

Fig. S1. Identification of the Psb35 protein in the RC47 assembly complex isolated using Psb28-FLAG from the strain expressing Psb28-1-FLAG. The preparation isolated using a single step FLAG-specific affinity chromatography was analysed by 2D-CN/SDS-PAGE. After the first dimension the gel was photographed (1D color) and scanned for Chl fluorescence (1D fluor). After the separation in the second dimension the 2D gel was stained using Sypro Orange (2D SYPRO stain), blotted to a PVDF membrane (2D blot) and Psb35 was detected by the specific antibody. PSII(I) is the monomeric PSII core complex, RC47 is a PSII core complex lacking CP43 and RC47(2) is its dimeric form. 1 µg of Chl was loaded. As a control, the identical procedure was performed using WT strain (WT control).

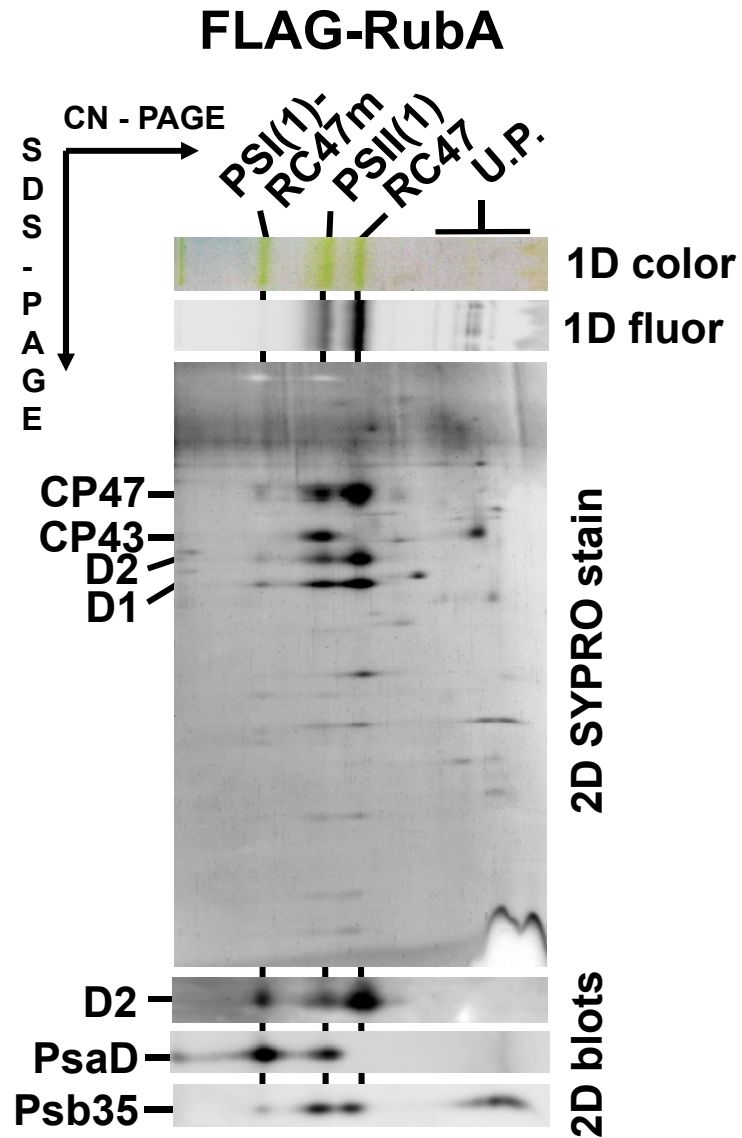


Fig. S2. Two-dimensional protein analysis of preparation isolated from the strain expressing FLAG-tagged RubA protein using FLAG-specific resin. Preparation was analysed by CN PAGE in the first dimension, the native gel was photographed (1D color) and scanned by LAS 4000 for Chl fluorescence (1D fluor), after SDS-PAGE in the 2nd dimension the gel was stained by SYPRO orange (2D SYPRO stain) and designated proteins were immunodecorated using specific antibodies. Designations of complexes as in Figs. 1 and 2. PSI(1)-RC47 is a complex containing monomeric PSI and RC47. 1 µg of Chl was loaded.

