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Structural revelations of photosynthesis' membrane protein complexes

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Supplementary Material:

The composition and structure of photosystem I in the moss *Physcomitrella patens*

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PpPsaD-1      MAALAATSAAIALAIPQASSGASTS-RSVNLRLSGIQGQKLAAVCNGSR
PpPsaD-2      MAALAATSAAASVALIAPQASSGAAVSTRGVSLRVSSLQGQRLVAVCNHR
PpPsaD-3      MAAFASTAATTLAIAAPSSVAASQLR-VNGSLKVSFAFQGQKFAAACNGSR
PpPsaD-4      MAAIASTAITTTVAIASPSSVAVSRPR-AAASLAISGFQGQKLASVCNGGR
                ***:***: :::*  *.: : :      . * :*.:***:..:..** . *
PpPsaD-1      VVAKAAGAAPDATA-----EAPKGFTPPTLNADTPAPIFGGSTGGLLR
PpPsaD-2      VVA-KAAAAPDAAA-----EAPKGFTPPTLNADTPAPIFGGSTGGLLR
PpPsaD-3      LVMAQAGAAPDGVADQKPDKEAPKGFTPPTLNADTPAPIFGGSTGGLLR
PpPsaD-4      VTMAQAGAVPDGVADRKPDKEAPKGFTPPTLNADTPAPIFGGSTGGLLR
                :.  *.*.*.*.*      *****
PpPsaD-1      KAQVEEFYVITWESPKEQIFEMPTGGAAIMRSGPNLLKLARKEQCLALGA
PpPsaD-2      KAQVEEFYVITWESPKEQIFEMPTGGAAIMRSGPNLLKLARKEQCLALGA
PpPsaD-3      KAQVEEFYVITWESPKEQIFEMPTGGAAIMRSGPNLLKLARKEQCLALGA
PpPsaD-4      KAQVEEFYVITWESPKEQIFEMPTGGAAIMRSGPNLLKLARKEQCLALGA
                *****
PpPsaD-1      RLRTKFKIQYQFYRVFPNGEVQYLHPKDGVPYPEKVNAGRSTAVGVNNRSIG
PpPsaD-2      RLRTKFKIQYQFYRVFPNGEVQYLHPKDGVPYPEKVNAGRSPVGVNNRSIG
PpPsaD-3      RLRTKFKIQYQFYRVFPNGEVQYLHPKDGVPYPEKVNAGRSPVGVNNRSIG
PpPsaD-4      RLRTKFKIQYQFYRVFPNGEVQYLHPKDGVPYPEKVNAGRSPVGVNNRSIG
                *****:.* *****
PpPsaD-1      QNANPAELKFAHKQAYDL
PpPsaD-2      KNANPAELKFAQKQAYDL
PpPsaD-3      KNANPAELKFAQKQAYDL
PpPsaD-4      KNANPAELKFAHKQAYDL
                : *****:*****

```

Fig. S1 Sequence comparison of PsaD isoforms of *P. patens*

Alignment of full length protein sequences of the four different isoforms for PsaD of *P. patens*. Sequence data were obtained from www.cosmos.org, v1.6 PpPsaD-1 (Pp1s4_321V6.1), PpPsaD-2 (Pp1s77_69V6.1), PpPsaD-3 (Pp1s1_788V6.1), PpPsaD-4 (Pp1s107_188V6.1). Sequence alignment was created using ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) with default settings.

Green indicates the chloroplast localization sequence as predicted by ChloroP (Emanuelsson et al., 1999).

