to determine which excitation powers were sufficient to measure on DCMU-infiltrated leaves in fully closed state, while also avoiding singlet-singlet annihilation. Samples were excited at 468 nm using different excitation powers and fluorescence was detected at 680 nm. Traces are representative of three biologically independent replicas with similar results. Powers of 2, 1.5, and 20 μW were selected for further experiments on WT, koLHCII, and Ch1lhcb5, respectively.

Supplementary Table 1. Global fitting of the fluorescence decay curves^a.

WT open-state				WT closed-state			
relative amplitudes (%)				relative amplitudes (%)			
τ (ps)	680 nm	700 nm	720 nm	τ (ns)	680 nm	700 nm	720 nm
103 ± 6	60 ± 15	70 ± 12	76 ± 11	0.09 ± 0.01	35 ± 6	52 ± 5	64 ± 3
353 ± 12	40 ± 15	30 ± 12	24 ± 11	1.06 ± 0.06	18 ± 4	14 ± 4	11 ± 3
τ_{avg} (ps)	202 ± 39	179 ± 32	162 ± 30	2.38 ± 0.07	45 ± 3	34 ± 4	25 ± 3
				τ_{avg} (ns)	1.34 ± 0.1	1.01 ± 0.09	0.79 ± 0.06
kolhcll open-state				kolhcll closed-state			
relative amplitudes (%)				relative amplitudes (%)			
τ (ps)	680 nm	700 nm	720 nm	τ (ns)	680 nm	700 nm	720 nm
66 ± 4	86 ± 3	88 ± 3	88 ± 4	0.07 ± 0.01	57 ± 2	73 ± 4	77 ± 6
321 ± 86	13 ± 3	11 ± 2	11 ± 4	0.6 ± 0.15	11 ± 1	10 ± 2	10 ± 3
1243 ± 151	1 ± 1	1 ± 0	0 ± 0	2.05 ± 0.09	32 ± 2	17 ± 3	13 ± 3
τ_{avg} (ps)	105 ± 13	97 ± 9	96 ± 10	τ_{avg} (ns)	0.77 ± 0.05	0.47 ± 0.05	0.38 ± 0.06
Ch1lhcb5 open-state				Ch1lhcb5 closed-state			
relative amplitudes (%)				relative amplitudes (%)			
τ (ps)	680 nm	700 nm	720 nm	τ (ns)	680 nm	700 nm	720 nm
64 ± 6	76 ± 4	76 ± 6	78 ± 6	0.08 ± 0	55 ± 5	69 ± 9	73 ± 9
231 ± 34	20 ± 2	20 ± 4	18 ± 4	0.56 ± 0.04	25 ± 2	17 ± 1	14 ± 0
1059 ± 108	4 ± 1	4 ± 2	3 ± 2	1.94 ± 0.12	19 ± 2	11 ± 1	9 ± 1
τ_{avg} (ps)	134 ± 26	136 ± 30	129 ± 30	τ_{avg} (ns)	0.55 ± 0.06	0.37 ± 0.05	0.32 ± 0.04

^a Samples were excited at 468 nm and fluorescence was detected at 680, 700 and 720 nm. The average fluorescence lifetime is calculated according to $\tau_{avg} = \sum A_i \tau_i / \sum A_i$, where A_i is the amplitude associated with τ_i . Data in the open and close state are mean ± s.d (n = 6, 6 and 5 biologically independent replicas for WT, kolhcll, and Ch1lhcb5, respectively). Note that both PSI and PSII contribute to the fluorescence.