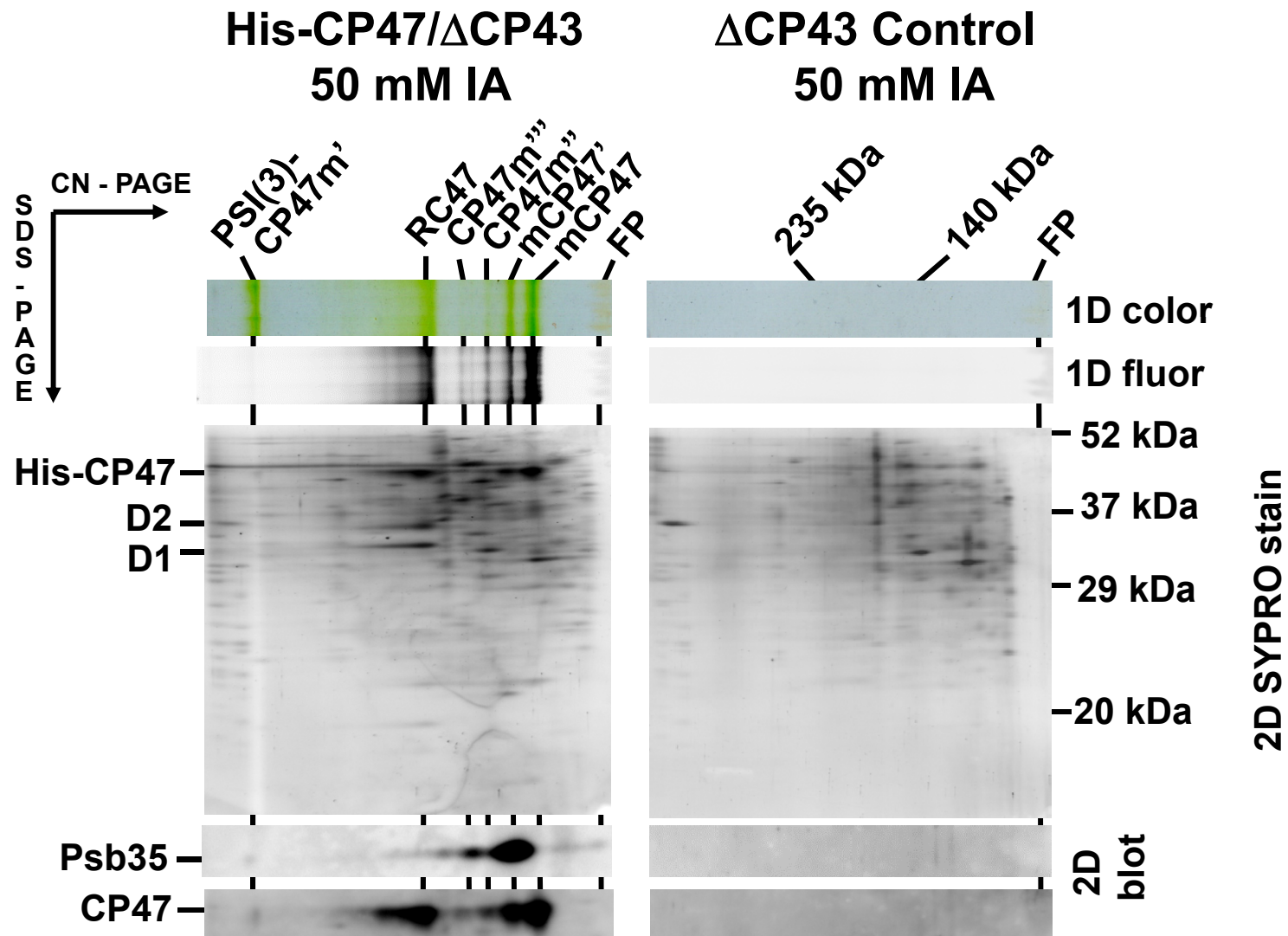


Fig. S3. Isolation of PSI(3)-His-CP47 complex from the preparation obtained by Ni-affinity chromatography of membrane complexes solubilized from membranes of a strain expressing the His-tagged CP47 in the CP43-less background. The preparation was analysed by 2D-CN/SDS-PAGE. After the first dimension the gel was photographed (1D color) and scanned for Chl fluorescence (1D fluor). After the separation in the second dimension the 2D gel was stained using Sypro Orange (2D SYPRO stain), blotted to a PVDF membrane (2D blot) and Psb35 and CP47 were detected by the specific antibody. As a control, the identical procedure was performed using the strain lacking CP43 (control). Designation of the complexes as in Figs. 1 and 2, CP47m' and CP47m'' are larger variants of CP47m associated with unknown components. The same volume of eluates corresponding to 1 μ g of Chl of the His-CP47/ Δ CP43 preparation were loaded.

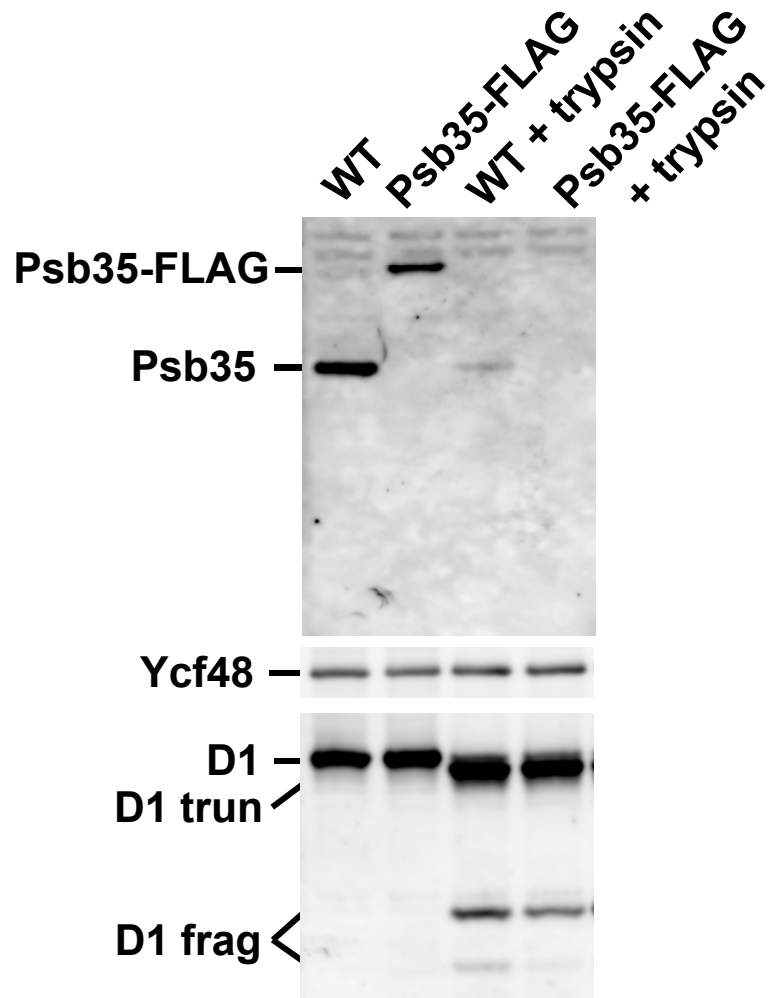
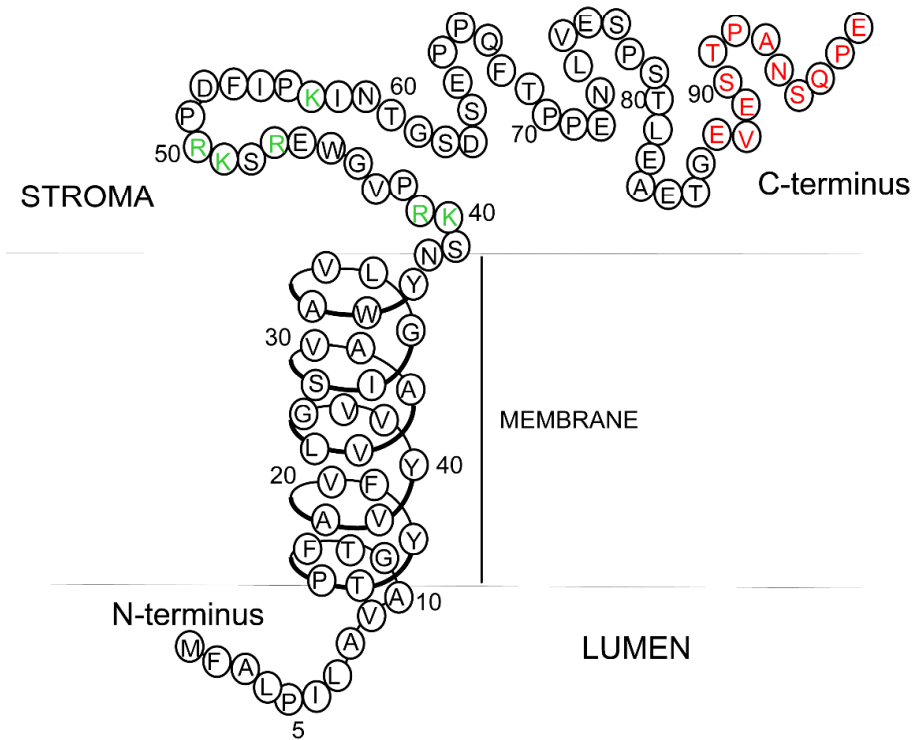
A**B**

