

Supplementary Material for

**Molecular evolution of far-red light-acclimated photosystem II**

Christopher J. Gisriel<sup>1,†</sup>, Tanai Cardona<sup>2,†</sup> Donald A. Bryant<sup>3</sup>, and Gary W. Brudvig<sup>1,4,\*</sup>

**Affiliations:**

<sup>1</sup>Department of Chemistry, Yale University, New Haven, CT 06520, USA.

<sup>2</sup>Department of Life Sciences, Imperial College London, London SW7 2AZ, UK

<sup>3</sup>Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA.

<sup>4</sup>Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA.

†These authors contributed equally to this work.

\*Correspondence: [gary.brudvig@yale.edu](mailto:gary.brudvig@yale.edu)

**Supplementary Figure S1.** Phylogenetic tree of PsbA sequences focused on the FRL sequences and basal clades.

**Supplementary Figure S2.** Phylogenetic tree of PsbD sequences focused around the FRL sequences and basal clades.

**Supplementary Figure S3.** Phylogenetic tree of PsbC sequences focused around the FRL sequences and basal clades.

**Supplementary Figure S4.** Phylogenetic tree of FRL-PsbB and neighboring sequences.

**Supplementary Figure S5.** Phylogenetic tree of FRL-PsbH and neighboring sequences, and helical prediction.

**Supplementary Figure S6.** Alignments of ancestral FRL-PSII sequences and selected extant FRL- and VL-specific PSII sequences.

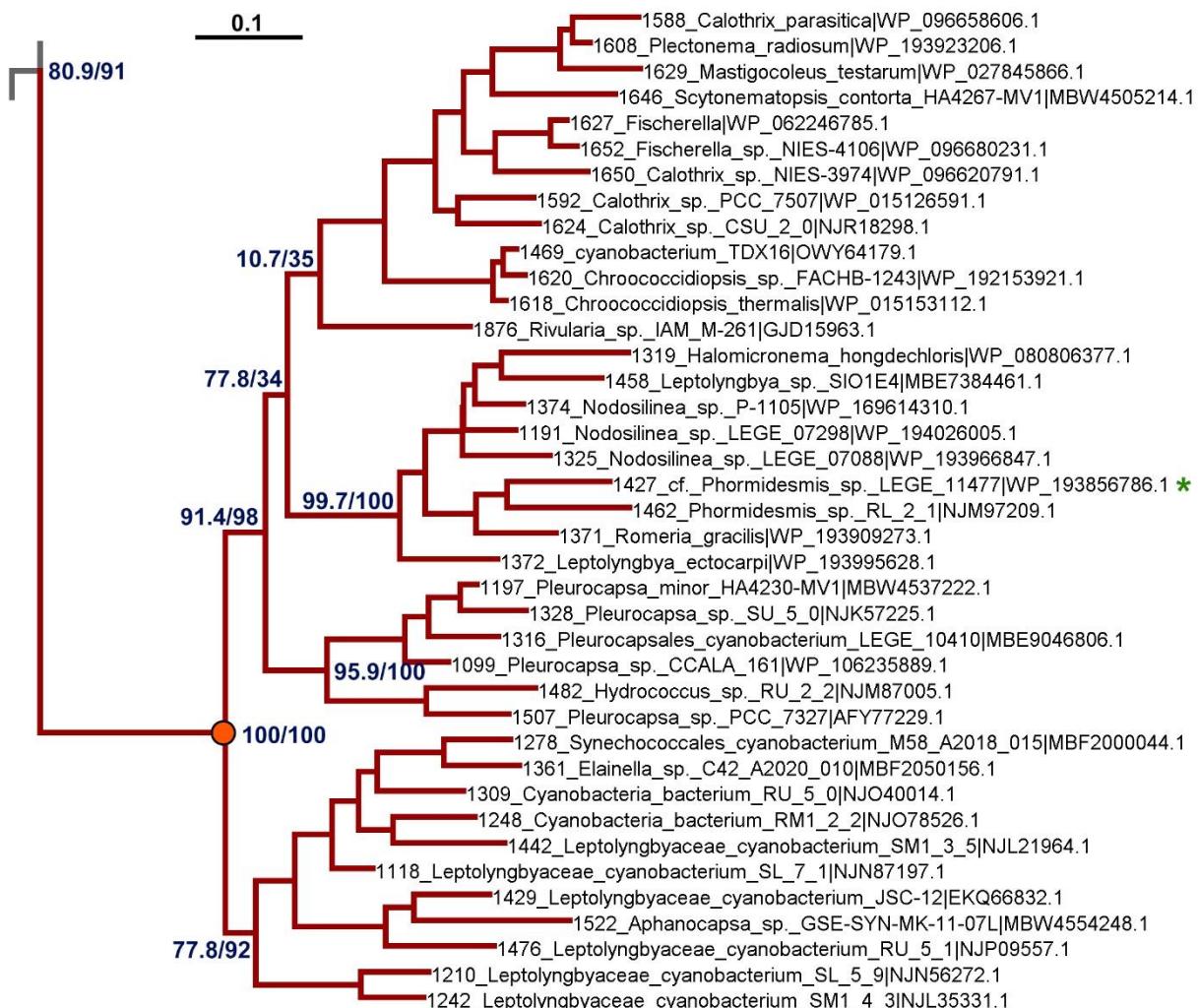
**Supplementary Figure S7.** Location of the FRL-PsbH2 homology model.

**Supplementary Figure S8.** Conservation of FRL-PsbD interactions near P<sub>D2</sub>.

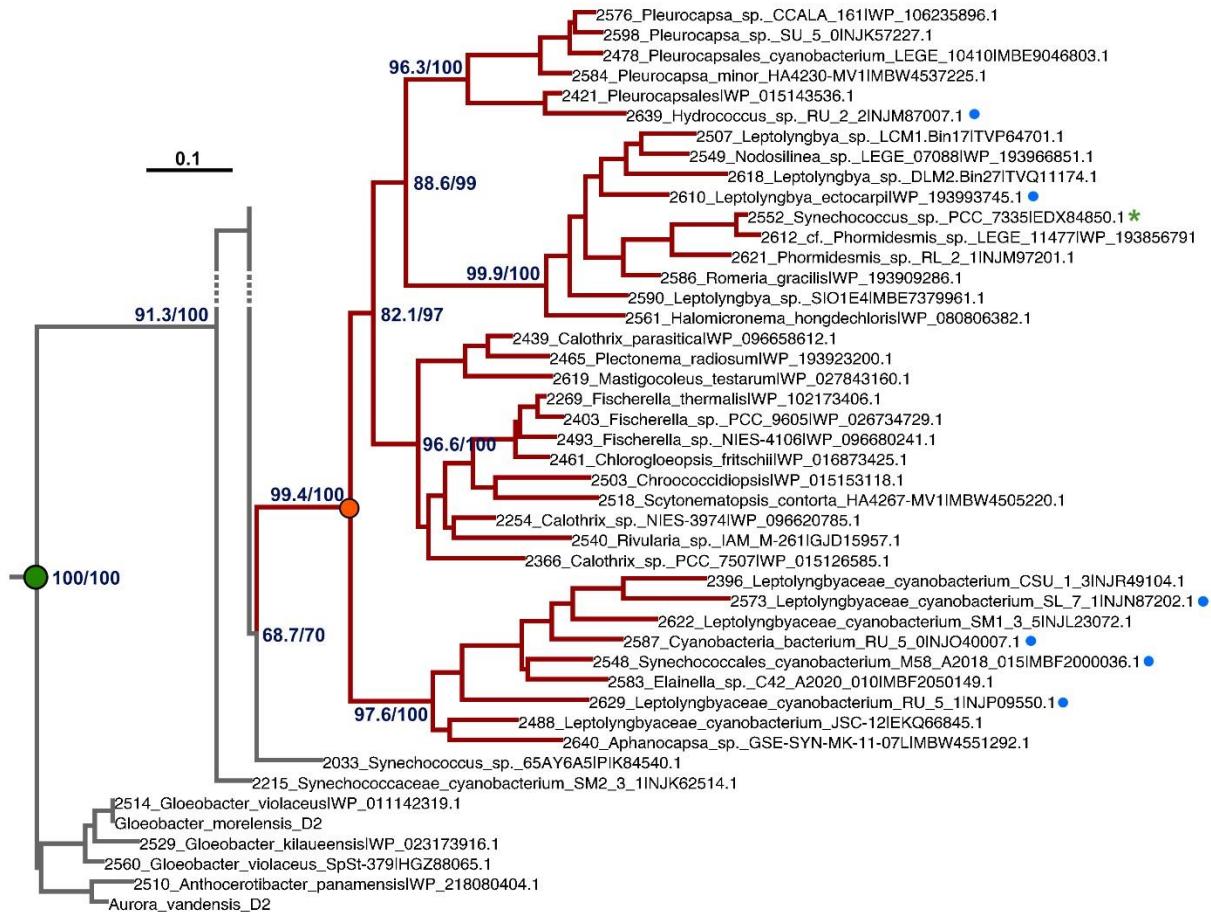
**Supplementary Figure S9.** Symmetry-related locations of Chl sites 507 and 611.