Supplementary Note 1. Calculating the intrinsic rate of NPQ

All Chl excited state decay pathways have an associated first-order rate constant (k) which is inversely proportional to the corresponding lifetime:

k_f is the rate constant of fluorescence, also called the radiative decay rate.

k_d is the rate constant of static quenching processes (internal conversion and intersystem crossing)

k_{NPQ} is the rate constant of non-photochemical quenching

The yield of any excited state decay pathway is the ratio between its specific rate and the sum of the rate constants for all pathways of excitation decay. Therefore, the fluorescence yields of F_m and F_m' can be expressed in terms of kinetic parameters and substituted into the Stern-Volmer equation:

$$F_m = k_f / (k_f + k_d) \quad F_m' = k_f / (k_f + k_d + k_{NPQ}) \quad NPQ = (F_m - F_m') / F_m'$$

$$NPQ = \frac{\frac{k_f}{k_f + k_d} - \frac{k_f}{k_f + k_d + k_{NPQ}}}{\frac{k_f}{k_f + k_d + k_{NPQ}}}$$

$$NPQ = \frac{k_{NPQ}}{(k_f + k_d)}$$

The outcome is that $k_{NPQ} = NPQ \cdot (k_f + k_d) = NPQ / \tau_m$ or, alternatively, $\tau_{NPQ} = \tau_m / NPQ$. In order to combine data from two different experimental set-up (i.e. Dual Pam and TCSPC), they have to be obtained with similar excitation and detection wavelengths. Steady-state fluorescence comes from excitation at 460 nm and detection at wavelengths above 700 nm. For consistency, τ_m and τ_o are obtained with excitation at 468 nm and reflect the average of the fluorescence lifetimes detected at 700 and 720 nm. For all calculations the measured τ_o is corrected for the presence of the natural decay of the chlorophylls in the membrane (2 ns).

In the main text we made the assumption that all Chl *a* molecules are isoenergetic, mainly for presenting a simplified calculation, but the assumption is not strictly required. Although the assumption is approximately correct (the Chl *a* absorption bandwidth is slightly larger than the thermal energy $k_B T$), it is not required for obtaining the presented results. If the population probability of the quenched Chl *a* is

for example twice as high as for the other pigments (because of a lower energy) then the calculated intrinsic rate of quenching would be half. However, the overall quenching efficiency by this pigment would essentially remain the same, because it is the product of this probability and the intrinsic quenching rate that counts in the end. Although this product is not exactly identical, in all realistic cases it will not deviate by more than 1-1.5 %.

Supplementary Table 2. Input values for the calculation of k_{NPQ} .

	τ_o (ns) ^a	τ_m (ns) ^a	NPQ ^a	k_{NPQ} (ns ⁻¹) ^b	N Chl a^c
WT	0.17 ± 0.03	0.89 ± 0.08	1.8 ± 0.21	2.03 ± 0.15	120
kolhcll	0.10 ± 0.01	0.42 ± 0.06	0.49 ± 0.06	1.16 ± 0.06	60
Ch1hcb5	0.13 ± 0.02	0.35 ± 0.04	0.4 ± 0.09	1.18 ± 0.14	35

^a Data are mean ± s.d (n ≥ 3 biologically independent replicas).

^b Error represents propagated standard error

^c Based on the Chl a/b ratio of the plants analyzed and the known pigment and protein stoichiometries¹⁻⁵

Supplementary Note 2. Correcting for the contribution of PSI to fluorescence emission

Fluorescence emission from leaves and thylakoid membranes originates from PSI and PSII. In the open state, both PSI and PSII are contributing to the fast component (~100 ps) of fluorescence decay. In the closed state, the lifetime of PSI remains approximately the same whereas the lifetime of PSII increases, meaning the fast component is primarily due to PSI. This contribution ($\tau_1 \cdot A_1$, averaged for detection at 700 and 720 nm) can be subtracted from $\tau_{o/m/m'}$, allowing the extraction of PSII lifetimes (PSII $\tau_{o/m/m'}$), and the recalculation of NPQ based on quenching of PSII fluorescence only. For example:

