Supplementary Information

Crystal structure of plant light-harvesting complex shows the active, energy-transmitting state

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1. Aggregation quenching of LHC-II mutants

Table SI. Chlorophyll pairs in LHC-II and respective mutants.

Chl pairs	π-π distance	Mg-Mg distance[1]	Mutant
Chl 2/Chl 7	3.8 Å	9.8 Å	N183A (ΔChl 2)
Chl 3/Chl 8	3.9 Å	9.4 Å	H212A (ΔChl 8)
Chl 5/Chl 12	3.9 Å	9.8 Å	E139A (ΔChl 12)
Chl 10/Chl 13	3.7 Å	9.5 Å	Q131A (ΔChl 13)

[1] Distance taken from the atomic coordinates of the pea LHC-II structure (PDB code: 2BHW)

Table SII. Pigment composition of refolded WT LHC-II and mutant ΔChl 13[1]

	WT	**ΔChl 13**
Chl *a*	7.99	8.76
Chl *b*	6.01	4.24
Lut	2.30	2.23
Neo	1.03	0.14
Vio	0.15	0.13

[1] Number of pigments normalised to 14 or 13 Chls per monomer for WT and ΔChl 13 mutant, respectively.

3. Emission spectra of single LHC-II crystals

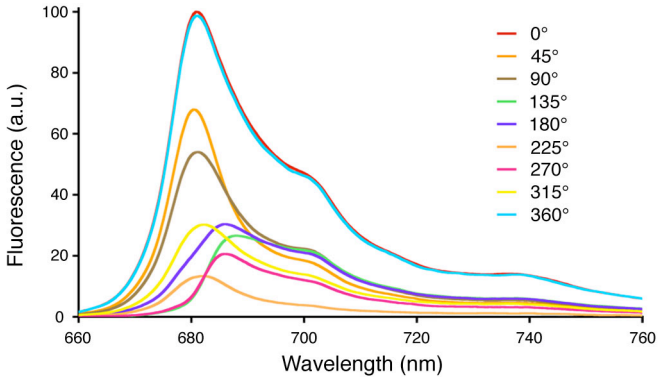

Figure S1. 100 K fluorescence emission spectra of a single type-I crystal of pea LHC-II in different orientations. Each spectrum was recorded after rotating the crystal in 45° steps around an axis perpendicular to the incident laser beam.

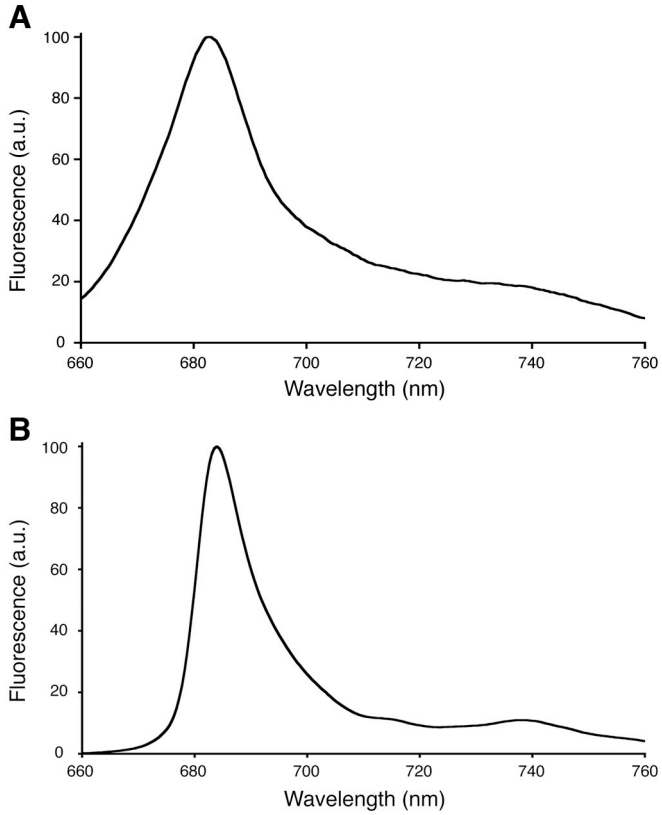

Figure S2. (A) Room temperature fluorescence emission spectrum of a single LHC-II crystal. (B) Low-temperature fluorescence emission spectrum of a concentrated LHC-II solution (3.5 mg(Chl)/ml). Both spectra indicate significant emission at 700 nm.