Fig.S4. Identification of membrane orientation of the Psb35 protein (A) and its model (B). **A:** Right-side-out membrane vesicles of WT and Psb35-FLAG expressing strain before (left two lanes) and after (right two lanes) one hour trypsinization were analysed by SDS-PAGE, the gel was electroblotted and the Psb35, luminal Ycf48 and D1 were detected using specific antibodies. Each loaded sample contained 3 μ g of Chl. **B:** model of the Psb35 protein with basic amino acid residues cleavable by trypsin in green and sequence used as a peptide for the specific antibody production in red.

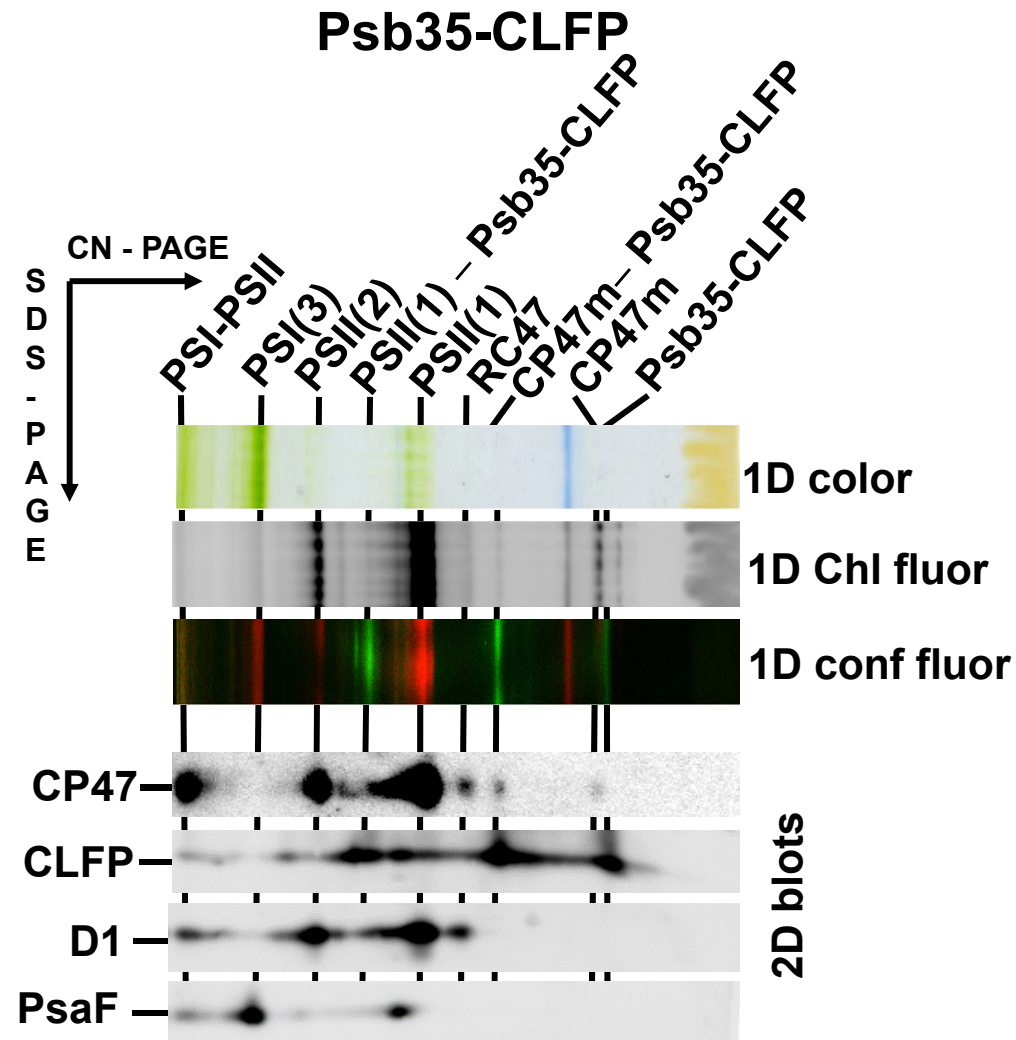


Fig. S5. Identification of the Psb35-CLFP protein in the membranes of the strain expressing Psb35-CLFP instead of Psb35. The membranes isolated from the strain were analysed by 2D-CN/SDS-PAGE. After the first dimension the gel was photographed (1D color) and scanned for Chl (1D Chl fluor) and CLFP (1D conf fluor). After the separation in the second dimension the 2D gel was stained using Sypro Orange, blotted to a PVDF membrane (2D blots) and CP47, CLFP, D1 and CP47 were detected by the specific antibodies. Designation of complexes as in Figs. 1 and 2. 4 μ g of Chl were loaded.

