

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

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**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.

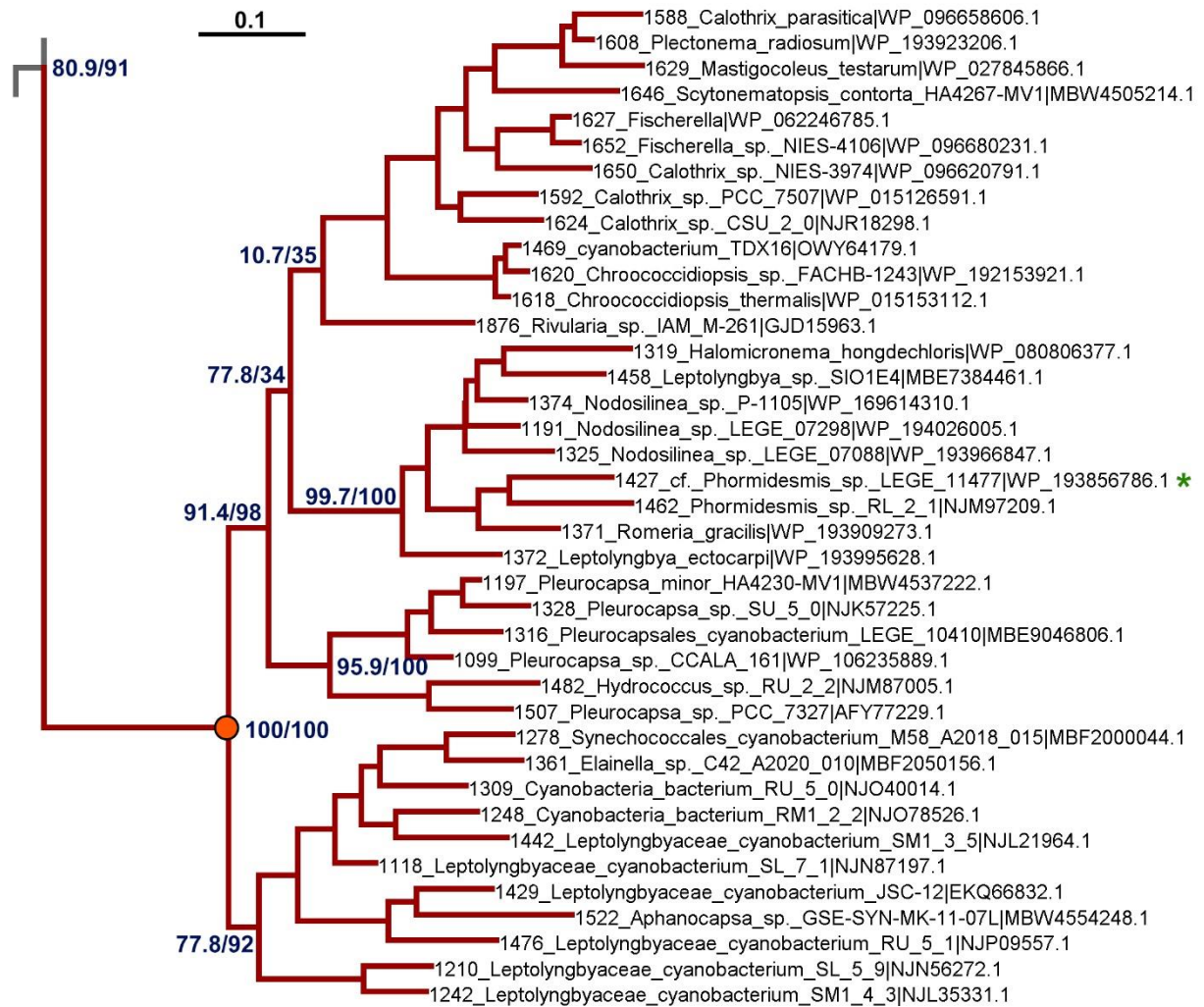
**Supplementary Figure S7.** Location of the FRL-PsbH2 homology model.

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

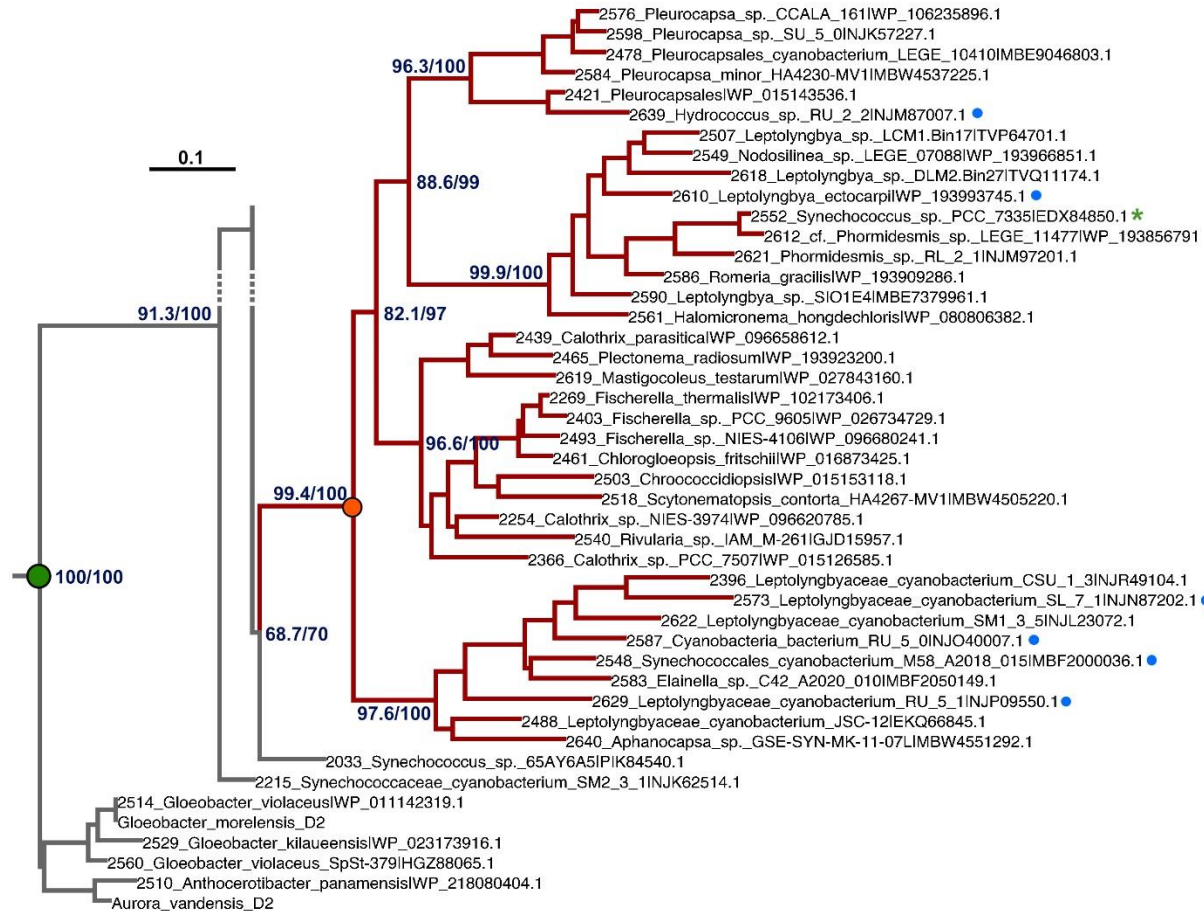
**Supplementary Figure S9.** Symmetry-related locations of Chl sites 507 and 611.