**Supplementary Data S1.** Sequence alignments with ancestral sequence reconstructions, and phylogenetic trees used in this study. (external)

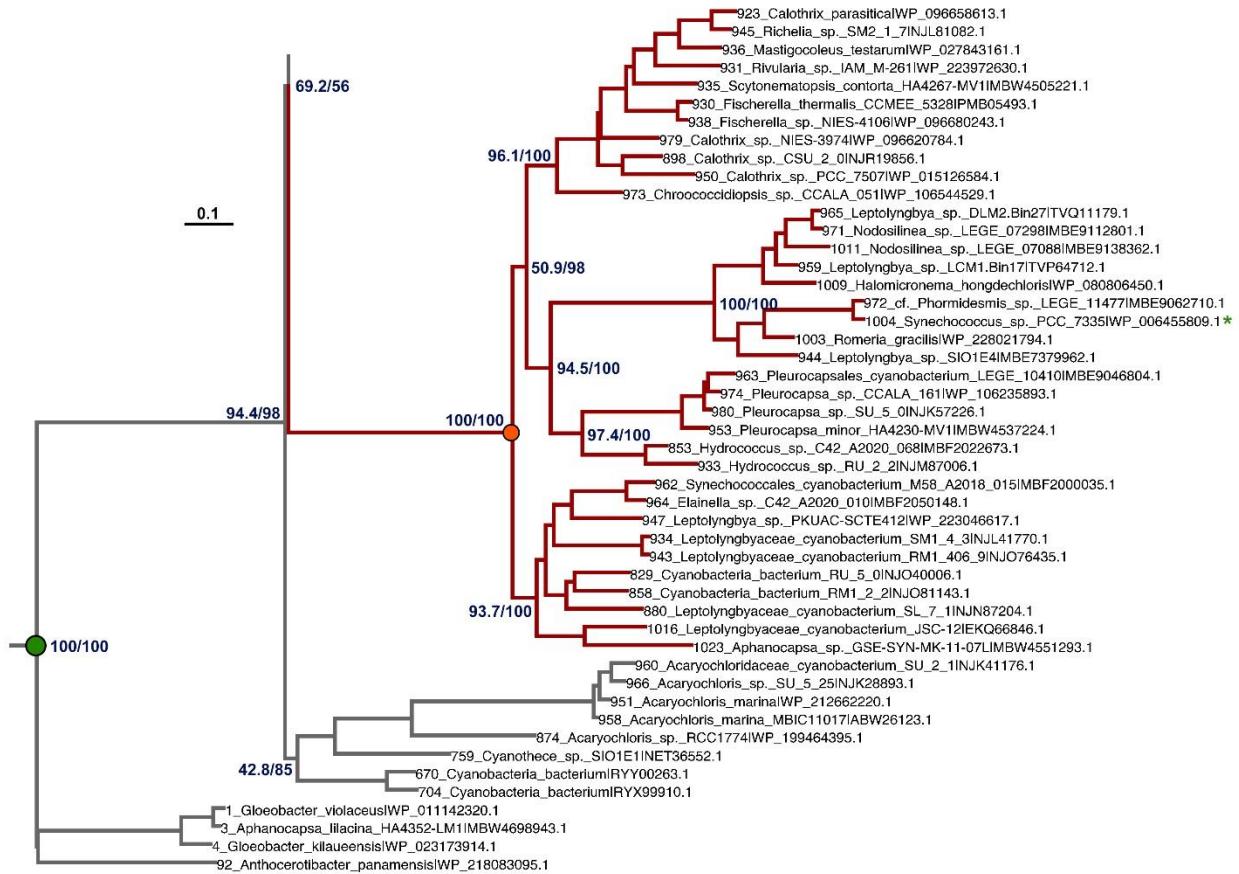
## Supplementary Figures



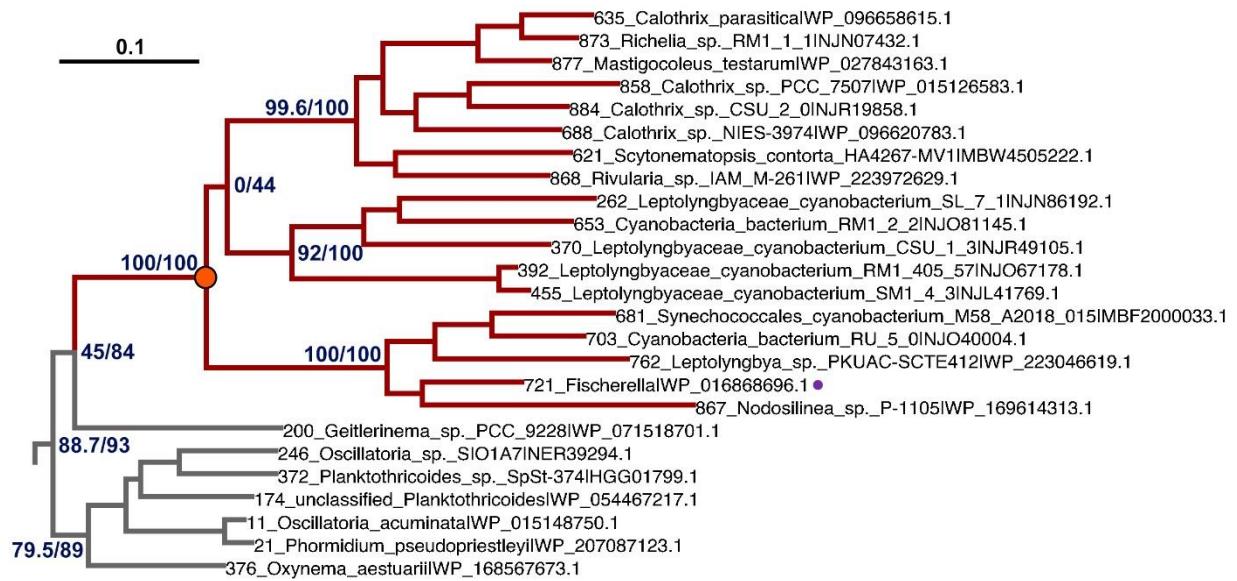
**Supplementary Figure S1.** Phylogenetic tree of PsbA sequences focused on the FRL sequences and basal clades. The scale bar represents the number of substitutions per site. Blue numbers are branch support values calculated using the average likelihood ratio test and ultrafastbootstrap methods, respectively (aLRT/UFbootstrap). Only those on the major subgroups are shown for clarity. The sequence marked with an asterisk has over 98% sequence identity to that of *Synechococcus* 7335, which is not shown in here due to redundancy curation. The orange circle denotes the most recent common ancestor of FRL sequences used in the FaRLiP response.



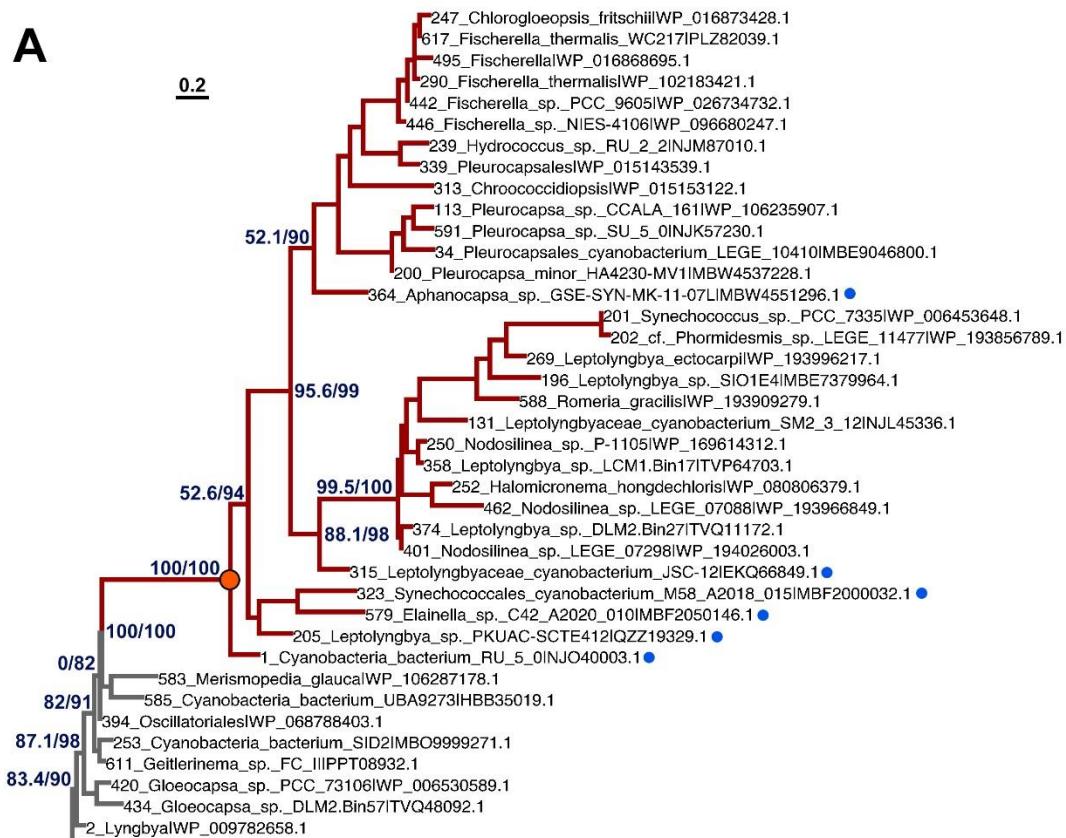
**Supplementary Figure S2.** Phylogenetic tree of PsbD sequences focused around the FRL sequences and basal clades. The scale bar represents the number of substitutions per site. Blue numbers are support branch values (aLRT/UFbootstrap). Only those on the major subgroups are shown for clarity. The green circle denotes the most recent common ancestor of cyanobacteria, and the smaller orange circle denotes the most recent common ancestor of FRL sequences used in the FaRLiP response. The sequence with an asterisk is that from *Synechococcus* 7335, for which a structure is available. Sequences marked with blue dots represent independent reversals from PsbD-Tyr161 to Phe161.