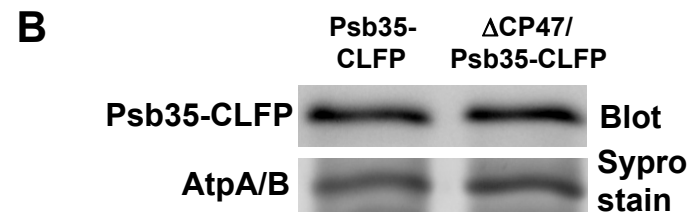
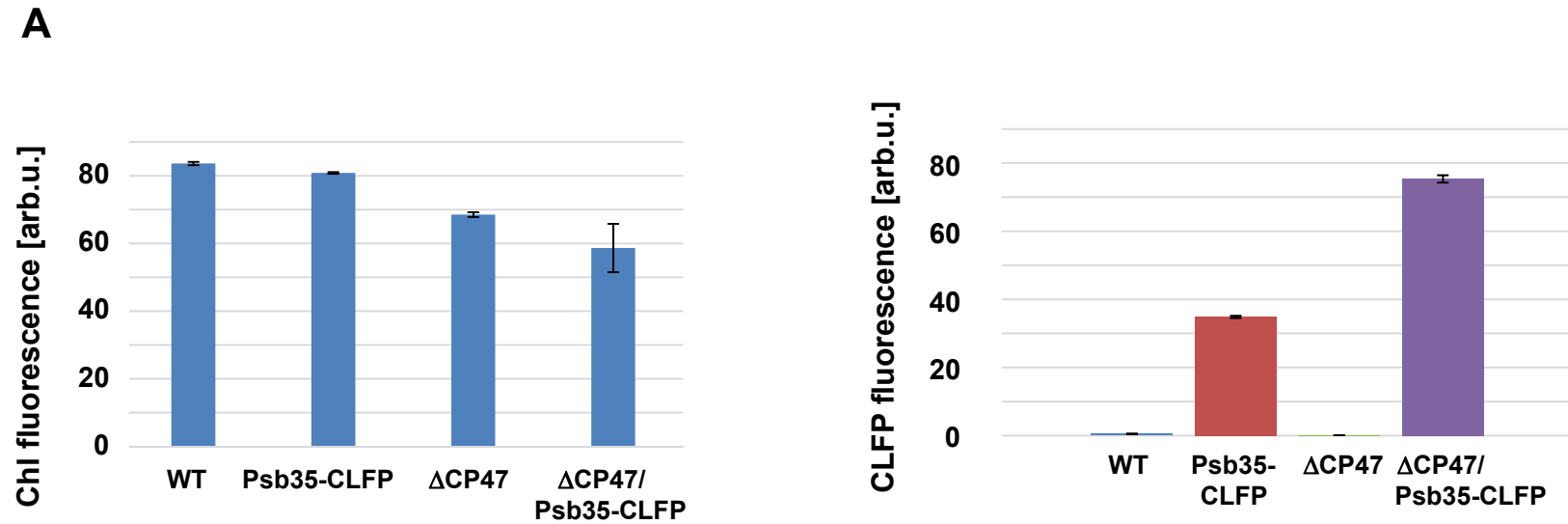


Fig. S6. Intensity of Chl and Psb35-CLFP fluorescence signals in WT, Psb35-CLFP, ΔCP47 and ΔCP47/Psb35-CLFP strains (A), and level of Psb35-CLFP in the single Psb35-CLFP and double ΔCP47/Psb35-CLFP mutants (B). **A:** The fluorescence intensity was calculated from the peaks obtained using the confocal microscope; **B:** The membranes isolated from the strains were analyzed using 1D SDS-PAGE, gel stained with SYPRO Orange and was electroblotted. The blot was probed using anti Psb35 antibody, stained AtpA/B band was used as a loading control. Sample were loaded on the same OD_{750 nm} basis.

A

Size of PCR product:

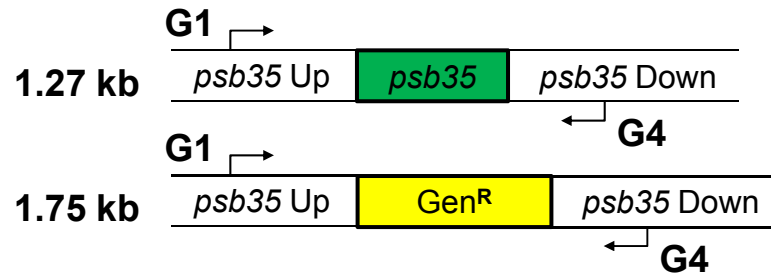
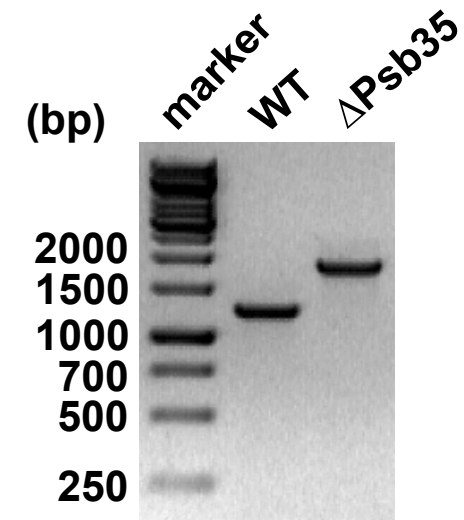
**B**

Fig. S7. Construction (A) and validation (B) of the *psb35* deletion strain Δ Psb35. **A:** Cartoons show the *psb35* locus before and after replacement of *psb35* with the gentamycin resistance cassette. Positions of the forward (G1) and reverse (G4) primers (Table S3) used for PCR genotyping are indicated. **B:** An agarose gel of PCR fragments is shown confirming the genotype of the strain.