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

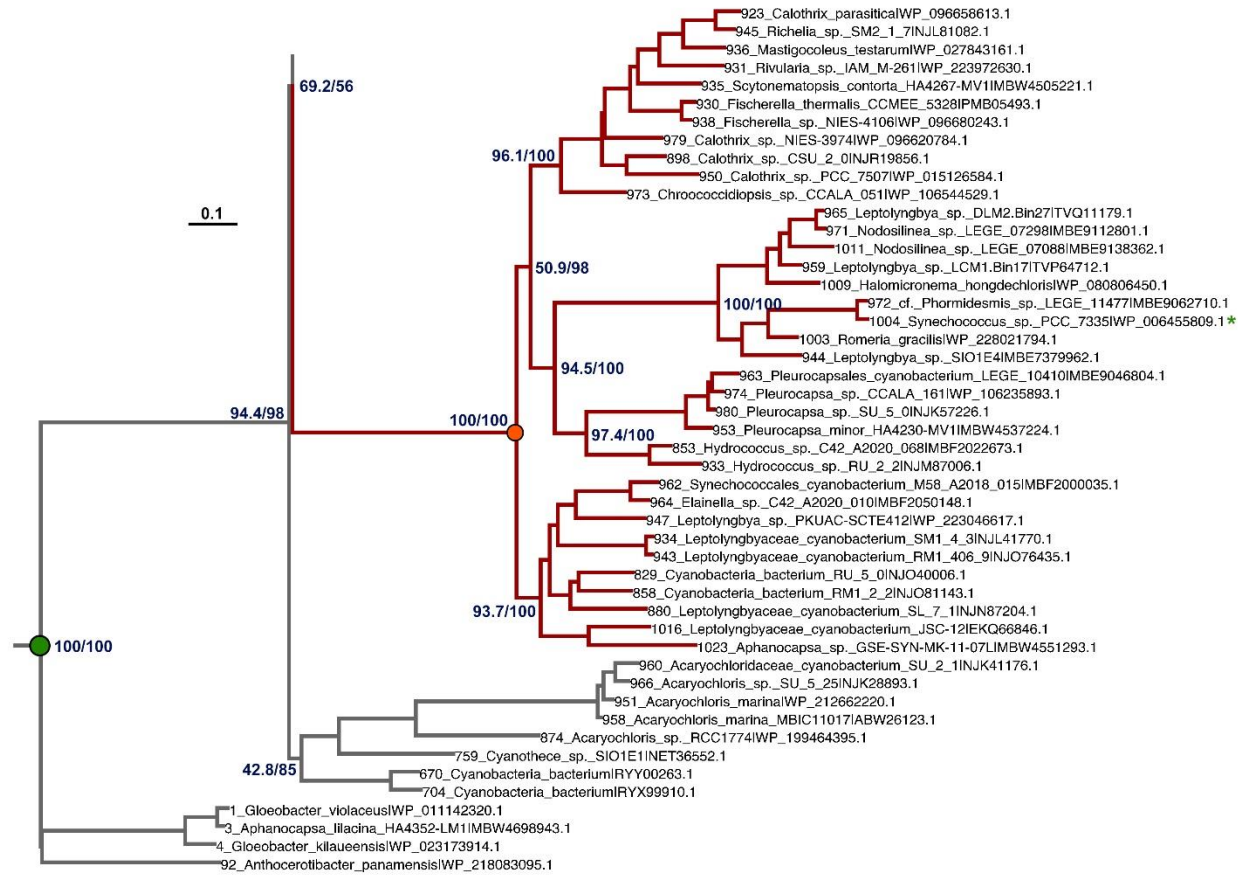
## Supplementary Figures



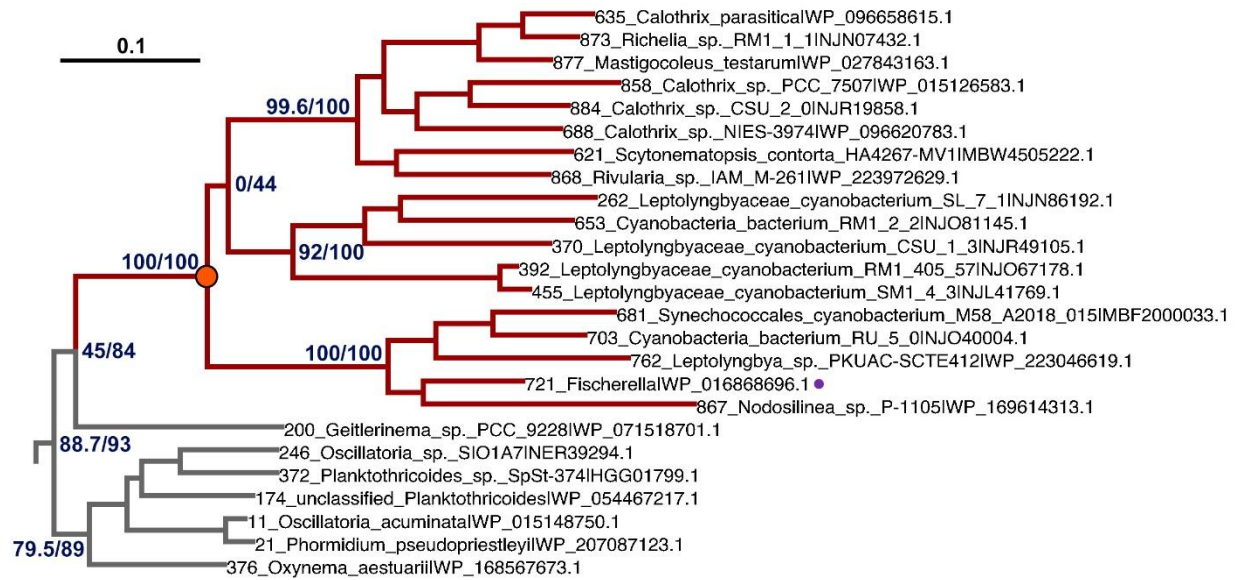
**Supplementary Figure S1.** Phylogenetic tree of PsbA sequences focused on the FRL sequences and basal clades. The scale bar represents the number of substitutions per site. Blue numbers are branch support values calculated using the average likelihood ratio test and ultrafastbootstrap methods, respectively (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 support 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.