$$\tau_m' = \frac{\tau_m}{NPQ + 1}$$

$$PSII \tau_{o/m/m'} = \frac{\tau_{o/m/m'} - (\tau_1 \times A_1)}{1 - A_1}$$

$$PSII NPQ = \frac{PSII \tau_m - PSII \tau_{m'}}{PSII \tau_{m'}}$$

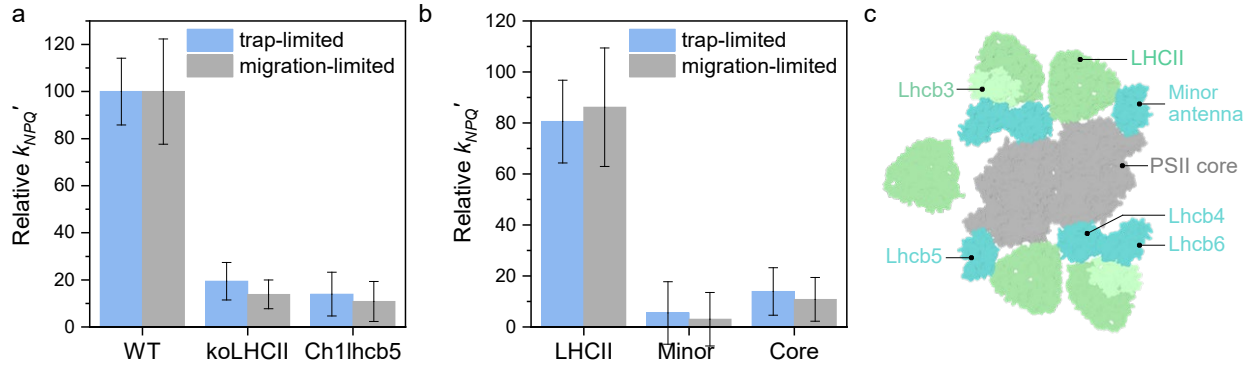
Note that the contribution of PSI is similar in all plants (Supplementary Table 3, first column) despite the smaller PSI/PSII protein ratios observed in the antenna mutants (Supplementary Fig. 5). Indeed, the PSII core binds only 35 Chls and PSII-LHCII binds approximately 150 Chls per monomer, whereas the PSI core contains 100 Chls and PSI-LHCI contains 156 Chls. As a result, the antenna mutants require a substantial (40-45%) reduction in the relative PSI protein content in order to maintain the excitation balance between photosystems (this balance is manifested in the similar PSI contributions – which depend on the product of the number of complexes and their antenna size – to the time-resolved fluorescence data of all plants).

Supplementary Table 3. Input values for the calculation of k_{NPQ}' corrected for PSI contribution. Error represents propagated standard error.

	PSI ($\tau_1 \cdot A_1$)	PSII τ_o (ns)	PSII τ_m (ns)	PSII τ_m' (ns)	PSII NPQ	PSII k_{NPQ} (ns ⁻¹)	N Chl <i>a</i>
WT	0.052 ± 0.002	0.28 ± 0.03	2 ± 0.1	0.64 ± 0.04	2.1 ± 0.3	1.1 ± 0.2	120
kolhcll	0.056 ± 0.005	0.16 ± 0.03	1.5 ± 0.2	0.9 ± 0.1	0.6 ± 0.2	0.4 ± 0.2	60
Ch1lhcb5	0.054 ± 0.003	0.27 ± 0.05	1 ± 0.2	0.7 ± 0.1	0.5 ± 0.3	0.5 ± 0.3	35

Supplementary Table 4. Comparison of the intrinsic rates of NPQ with and without PSI contribution. Error represents standard error.

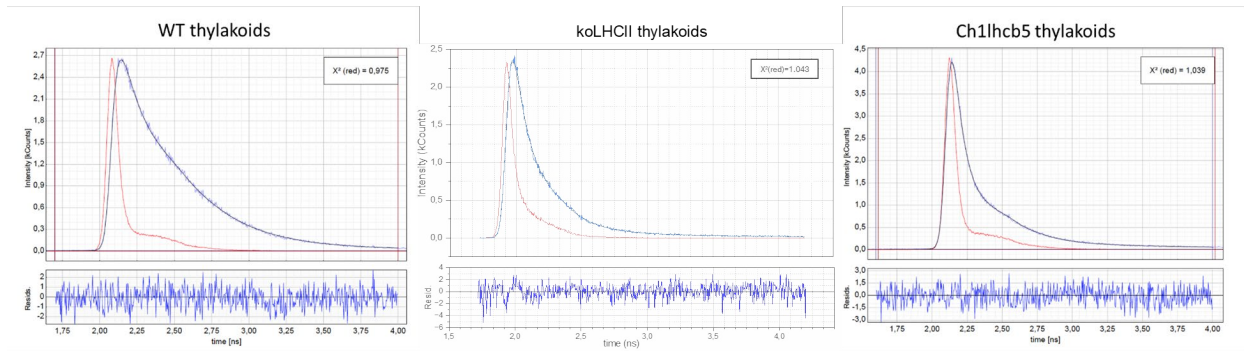
	trap-limited k_{NPQ}' (ns ⁻¹)		migration-limited k_{NPQ}' (ns ⁻¹)	
	PSII	PSII + PSI	PSII	PSII + PSI
WT	127 ± 18	244 ± 18	194 ± 43	392 ± 51
kolhcll	25 ± 10	70 ± 4	27 ± 12	79 ± 5
Ch1lhcb5	18 ± 12	41 ± 5	21 ± 16	50 ± 7