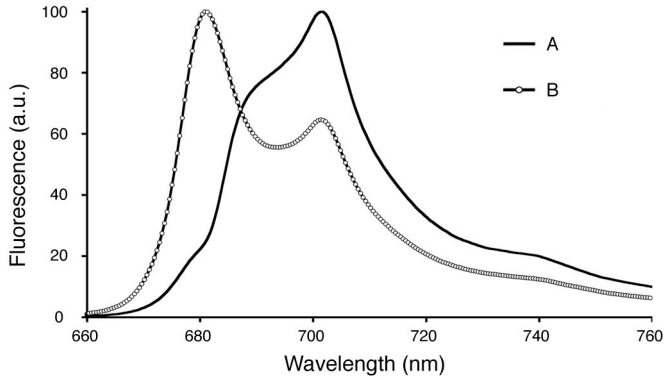

Figure S3. Low-temperature fluorescence spectra of a single crystal of pea LHC-II in two different orientations. By changing the position of the laser spot within the crystal, the spectrum changes from a broad band at 700 nm with very little emission at 680 nm (A), to a spectrum showing an emission peak at 680 nm characteristic of unquenched LHC-II, with a second band at 700 nm (B). (A) is very similar to previously published crystal spectra (Pascal et al, 2005; Yan et al, 2007).

3. Fluorescence lifetimes

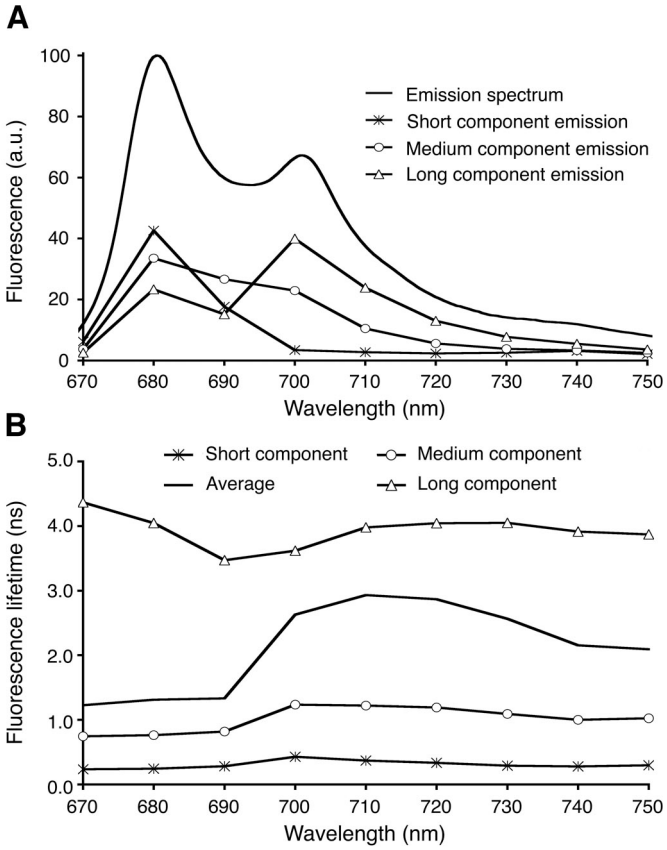

Figure S4. Low-temperature time-resolved fluorescence of a single LHC-II crystal. (A) Total emission spectrum and relative emission of the 3 components used to fit the fluorescence decay. (B) Wavelength dependence of average and component lifetimes.

Table SIII. Fluorescence lifetime of LHC-II in different aggregation states at 100K.

	[Chl] mg/ml	t_1 ns	a_1	f_1	t_2 ns	a_2	f_2	t_3 ns	a_3	f_3	<t> ns
Total emission											
solution	0.01	2.89	0.22	0.13	5.57	0.78	0.87	-		-	5.22
single crystal	~150	0.27	0.65	0.24	0.90	0.27	0.34	3.78	0.08	0.43	1.98
aggregates	n.a.	0.23	0.77	0.36	0.85	0.17	0.29	3.16	0.06	0.35	1.43
680 nm band											
solution	0.01	2.73	0.22	0.12	5.53	0.78	0.88	-	-	-	5.19
single crystal	~150	0.24	0.78	0.43	0.76	0.20	0.34	4.05	0.03	0.23	1.31
aggregates	n.a.	0.19	0.88	0.69	0.56	0.12	0.27	2.94	<0.01	0.03	0.39
700 nm band											
solution	0.01	3.04	0.26	0.16	5.54	0.74	0.84	-	-	-	5.14
single crystal	~150	0.43	0.21	0.05	1.24	0.50	0.35	3.62	0.29	0.60	2.63
aggregates	n.a.	0.30	0.44	0.15	0.92	0.43	0.45	2.68	0.13	0.40	1.52