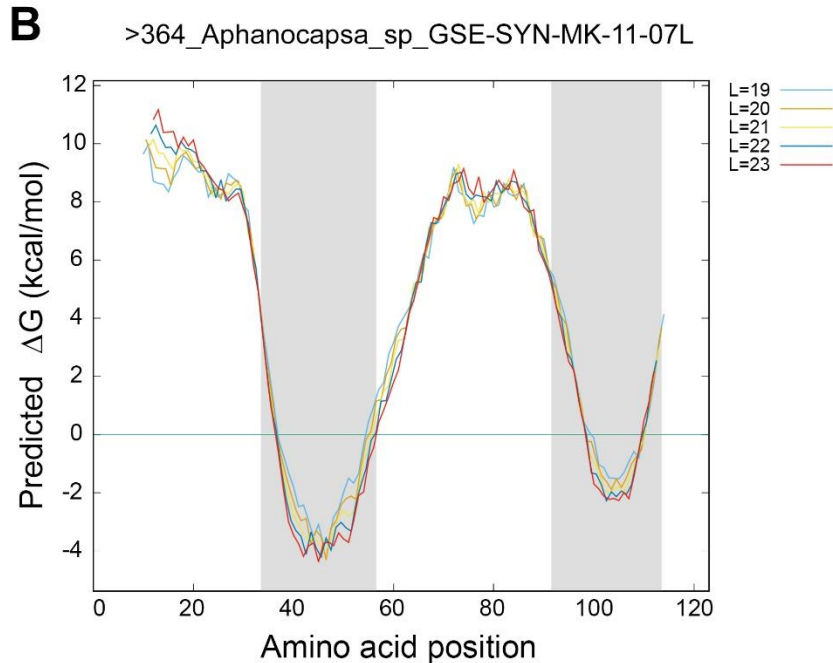
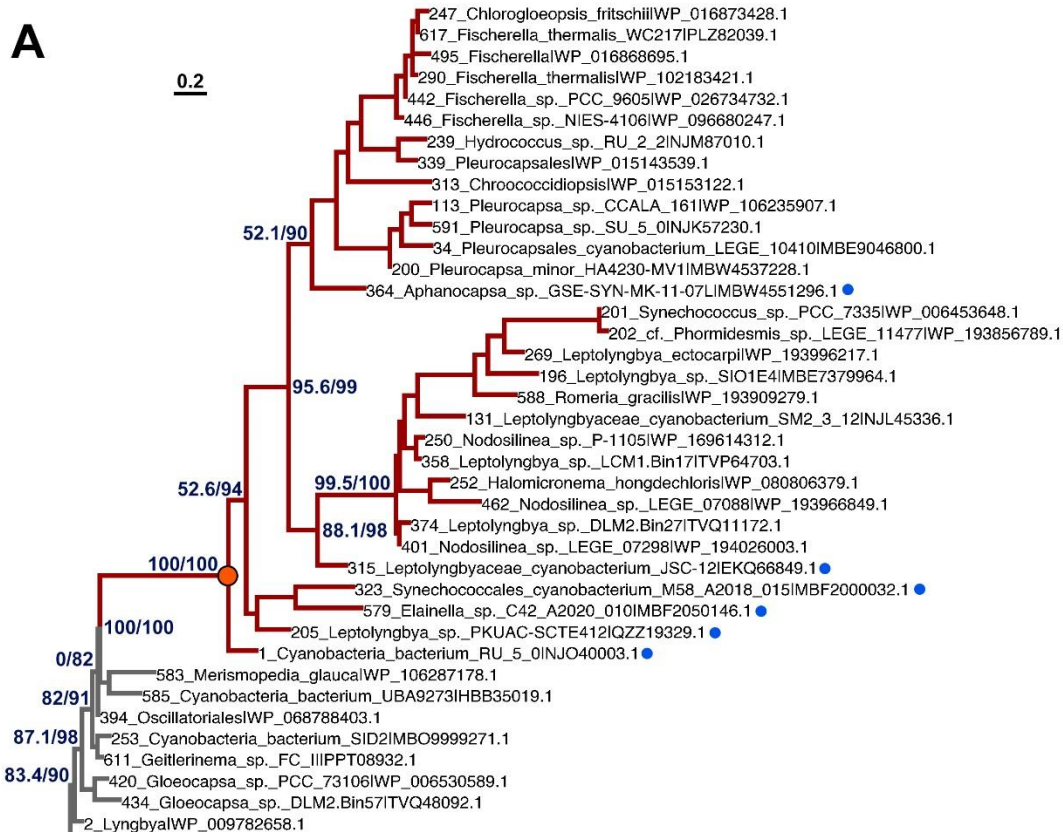


**Supplementary Figure S3.** Phylogenetic tree of PsbC sequences focused around the FRL sequences and basal clades. The scale bar represents the number of substitutions per site. Blue numbers are branch support values (aLRT/UFbootstrap). Only those on the major subgroups are shown for clarity. The sequence 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*).