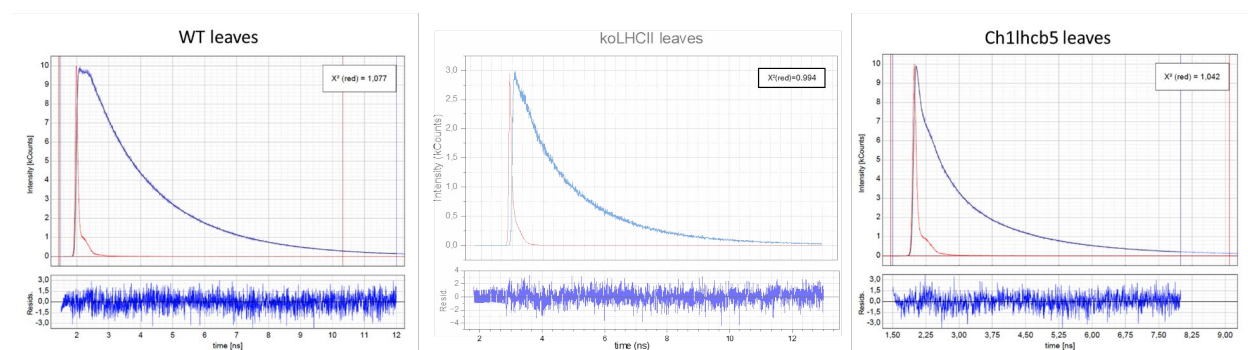
Supplementary Fig. 3. Intrinsic rates of NPQ corrected for PSI contribution. (a) The k_{NPQ}' of each antenna mutant relative to WT (b) the percentage of k_{NPQ}' occurring in each compartment in WT. Error bars represent propagated standard error.

Supplementary Table 5. Intrinsic rates of NPQ assuming 2, 2.5 or 3 LHCII trimers per PSII RC with and without PSI contribution. (a) The k_{NPQ}' of each antenna mutant relative to WT (b) the percentage of k_{NPQ}' occurring in each compartment in WT. Errors represent standard error.