```

AtPsaF      -MSLTIPAN-----LVLNP-----RSNKSLTQS---VPKSSARFVCSDD--KSSSSTP 42
GsPsaF      -----MM 2
CrPsaF      -MALTMRN-----PAVKASSRVAPSSRRALRVACQAQ---KNETAS 37
S_PCC6803   -----
PpPsaF-1    MASFTMAAA-IAPAGLVAPL--DLSSRTAISGSRASAFVKNSKARTTCSASSVDETTVA 57
PpPsaF-2    MASVTMAAAIAPVGLAANL--DLSSRAAISGTRTSMFVKNTKARTVCSAS-ADETATVA 57
PpPsaF-3    MASVTMAA--IAPAGLVAPLCKDVSSRTAISGARASVFKSSKARIVCSTS-ADETSTVA 57
PpPsaF-4    -----

AtPsaF      QSMKAFSAVALSSILLSAPMP---AVADISGLTPCKDSKQFAKREKQQIKKLESSLKLY 99
GsPsaF      KINRFILYVILFSSILSFSTNN--LVQAEFNNLIPCKESKIFNKRLESTIKKLENKLSKY 60
CrPsaF      KVGTLAASALAAAVSLSAPSA---AMADIAGLTPCSESKAYAKLEKKELKTLEKRLKQY 94
S_PCC6803   -MKHLLALLLAFTLWFNFAPSA---SADDFANLTPCSENPAYLAKSKNFLNTTN----- 50
PpPsaF-1    QAAGKFATALALAAALVGGSDMVVPEAKADVAGLTPCKESKGFAPKREKQEIKKLESRLKLY 117
PpPsaF-2    QTAGKFATALALAAIVGGSDMVVPEARADVAGLTPCKESKGFAPKQKQEIKKLESRLKLY 117
PpPsaF-3    ETAGKFATALALAAVVGSSDFVVPDARADVAGLTPCKESKGFAPKQKQEIKKLEGLRLKLY 117
PpPsaF-4    -----MSHRLQGFAPKQKQEIKKLEGLRLKLY 26

AtPsaF      APESAPALALNAQIEKTKRRFDNYGKYGLLCGSDGLPHLIVNG----DQRHWGEFITPGI 155
GsPsaF      EVGSSSYLAIKNTINKTNNRFHKYMEGVLGCKDGLPHLIADG----RWSHAGEFVIPS 116
CrPsaF      EADSAPAVALKATMERTKARFANYAKAGLLCGNDGLPHLIADPGLALKYGHAGEVFIP 154
S_PCC6803   DPNSG-----KIRAERYAS--ALCGPEGYPHLIVDG----RFTHAGDFLIPSI 92
PpPsaF-1    APDSAPALALNATIEKTKRRFAFYGNEGLLCGTDGLPHLIVDG----DQAHLGEFVYPGL 173
PpPsaF-2    APDSAPALAINATIEKTKRRFEFYGNQGLLCGTDGLPHLIVDG----DQAHLGEFVYPGL 173
PpPsaF-3    APDSAPALAINATIEKTKRRFEFYGNQGLLCGTDGLPHLIVDG----DQAHLGEFVYPGL 173
PpPsaF-4    APDSAPALAINATIEKTKRRFEFYGNQGLLCGTDGLPHLIVDG----DQAHLGEFVYPGL 82
          * . : * * . *** : * ****. : * * :.. * :
AtPsaF      LFLYIAGWIGWVGRSYLIAISGEKKPAMKEIIIDVPLASRIIFRGFIWPVAAAYREFLNGD 215
GsPsaF      LFIYISGWIGWVGRGYLSAIKNTNKAIENEIIIDVPLALKFMSSGFIWPLSALREYTKGD 176
CrPsaF      GFLYVAGYIGYVGRQYLIAVKGAEKPTDKEIIIDVPLATKLAWQGAGWPLAAVQELQRGT 214
S_PCC6803   LFLYIAGWIGWVGRSYLIEIRESKNPEMQEVVINVPLAIKKMLGGFLWPLAAVGEYTS 152
PpPsaF-1    VFLYIAGWIGWVGRAYLIDVRTSKKPTEKEIIIDVPLALRIMSKGLTWPVAAIGELRSGK 233
PpPsaF-2    VFLYIAGWIGWVGRAYLIDVRTSKKPTEKEIIIDVPLALRVMSKGLTWPLAAIGELRSGK 233
PpPsaF-3    VFLYIAGWIGWVGRAYLIDVRTSKKPTEKEIIIDVPLALRIMSKGLTWPVAAIGELRSGK 233
PpPsaF-4    VFLYIAGWIGWVGRAYLIDVRTSKKPTEKEIIIDVPLALRVMSKGLTWPLAAIGELRSGK 142
          *: * : : * : * : * : * : * : * : * : * : * : * : * : * :
AtPsaF      LIAKDV----- 221
GsPsaF      LLMKDSEVTISPR 189
CrPsaF      LLEKEENITVSPR 227
S_PCC6803   LVMKDSEIPTSPR 165
PpPsaF-1    LVEKSANITVSPR 246
PpPsaF-2    LVEKSGNITVSPR 246
PpPsaF-3    LVEKSSNITVSPR 246
PpPsaF-4    LVEKSANITVSPR 155
          * : * .