All values are the average of 5 individual TCSPC histograms recorded for each aggregation state (solution, crystals, aggregates) under identical experimental conditions. t_i – lifetime of component i; a_i – relative amplitude; f_i – fractional contribution to the steady state intensity; <t> - average lifetime. n. a. – not available.

4. Implications for the mechanism of qE

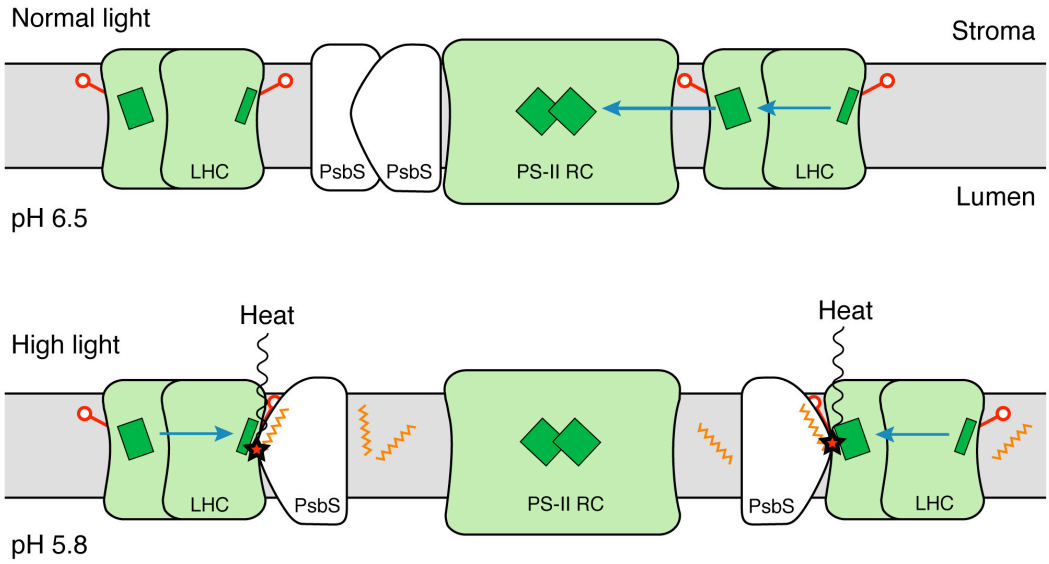

Figure S5. Proposed model for qE in thylakoids. Light-harvesting complexes (LHC) normally transmit the absorbed excitation energy to the reaction centres of photosystem II (PSI-II RC). Under high-light conditions, Vio is converted into Zea in the xanthophyll cycle. Zea is proposed to bind to PsbS, which monomerizes as the pH in the thylakoid lumen drops (pH values according to (Kramer et al, 1999)). Upon interaction with LHC, a Zea/Chl heterodimer is created which converts excess excitation energy into heat. Green rectangles, representative Chls; orange, Zea; red, flexible part of Neo; blue arrows, radiation-less energy transfer; stars, quenching centres.

Supplementary Information References

Kramer DM, Sacksteder CA, Cruz JA (1999) How acidic is the lumen? *Photosynthesis Research* **60**(2 - 3)**:** 151-163

Pascal AA, Liu Z, Broess K, van Oort B, van Amerongen H, Wang C, Horton P, Robert B, Chang W, Ruban A (2005) Molecular basis of photoprotection and control of photosynthetic light-harvesting. *Nature* **436**(7047)**:** 134-137

Yan H, Zhang P, Wang C, Liu Z, Chang W (2007) Two lutein molecules in LHCII have different conformations and functions: Insights into the molecular mechanism of thermal dissipation in plants. *Biochemical and Biophysical Research Communications* **355**(2)**:** 457-463