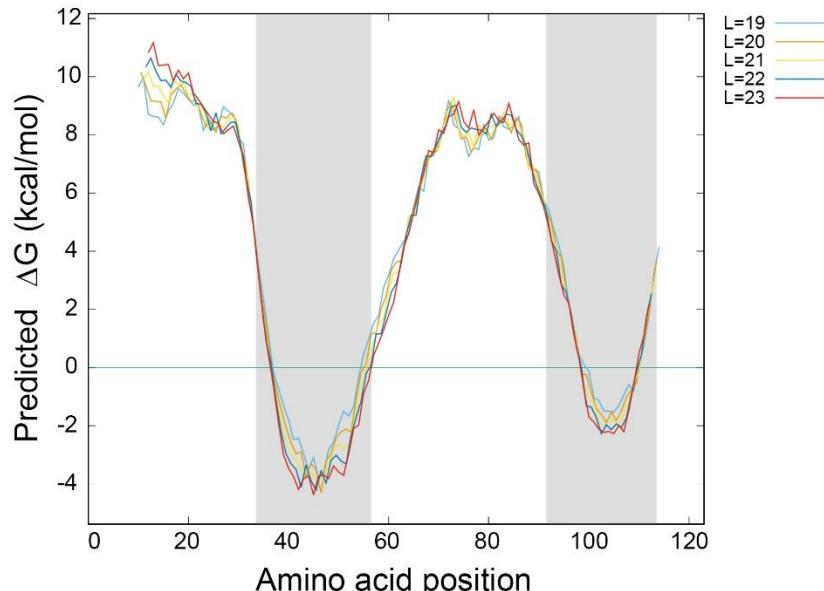
**Supplementary Figure S3.** Phylogenetic tree of PsbC sequences focused around the FRL sequences and basal clades. The scale bar represents the number of substitutions per site. Blue numbers are branch support values (aLRT/UFbootstrap). Only those on the major subgroups are shown for clarity. The sequence with an asterisk is that from *Synechococcus* 7335, for which a structure is available. The green circle denotes the most recent common ancestor of cyanobacteria, and the smaller orange circle denotes the most recent common ancestor of FRL sequences used in the FaRLiP response.



**Supplementary Figure S4.** Phylogenetic tree of FRL-PsbB (red) and neighboring (gray) sequences. The scale bar represents the number of substitutions per site. Blue numbers are branch support values (aLRT/UFbootstrap). Only those marking the major subgroups are shown for clarity. The orange circle denotes the most recent common ancestor of FRL sequences used in the FaRLiP response. The purple circle highlights the sequence from *Fischerella* (multispecies), which does not group with other heterocystous sequences (e.g., *Calothrix*, *Rivularia*, *Mastigocoleus*).

**A****B**

&gt;364\_Aphanocapsa\_sp\_GSE-SYN-MK-11-07L



## Predicted TM helices

Position	$\Delta G$	Sequence
34-56 (23)	-4.377	TAPLMVVLMLLFLVFLLIILQIF
92-113 (22)	-2.272	TGTSIFIGLVVFALTCLALIFY

**Supplementary Figure S5.** Phylogenetic tree of FRL-PsbH and neighboring sequences, and helical prediction. **(A)** shows a phylogenetic tree of FRL-PsbH (red) and neighboring (gray) sequences. The scale bar represents the number of substitutions per site. Blue numbers are branch support values (aLRT/UFbootstrap). Only those on the major subgroups are shown for clarity. The orange circle denotes the most recent common ancestor of FRL sequences used in the FaRLiP response. The blue circle highlights the sequences that contain a second transmembrane helix at the C-terminus. **(B)** shows the prediction of  $\Delta G$  for transmembrane helix insertion for a representative sequence [1], FRL-PsbH from *Aphanocapsa* GSE. Y axis shows  $\Delta G$  and X axis the amino acid position in the sequence. L=19 to 23 represent calculations using different transmembrane helix lengths.

## PsbA

FRL Ancestral --MTTLLRKRSANLWERFCNWVITSTENRLYIGWFGVLMIPLLSVSTCVFIAFIAAPP 58  
FRL Synechococcus 7335 ---MTLIQRPNIQSWERFCQWITSTENRLYIGWFGVLMPLPGLGSIITVFVTAFIAAPP 56  
FRL Aphanocapsa GSE ---MNTLIQQGKIVFPOWERFCNWVITSTENRLYIGWFGVLMIPLLLVSLSFIAFIAAPP 57  
FRL Pleurocapsa 7327 ---MTTLQKREIIPNLWEQFCQWVITSTENRLYIGWFGVLMIPLLGVSTTVFLAIIAAPP 57  
FRL Fischerella 7521 --MTTISTRPTSRFPTWDRFCNWVITSTENRLYIGWFGVLMIPLLGSICVFTIAFIAAPP 58  
VL Synechococcus 7335 --MTTLQQRQSAASLWEQFCQWVITSTENRLYVGWFGVLMIPTLAAATACFVIAFIAAPP 57  
VL Synechococcus 7335 MVSTTTLQRSEANLWEQFCQWVITSTENRLYVGWFGVLMIPTLAAATACFVIAFIAAPP 60  
VL Aphanocapsa GSE ---MTTLQRRESANLWERFCNWVITSTDNRIYIGWFGVIMVPTLAAATCFLIAFVAAPP 57  
VL Pleurocapsa 7327 ---MTTLQTREKASLWEQFCEWITSTNNRIYIGWFGVIMIPTLLTATICYIIAFIAAPP 57  
VL Pleurocapsa 7327 ---MTTLQQRESASLWEQFCQWVITSTNNRIYIGWFGVIMIPTLLTATACFIIAFIAAPP 57  
VL Pleurocapsa 7327 ---MTTLQQRESASLWEQFCQWVITSTNNRIYIGWFGVIMIPTLLTATTCFIIAFIAAPP 57  
VL Fischerella 7521 ---MTTLQRRAASGNVWERFCNWVITSTENRLYIGWFGVLMIPTLAAATTCFIIAFIAAPP 57  
VL Fischerella 7521 ---MTATLQRAQSANWFERFCNWVITSTENRLYIGWFGVLMIPTLAAATTCFIIAFIAAPP 57  
VL Fischerella 7521 ---MTTLTRGESGSLWDRFCEWITSTNNRLYIGWFGVLMIPTLTTATICFIIAFIAAPP 57  
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FRL Ancestral VDIDGIREPVGSLLYGNNIITAAVVPSSNAIGLHFYPIWEAASMDEWLYNGGPYQMIAF 118  
FRL Synechococcus 7335 VDIDGIREPVLGSLLYGNNIITAAVVPSSNAIGLHFYPIWEAAATLDEWLYNGGPYQMIAF 116  
FRL Aphanocapsa GSE VDIDGIREPVCGLLCGNNIITGAVVPPSSNAIGLHFYPIWDAASIDEWLYNGGPYQMIGA 117  
FRL Pleurocapsa 7327 VMDGLREPIGSLLYGNNIITAAVVPSSNAIGLHFYPIWEAASMDEWLYNGGPYQMIAL 117  
FRL Fischerella 7521 VDIDGIREPVGSLLYGNNIITAAVVPSSNAIGLHFYPIWEAASMDEWLYNGGPYQMIGF 118  
VL Synechococcus 7335 VDIDGIREPVGASLMLYGNNIISGAVVPSSNAIGLHFYPIWEAASLDIWLYNGGPYQLVIF 117  
VL Synechococcus 7335 VDIDGIREPVGASLMLYGNNIISGAVVPSSNAIGLHFYPIWEAASLDIWLYNGGPYQLVIF 120  
VL Aphanocapsa GSE VDIDGIREPVGASLIFGNNIITGAVIPSSNAIGLHFYPIWEAASLDIWLYNGGPYQLIIF 117  
VL Pleurocapsa 7327 VDIDGIREPVGSLLYGNNIISGAVVPSSNAIGLHFYPIWEAASLDIWLYNGGPYQLILF 117  
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VL Fischerella 7521 VDIDGIREPVGASLLYGNNIISGAIVPTSNAIGLHFYPIWEAASSLDIWLYNGGPYELIVF 117  
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FRL Synechococcus 7335 HYIPALCYLGREWELSYRLGMRPWICIAYSAPVAAISVFLYPIQGGSFSDGIPMGIS 176  
FRL Aphanocapsa GSE HYIPALACYMGROWELSYRLGMRPWICVAYSAPLISTTSVFLYPIQGGSFSDGIPMGIS 177  
FRL Pleurocapsa 7327 HYVPALCCYLGREWELSYRLGMRPWICVAFSAPLAATTTSVFLYPIQGGSFADGLPMIS 177  
FRL Fischerella 7521 HYIPALACYMGREWELSYRLGMRPWIAVAYSAPLAATTTSVFLYPIQGGSFSDGIPMGIS 178  
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 VL Pleurocapsa 7327 ING----- 360  
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 FRL Aphanocapsa GSE MGLPWYRVHTVVLNDPGRLIAVHLMHNALCAGFAGSMLLYELALFDPTDPVLPNPMWRQGM 60  
 FRL Pleurocapsa 7327 MGLPWYRVHTVVLNDPGRLIAVHLMHNALCAGFAGSMLLFELALYDPSDPVLPNPMWRQGM 60  
 FRL Fischerella 7521 MGLPWYRVHTVVLNDPGRLIAVHLMHNALCAGFAGSMLLFELALYDPSDPVLPNPMWRQGM 60  
 VL Synechococcus 7335 MGLPWYRVHTVVLNDPGRLISVHLMHTALVAGWAGSMALFELATFDPSPDVLPNPMWRQGM 60  
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 VL Fischerella 7521 FVLPFMARLGVGWSWGGWNVTGAANYDPGFWSFEGVAAAHVLSGLLFLAAVWHWVYWDL 120  
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 FRL Fischerella 7521 ATFFDSKTGEPTLDPKIFGIHLFLAGLCLCFGFGAFHLTGIFGPGMWSDPYGLTGHVQP 180