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Fig. S2 Alignment of full length PsaF proteins from different species

Sequences for *Physcomitrella* are obtained from www.cosmoss.org, v1.6 PpPsaF-1 (Pp1s19_276V6.1), PpPsaF-2 (Pp1s345_25V6.1), PpPsaF-3 (Pp1s121_54V6.1), PpPsaF-4 (Pp1s80_23V6.1). Other sequences obtained from NCBI *Arabidopsis thaliana* :AtPsaF (NP_174418), *Galdieria sulphuraria* GsPsaF (ADO32970.1), *Chlamydomonas reinhardtii* CrPsaF (P12356), *Synechocystis* PCC6803 S_PCC6803 (P29256).

Alignment was created using ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) using default settings.

```

Pp1s74_135V6.1      MAAMATTMSVATVRQFEGLKATTSSFSKPLPSLALRKTAGKGALGARC  

Pp1s5_384V6.1      MAAVAATMSVATVRQFEGLKATTS-FSKPVPSLVMKKSSGKGALGARC  

Pp1s5_396V6.1      MATVAATMSVATVHLFEGLKSTTC-FSKPIPSLAS-----CDYIGS  

                    *:.*:*****: *****:*. ***:***. *****
Pp1s74_135V6.1      ASTTLMFAGRFGLAPSANRKSTAGLKLVDKDSGLQTGDPAGFTATDTLACGALGHVIGV 120
Pp1s5_384V6.1      ASTTLMFAGRFGLAPSANRKSTAGLKLVDKDSGLQTGDPAGFTATDTLACGAMGHVIGV 119
Pp1s5_396V6.1      ASTTLMFAGRFGLAPSANRKSTAGLKLVDKDSGLQTGDPAGFSATDTLACGAMGHVIGV 107
                    *****:*****:*****
Pp1s74_135V6.1      GIVLGLKATAGL 132
Pp1s5_384V6.1      GIVLGLKATAGL 131
Pp1s5_396V6.1      GIVQGLKATGGL 119
                    *** *****.*

```

Fig. S3 Alignment of the three different isoforms of PsaK of *P. patens*

Green indicates predicted chloroplast localization sequence (predicted by ChloroP, Emanuelsson et al., 1999). Yellow indicates tryptic peptides that were identified with MS/MS analysis (see Supplemental Table 1). Arginine and lysin residues in bold, indicating potential tryptic cleavage sites.

Alignment was done with ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) using default settings. Sequences were obtained from www.cosmos.org, V1.6

