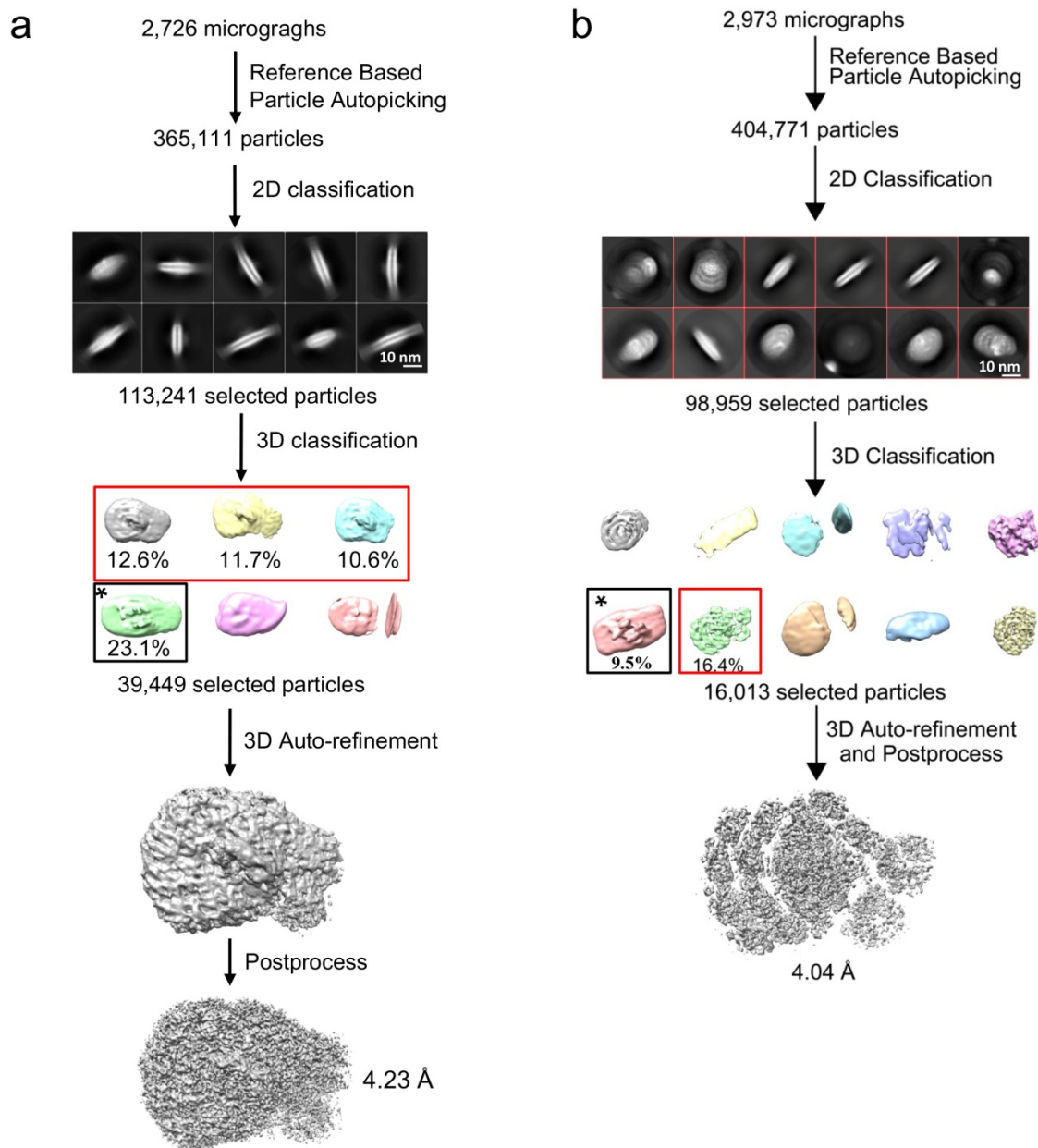

Supplementary information

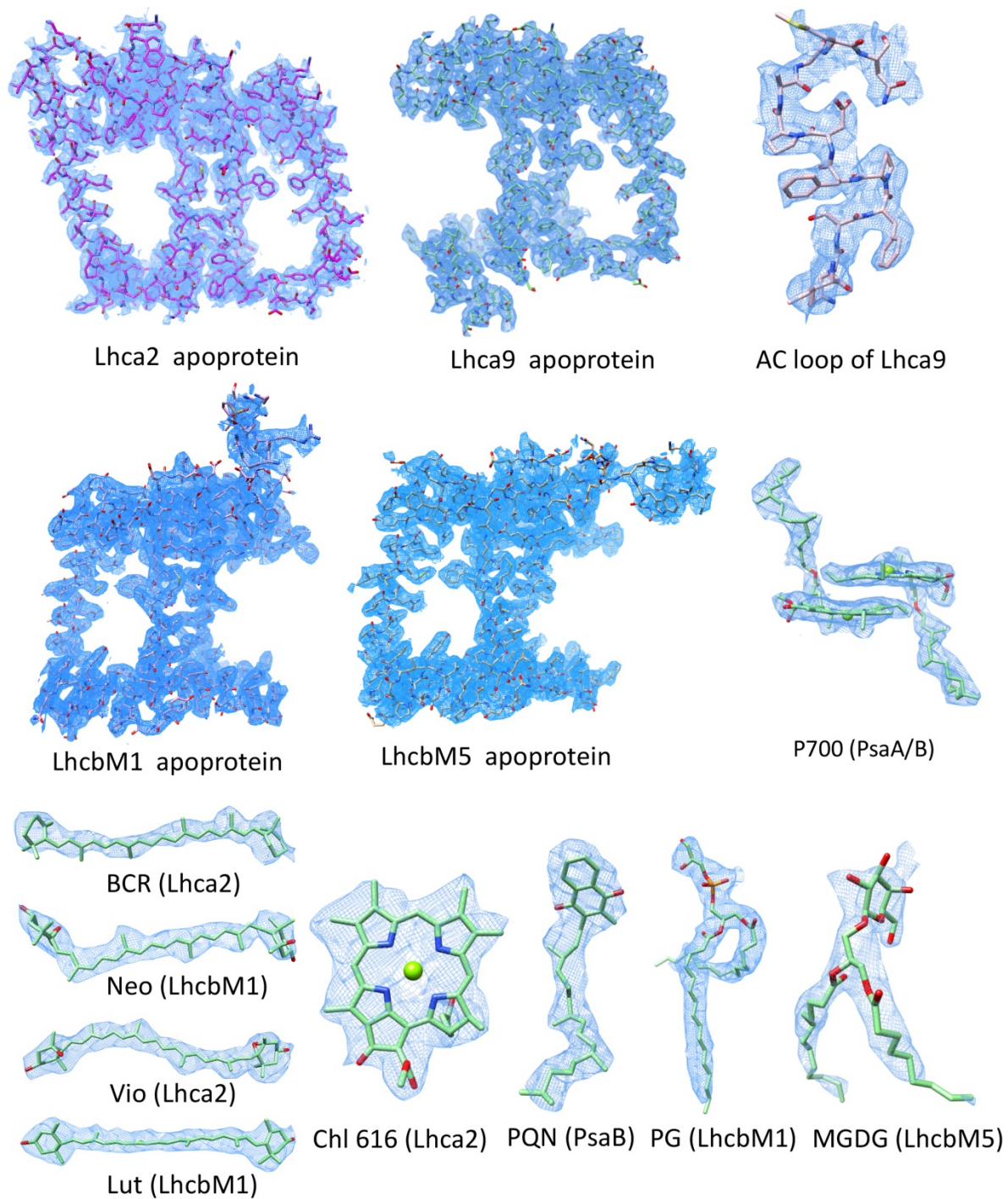
Structural basis of LhcbM5-mediated state transitions in green algae