VL Synechococcus 7335 ELF RD PRT GE PA LD LP KM FG IHL FLS GLL C FG FG A FH LT GL WGP GMW VSD PY GL TGH VQG 180  
 VL Aphanocapsa GSE ELF RD PRT GE PA LD LP KM FG IHL FLS GLL C FG FG A FH LT GL WGP GMW VSD PY GL TGS IQP 180  
 VL Pleurocapsa 7327 ELF TD PRT GE PA LD LP KM FG IHL FLS GLL C FG FG A FH LT GL WGP GMW VSD PY GL TGH VQP 180  
 VL Fischerella 7521 ELF QD PRT GE PA LD LP KM FG IHL FLS GLL C FG FG A FH LT GL WGP GMW VSDAY GLT GHIAPI  
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 FRL Ancestral VAPEWGPDGFPNPNPGVVVAHHIAAGIVGII EGLFHITVRPENLYRALRGMNIETVLAS 240  
 FRL Synechococcus 7335 VAPEWGAAAGFDPHNPGVVVAHHIAALGIVAI EGLFHIVRPPPEYLYKGLRMGNIEGTLS 240  
 FRL Aphanocapsa GSE VAPDWGASGFNPNGGVVVAHHIAAGIVGIVGGFLHMNRVPSENLYKGLRMGNLETVLAS 240  
 FRL Pleurocapsa 7327 VAPVWGPEGFPNPNGGVVVAHHIAAGIVGIVGIIGGLFHIVVRPSEGLYRLLRMGNIEGVLAS 240  
 FRL Fischerella 7521 VAPVWGPEGFPNPNGGVVVAHHIAAGIVGIVGIIGGLFHIVVRPPEVLYRGLRMGNIEGVLAS 240  
 VL Synechococcus 7335 VAPEWPAGFPNPNPGVVVAHHIAAGIVGIVAGLFHLTVRP PQRLYKALRGMNIETVLSS 240  
 VL Aphanocapsa GSE VAPA WPDPGFPNPNPGGIVAH HIAAGVGVIIAGLFH LIVRP PQRLYKALRGMNIETVLSS 240  
 VL Pleurocapsa 7327 VAPEWPAGFPNPNPGVVVAHHIAAGIVGIIAGLFH LT VRP PERLYR ALRGMNIETVLSS 240  
 VL Fischerella 7521 VAPEWPDPGFPNPNPGGVVVAHHIAAGIVGIIAGLFHLSVRP PERLYKALRGMNIETVLSS 240  
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 FRL Aphanocapsa GSE GLATFFFAAFVASGSMWYGTATTPIELFGPTRYQWDSGYFQQEIDRRVQANLAEGKSLSQ 300  
 FRL Pleurocapsa 7327 SLAVFFAGFIASASMWYGTATTPIELFGPTRYQWDRGYFQQEIDRRVQAGLAEGKSLNQ 300  
 FRL Fischerella 7521 ALATFFAGFVAAGSMWYGTATTPIELFGPTRYQWDRGYFQQEIDRRVQAGLDEGKTLSQ 300  
 VL Synechococcus 7335 SIAAVFAAFIVAGTMWYGSATTPIELFGPTRYQWDSGYFAEEIDRRVQRDIANGASEED 300  
 VL Aphanocapsa GSE SIAAVFAAFVVAAGTMWYGSATTPIELFGPTRYQWDSGYFNQEINRRVQTSLAQGASLSE 300  
 VL Pleurocapsa 7327 SIAAVFAAFVVAAGTMWYGSATTPIELFGPTRYQWDKGYFQQEIQRRVEANLAAGDTLSE 300  
 VL Fischerella 7521 SIAAVFAAFVVAAGTMWYGSATTPIELFGPTRYQWDQGYFQQEIQRRVQASLASGANLSE 300  
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 FRL Ancestral AWSSIPEKLAFYDYVGNSPAKGGIFRVPGPMTKG D GIAQS WL GH PVF QDAE GRELT VRRMP 360  
 FRL Synechococcus 7335 AWSEIPTKLAFYDYIGNSPA KGGIFRVG R M V GDGLPTGWLGH PVFKDGE GRELT VRRMP 360  
 FRL Aphanocapsa GSE AWSAIPKLAFYDYIGNSPA KGGIFRVRGMVDGDGLATGWLGHPVFKDGE GRELT VRRMP 360  
 FRL Pleurocapsa 7327 AWSAIPPKLAFYDYIGNSPA KGGIFRVRGMVDGDGLATGWLGHPVFKDGE GRELT VRRMP 360  
 FRL Fischerella 7521 SWSAIPEKLAFYDYIGNSPA KGGIFRVRGMVDGDVAQS WL GH PVFKDREGRELT VRRMP 360  
 VL Synechococcus 7335 AYAAIPEKLAFYDYVGNSPAKGGIFRVP GPMT KG DIA TAWL GH PVF HDGDGRELT VRRLP 360  
 VL Aphanocapsa GSE AWSAIPKLAFYDYIGNSPA KGGIFRVRGMVDGDGLATGWLGHPVFKDREGRELE VRRLP 360  
 VL Pleurocapsa 7327 AWSKIPEKLAFYDYVGNSPAKGGIFRVTGAMNSGDGIARAWL GH AVFRDGEGRELS VRRMP 360  
 VL Fischerella 7521 AWSQIPEKLAFYDYVGNSPAKGGIFRVTGP MVKG D GIAQS WDGH P VFKDAE GRELE VRRLP 360  
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 FRL Ancestral NFFETFPVVLTDKD GIVRADIPF RRAESKYSF E Q TGVTVSF YGG ELDG QFT DPA TVK KY 420  
 FRL Synechococcus 7335 NFFENFPVVLFDQDGIVRADIPF RRAESKYSF E Q TGVTVSF YGG ELDG QFT DPGM V KRY 420  
 FRL Aphanocapsa GSE SFFENFPVIVFDNDGVVRADIPF RRAAKYSV E Q TGVNTF YGG ELDG QFT DPGM V KRY 420  
 FRL Pleurocapsa 7327 NFFETFPVVM TDKG D VVRADIPF RRT DAKY S ID QT G T V SF YGG ILL DG QFT IS DP ALV K QY 420  
 FRL Fischerella 7521 TFFETFPVVLTDKG D VVRADIPF Q RAE AKY S F E Q TGVNV SF FCG ILL DG QFT TDP MT V K KY 420  
 VL Synechococcus 7335 NFFETFPVVLVDKDLNRADIPF RRAESKYSF E Q TGVNV SF FCG ILL DG QFT TDP MT V K KY 420  
 VL Aphanocapsa GSE NFFETFPVILTDKG D VVRADIPF RRAESKYSF E Q QGV NV SF YGG ELDG QFT FK DAP T V K KY 420  
 VL Pleurocapsa 7327 NFFETFPVVLTDSDGIVRADIPF RRAESKTSIE Q TGT KV SF YGG ILL DG QFT FSDP AT V K QF 420  
 VL Fischerella 7521 NFFETFPVILTDKG D VVRADIPF RRAESQNSF E Q TGVTVSF YGG NLDG QFT FSDP ADV K KW 420  
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 FRL Ancestral ARKAQLGEPF E FDR QI LNS DGV F RT STR GWF AFA HAC F ALL W FFG H IWH G SRT L F RDV FA 480  
 FRL Synechococcus 7335 ARRAQLGEPF E FDR S VY D S D GL F RT SRG FF FHV IF GL L FFG H IWH G L RAL F QDV FS 480  
 FRL Aphanocapsa GSE ARQAQLGEV FNF NR E TY NS DGV F RT SNRG FFA F HAC F ALV WF FGH LWH G SRT L F RDV FA 480  
 FRL Pleurocapsa 7327 ARKAQLGEPF D FDR QAY NS DGV F RT SNRG W F A F HAC F ALV WF FGH VWH G ART L F RDV FA 480  
 FRL Fischerella 7521 ARQAQLGEPF PKF D RT IHN SDG V F RT SNRG F F A F HTC F ALV WF FGH IWH G SRT IY RDV FA 480  
 VL Synechococcus 7335 ARKAQLGEPF S F D R E TL SDG V F R S S P R G W F T Y GH AV F ALL F G H IWH G ART L F RDV FA 480  
 VL Aphanocapsa GSE ARKAQLGEQ F E F D Q E T L S S D G I L R T S P R G W F T Y GH AC F ALL F G H IWH G ART L F RDV FS 480  
 VL Pleurocapsa 7327 ARKAQLGESF D F D R E TL SDG V F RT S P R G W F T F A H AC F ALL F G H IWH G SRT L F RDV FA 480  
 VL Fischerella 7521 ARKAQLGEV F E F D R E TL SDG V F RT S P R G W F T F G H AC F ALL F G H IWH G ART IY RDV FA 480  
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 FRL Ancestral GIDPD L D E E Q V E F G V F Q K V G D T T R K K E A V A V ----- 512  
 FRL Synechococcus 7335 GIDPSL SAE Q V E W G Y F K K V G D P T S Q Q T P A ----- 509  
 FRL Aphanocapsa GSE GIDPEL D E E Q V E W G Y F K Q V G D K S R K Q R S L E P I V P V S A L P S Q V S P Q E 528  
 FRL Pleurocapsa 7327 GINPD L D E E Q V E F G V W Q K V G D V T S R K R Q V A ----- 510  
 FRL Fischerella 7521 GIDPEL D E - Q V E F G V F Q K V G D T T R K K Q P V I ----- 510  
 VL Synechococcus 7335 GVDPD L S P Q Q V E W G F Y Q K V G D F S T K A S K ----- 508  
 VL Aphanocapsa GSE GIDPEL S E E Q V E W G F Y Q K L G D T S T R K E T V ----- 510  
 VL Pleurocapsa 7327 GIDPD LG - E Q V E F G L F A K V G D V S T R K E G V ----- 508  
 VL Fischerella 7521 GIDPD LE - E Q V E F G V F A K V G D L S T R K E A V ----- 509  
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## PsbC