#### 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.

[illegible]





VL Synechococcus 7335 ELFRDPRTGEPALDLPKMFGIHLFLSGLLCFGFGAFHLTGLWGPGMWVSDPYGLTGHVQG 180  
VL Aphanocapsa GSE ELFRDPRTGEPALDLPKMFGIHLFLSGLLCFSFGAFHLTGLFGPGMWVSDPYGLTGSIQP 180  
VL Pleurocapsa 7327 ELFTDPRTGEPALDLPKMFGIHLFLSGLLCFGFGAFHLTGLWGPGMWVSDPYGLTGHVQP 180  
VL Fischerella 7521 ELFQDPRTGEPALDLPKMFGIHLFLSGLLCFGFGAFHLTGLWGPGMWVSDAYGLTGHIAF 180  
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FRL Ancestral VAPEWGPDPGNPFPNPGGVVAHHIAAGIVGII<sup>1</sup>GLFHITVRPPENLYRALRMGNIETVL<sup>2</sup>AS 240  
FRL Synechococcus 7335 VAPEWGAAGFDPHNPGGVVAHHIALGIVAI<sup>1</sup>GLFHIFVRPPEYLYKGLRMGNIETL<sup>2</sup>AS 240  
FRL Aphanocapsa GSE VAPDWGASGFNPFNPGGVVAHHIAAGIVGIVGGLFHMNVRPSENLYKGLRMGNLETVLAS 240  
FRL Pleurocapsa 7327 VAPVWGPEGFPNPNPGGVVAHHIAAGIVGII<sup>1</sup>IGGLFHIVVRPSEGLYRLLRMGNIEGVLAS 240  
FRL Fischerella 7521 VAPVWGPEGFPNPQNPGGVVAHHIAAGIVGII<sup>1</sup>IGGLFHIVVRPPEVLYRGLRMGNIETVLAS 240  
VL Synechococcus 7335 VAPEWGPAGFNPFPNPGGVVAHHIAAGIVGIVAGLFHITVRPPQRLYKALRMGNIETVLSS 240  
VL Aphanocapsa GSE VAPAWGPDGFNPFNPGGIVAAHIAAGVVGII<sup>1</sup>IAGLFHLIVRPPQRLYKALRMGNIETVLSS 240  
VL Pleurocapsa 7327 VAPEWGPAGFNPFPNPGGVVAHHIAAGIVGII<sup>1</sup>IAGLFHITVRPPERLYRALRMGNIETVLSS 240  
VL Fischerella 7521 VAPEWGPDPGNPFPNPGGVVAHHIAAGIVGII<sup>1</sup>IAGLFHLSVRPPERLYKALRMGNIETVLSS 240  
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FRL Ancestral ALAT<sup>1</sup>FFAGFV<sup>2</sup>VAGTMWYGSATTP<sup>3</sup>IELFGPTRYQWD<sup>4</sup>SGYFQ<sup>5</sup>Q<sup>6</sup>EIDRRVQANLAEG<sup>7</sup>KS<sup>8</sup>LS<sup>9</sup>E 300  
FRL Synechococcus 7335 GLAV<sup>1</sup>FSGAFI<sup>2</sup>AGTMWY<sup>3</sup>GATTP<sup>4</sup>IEL<sup>5</sup>GPTRYQWDQ<sup>6</sup>GFFQQAIS<sup>7</sup>RQVKASISD<sup>8</sup>G<sup>9</sup>SP<sup>10</sup>E 300  
FRL Aphanocapsa GSE GLATFFFAAFV<sup>1</sup>ASGSMWYGTATTP<sup>2</sup>IELWGPT<sup>3</sup>RFQWDKGYFKHEIDRRVQASLAEGKSL<sup>4</sup>SQ 300  
FRL Pleurocapsa 7327 SLAVFFFAGFIASASMWYGTATTP<sup>1</sup>IELWGPT<sup>2</sup>RYQWD<sup>3</sup>RGYFQ<sup>4</sup>Q<sup>5</sup>EIDRRVQAGLAEGKSLN<sup>6</sup>Q 300  
FRL Fischerella 7521 ALATFFFAAGFVAAGSMWYGTATTP<sup>1</sup>IELWGPT<sup>2</sup>RYQWDQ<sup>3</sup>NYFKQ<sup>4</sup>EIDRRVQAGLDEGKTL<sup>5</sup>SQ 300  
VL Synechococcus 7335 SIAAVFFAA<sup>1</sup>FIVAGTMWYGSATTP<sup>2</sup>IELFGPTRYQWD<sup>3</sup>GSYFAEEIDRRVQ<sup>4</sup>RDIA<sup>5</sup>NGASEE<sup>6</sup> 300  
VL Aphanocapsa GSE SIAAVFFAA<sup>1</sup>FVAGTMWYGSATTP<sup>2</sup>IELFGPTRYQWD<sup>3</sup>GSYFNQ<sup>4</sup>EINRRVQ<sup>5</sup>TSLAQ<sup>6</sup>QASL<sup>7</sup>E 300  
VL Pleurocapsa 7327 SIAAVFFAA<sup>1</sup>FVAGTMWYGSATTP<sup>2</sup>IELFGPTRYQWD<sup>3</sup>KGYFQ<sup>4</sup>Q<sup>5</sup>EIQRRVEANLAAGDTL<sup>6</sup>SE 300  
VL Fischerella 7521 SIAAVFFAA<sup>1</sup>FVAGTMWYGSATTP<sup>2</sup>IELFGPTRYQWD<sup>3</sup>QGYFKQ<sup>4</sup>EIQRRVQASLASGANL<sup>5</sup>SE 300  
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FRL Ancestral AWSS<sup>1</sup>IPEKLA<sup>2</sup>FYDYVGN<sup>3</sup>SPAKGGLFRV<sup>4</sup>GPMTKGD<sup>5</sup>GIAQSWLGH<sup>6</sup>VPV<sup>7</sup>FQDAEGRELT<sup>8</sup>VRRMP 360  
FRL Synechococcus 7335 AWSEIPTKLA<sup>1</sup>FYDYIGN<sup>2</sup>SPAKGGLFRV<sup>3</sup>G<sup>4</sup>LV<sup>5</sup>GDGLPTGWLGH<sup>6</sup>VPVKDGE<sup>7</sup>GRELT<sup>8</sup>VRRMP 360  
FRL Aphanocapsa GSE AWSA<sup>1</sup>IPEKLA<sup>2</sup>FYDYTGNN<sup>3</sup>PAKGG<sup>4</sup>FRVGRMVD<sup>5</sup>GDGVAQ<sup>6</sup>DWLGH<sup>7</sup>VPFTDQEGRELT<sup>8</sup>VRRMP 360  
FRL Pleurocapsa 7327 AWSA<sup>1</sup>IPKLA<sup>2</sup>FYDYIGN<sup>3</sup>SPAKGGLFRVGRMVD<sup>4</sup>GDGLATGWLGH<sup>5</sup>VPVKDGE<sup>6</sup>GRELT<sup>7</sup>VRRMP 360  