In the format provided by the
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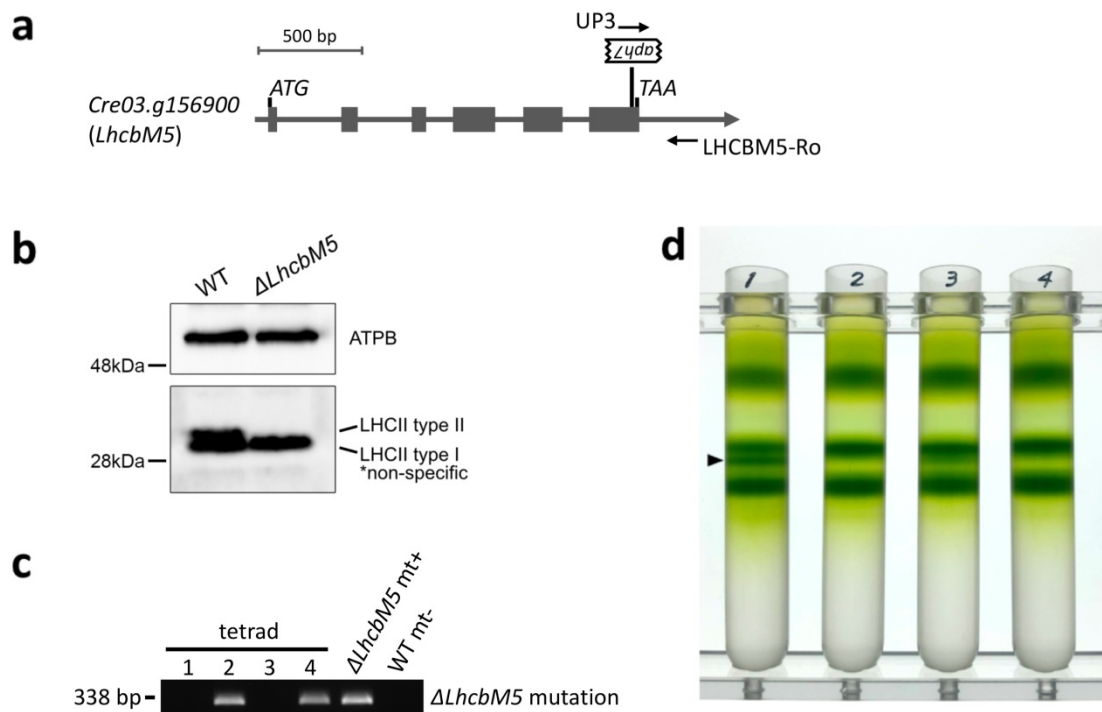


Supplementary Fig. 1. Single particle cryo-EM data processing of *Cr*PSI-LHCI-LHCII supercomplex obtained by two different methods from *C. reinhardtii* wild type strain.

(a) Single particle cryo-EM data processing procedure of WT supercomplex purified using amphipols. (b) Single particle cryo-EM data processing procedure of WT supercomplex purified in α -DDM. The class corresponding to PSII-LHCII complex is framed by black box and indicated by asterisk.