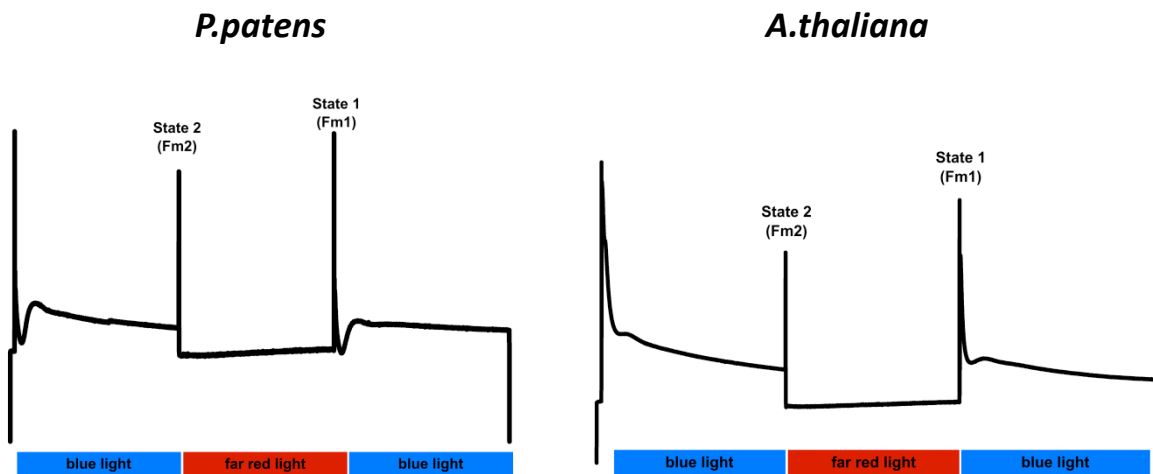


Fig. S4: Traces of state transition experiment

Chlorophyll fluorescence measurements to assess the state 1/state 2 transition. State II was induced by illumination of either *Arabidopsis* leafs or *Physcomitrella* protonema with blue light for 20 min followed by a saturation pulse to determine the maximal fluorescence (Fm2). State I was induced by illumination of the sample with far red light for 20 min followed by a saturation pulse to determine the maximal fluorescence (Fm1).

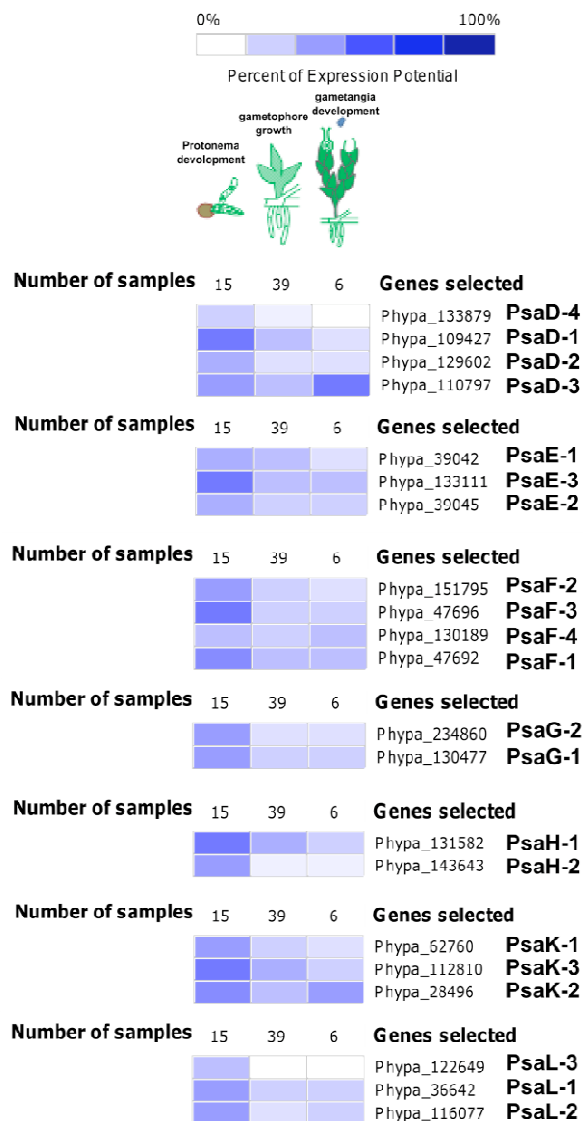
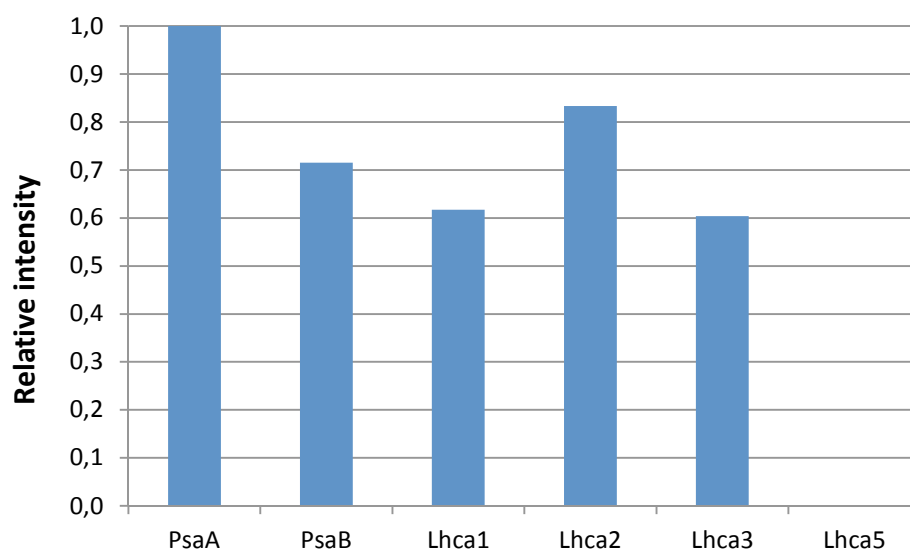