FRL Ancestral	METPFDRSVVTETMSTTI <del>PDKTKGTSITTAREGRD</del> <b>EASTGYAWWAGNARFINTELSG</b> 60
FRL Synechococcus 7335	<b>METPLETIP</b> ---- <b>D</b> -- <b>LSLSPTAEGSILAPASPGYDEATSGYAWWAGNARLITPELTG</b> 53
FRL Aphanocapsa GSE	----- <b>MTTSRDRGRDEASTGYAWWAGNARFIN--LSG</b> 29
FRL Pleurocapsa 7327	METPFNSSTV <b>KIG</b> --- <b>TTVQDAPTRDVFDLAREGRDEASTGYAWWAGNARFIN--LSG</b> 54
FRL Fischerella 7521	METPFDSKV <b>SKPKD</b> ----- <b>EVQKPAYIVNSPSEGREGDEASTGYAWWAGNARFIN--QSG</b> 51
VL Synechococcus 7335	----- <b>MVTLSSNNSFVGGRDQPSTGYAWWSGNARLID--LSG</b> 35
VL Aphanocapsa GSE	----- <b>MVTLSSNSIVAGNRDQEESGGFAWWAGNARLIN--LSG</b> 35
VL Pleurocapsa 7327	----- <b>MVTLSSNTSYAGGGRDLSSSTGFAWWAGNARLIN--LSG</b> 35
VL Fischerella 7521	----- <b>MVTLSRPGVILGAGRQDSTGFAWWAGNARLIN--LSG</b> 35
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FRL Ancestral	<b>RELGAHVAHAGLIAFWAGAMLLFEVAHYVPEKPMYEQGLILMPHIATLGFGVGHGGEVVD</b> 120
FRL Synechococcus 7335	<b>R</b> <b>LGAHVAHAGLIVLWAGGMJLFEVSHFNLSKPMYEQGCIL</b> <b>JPHIATLGIGVQGSGEITS</b> 113
FRL Aphanocapsa GSE	<b>RFLGAHIAHAGLMAFWAGAMLLFEVSHFVPEKPMYEQGLLMPHVATLGFGVGVPGVVND</b> 89
FRL Pleurocapsa 7327	<b>KFLGAHVAHAGLIAFWAGAMLLFEVAHYVPEKPMYEQGCILMPHIATLGFGVGSQQVVD</b> 114
FRL Fischerella 7521	<b>RFLGAHVAHAGLIAFWAGAMLLFEVAHYVPEKPMYDQGLILMPHIAALGFGVGVPGQVVD</b> 111
VL Synechococcus 7335	<b>KLLGAHVAHAGLIVLWTGAMTLEFVSHYIPEKPMYEQGCILMPHIATLGFGVGVPGGEVIN</b> 95
VL Aphanocapsa GSE	<b>KLLGAHVAHAGLIVFWAGAMTLFEVAHFPEKPMYEQGCILMPHIATLGFGVGVPGGEVTD</b> 95
VL Pleurocapsa 7327	<b>KLLGAHVAHAGLIVFWAGAMTLFEVAHFPEKPMYEQGLLMPHIATLGFGVGVPGGEVVD</b> 95
VL Fischerella 7521	<b>KLLGAHVAHAGLIVFWAGAMTLFEVAHFVPEKPLYEQGLLMPHIATLGFGVGVAGGEVVD</b> 95
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FRL Ancestral	<b>IFPPFFIAAVIHLICSAVLGFGLLYHSLRGPEKL</b> --- <b>SGFFDFDWSDKDKITILGYHLIA</b> 177
FRL Synechococcus 7335	<b>MFPFFFAIGVAHLICSAVLGIGGMYHAIKGPEKL</b> --- <b>YQFFQFDWTDRAKVAQILGFHIAI</b> 170
FRL Aphanocapsa GSE	<b>IFPPFFIAAVIHLIGSAVLGFGGVFSHSKGPAIL</b> --- <b>PGYYDIDYADKDKITDILAYNLFW</b> 146
FRL Pleurocapsa 7327	<b>TFPYFAIAVIHLIGSAVLGIGGLYHSLRGPEKL</b> --- <b>AGFFDFDWSDKDKMTSIIGYHLIA</b> 171
FRL Fischerella 7521	<b>IFPPFFIAAVAHLLIGSAVLGFGGIYHSLKGPKQL</b> --- <b>PGFFNFDFWSDKDKVTSILGYHLIA</b> 168
VL Synechococcus 7335	<b>LFPYFVVGVLHLVSSAVLGLGGVYHALRGPETLEEYSSFFSQDWKDKNQMTNIIGYHLIL</b> 155
VL Aphanocapsa GSE	<b>IFPFVFVGVVHLISSLASAVLGLGGIYHAVRGPEVILEEYSSFFGYDWKDKNQMTSILGFHLIV</b> 155
VL Pleurocapsa 7327	<b>TFPYFVVGVLHLISSLASAVLGFGGIYHALRGPETLEEYSSFFGYDWKDKNQMTNIIGYHLIL</b> 155
VL Fischerella 7521	<b>TYPYFVIGVLKAMFFGGVYHAIRGPEVILEEYSSFFGYDWKDKNQMTNIIGFHLII</b> 155
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FRL Ancestral	<b>LGIAALLLVCKAMFWGLYDTWAPGGGDVRLLTNPNTLNPLVIFGYLFRSPFGGSGWIVSV</b> 237
FRL Synechococcus 7335	<b>LGIFALLFAAKAMYGGLYDPWAPGGGDVRLLTNPNTLDPRIIFGYLIKRTGGEWIVSV</b> 230
FRL Aphanocapsa GSE	<b>LGVGAWLLVGKAMLWGGLYDTWAPGGGDVRLLISHPTLNPLTIFGYLVRSPYQEGWIVGV</b> 206
FRL Pleurocapsa 7327	<b>LGIGAFLLVGKAMFWGGLYDTWLEPGGGGVRLVTNPNTLDPRVIFGYFKSPLGSGWIVSV</b> 231
FRL Fischerella 7521	<b>LGVAALLLVGKAMLWGGLYDTWAPGGGDVRLLTNPNTLDPRVIFGYLFKGFTGGAGNIASV</b> 228
VL Synechococcus 7335	<b>LGLGAFLLVIKACFLGGVYDTWAPGGGDVRVITNPNTLPNGVIFGYLASSPFGGEGWIVGV</b> 215
VL Aphanocapsa GSE	<b>LGFGALLLVVIKAMFGGLYDTWAPGGGDVRVITNPNTLPNAVIFGYLVKSPFGGDGWAVSV</b> 215
VL Pleurocapsa 7327	<b>LGFGALLLVFKAMFFGGVYDTWAPGGGDVRVITNPNTLPNAVIFGYLIKAPFGGEWIISV</b> 215
VL Fischerella 7521	<b>LGLGAFLLVLKAMFFGGVYDTWAPGGGDVRVITNPNTLPNAVIFGYLLKSPFGGDGWIIGV</b> 215
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FRL Ancestral	NNLEDIVGGHIWMGSLIAGGIWHILTKPFKWHKFVFIWSGEAYLSQLGNIAGQAFIAT 297
FRL Synechococcus 7335	NNLEDIIGGGHIWIGCILIAAGGIWHILVPPLRW <del>YNLFPWTGETYLW</del> <b>SQLGNIAGQAFIAT</b> 290
FRL Aphanocapsa GSE	NNMEDLVGGHIWIGTILIGGIWHYFTTPSKWTHKVFWSGEAYLAQSLGNVCQALIAT 266
FRL Pleurocapsa 7327	DNLEDIVGGHIWMGSLIAGGIWHIFTKPWKWTDKVFIWSGEAYLSQLGNIAGQAFIAT 291
FRL Fischerella 7521	DNLEDLVGGHIWIGSLLILGGIWHITKPFKWTBKAFIWSGEAYLSQLGNIAGQAFIAT 288
VL Synechococcus 7335	NNMEDIIGGGHIWIGLICIFGGVFHILTKPFGWARRALIWNGEAYLSSIGAVSLMAFICS 275
VL Aphanocapsa GSE	DNLEDVVGHHIWIGLICIAAGGIWHILTKPFAWARRAFVWSGEAYLSSLGALSLMAFIAT 275
VL Pleurocapsa 7327	DNMEDIIGGGHIWVGLCIAGGIWHILTKPFGWARRAFIWSGEAYLSSLGALSLMGFIAS 275
VL Fischerella 7521	DNMEDIIGGGHIWVGLCIAGGIFHILTKPFGWARRAFIWSGEAYLSSLGALSLMGFIAS 275
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FRL Ancestral	<b>CFLWFNNTAYPSEFYGPTAPEASQAQALTFLVRDQKLGANVASAQGPTGLGKYLMRSPTG</b> 357
FRL Synechococcus 7335	<b>AFLWFNNTAYPSVFYGPTVPESSQAQSFVFLMRDQGMGADVASAQGPTGLGKYLMRSPTG</b> 350
FRL Aphanocapsa GSE	<b>AFIWFNNTAYPSEFYGPTAPESSQAQALTFLVRDQSLGANVASAQGPTGLGKYLMRSPTG</b> 326
FRL Pleurocapsa 7327	<b>MFIWFNNTAYPSEFYGPTVPEASQAQALVFLARDQRLGADIGTAQSVTGLGKYLMRSPTG</b> 351
FRL Fischerella 7521	<b>MFIWFNNTAYPSEFYGPTVAESSNAQALFLVRDQNLGANVASAQGPTGLGKYLMRSPTG</b> 348
VL Synechococcus 7335	<b>CYVWFNNTAYPSEFYGPTNAEASQAQAMTFLVRDQRLGANIGSAQGPTGLGKYLMRSPTG</b> 335
VL Aphanocapsa GSE	<b>CFVWFNNTVYPSEFYGPTGPEASQAQAMTFLVRDQRLGANVSAQGPTGLGKYLMRSPTG</b> 335
VL Pleurocapsa 7327	<b>VYVWFNNTAYPSEFYGPTGMEASQAQAFTFLVRDQRLGANIASAQGPTGLGKYLMRSPTG</b> 335
VL Fischerella 7521	<b>CFVWFNNTAYPSEFYGPTNAEASQAQSFIFLVRDQKLGANVASAQGPTGLGKYLMRSPTG</b> 335
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FRL Ancestral	EIIFGGETMRFWDFRGPWLEPLRGPNGLDLDKLQNDIQPWQIRRAEYMTAHLGLSLSNV 417
FRL Synechococcus 7335	EIIFGGETMRFWDARAPWLEPLRGKNGLDLDKLQHDVQPWLRRAAEYMTHSPIGSLNSV 410