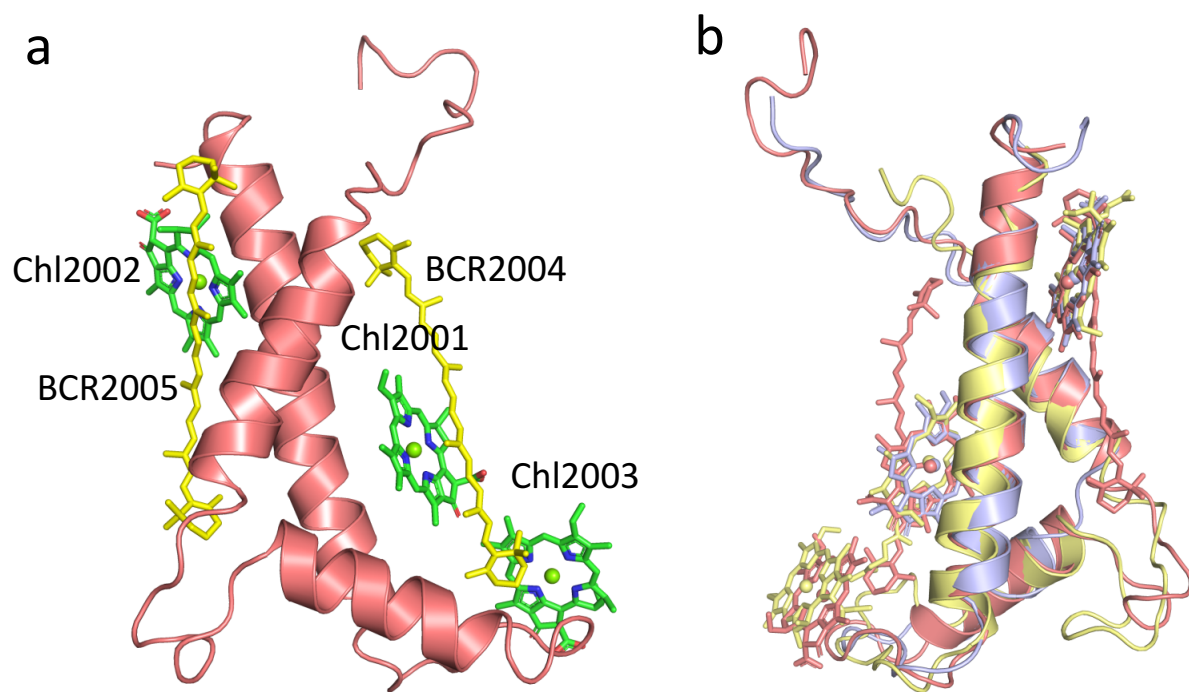


Supplementary Fig. 2. Representative densities of protein subunits and cofactors in the structure of CrPSI-LHCI-LHCII supercomplex from the *pph1;pbcp* mutant strain.



Supplementary Fig. 3. Characterization of the mutations in $\Delta LhcbM5$.

(a) A schematic diagram of the gene structure (chromosome) of *LhcbM5* (*Cre03.g156900*). The translation start and stop codons, positions of primers for detecting the mutation and the position and orientation of the inserted *aph7* tag with partial deletions in the mutant identified are all indicated. (b) Immunoblot analysis of LhcbM5 in the $\Delta LhcbM5$. ATPB proteins were included as loading controls. A representative result of 4 biological repeats is shown. (c) Genomic PCR analysis detecting the tag insertion in *LhcbM5* in the progeny #2 and #4 in a complete tetrad from a cross of $\Delta LhcbM5$ mt⁺ and WT mt⁻. A representative result of 2 biological repeats is shown. (d) Formation of the PSI-LHCI-LHCII supercomplex on the sucrose density gradients in a complete tetrad showing a co-segregation of the $\Delta LhcbM5$ mutation and the loss of PSI-LHCI-LHCII supercomplex (arrow head) in the progeny #2 and #4.



Supplementary Fig. 4. Overall structure of *CrPsaO*.

(a) Cartoon representation of *CrPsaO* from the *pph1:pbcp* mutant strain, viewed from the membrane plane. *CrPsaO* possesses two TMHs and one amphiphilic helix along the luminal surface, and binds three chlorophyll and two β -carotene molecules. The two β -carotenes are located at grooves formed by the two TMHs of PsaO from opposite sides. Three chlorophylls and two β -carotenes (BCRs) are shown in stick mode and labeled. (b) Structural comparison of *CrPsaO* (salmon) with PsaO from maize (light-blue, PDB code 5ZJI) and red alga (yellow, PDB code 5ZGH).