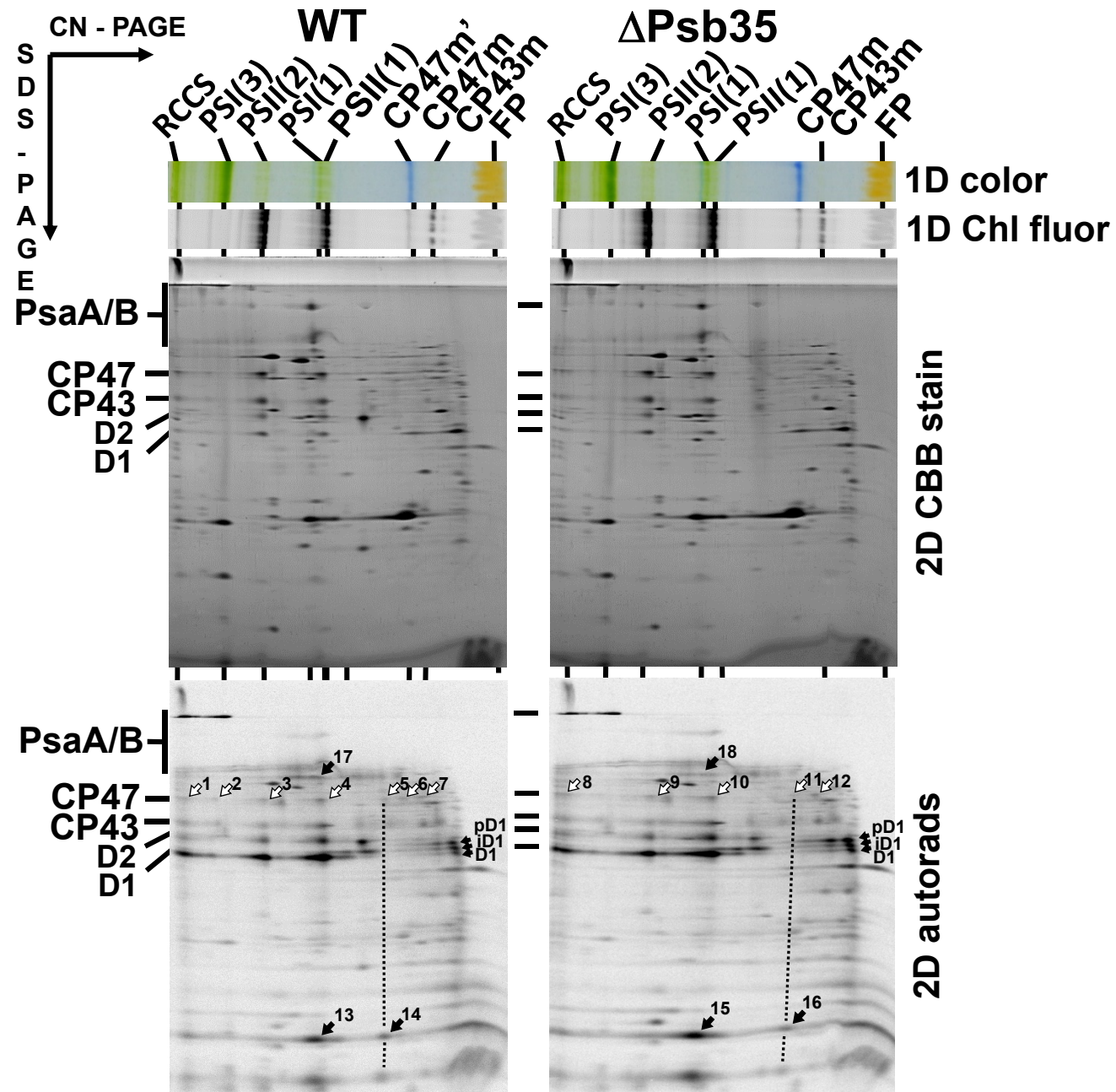
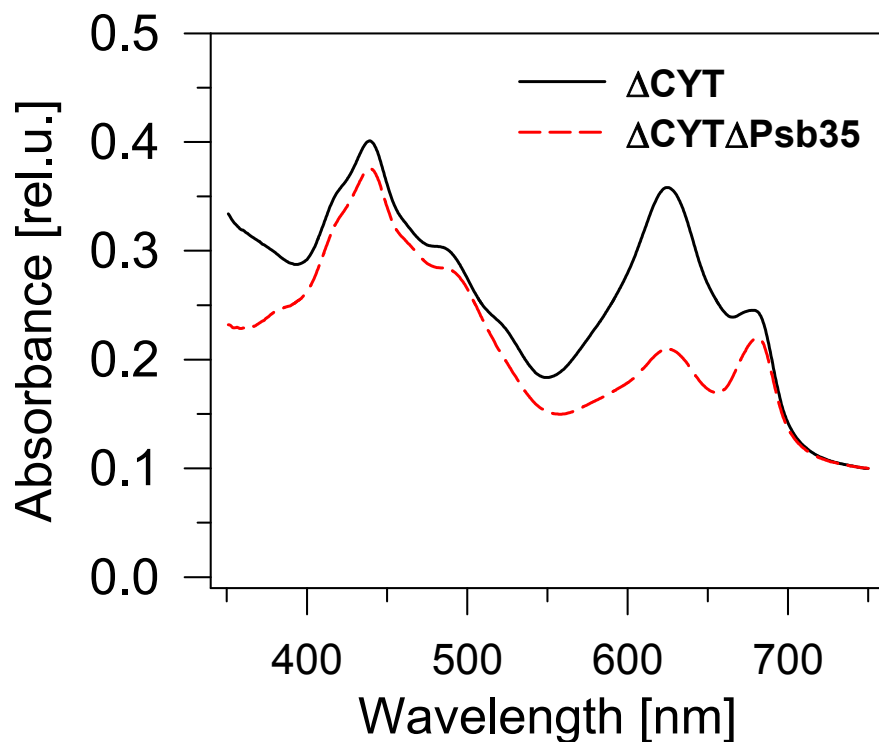