FRL Fischerella 7521 SWSA<sup>1</sup>IPEKLA<sup>2</sup>FYDYIGN<sup>3</sup>NPAGG<sup>4</sup>FRVGRMVD<sup>5</sup>GDGVAQ<sup>6</sup>SWLGH<sup>7</sup>VPVKDREGRELT<sup>8</sup>VRRMP 360  
VL Synechococcus 7335 AYA<sup>1</sup>AIPEKLA<sup>2</sup>FYDYVGN<sup>3</sup>SPAKGGLFRVGPMT<sup>4</sup>GDGIATAWLGH<sup>5</sup>VPV<sup>6</sup>FHDGDGRELT<sup>7</sup>VRRRLP 360  
VL Aphanocapsa GSE AWSA<sup>1</sup>IPEKLA<sup>2</sup>FYDYIGN<sup>3</sup>NPSKGG<sup>4</sup>LFRGTAMDN<sup>5</sup>GDGIAGK<sup>6</sup>WIGHATFRDAEGTEL<sup>7</sup>FVRRMP 360  
VL Pleurocapsa 7327 AWSK<sup>1</sup>IPEKLA<sup>2</sup>FYDYVGN<sup>3</sup>SPAKGGLFRGTAMNS<sup>4</sup>GDGIARAWLGH<sup>5</sup>AVFRDGE<sup>6</sup>GRELT<sup>7</sup>VRRMP 360  
VL Fischerella 7521 AWSQ<sup>1</sup>IPEKLA<sup>2</sup>FYDYVGN<sup>3</sup>SPAKGGLFRGT<sup>4</sup>PMVKGD<sup>5</sup>GIAQSWDGH<sup>6</sup>VPVKDAEGRELE<sup>7</sup>VRRRLP 360  
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FRL Ancestral NFFETFPVVLTDKDGIVRADIPFRAESKYSFEQTGVTVS<sup>1</sup>FYGGELD<sup>2</sup>GGQTF<sup>3</sup>TDPA<sup>4</sup>TVKKY 420  
FRL Synechococcus 7335 NFFENFPVVLFDQDGIVRADIPFRAESKYGIEQTGVTVS<sup>1</sup>FYGGELD<sup>2</sup>GGQTF<sup>3</sup>SDPK<sup>4</sup>DVKKY 420  
FRL Aphanocapsa GSE SFFENFPVIFVDNDGVVRADIPFRAAAKY<sup>1</sup>SVEQTGVNVTFYGGELD<sup>2</sup>GGQTF<sup>3</sup>TDPM<sup>4</sup>GMVKY 420  
FRL Pleurocapsa 7327 NFFETFPVMTDKDGVVRADIPFRRTDAKYSIDQ<sup>1</sup>GVTVS<sup>2</sup>FYGGLLD<sup>3</sup>GGQTF<sup>4</sup>ISDPAL<sup>5</sup>VKKY 420  
FRL Fischerella 7521 TFFETFPVVLTDKDGIVRADIPFQRAEAKYSFEQTGVNV<sup>1</sup>SFFGGILD<sup>2</sup>GGQTF<sup>3</sup>TDPM<sup>4</sup>TVKKY 420  
VL Synechococcus 7335 NFFETFPVVLVDKDDNLRADIPFRAESKYSFEQTGVVVD<sup>1</sup>FYGGQLD<sup>2</sup>GGQHIT<sup>3</sup>DPAA<sup>4</sup>VKKY 420  
VL Aphanocapsa GSE NFFETFPVILTDKDGIVRADIPFRAESKYSFEQQGVNV<sup>1</sup>SFYGGELD<sup>2</sup>GGQTF<sup>3</sup>FKDAP<sup>4</sup>TVKKY 420  
VL Pleurocapsa 7327 NFFETFPVVLTDSDGIVRADIPFRAESKTSIEQTG<sup>1</sup>TKVSFYGGILD<sup>2</sup>GGQTF<sup>3</sup>SDPAT<sup>4</sup>VKKF 420  
VL Fischerella 7521 NFFETFPVILTDKDGIVRADIPFRAESQNSFEQTGVTVS<sup>1</sup>FYGGNLD<sup>2</sup>GGQTF<sup>3</sup>TDPA<sup>4</sup>ADVKKW 420  
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FRL Ancestral ARKAQLGEPFEFDRQILNSDGVFRTSTRGW<sup>1</sup>FAHACFALL<sup>2</sup>WFFGHIWHGSRTLFRDVFA 480  
FRL Synechococcus 7335 ARRAQLGEPFEFDRSVYDSGLFRTS<sup>1</sup>RGFF<sup>2</sup>FF<sup>3</sup>HVIFGLL<sup>4</sup>FFGHIWHGLRALFQDVFS 480  
FRL Aphanocapsa GSE ARQAQLGEVFNFNRETYNSDGVFRTSNRGFFAFFHACFALVWFFGHLWHGSRTLFRDVFA 480  
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VL Synechococcus 7335 ARKAQLGEPFSFDR<sup>1</sup>ETLSDGVFRSSPRGWFTYGHAVFALLFFFGHIWHGARTLFRDVFA 480  
VL Aphanocapsa GSE ARKAQLGEQFEFDQETLSSDGILRTSPRGWFTYGHACFALLFFFGHIWHGARTLFRDVFS 480  
VL Pleurocapsa 7327 ARKAQLGESFDFDRET<sup>1</sup>LNSDGVFRTSPRGWFTFAHACFALLFFFGHIWHGSRTLFRDVFA 480  
VL Fischerella 7521 ARKAQLGEVFEFDRET<sup>1</sup>LNSDGVFRTSPRGWFTFGHACFALLFFFGHIWHGARTIYRDVFA 480  
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FRL Ancestral GIDPDLDEEQVEFGVFQKVGDTTTRKKEAVAV----- 512  
FRL Synechococcus 7335 GIDP<sup>1</sup>SLSAEQVEWGYFKKVGDP<sup>2</sup>TSQQT<sup>3</sup>PA----- 509  
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FRL Pleurocapsa 7327 GINPDLDEEQVEFGVWQKVGDTVSRKRQVA----- 510  
FRL Fischerella 7521 GIDPELDE-QVEFGVFQKVGDTTTRKKQ<sup>1</sup>PVI----- 510  
VL Synechococcus 7335 GVD<sup>1</sup>PDLSPQQVEWGYFQKVGDFSTKASK----- 508  
VL Aphanocapsa GSE GIDPELSEEQVEWGYFQKLGDTSTRKETV----- 510  
VL Pleurocapsa 7327 GIDPDLG-EQVEFGLFAKVGDVSTRKEGV----- 508  
VL Fischerella 7521 GIDPDLE-EQVEFGVFAKVGDLSTRKKEAV----- 509  
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## PsbC