Supplementary Table 1. Summarization of the structural model of Native *Cr*PSI-LHCI-LHCII structure

Sub unit	Traced residues	Chlorophylls	Carotenoids	Lipids	Others
PSI-LHCI					
PsaA	741 (11-751)	45 Chl	6 BCR	2 PG, 1 MGDG	1 PQN, 1 SF4,
PsaB	733 (2-734)	40 Chl	10 BCR	1 DGDG, 2 PG	1 PQN
PsaC	80 (2-81)				2 SF4
PsaD	143 (53-195)				
PsaE	63 (34-96)				
PsaF	165 (63-227)	3 Chl	1 BCR		
PsaG	94 (32-125)	2 Chl	1 BCR		
PsaH	100 (31-130)	2 Chl		1 PG, 1 MGDG	
PsaI	42 (63-104)				
PsaJ	41 (1-41)	1 Chl	1 BCR	2 MGDG	
PsaK	86 (28-113)	4 Chl	2 BCR		
PsaL	159 (38-196)	5 Chl	4 BCR	1 MGDG	
PsaO	97 (30-126)	3 Chl	2 BCR	1 PG	
Lhca1 _{in}	194 (35-228)	14 Chl	1 Lut, 1 Vio, 1 BCR	1 PG	
Lhca1 _{out}	194 (35-228)	14 Chl	1 Lut, 1 Vio, 1 BCR	1 PG	
Lhca2	217 (28-244)	13 Chl	1 Lut, 1 Vio, 1 BCR	1 PG	
Lhca3	220 (44-263)	14 Chl	1 Lut, 1 Vio, 3 BCR	2 PG	
Lhca4	210 (55-264)	15 Chl	1 Lut, 1 Vio, 1 BCR	1 PG, 2 MGDG	
Lhca5	227 (31-257)	17 Chl	1 Lut, 1 Vio, 1 Neo, 1 BCR	2 PG, 1 MGDG	
Lhca6	230 (28-257)	17 Chl	1 Lut, 1 Vio, 1 Neo, 1 BCR	1 PG	
Lhca7	213 (29-241)	15 Chl	1 Lut, 1 Vio, 2 BCR	1 PG	
Lhca8	217 (28-244)	14 Chl	1 Lut, 1 Vio, 1 BCR	2 PG, 1 MGDG	
Lhca9	183 (28-210)	12 Chl	1 Lut, 1 Vio, 1 BCR	3 PG, 1 MGDG	
LHCII-1					
LhcbM1	232	8 Chl _a , 6 Chl <i>b</i>	2 Lut, 1 Vio, 1 Neo	1 PG	
LhcbM2	220	8 Chl _a , 6 Chl <i>b</i>	2 Lut, 1 Vio, 1 Neo	1 PG	
LhcbM3	220	8 Chl _a , 6 Chl <i>b</i>	2 Lut, 1 Vio, 1 Neo	1 PG	
LHCII-2					
LhcbM2	220	8 Chl _a , 6 Chl <i>b</i>	2 Lut, 1 Vio, 1 Neo	1 PG	
LhcbM3	219	8 Chl _a , 6 Chl <i>b</i>	2 Lut, 1 Vio, 1 Neo	1 PG	
LhcbM5	237	8 Chl _a , 6 Chl <i>b</i>	2 Lut, 1 Vio, 1 Neo	1 PG, 1 MGDG	
Total	5992	334	87	39	5