FRL Aphanocapsa GSE	EIIFFGETMRFWDFRGPWLEPLRGPNGLDDKLRLYDVQWPQIRRAEYMTA	PGLSINSV	386		
FRL Pleurocapsa 7327	EIIFFGETMRFWDFRGPWLEPLRGTNGLDLKKLQNDIQPWQIRRASEYM	VHAPLGSINSV	411		
FRL Fischerella 7521	EIIFFGETMRFWDKAPWLEPLRGPNGLDIDKLQHDVQPWQIRRASEYM	HAPIGSINSV	408		
VL Synechococcus 7335	EIIFFGETMRFWDFGPWLAPLRGTNGLDLKLKDNIQPWQVRRAEYMTA	HAPNASINSV	395		
VL Aphanocapsa GSE	EIIFFGETMRFWDFRGPWLEPLRGPNGLDLNKIKNDIQPWQARRAAEY	MTA	395		
VL Pleurocapsa 7327	EIIFFGETMRFWDFRGPWLEPMRGPNGLDDKLRLNDVQPWQVRRAEY	MTA	395		
VL Fischerella 7521	EIIFFGETMRFWDFRGPWLEPLRGPNGLDDKLKDNDVQPWQIRRAEY	MTA	395		
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FRL Ancestral	GGVATEINSFNFSVPRTWLAAHFIF	AFLFLVGH	LWHAGRARAAGFERGIDREDEPVL	477	
FRL Synechococcus 7335	AGLATESNAFNVYVPRTWLASHFI	GFFF	LVGH	LWHAGRARAAGFETGLDREDEPVL	470
FRL Aphanocapsa GSE	GGVATEVSFNFVSPRSWLAASHFVFAFLFLVGH	HLWHAGR	RASAAAAGFARGIDPEDEPVL	446	
FRL Pleurocapsa 7327	AGLATDVSFNFVSPRTWMATSHLI	FAL	FFLVGH	WWHAGRARAAGFVRGINREDEPV	471
FRL Fischerella 7521	GGLATELNSFNFGPRAWLASAHFV	FALL	FLVGH	LWHAGRARAAGFERGIDREDEPVL	468
VL Synechococcus 7335	GGIITEVNSVNFVNPRQWLASFHVMAFFFLV	GHLWHAGRARA	AECCFERGLNREAEPVL	455	
VL Aphanocapsa GSE	GGVATEINSNFVSPRSWLSTHFVLAFFFLV	GHLWHAGR	ARAAGFEKGIERETEPVL	455	
VL Pleurocapsa 7327	GGVITDVSFNFVSPRAWLATSHFVLGFFF	VGH	LWHAGRARA	AAAGFEKGIDRETEPV	455
VL Fischerella 7521	GGVITEPNSFNYVNPRAWLATSHFVLAFFFLV	GHLWHAGR	ARAAGFEKGIDRETEPV	455	
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FRL Ancestral	SMPDLD-----	483			
FRL Synechococcus 7335	SMAPIDPSLRSD	482			
FRL Aphanocapsa GSE	SMADIDS-----	453			
FRL Pleurocapsa 7327	SMGDID-----	477			
FRL Fischerella 7521	SMPPLD-----	474			
VL Synechococcus 7335	SMPDLD-----	461			
VL Aphanocapsa GSE	SMPNLD-----	461			
VL Pleurocapsa 7327	AMPDLD-----	461			
VL Fischerella 7521	FMKDLD-----	461			
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FRL Ancestral	MTIAIGRASQQNRFWFSTLDDWLKRDRFVFIGWSGLL	FP	CAYLAIGGWFTGTTFVT	TSWY	60							
FRL Synechococcus 7335	MTITMGSLSARDWI	KQLDDWLKRD	RFVFIGWSGLL	FP	CSFLAIGAWTGTTFVT	TSWY	59					
FRL Aphanocapsa GSE	MTIALKPARQ-WGDWVFTLDDWLKRD	RFVFIGWSGLL	FP	CSFLAL	GGLWTGTTFVT	SSWY	59					
FRL Pleurocapsa 7327	MTIAITR-RQPTQGWFR	TLLDDWLKRD	RFVFIGWSGLL	FP	CSFLAIGGWFTGTTFVT	AWY	59					
FRL Fischerella 7521	MTIAIRPSRTGFEW	FYVLLDDWLKRD	RFVFIGWSGLL	FP	CSFLAIGGWFTGTTFVT	TSWY	60					
VL Synechococcus 7335	MTIAMGQA-PAARGRFDVL	DDWLKRD	RFVVGWSGLL	FP	CAFLAIGGWMTGTTFVT	TSWY	59					
VL Aphanocapsa GSE	MTI	AVGRA-QPTRGWFDV	LDDWLKRD	RFVVGWS	GLLLFP	CAFLALGGWTGTA	FVSSWY	59				
VL Pleurocapsa 7327	MTIAMGRA-QAQRGWF	DV	LDDWLKRD	RFVFIGWSGLL	FP	CSFLAIGGWLTGTTFVT	TSWY	59				
VL Fischerella 7521	MTIAIGR--STTRGWF	DVLLDWL	WLKRD	RFVVGWSGLL	FP	CSFLAIGGWLTGTTFVT	TSWY	58				
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FRL Ancestral	THGLASSYLEGCNFLTAAVSTPADSMGH	SLLL	LWGPEAQGDF	TRWCQIG	GLWTFVAFHGA	120						
FRL Synechococcus 7335	THGLVSSYLEGCNFL	TAAVSTPA	ESMGH	SLLL	LWGPEASGDF	VRCQIG	GLWTFTALHGA	119				
FRL Aphanocapsa GSE	SHGLATSYLEGCN	FLTAAVSTPPD	SMGH	SLLL	LWGPEAQGDF	TTRWCQ	LGGLWTFVALHGA	119				
FRL Pleurocapsa 7327	THGLVSSYLEGCN	FLTAAVSTPAD	SMGH	SLLL	LWGPEANWN	FTRWCQIG	GLWTFTALHGA	119				
FRL Fischerella 7521	THGIVSSYLEGCN	FLTAAVSTPADSMGH	SLLL	LFLW	GPEAQGDF	TRWCQIG	GLWNFVALHGA	120				
VL Synechococcus 7335	THGLASSYLEGCNFL	TAVSTPADSMGH	SLLL	LWGPEAQGDF	VRCQIG	GLLWAF	VALHGA	119				
VL Aphanocapsa GSE	THGLASSYLEGCNFL	TAVSTPANS	MGH	SLLL	LWGPEAQGDF	TTRWCQ	LGGLWTFVALHGA	119				
VL Pleurocapsa 7327	THGLASSYLEGCNFL	TAVSTPADA	FGH	SLLL	FLW	GPEAQGN	FTRWCQIG	GLWPVALHGA	119			
VL Fischerella 7521	THGLASSYLEGCNFL	TAVSTPADAMGHS	SLLL	LWGPEAQGDF	TTRWCQ	LGGLWTFVALHGA	118					
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FRL Ancestral	LGLIGFMLRQIE	IA	LRVG	I	PYNAIAFS	APIAV	FVSVFLIYPL	PGSSWF	FPSFGVAAIF	180		
FRL Synechococcus 7335	FGLIGFMLRQIE	IA	LRVG	I	PYNAIAFS	APIAVYC	CATFLIYPL	PGSSWF	FPGFGVSAIF	179		
FRL Aphanocapsa GSE	FSLIGFCLROLEI	ARLIG	V	PYNAIAFS	APIAVV	SVSFL	YPLM	PGSSWF	FPSFGVTAIF	179		
FRL Pleurocapsa 7327	LGLMGFMLRQIE	IA	LRVG	I	PYNAIAFS	APIAVV	SVSFL	YPLM	PGSSWF	FAPSFGVAGIF	179	
FRL Fischerella 7521	FGLIGFMLRQFEI	ARAVG	VG	PYNAIAFS	APIVS	VFSV	FLIYPL	QSSWFF	FAPSFGVAAIF	180		
VL Synechococcus 7335	FGLIGFMLRQFEI	SR	LG	VG	IPYNAIAFS	APIAVF	SVSFL	M	PGSSWF	FAPSFGVAAIF	179	
VL Aphanocapsa GSE	FGLIGFMLRQFEI	ARL	VG	LRP	YNAIAFS	APIAVF	SVSFL	M	PGSSWF	FAPSFGVAGIF	179	
VL Pleurocapsa 7327	FGLIGFMLRQFEI	ARLV	VG	RPY	YNAIAFS	APIAVF	SVSFL	M	PGSSWF	FAPSFGVAGIF	179	
VL Fischerella 7521	FGLIGFMLRQFEI	ARLVG	VG	IPY	YNAIAFS	APIAVF	SVSFL	M	PGSSWF	FAPSFGVAAIF	178	
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FRL Ancestral	RFL	LLFFQGFHN	YTLP	NPF	HM	MGVAG	VLG	GALLCAI	HGATVENTL	FKDTKNFNTFR	PTQ	240
FRL Synechococcus 7335	RFL	LLFFQGFHN	YTLP	NPF	HM	MGVAG	VLG	GALLCAI	HGATVN	TFLFRDNQSNTFK	PTQ	239
FRL Aphanocapsa GSE	RFL	LLFFQGFHN	YTLP	NPF	HM	MGVAG	VLG	GALLCAI	HGATVENT	LF	EKFTRGFNTFSGFSPTQ	239
FRL Pleurocapsa 7327	RFL	LLFFQGFHN	YTLP	NPF	HM	MGVAG	VLG	GALLCAI	HGATVN	T	TFEDTKSFTFGGSPTQ	239
FRL Fischerella 7521	RFL	LLFFQAFHN	YTLP	NPF	HM	MGVAG	VLG	GALLCAI	HGATVENT	LF	RDTKSFNTFGGSPTQ	240