Figure S8. 2D analysis of radioactively labelled membrane proteins of WT and Δ Psb35 autotrophic strains. Membranes isolated from radioactively labelled cells were analysed by CN-PAGE in the first dimension (1D color; 1D fluor). After 2D SDS-PAGE the gel was stained (2D CBB stain) and the radiolabelled proteins were subsequently detected by autoradiography (2D autorads). Designation of complexes as described in Figs. 1 and 2; the white arrows (1-12) designate various labelled CP47 within various complexes, black single arrows (13-16) designate labelled HliA/B while other black arrows (17-18) designate labelled PsaA/B; arrow triplet designates different unassembled D1 forms, Each loaded sample contained 5 μ g of Chl.

A**B**

Compounds	$\Delta\text{CYT}/\Delta\text{Psb35}$ (% of ΔCYT)
Chl	76 ± 6
heme b	65 ± 16
CoPP	66 ± 16
PPIX	108 ± 20
MgP	70 ± 5
MgPME	135 ± 40
MV Pchlde	48 ± 9
DV Pchlde	42 ± 5
MV Chlide	86 ± 50
DV Chlide	95 ± 29

Fig. S9. The whole cell absorption spectra (A) and content of heme, chlorophyll and its biosynthesis precursors (B) of ΔCYT and $\Delta\text{CYT}\Delta\text{Psb35}$ strains normalized to the same optical densities at 750 nm. **A:** The spectra of liquid cultures of ΔCYT (solid black line) and $\Delta\text{CYT}\Delta\text{Psb35}$ (dashed red line) were measured using Shimadzu UV 3000 after their dilution to $\text{OD}_{750\text{ nm}} = 0.1$. **B:** The content of individual compounds determined by HPLC was normalized to the same $\text{OD}_{750\text{ nm}}$ and expressed as % of the control ΔCYT strain, numbers represent means of 3 of three biological replicates \pm SD. Abbreviations: Chl, chlorophyll; CoPP, coproporphyrin; PPIX, protoporphyrin IX; MgP, magnesium protoporphyrin IX; MgPME, magnesium protoporphyrin IX methyl ester; MV Pchlde, mono-vinyl-protochlorophyllide; DV Pchlde, di-vinyl-protochlorophyllide; MV Chlide, mono-vinyl-chlorophyllide; DV Chlide, di-vinyl-chlorophyllide.