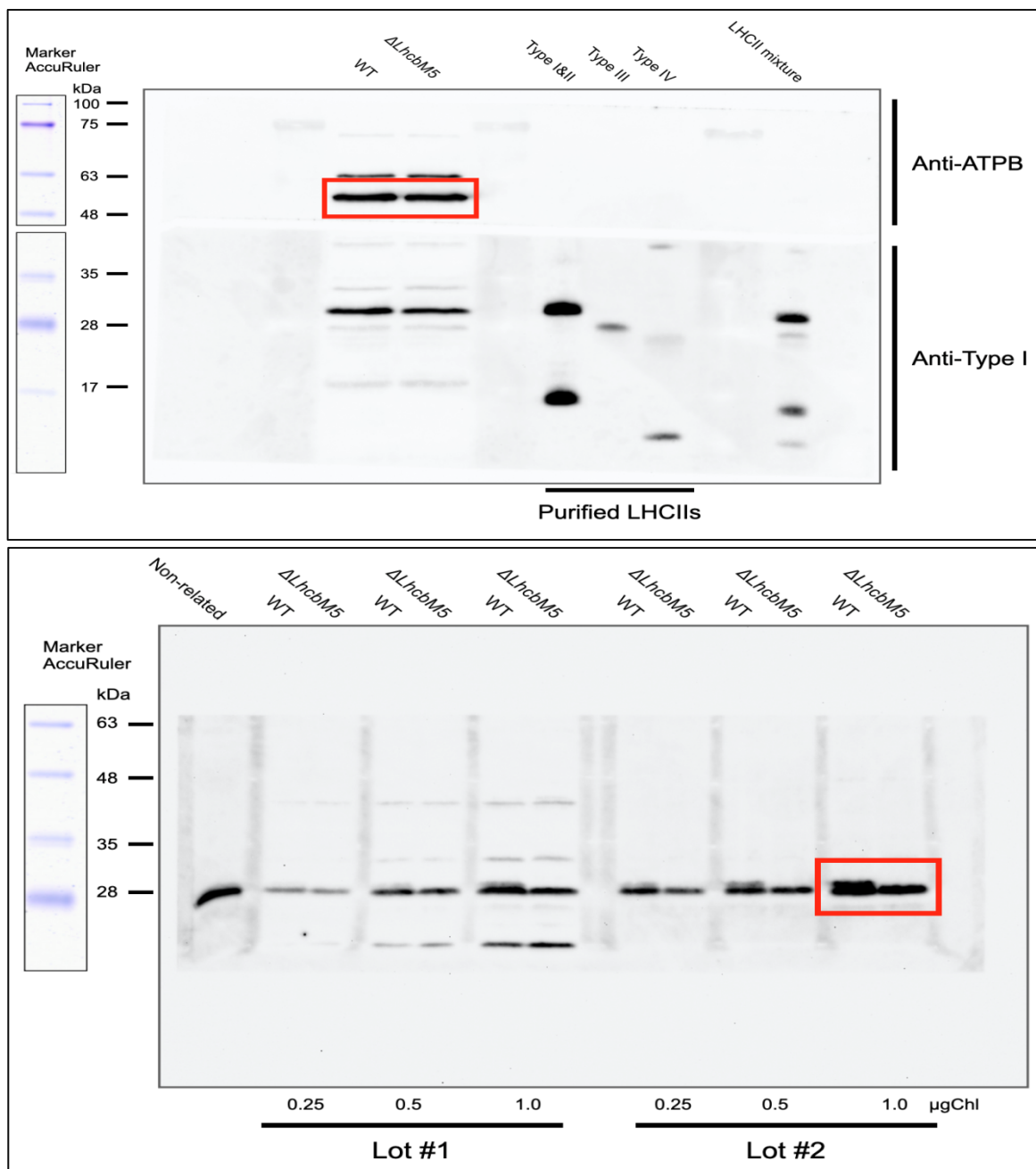
BCR, β -carotene; Lut, lutein; Vio, violaxanthin; Neo, neoxanthin; PG, phosphatidyl glycerol; DGDG, digalactosyldiacyl glycerol; MGDG, monogalactosyldiacyl glycerol; PQN, phyloquinone; SF4, sulphur-iron cluster.