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FRL Ancestral      METPFDRSVVTTETMSTTTIPDKTKGTSITTAREGRDEASTGYAWWAGNARFINTEL 60
FRL Synechococcus 7335 METPLETIP-----D---LSLSPTAEVGSILAPASPGYDSTGYAWWAGNARLITPELTG 53
FRL Aphanocapsa GSE -----MTTSRDGRDEASTGYAWWAGNARFIN--LSG 29
FRL Pleurocapsa 7327 METPFNSSTVKIG---TTVQDAPTRDVTFDLAREGRDEASTGYAWWAGNARFIN--LSG 54
FRL Fischerella 7521 METPFDSKVSKPKD-----EVQKPAYIVNSPSEGRDEASTGYAWWAGNARFIN--QSG 51
VL Synechococcus 7335 -----MVTLSNNSFVGGGRDQSTGYAWWSGNARLID--LSG 35
VL Aphanocapsa GSE -----MVTLSNSIVAGNRDQESSGFAWWAGNARLIN--LSG 35
VL Pleurocapsa 7327 -----MVTLSNTSYAGGGRDLSSTGFAWWAGNARLIN--LSG 35
VL Fischerella 7521 -----MVTLSRPGVLGAGRQDQSTGFAWWAGNARLIN--LSG 35
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FRL Ancestral      RFLGAHVAHAGLIAFWAGAMLLFEVAHYVPEKPMYEQGLILMPHIATLGFVGVGHGGEVVD 120
FRL Synechococcus 7335 RFLGAHVAHAGLVFWAGMMLFEVSHFNLSKPMYEQGCILLPHIATLIGIVGQSGEITS 113
FRL Aphanocapsa GSE RFLGAHIAHAGLMAFWAGAMLLFEVSHFVPEKPMYEQGLLLMPHVATLGFVGVPGGVVND 89
FRL Pleurocapsa 7327 KFLGAHVAHAGLIAFWAGAMLLFEVAHYVPEKPMYEQGCILLPHLATLGFVGVSGGQVVD 114
FRL Fischerella 7521 RFLGAHVAHAGLIAFWAGAMLLFEVAHYVPEKPMYDQGLILMPHIAALGFVGVPGGQVVD 111
VL Synechococcus 7335 KLLGAHVAHAGLIVLWTGAMTLFEVSHYIPEKPMYEQGCILLPHLATLWGVGVPGGEVIN 95
VL Aphanocapsa GSE KLLGAHVAHAGLIVFWAGAMTLFEVAHFPEKPMYEQGCILLPHIASLWGVGVPGGEVTD 95
VL Pleurocapsa 7327 KLLGAHVAHAGLIVFWAGAMTLFEVAHFPEKPMYEQGLILLPHLATLWGVGVPGGEVVD 95
VL Fischerella 7521 KLLGAHVAHAGLIVFWAGAMTLFEVAHFPEKPLYEQGLILLPHLATLWGVGVAGGEVVD 95
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FRL Ancestral      IFPFFAIAVIHLIGSAVLGFGGLYHSLRGPEKL---SGFFDFDWSDKDKITILGYHLIA 177
FRL Synechococcus 7335 MFPFFAIGVAHLIGSAVLGIGGMYHAIKGEPEL---YFFQFDWTDRAKVAQILGFHIAI 170
FRL Aphanocapsa GSE IFPFFAIAVIHLIGSAVLGFGGVFHSIKGPAIL---PGYDIDYADKDKITDILAYNLFW 146
FRL Pleurocapsa 7327 TFPYFAIAVIHLIGSAVLGIGGLYHSLRGPEKL---AGFFDFDWSDKDKMTSIIIGYHLIA 171
FRL Fischerella 7521 IFPFFAIAVAHLIGSAVLGFGGIYHSLKGPQKL---PGFFNFDWSDKDKVTSILGYHLIA 168
VL Synechococcus 7335 LFPPYFVVGVLHLVSSAVLGLGGVYHALRGPETLEEYSSFFSQDWKDKNQMTNIIIGYHLIL 155
VL Aphanocapsa GSE IFPFFYVVGVLHLISSAVLGLGGIYHAVRGPEVLEEYSSFFGYDWKDKNQMTSILGFHLIV 155
VL Pleurocapsa 7327 TFPYFVVGVLHLISSAVLGFGGIYHALRGPETLEEYSSFFGYDWKDKNQMTNIIIGYHLIL 155
VL Fischerella 7521 TYPYFVIGVLHLISSAVLGFGGIYHAIRGPEVLEEYSSFFGYDWKDKNKMNTNIIIGFHLII 155
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FRL Ancestral      LGIAALLLVCKAMFGGLYDTWAPGGGDVRLITNPTLNPLVIFGYLFRSPFGGSGWIVSV 237