Fig. S5: Expression data from GENEVESTIGATOR for selected isoforms

Visualization of the expression profile of isoforms of PSI core subunits using the “Development” tool of Genevestigator (www.genevestigator.com). All 63 available datasets for *P. patens* were included.



Protein name	Area	Relative intensity %
PsaA	5.39E+09	1.000000
PsaB	3.85E+09	0.715161
Lhca1	3.33E+09	0.617239
Lhca2	4.49E+09	0.833364
Lhca3	3.25E+09	0.603952
Lhca5	1.77E+06	0.000329

Fig. S6 Label free quantification of PSI-LHCI proteins in *P. patens*

The label free quantification (Wong and Cagney 2010) were based on unique peptides from PsaA, PsaB, Lhca1, Lhca2, Lhca3 and Lhca5 (including isoforms).

Materials and Methods:

The preliminary label free quantification was achieved using the Proteome Discoverer software (version 1.3.0.339). Peptide areas were manually extracted from the data using only protein unique peptides as extraction criteria. Areas from the different isoforms of Lhca1, Lhca2, Lhca3 and Lhca5 were pooled so only one Lhca1, Lhca2, Lhca3 and Lhca5 appeared. The data analysis was based on the eleven raw files generated from the LC-MS/MS of peptides extracted from the in-gel digested PSI proteins. The samples were analysed once and from only one replica and the quantification data is therefore to be considered preliminary.

References:

Wong, J. W., and G. Cagney. 2010. An overview of label-free quantitation methods in proteomics by mass spectrometry. *Methods Mol Biol* 604:273-83.

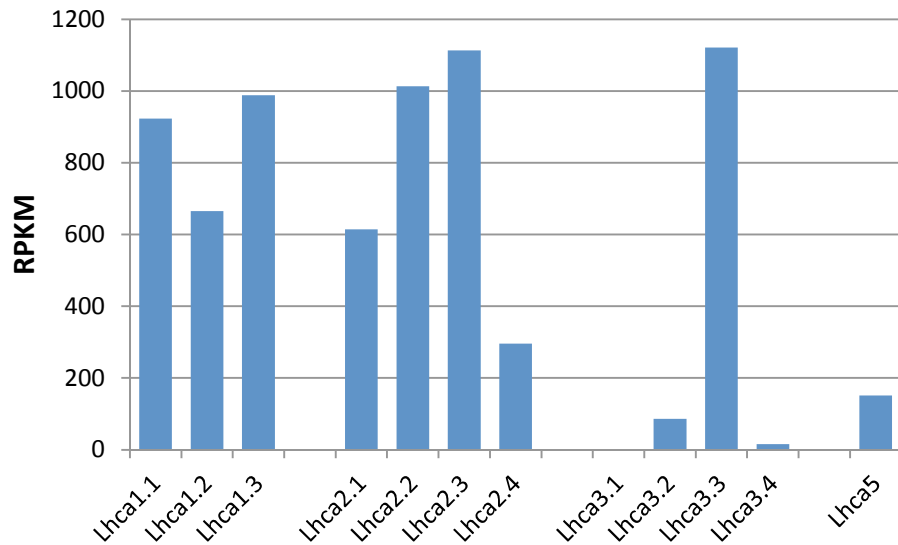


Figure S7: Expression profile of light harvesting proteins in *Physcomitrella patens*.