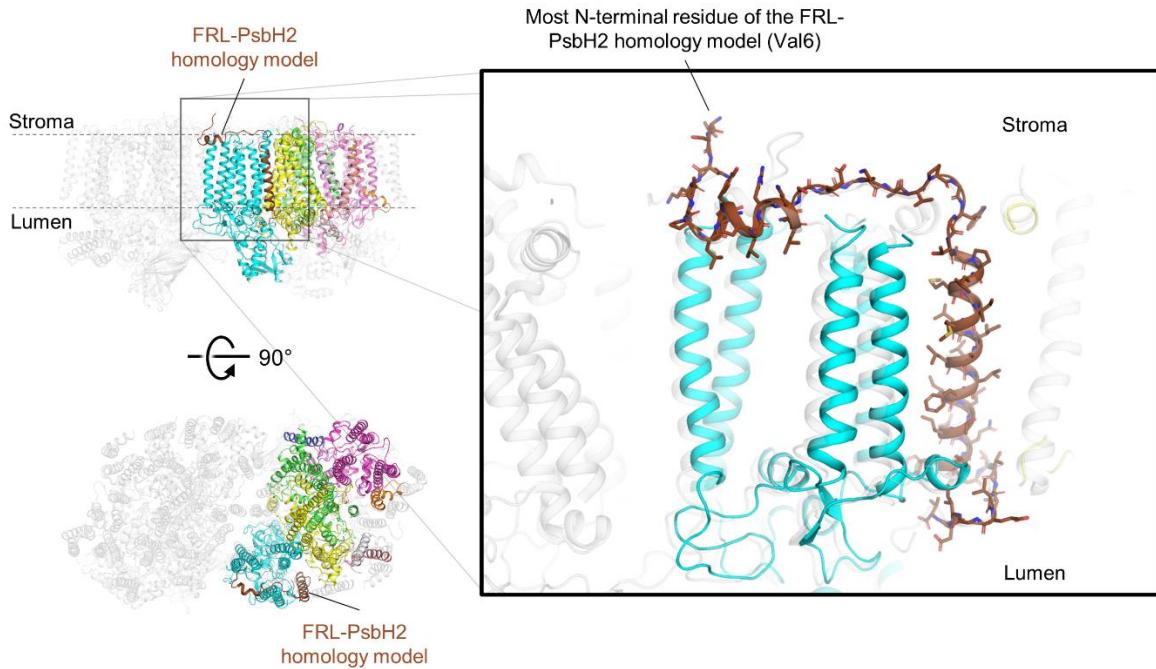
VL	Synechococcus	7335	RFLFLQGFHNWTLNPFHMMGVAGILGGALLCAIHGATVENTLFQDDENANTFRAFEPTQ	239
VL	Aphanocapsa	GSE	RFLFFQGFHNWTLNPFHMMGVAGILGGALLCAIHGATVENTLFEDGEKSNTFSAFNPTQ	239
VL	Pleurocapsa	7327	RFLFVQGFHNFTLNPFHMMGVAGVLGGALLCAIHGATVENTLFQDSQANTFRAFQPTQ	239
VL	Fischerella	7521	RFLFLQGFHNWTLNPFHMMGVAGVLGGALLCAIHGATVENTLFEDGEGANTFRAFNPTQ	238
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FRL	Ancestral		AEETYSMVTANRFWSQIFGIAFSNKRWLHFFMLFVPTGLWMSAIGMVGLAFNLRAYDFV	300
FRL	Synechococcus	7335	GEETYSMVTANRFWSQIFGIAFSNKRWLHFFMLFVPTGLWMSAIGMVGLAFNLRAYDFV	299
FRL	Aphanocapsa	GSE	AEETYSMVTANRFWSQIFGIAFSNKRWLHFFMLFVPTGLWMSAIGMVGLAFNLRSYDFV	299
FRL	Pleurocapsa	7327	EEETYSMVTANRFWSQIFGIAFSNKRWLHFFMLFVPTGLWMSAIGMVGLAFNLRAYDFV	299
FRL	Fischerella	7521	AEETYSFVTANRYWSQIFGIAFSNKRWLHFFMLFVPTGLWMSSIGMVGLAFNLRAYDFV	300
VL	Synechococcus	7335	SEETYSMVTANRFWSQIFGIAFSNKRWLHFFMLFVPTGLWMSSIGIVGLALNLRAYDFV	299
VL	Aphanocapsa	GSE	AEETYSMVTANRFWSQIFGIAFSNKRWLHFFMLFVPTGLWMASIGVVGALNLRSYDFI	299
VL	Pleurocapsa	7327	AEETYSMVTANRFWSQIFGIAFSNKRWLHFFMLFVPTGLWMASIGIIGIALNLRAYDFV	299
VL	Fischerella	7521	AEETYSMVTANRFWSQIFGIAFSNKRWLHFFMLFVPTGLWMSAIGIVGLALNLRAYDFV	298
			*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:	
FRL	Ancestral		SQELRAAEDPEFETFYTKNILLNEGLRAWMAQPQDPHEHFVFPEEVLPGRNAL-	353
FRL	Synechococcus	7335	SQEIRAAEDPEFETFYTKNILLNEGLRAWLSEMOPQPAKKFVFPDEVLPGRFSE-	352
FRL	Aphanocapsa	GSE	SQELRAAEDPEFETFYTKNILLNEGLRAWMAPEDQPHEHFVFPEEVLPGRNAL-	352
FRL	Pleurocapsa	7327	SQELRAAEDPEFETFYTKNILLNEGLRAWMATMDQPHERFQFPDEVLPGRNAK	353
FRL	Fischerella	7521	SQEIRAAEDPEFETFYTKNILLNEGLRAWMAAQDQPHEKFQFPPEEVMPRGNAE-	353
VL	Synechococcus	7335	SQEIRAAEDPEFETFYTKNILLNEGLRAWMAQPQDPHEKFVFPPEEVLPGRNAL-	352
VL	Aphanocapsa	GSE	SQEIRAAEDPEFETFYTKNILLNEGLRAWMAQPQDPHENLSFPEEVLPGRNAL-	352
VL	Pleurocapsa	7327	SQELRAAEDPEFETFYTKNILLNEGLRAWMAQPQDPHENFVFPPEEVLPGRNAL-	352
VL	Fischerella	7521	SQELRAAEDPEFETFYTKNILLNEGLRAWMAQPQDPHEKFVFP-----	342
			*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:	