FRL Synechococcus 7335 LGIFALLFAAKAMYGGLYDPWAPGGGDVRLVTNPTLDPRIIFGYLIKRPFGGEGWIVSV 230
FRL Aphanocapsa GSE LGVGAWLLVGKAMLVGGGLYDTWAPGGGDVRLISHPTLNPLTIFGYLVRSPYGGEGWIVSV 206
FRL Pleurocapsa 7327 LGIGAFLLVGKAMFWGGLYDTWLPGGGGVRLVTNPTLDPRVIFGYFFKSPLGGSGWIVSV 231
FRL Fischerella 7521 LGVAAFLLVGKAMLVGGGLYDTWAPGGGGVRLVTNPTLDPRVIFGYLFGKFTGGAGNIASV 228
VL Synechococcus 7335 LGLGAFLLLVIKACFLGGVYDTWAPGGGDVRLVTNPTLNPGVIFGYLASSPFGGEGWIVSV 215
VL Aphanocapsa GSE LGFGALLLVKAMFVGGLYDTWAPGGGDVRLVTNPTLNPAVIFGYLVKSPFGGDGVAVS 215
VL Pleurocapsa 7327 LGFGALLLVKAMFFGGVYDTWAPGGGDVRLVTNPTLNPAVIFGYLIKAPFGGEGWIIISV 215
VL Fischerella 7521 LGLGAFLLLVKAAMFFGGVYDTWAPGGGDVRLVTNPTLNPAVIFGYLLKSPFGGDGWIIGV 215
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FRL Ancestral      NNLEDIVGGHIWMSGILIAGGIWHILTKPKFWTHKVFIVSSEAYLSQSLGNIAGQAFIAT 297
FRL Synechococcus 7335 NNLEDIIGGHIWIGCI IAGGIWHILVPPLRWYNLFPWTGETYLSQSLGNIAGQAFIAA 290
FRL Aphanocapsa GSE NNMEDLVGGHIWIGTILILGGIWHYFTTPSKWTHKVFIVSSEAYLAQSLGNVCGQALIAI 266
FRL Pleurocapsa 7327 DNLEDIVGGHIWMSGILIAGGIWHIFTKPKWTKDKVFIWSSEAYLSQSLGNIAGQAFIAA 291
FRL Fischerella 7521 DNLEDLVGGHIWIGSLILGGIWHIVTKPKFWTHKAFIWSSEAYLSQSLGNVAGQAFIAT 288
VL Synechococcus 7335 NNMEDIIIGGHIWIGLICIFGGVFHILTKPFGWARRALIWNSEAYLSYSIGAVSLMAFICS 275
VL Aphanocapsa GSE DNLEDVVGHHIWIIGLICIAGGIWHVLTKPFARWARAFVWSSEAYLSYSLGALSMLMAFIAT 275
VL Pleurocapsa 7327 DNMEDIIIGGHIWVGLICIAAGGIWHILTKPFGWARRAFIWSSEAYLSYSLGALSMLMGFIAS 275
VL Fischerella 7521 DNMEDIIIGGHIWVGLICIFGGIFHILTKPFGWARRAFIWSSEAYLSYSLGALSMLMGFIAS 275
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FRL Ancestral      CFVWFNNTAYPSEFYGPTAPEASQAQALTFLVRDQKLGANVASAQGPTGLGKYLMRSPTG 357
FRL Synechococcus 7335 AFVWFNNTAYPSVFYGPTVPESSQAQSFVFLMRDQGMGADVASAQGPGLGKYLMRSPTG 350
FRL Aphanocapsa GSE AFIWFNNTAYPSEFYGPTAPESSQAQALTFLVRDQSLGANVASAQGPTGLGKYLMRSPTG 326
FRL Pleurocapsa 7327 MFIWFNNTAYPSEFYGPTVPEASQAQALVFLARDQRLGADIGTAQSVTGLGKYLMRSPTG 351
FRL Fischerella 7521 MFIWFNNTAYPSEFYGPTVAESSNAQALVFLVRDQNLGANVASAQGPGLGKYLMRSPTG 348
VL Synechococcus 7335 CYVWFNNTAYPSEFYGPTNAEASQAQAMTFLVRDQRLGANIGSAQGPGLGKYLMRSPTG 335
VL Aphanocapsa GSE CFVWFNNTVYPSEFYGPTGPEASQAQAMTFLVRDQRLGANVGSAQGPGLGKYLMRSPTG 335
VL Pleurocapsa 7327 VYVWFNNTAYPSEFYGPTGMEASQAQAFVFLVRDQRLGANIASAQGPGLGKYLMRSPTG 335
VL Fischerella 7521 CFVWFNNTAYPSEFYGPTNAEASQAQSFVFLVRDQKLGANVASAQGPGLGKYLMRSPTG 335
      :*:***.*.*** ***** :*:***: ** *** :*: : :*. ***** **:*