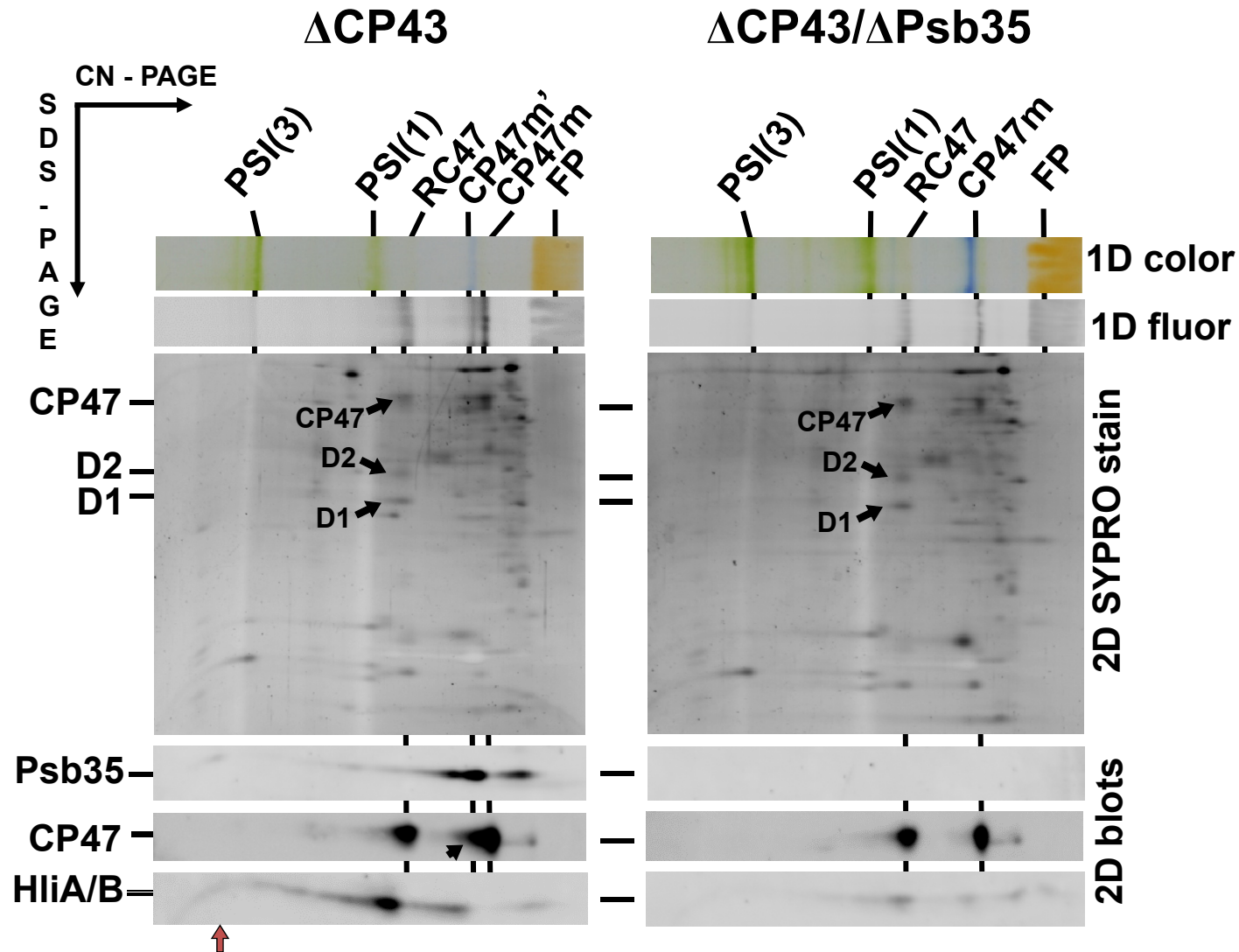


Figure S10. 2D analysis of membrane proteins of Δ CP43 and Δ CP43/ Δ Psb35. Membranes were analysed by 2D-CN/SDS-PAGE. After the first dimension the gel was photographed (1D color) and scanned for Chl fluorescence (1D fluor). After the separation in the second dimension the 2D gel was stained using Sypro Orange (2D SYPRO stain), blotted to a PVDF membrane (2D blots) and Psb35, CP47 and HliA/B were detected by the specific antibodies. Designation of complexes as described in Figs. 1 and 2; the black arrow designates CP47 within the CP47m', the red arrow PSI(3)-CP47m' complex. Each loaded sample contained 4 μ g of Chl.

A

MFALPILAVAGTFPTYFVAVVYVGLVAASIGLVAWYNSKRYPVWERSKRPDFIPKINTGSDSEPPQFPPENLVESPSTLEAETGEVESTPANSQPESAGGSGGSVSKGEELFTG
 VVPILVELDGDVNGHKFSVRGEGEGDATNGKLTGKLPVPWPTLVVTFYGVACFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGYKTRAEVKFEGDTLVNRIEL
 KGIDFKEDGNILGHKLEYNFNHSHYVYITADKQKNCIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSHQSKLSKDPNEKRDHMLLEFVTAAGITHGMDELKY

B

CATCATTGGCGGAGGAGCTGATAGGGGCGCTGATGAGGGTGGGATGCATAGAAAAAGACAGTCAGGGGGACTTCGTAGGGTAGCGGAACAAACTGACAGGGTCAA
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 GCACCAAGTAAGCATACATGCCCCCGTTTGGGCTTCAAATGCGCCCCAGGGGCCGTTAGCGGACCACCTCTGTGCCATCCCCCTACCGCCACTGCCCAAC

Fig. S12. Amino acid sequence of the Psb35-CLFP protein (A) and the nucleotide sequence of the synthetic DNA construct used for the transformation (B). **A:** The Psb35 protein (in magenta) was fused with the mClover3 fluorescent protein (Bajar et al. Sci Rep. 16, 20889, 2016; in yellow) via SAGGSGGSV linker (in bluegreen). **B:** The synthetic construct (Genscript, USA) contained 500 bp of flanking upstream region of the *psb35* gene (no marking) followed by the nucleotide sequence of the *psb35* gene (in magenta), linker (in bluegreen), mClover3 (in yellow), Ery cassette including promoter (in grey) and 500 bp of flanking downstream region of the *psb35* gene (no marking).

Supplemental Table S1. The list of *Synechocystis* strains used in the study (related to the Material and Methods section). Gene IDs are derived from the cyanobacterial genome database (cyanobase; <http://genome.annotation.jp/>; CyanoBase)

strain	description	reference
WT	<i>Synechocystis</i> GT-P	(Tichy, Beckova et al. 2016)
ΔPsb35	Δ <i>ssl2148</i>	this study
Psb35-FLAG	Δ <i>ssl2148</i> / <i>psbA2_{pro}</i> : <i>ssl2148-flag</i>	this study
Psb35-CLFP	Δ <i>ssl2148</i> / <i>psbA2_{pro}</i> : <i>ssl2148-mClover gfp</i>	this study
ΔCP47	Δ <i>sll0906</i>	(Eaton-Rye and Vermaas 1991)
ΔCP47/Psb35-FLAG	Δ <i>sll0906</i> /Δ <i>ssl2148</i> / <i>psbA2_{pro}</i> : <i>ssl2148-flag</i>	this study
ΔCP47/Psb35-CLFP	Δ <i>sll0906</i> /Δ <i>ssl2148</i> / <i>psbA2_{pro}</i> : <i>ssl2148-mClover gfp</i>	this study
ΔCP47/ΔCP43/His-CP47	Δ <i>sll0906</i> /Δ <i>sll0851</i> / <i>his-sll0906</i>	(Boehm et al. 2012)
ΔD1/ΔPsbH/ΔCYT/ΔFtsH2/His-PsbH	Δ <i>slr1311</i> /Δ <i>slr1867</i> /Δ <i>slr0228</i> /Δ <i>ssr3451</i> /Δ <i>smr0006-8</i> / <i>his-ssl2598</i>	(D'Haene et al. 2015)
ΔCP47/ΔD1//ΔPsbH/ΔFtsH2/His-CP47	Δ <i>sll0906</i> /Δ <i>ssl2598</i> /Δ <i>slr1311</i> /Δ <i>slr1867</i> /Δ <i>slr0228</i> // <i>his-sll0906</i>	(D'Haene et al. 2015)
ΔCP43	Δ <i>sll0851</i>	(Vermaas et al. 1988)
ΔCP43ΔPsb35	Δ <i>sll0851</i> Δ <i>ssl2148</i>	(Boehm et al. 2011)
ΔCP43/ΔD1/His-CP43	Δ <i>sll0851</i> /Δ <i>slr1311</i> /Δ <i>slr1867</i> / <i>His-sll0851</i>	this study
FLAG-RubA	Δ <i>slr2033</i> / <i>psbA2_{pro}</i> : <i>flag-slr2033</i>	(Kiss et al. 2019)
Psb28-FLAG	Δ <i>sll1398</i> / <i>sll1398-flag</i>	(Bečková et al. 2017)
ΔPSI	Δ <i>slr1834</i> /Δ <i>slr1835</i>	(Shen et al. 1993)
ΔCYT	Δ <i>ssr3451</i> /Δ <i>smr0006-8</i>	(Pakrasi et al. 1988)
ΔCYTΔPsb35	Δ <i>ssr3451</i> Δ <i>smr0006-8</i> /Δ <i>ssl2148</i>	this study