Shown are reads per million per kb (RPKM) of the different genes encoding light harvesting proteins of *P. patens*. Samples are derived from *P. patens* cultures grown under standard conditions.

Material and Methods:

Six days old protonema was transferred on PhyB media and incubated for 8 hours under standard conditions (50 μ E white light, 22°C). Subsequently protonemal tissue was harvested, excess water was removed by pressing the tissue between tissue and the material was frozen in liquid nitrogen and stored at -80°C until further use. RNA was isolated using the RNeasy Plant Mini Kit (Qiagen) following the suppliers manual.

Isolated RNA was send to GATC Biotech (Germany) for cDNA library synthesis and Illumina sequencing (Illumina HiSeq 2000, 50bp single runs). Raw data were processed and mapped against the *P. patens* transcriptome (P.patens.V1.2.2_CGI_transcripts.seq, downloaded from www.cosmos.org) by GATC Biotech.

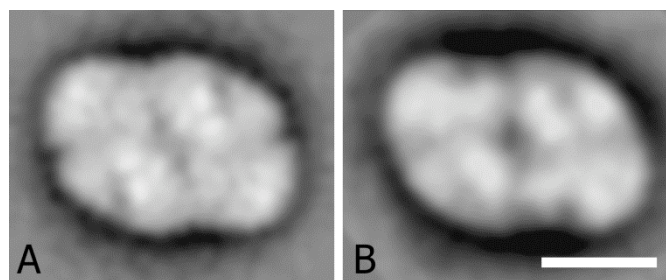


Fig. S8 Transmission electron microscopy and single particle analysis of isolated PSII-LHCII complex of *P. patens*

(A) Averaged projection map of *P. patens* PSII core particles, derived from 180 particles. (B) cyanobacterial PSII for comparison, from a previous investigation (Kuhl et al. 1999). Two-fold rotational symmetry was imposed on the images after analysis.

Reference:

H. Kuhl, M. Rögner, J.F.L. van Breemen and E.J. Boekema (1999) Localization of cyanobacterial PS II donor-side subunits by electron microscopy and the supramolecular organization of PS II in the thylakoid membrane. Eur. J. Biochem. 266, 453-460.

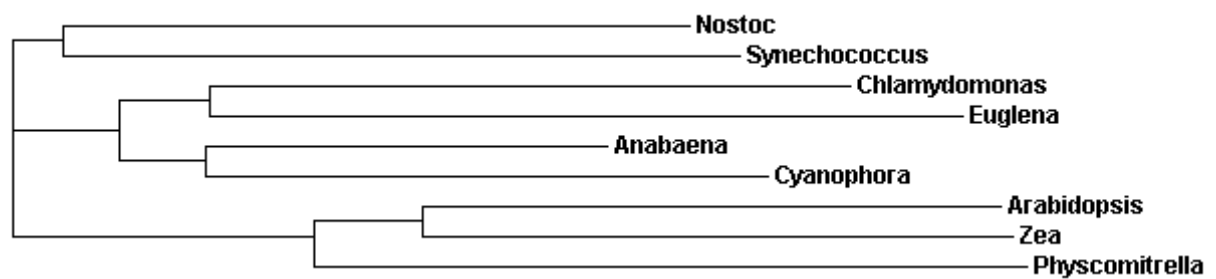


Fig. S9: Phylogenetic tree based on the protein sequence of cytochrome c6 sequences
Tree was constructed using ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) using default settings with sequences obtained from NCBI.