Supplementary Table 2: Variation of the chlorophyll-chlorophyll distances at the interfaces between LHCII-1/LHCII-2 and PSI-LHCI in the top three multi-body motion components.

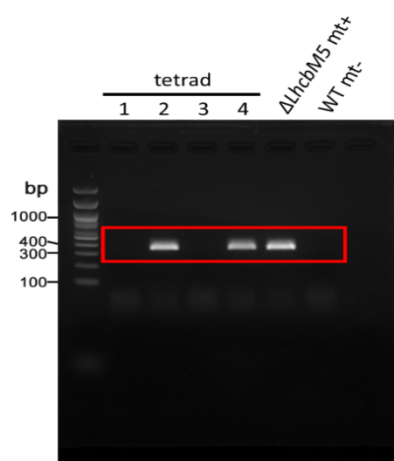
	Interfacial chlorophyll Pairs	Mg-Mg Distances (Å)		Distance(Bin1) - Distance(Bin10) (Å)
		Bin1	Bin10	
Component 1	604(a2)-605(bM5)	20.0	16.1	3.90
	606(a2)-604(bM5)	22.6	17.8	4.80
	609(a2)-610(bM5)	29.4	25.1	4.30
	203(H)-610(bM5)	27.5	25.4	2.10
	307(L)-611(bM5)	21.4	22.5	1.10
	307(L)-612(bM5)	22.5	21.9	0.60
	2001(O)-612(bM1)	18.5	20.3	1.80
	2001(O)-611(bM1)	19.5	19.8	0.30
	2001(O)-614(bM1)	19.5	18.4	1.10
	2002(O)-611(bM1)	19.9	23.7	3.80
Component 2	604(a2)-605(bM5)	17.8	18.3	0.50
	606(a2)-604(bM5)	20.2	20.6	0.40
	609(a2)-610(bM5)	27.6	27.3	0.30
	203(H)-610(bM5)	26.5	26.4	0.10
	307(L)-611(bM5)	21.8	21.7	0.10
	307(L)-612(bM5)	22.7	22.6	0.10
	2001(O)-612(bM1)	18.5	19.8	1.30
	2001(O)-611(bM1)	18.8	20.8	2.00
	2001(O)-614(bM1)	18.8	18.9	0.10
	2002(O)-611(bM1)	21.5	21.2	0.30
Component 3	604(a2)-605(bM5)	18.1	18.3	0.20
	606(a2)-604(bM5)	20.7	20.4	0.30
	609(a2)-610(bM5)	27.6	27.5	0.10
	203(H)-610(bM5)	26.4	26.7	0.30
	307(L)-611(bM5)	21.2	22.4	1.20
	307(L)-612(bM5)	22.3	23.2	0.90
	2001(O)-612(bM1)	19.6	18.7	0.90
	2001(O)-611(bM1)	20.4	19.2	1.20
	2001(O)-614(bM1)	18.8	18.9	0.10
	2002(O)-611(bM1)	20.8	21.7	0.90

Supplementary Movie 1: Analysis of the multi-body refinement result reveals the motion of two LHCII trimers relative to PSI-LHCI.

The movie contains three segments showing the three major types of motion (components 1-3) along eigenvectors #1-3. The densities of reconstructed bodies of LHCII-1, LHCII-2 and PSI-LHCI are repositioned along the eigenvectors to fit the relative motion as in the `relion_flex_analyse` program. The maps are superposed on the region of PSI-LHCI. Component 1 describes the in-plane rotation of the two LHCII trimers around their anchoring subunits (PsaH and PsaL) in PSI, while components 2 and 3 describes two different types of pivoting movement of LHCII trimers away from the membrane plane.



Additional Supplementary Fig. 1. Uncropped scans of blots in Supplementary Fig. 3b.



Additional Supplementary Fig. 2. Uncropped scan of gel in Supplementary Fig. 3c.