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FRL Ancestral      EIIFGGETMRFWDFRGPWLEPLRGPNGLDLDKLQNDIQPWQIRRAAEYMTAPLGSLSNV 417
FRL Synechococcus 7335 EIIFGGETMRFWDARAPWLEPLRGKNGLDLDKLQHDVQPWQLRRAAEYMTSPIGLSNV 410

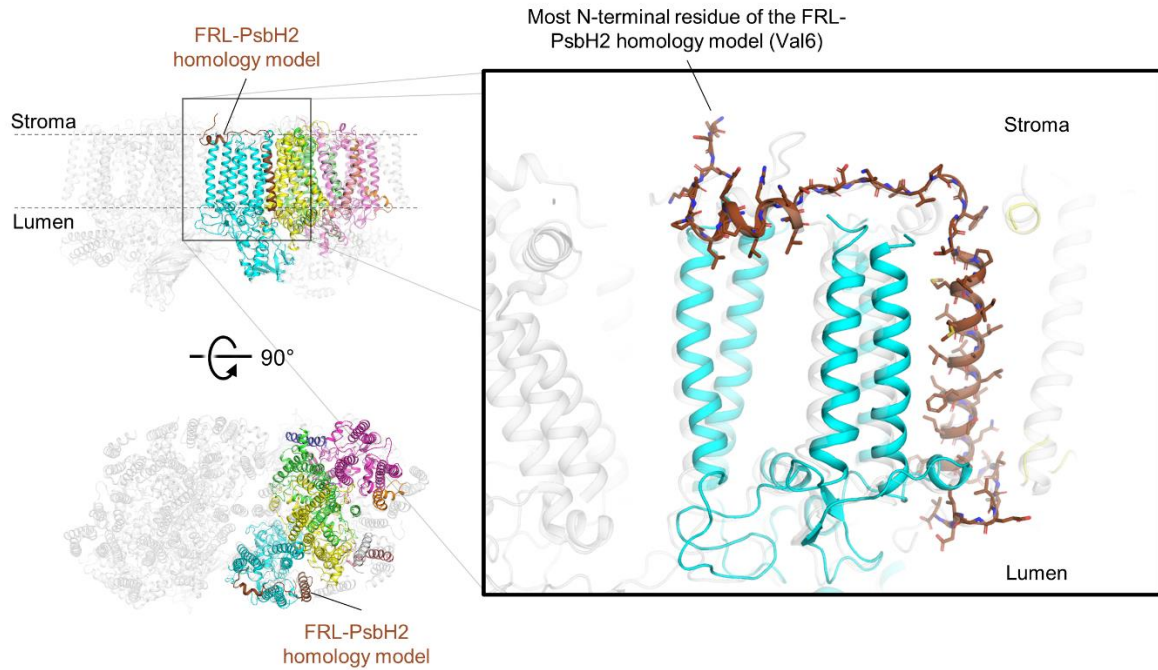
```



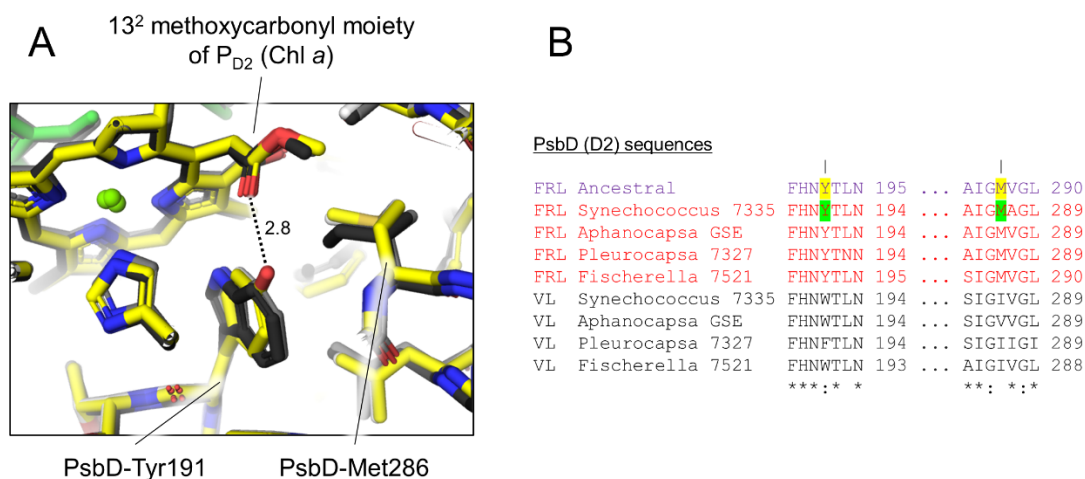


that is highlighted yellow in the ancestral sequence and green in the *Synechococcus* 7335 sequence is likely conserved from the FRL-PSII ancestor that is found in extant FRL-PSII, implying important functional significance. Residues that are highlighted green in the *Synechococcus* 7335 sequence but 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.

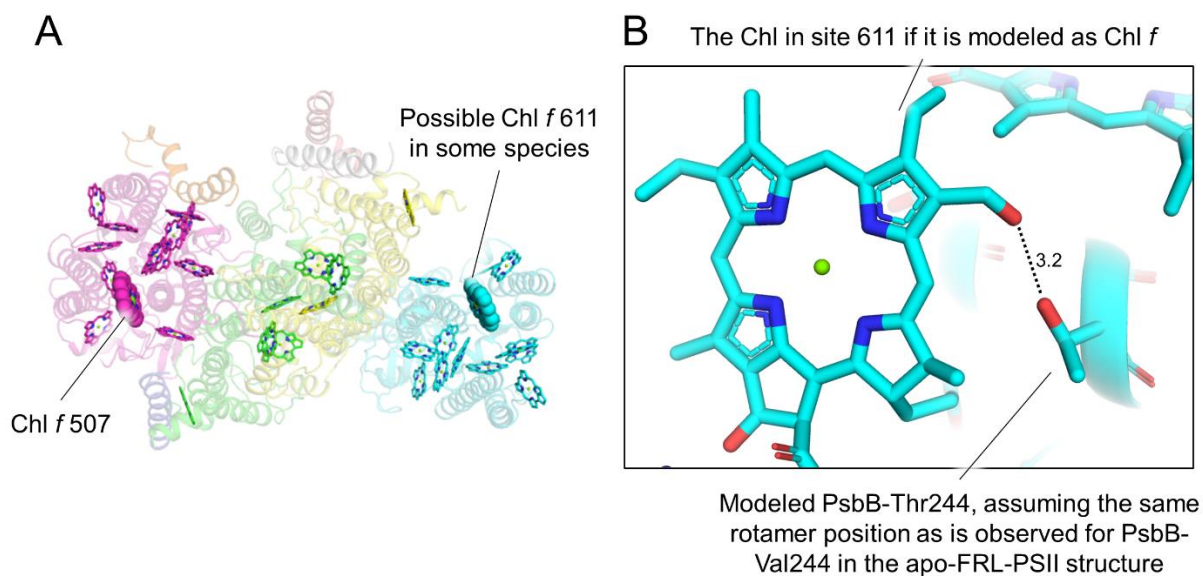




**Supplementary Figure S7.** Location of the FRL-PsbH2 homology model. On the left side, the *Synechococcus* 7335 apo-FRL-PSII structure is shown (colored) superimposed onto the structure of the PSII holocomplex from *Synechocystis* 6803 [5]. The FRL-PsbH homology model (brown) was generated using Swiss 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  $\text{P}_{\text{D2}}$ . 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  $^{13}\text{C}$  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)

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