## PsbH

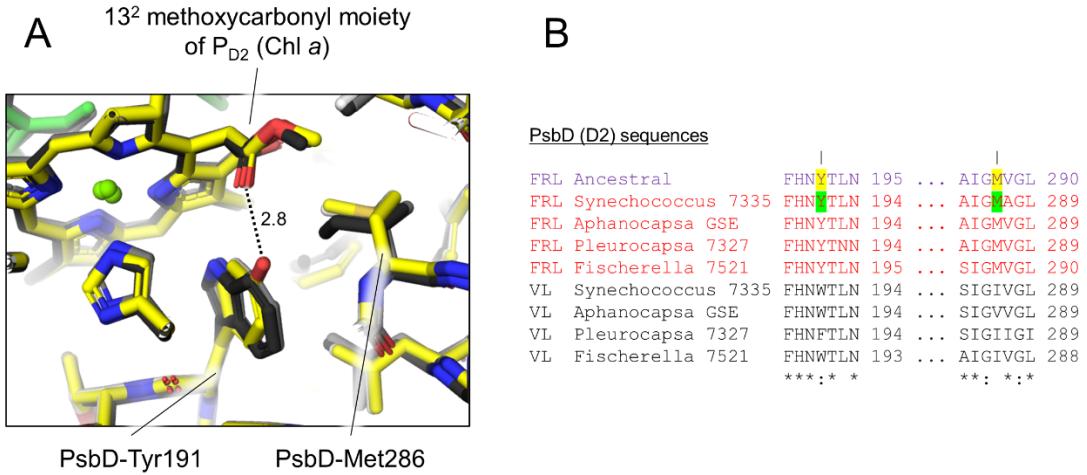
FRL	Ancestral		MQITNFQPKKVAFICQLLKQLNSDSGVVPGWGTTPLMAILMVLFFVLLIILQIFNSSI	60
FRL	Synechococcus	7335	-MRQKYVSNKAAPLQYPLRKLNSEAGKVVPGWGTAPlMGIMLIALLFILTILQLYNGTV	59
FRL	Aphanocapsa	GSE	MEQPNNFTKKVAPLQYFFRQFNSDAGKVVVRGWTAPLMVVLMLLFLVFLIIILQIFNASI	60
FRL	Pleurocapsa	7327	MQVRKFAPKKVAPLQYFFKRFNSQAGKVIPGWGTTPLMLALMFLFFFLLMLLEIVNASI	60
FRL	Fischerella	7521	MQSQNFPQPKKVAPVQYLLKQLNTEAGKVTGPGWGTTPLMAALMFLFILLMILEIVNSSI	60
VL	Synechococcus	7335	-----MAQRTKLGNLLKPLNSEYGVSPGVWGTTPMGVFMVLFLVFLIIILQLYNSSL	53
VL	Aphanocapsa	GSE	-----MAKQTWLGDLVLRPLNAYEGVAPGVWGTTPLMAVFMLGLFLVFLIIILQIYNSSL	53
VL	Pleurocapsa	7327	-----MAQRTRLGDILRPLNSEYGVVPGWGTTPLMGVFMLLFLVFLIIILQIYNSSL	53
VL	Fischerella	7521	-----MAQRTRLGDILRPLNSEYGVAPGVWGTTPLMAVFMLFFVFLIIILQLYNKSI	53
			: : : : : : : * *** * : * : : : : : : * : * : :	
FRL	Ancestral		LIEGVEDIAVDWSSLSK-----	76
FRL	Synechococcus	7335	IVEGIDV-----	66
FRL	Aphanocapsa	GSE	LLEGINN-IDWSTLSDYAEVAASTPYQGQGQFASTGTSIFIGLVVFALTCLALIFYGATTY	118
FRL	Pleurocapsa	7327	QLEGID--VDWKSLSY-----	74
FRL	Fischerella	7521	LLEGINMS-----	68
VL	Synechococcus	7335	IINNVD--VDWRSLGN-----	67
VL	Aphanocapsa	GSE	ILDGVN--VTWKSFAG-----	67
VL	Pleurocapsa	7327	LLEGFS--VDWRSLQ-----	67
VL	Fischerella	7521	IIQDVS--VDWRSLGR-----	67
			: : : :	
FRL	Ancestral		----- 76	
FRL	Synechococcus	7335	----- 66	
FRL	Aphanocapsa	GSE	PKDQR 123	
FRL	Pleurocapsa	7327	----- 74	
FRL	Fischerella	7521	----- 68	
VL	Synechococcus	7335	----- 67	
VL	Aphanocapsa	GSE	----- 67	
VL	Pleurocapsa	7327	----- 67	
VL	Fischerella	7521	----- 67	

**Supplementary Figure S6.** Alignments of ancestral FRL-PSII sequences and selected extant FRL- and VL-specific PSII sequences. The sequence with purple font (top) is the calculated ancestral FRL sequence. Sequences with dark red font are FRL-specific sequences from four extant FaRLiP-capable cyanobacteria (*Synechococcus* 7335, *Aphanocapsa* GSE, *Pleurocapsa* 7327, and *Fischerella* 7521). Sequences with black font are VL sequences from those organisms. A residue is highlighted yellow in the ancestral sequence if it is identical to the FRL sequences from extant cyanobacteria and dissimilar to the VL sequences. A residue is highlighted green in the *Synechococcus* 7335 sequences if it is identical to the other FRL sequences and dissimilar to the VL sequences. Thus, a residue position

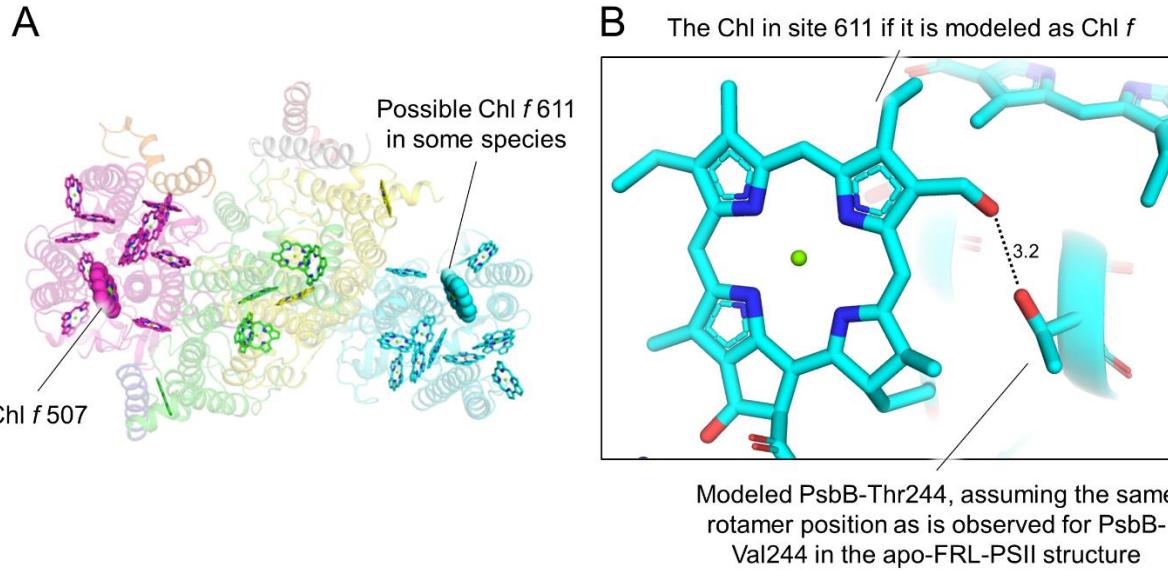
that is highlighted yellow in the ancestral sequence and green in the *Synechococcus* 7335 sequence is likely conserved from the FRL-PSII ancestor that is found in extant FRL-PSII, implying important functional significance. Residues that are highlighted green in the *Synechococcus* 7335 sequence but the same site is not highlighted in the ancestral sequence are likely to be later adaptations, possibly enhancing FRL absorption. In the FRL-PSII sequences from *Synechococcus* 7335, regions that were not modeled in the cryo-EM structure (PDB 7SA3 [2]) are shaded grey. In the PsbH sequences, those residues that were not modeled due to low homology are also shaded grey. In all sequence alignments, the Clustal Omega [3] sequence conservation identifiers are shown below the alignment.



**Supplementary Figure S7.** Location of the FRL-PsbH2 homology model. On the left side, the *Synechococcus* 7335 apo-FRL-PSII structure is shown (colored) superimposed onto the structure of the PSII holocomplex from *Synechocystis* 6803 [5]. The FRL-PsbH homology model (brown) was generated using Swiss Model [6] and superimposed onto PsbH of the *Synechocystis* 6803 structure to approximate its location in the FRL-PSII complex. The magnification on the right shows the residues of FRL-PsbH additionally as sticks. The N-terminal region is shown extending across the stromal surface.



**Supplementary Figure S8.** Conservation of FRL-PsbD interactions near  $P_{D2}$ . In (A), the *S. 7335* apo-FRL-PSII structure (yellow), the homology model of the FRL-ASR (white), and two non-FaRLiP holocomplex PSII structures (light and dark grey from *T. vulcanus* and *Synechocystis* 6803, respectively) are superimposed. It also shows the H-bonding interaction involving the  $^{13^2}$  methoxycarbonyl moiety with a dashed line with its distance in units of Å. In (B), two partial sequence alignments are shown that includes the FRL-ancestral sequence, FRL-specific sequences, and VL sequences from three FaRLiP-capable cyanobacteria. Conserved FRL-specific residues in extant cyanobacteria are highlighted in green in the sequence from *Synechococcus* 7335. If the same position is conserved in the FRL ancestral sequence, it is highlighted in yellow. Vertical lines above residue positions in (B) correspond to amino acids from the *Synechococcus* 7335 apo-FRL-PSII structure labeled in (A). The Clustal Omega [3] sequence conservation identifiers are shown below the alignment.



**Supplementary Figure S9.** Symmetry-related locations of Chl sites 507 and 611. In (A), a stromal view of the apo-FRL-PSII structure from *Synechococcus* 7335 is shown with transparent cartoons. Only Chl and pheophytin tetrapyrrole rings are shown, either as sticks, or spheres (507 and 611). In (B), the PsbB site 611 containing Chl *a* in the apo-FRL-PSII structure was instead fit with a Chl *f* molecule, and the Val in position 121 was instead modeled as a Thr with the same rotamer position. If the formyl moiety is directed toward the Thr sidechain, it is within H-bonding distance of the hydroxyl moiety of the Thr. The distance is shown in units of Å. Modeling was performed using Coot [4].

## **Supplementary Data**

**Supplementary Data S1.** Sequence alignments with ancestral sequence reconstructions, and phylogenetic trees used in this study. (external)

## Supplementary References

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