a	Intrinsic rates of NPQ assuming 2 LHCII trimers per RC				
		trap-limited k_{NPQ}' (ns ⁻¹)		migration-limited k_{NPQ}' (ns ⁻¹)	
		PSII	PSII + PSI	PSII	PSII + PSI
	WT	1 ± 0.14	1 ± 0.08	1 ± 0.22	1 ± 0.13
	kolhcll	0.22 ± 0.09	0.32 ± 0.02	0.15 ± 0.07	0.22 ± 0.01
	Ch1lhcb5	0.15 ± 0.1	0.19 ± 0.02	0.12 ± 0.09	0.14 ± 0.02
	Intrinsic rates of NPQ assuming 2.5 LHCII trimers per RC				
		trap-limited k_{NPQ}' (ns ⁻¹)		migration-limited k_{NPQ}' (ns ⁻¹)	
		PSII	PSII + PSI	PSII	PSII + PSI
	WT	1 ± 0.14	1 ± 0.08	1 ± 0.22	1 ± 0.13
	kolhcll	0.19 ± 0.08	0.29 ± 0.02	0.14 ± 0.06	0.2 ± 0.01
	Ch1lhcb5	0.14 ± 0.09	0.17 ± 0.02	0.11 ± 0.08	0.13 ± 0.02
	Intrinsic rates of NPQ assuming 3 LHCII trimers per RC				
		trap-limited k_{NPQ}' (ns ⁻¹)		migration-limited k_{NPQ}' (ns ⁻¹)	
		PSII	PSII + PSI	PSII	PSII + PSI
	WT	1 ± 0.14	1 ± 0.08	1 ± 0.22	1 ± 0.13
	kolhcll	0.18 ± 0.07	0.26 ± 0.01	0.13 ± 0.06	0.18 ± 0.01
	Ch1lhcb5	0.13 ± 0.08	0.15 ± 0.02	0.1 ± 0.08	0.12 ± 0.02
b	Intrinsic rates of NPQ assuming 2 LHCII trimers per RC				
		trap-limited k_{NPQ}' (ns ⁻¹)		migration-limited k_{NPQ}' (ns ⁻¹)	
		PSII	PSII + PSI	PSII	PSII + PSI
	LHCII	0.78 ± 0.17	0.68 ± 0.08	0.85 ± 0.23	0.78 ± 0.13
	Minor	0.06 ± 0.13	0.13 ± 0.03	0.03 ± 0.12	0.08 ± 0.02
	Core	0.15 ± 0.1	0.19 ± 0.02	0.12 ± 0.09	0.14 ± 0.02
	Intrinsic rates of NPQ assuming 2.5 LHCII trimers per RC				
		trap-limited k_{NPQ}' (ns ⁻¹)		migration-limited k_{NPQ}' (ns ⁻¹)	
		PSII	PSII + PSI	PSII	PSII + PSI
	LHCII	0.81 ± 0.16	0.71 ± 0.08	0.86 ± 0.23	0.8 ± 0.13
	Minor	0.06 ± 0.12	0.12 ± 0.03	0.03 ± 0.1	0.07 ± 0.02
	Core	0.14 ± 0.09	0.17 ± 0.02	0.11 ± 0.08	0.13 ± 0.02
	Intrinsic rates of NPQ assuming 3 LHCII trimers per RC				
		trap-limited k_{NPQ}' (ns ⁻¹)		migration-limited k_{NPQ}' (ns ⁻¹)	
		PSII	PSII + PSI	PSII	PSII + PSI
	LHCII	0.82 ± 0.16	0.74 ± 0.08	0.87 ± 0.23	0.82 ± 0.13
	Minor	0.05 ± 0.11	0.11 ± 0.02	0.03 ± 0.09	0.07 ± 0.02
	Core	0.13 ± 0.08	0.15 ± 0.02	0.1 ± 0.08	0.12 ± 0.02



Supplementary Fig. 4. Fitting of time-resolved fluorescence traces of thylakoids in the open state. Overlay of raw and globally fitted time-resolved fluorescence traces of thylakoids from different plants in the open state (excited at 468 nm and detected at 680 nm). The IRF profile is shown in red, and the fitting residuals are shown below each plot. The χ^2 parameter is also indicated in the top left of each plot. The raw traces correspond to those shown in the left panel of Fig. 2a in the manuscript, whereas the results from the exponential fittings of the traces (lifetimes and amplitudes) are displayed in Supplementary Table 1. Traces are representative of 6, 6, and 5 biologically independent replicas with similar results for WT, koLHCII, and Ch1hcb5, respectively.



Supplementary Fig. 5. Fitting of time-resolved fluorescence traces of leaves in the closed state. Overlay of raw and globally fitted time-resolved fluorescence traces of leaves from different plants in the closed state (excited at 468 nm and detected at 680 nm). The IRF profile is shown in red, and the fitting residuals are shown below each plot. The χ^2 parameter is also indicated in the top left of each plot. The raw traces correspond to those shown in the left panel of Fig. 2b in the manuscript, whereas the results from the exponential fittings of the traces (lifetimes and amplitudes) are displayed in Supplementary Table 1. Traces are representative of 6, 6, and 5 biologically independent replicas with similar results for WT, koLHCII, and Ch1hcb5, respectively.

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