Supplementary Table S2. List of protein bands identified in the isolated Psb35-FLAG preparation by MS and Western blotting after the analysis using 2D CN/SDS PAGE (see Fig.2).

Protein (Kazusa annotation)	Size (Da)	MS coverage (%)	Detected/theoretical no. of peptides	PLGS score	Western blot detection
CP47, PsbB (Slr0906)	55903				+
CP43, PsbC (Sll0851)	50303				+
D2, PsbD (Sll0849)	39493				+
D1, PsbA (Slr1311)	39721				+
Cyt <i>b</i>₅₅₉ α, PsbE (Ssr3451)	9442				+
Cyt <i>b</i>₅₅₉ β, PsbF (Smr0006)	4929				+
PsbH (Ssl2598)	7116				+
Psb35 (Ssl2148)	10556				+
Ycf48 (Slr2034)	37291				+
RubA (Slr2033)	12570	23	1/9	8	+
Psb27 (Slr1645)	14776	7	1/15	8	+
Psb28 (Sll1398)	12582	75	5/11	1161	-
Psb28-2 (Slr1739)	14103	29	3/11	246	-
PsaF (Sll0819)	18237	29	3/11	636	-

Supplementary Table S3. The list of primers used in the study

Strain	Primer name	Primer sequence
Δ Psb35	<i>psb35-G1</i>	tagggcgatggagttcactt
	<i>psb35-G2</i>	gttcggtcaaggttctggacCCAGGATGGGTAGAGCAAA C*
	<i>psb35-G3</i>	caattcgttcaagccgagatGAAACTGGGGAGGTGGAAT C*
	<i>psb35-G4</i>	cgacagagtgggccgtaac
Psb35-FLAG	<i>psb35_NdeI</i>	ctagagcatatgttgctctacctcctggc
	<i>psb35_NheI</i>	gaggacgctagcttccggctgagaattggcagg

*Capital letters indicate the sequence to amplify gentamycin cassette

Supplementary list of references:

1. Bajar, B.T., Wang, E.S., Lam, A.J., Kim, B.B., Jacobs, C.L., Howe, E.S., et al. (2016) Improving brightness and photostability of green and red fluorescent proteins for live cell imaging and FRET reporting. *Sci. Rep.* 16: 20889.
2. Bečková, M., Gardian, Z., Yu, J., Konik, P., Nixon, P.J. and Komenda, J. (2017) Association of Psb28 and Psb27 proteins with PSII-PSI supercomplexes upon exposure of *Synechocystis* sp. PCC 6803 to high light. *Mol. Plant* 10: 62-72.
3. Boehm, M., Romero, E., Reisinger, V., Yu, J., Komenda, J., Eichacker, L.A., et al. (2011) Investigating the early stages of photosystem II assembly in *Synechocystis* sp. PCC 6803: isolation of CP47 and CP43 complexes. *J. Biol. Chem.* 286: 14812-14819.
4. Boehm, M., Yu, J., Reisinger, V., Bečková, M., Eichacker, L.A., Schlodder, E., et al. (2012) Subunit composition of CP43-less photosystem II complexes of *Synechocystis* sp. PCC 6803: implications for the assembly and repair of photosystem II. *Phil. Trans. R. Soc. B.* 367: 3444-3454.
5. D'Haene, S.E., Sobotka, R., Bučinská, L., Dekker, J.P. and Komenda, J. (2015) Interaction of the PsbH subunit with a chlorophyll bound to histidine 114 of CP47 is responsible for the red 77K fluorescence of Photosystem II. *Biochim. Biophys. Acta* 1847: 1327-1334.
6. Eaton-Rye, J.J. and Vermaas, W.F. (1991) Oligonucleotide-directed mutagenesis of *psbB*, the gene encoding CP47, employing a deletion mutant strain of the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant. Mol. Biol.* 17: 1165-1177.
7. Kiss, E., Knoppová, J., Aznar, G.P., Pilný, J., Yu, J., Halada, P., et al. (2019) A photosynthesis-specific rubredoxin-like protein is required for efficient association of the D1 and D2 proteins during the initial steps of photosystem II assembly. *Plant Cell* 31: 2241-2258.
8. Pakrasi HB, Williams JGK, Arntzen CJ (1988) Targeted mutagenesis of the *psbE* and *psbF* genes blocks photosynthetic electron transport: evidence for a functional role of cytochrome b559 in photosystem II. *EMBO J.* 7: 325-332.
9. Shen, G., Boussiba, S. and Vermaas, W.F.J. (1993) Strains lacking photosystem I and phycobilisome function. *Plant Cell* 5: 1853-1863
10. Tichý, M., Bečková, M., Kopečná, J., Noda, J., Sobotka, R. and Komenda, J. (2016) Strain of *Synechocystis* PCC 6803 with aberrant assembly of photosystem II contains tandem duplication of a large chromosomal region. *Front. Plant Sci.* 7: 648.
11. Vermaas, W.F.J., Ikeuchi, M. and Inoue, Y. (1988) Protein composition of the photosystem II core complex in genetically engineered mutants of the cyanobacterium *Synechocystis* sp. PCC 6803. *Photosynth. Res.* 17: 97-113