

**Supplementary Figure 1.** Alignments of 1-Cys Prx (A), 2-Cys Prx (B), and Prx-BCP/PrxQ (C) from *Synechococcus* OS-B' (CYB), *Synechococcus* OS-A (CYA), *Synechococcus* sp. PCC 7942 (SynPCC7942), *Synechocystis* sp. PCC 6803 (SynPCC6803), *Synechococcus* sp. PCC 7002 (SynPCC7002), *Homo sapiens* (Hs), *Arabidopsis thaliana* (A. thaliana) and *Chlamydomonas reinhardtii* (Chlamy). Amino acids in the catalytic triad are indicated by number signs and grey shading. The secondary conserved cysteine in 2-Cys Prx is indicated by asterisk and yellow shading.

**Supplementary Figure 1A. 1-Cys Prx**

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CYB_0113      M--SLRLGDTAPNFTQKSTHGEIDFYSWAGDSWVVLFSHPADYTPVCTTELGTVAKLMPE
CYA_2849      M--ALRLGDTAPNFTQKSTHGEIDFYSWAGDSWVVLFSHPADYTPVCTTELGTVAKLMPE
SynPCC7942    M--SLRLGDTVPNFTQQSSEGEINFYDWAGDSWVILFSHPADYTPVCTTELGTVAKLKEE
SynPCC6803    M--ALQLGDVVPDFEQESSQGPISFHEWAGDSWVVLFSHPADYTPVCTTELGTVAKLKPE
SynPCC7002    M--SLRLGDVAPNFTQDSTIGEINFYDWAGDSWVVLFSHPADFTPVCTTELGEVARLKGE
Hs. PRDX6     MPGGLLLGDVAPNFEANTTVGRIRFHDGFLGDSWGILFSHPRDFTPVCTTELGRAAKLAPE
A. thaliana   MP-GITLGDTPVNLEVETTHDKFKLHDYFANSWTVLFSHPGDFTPVCTTELGAMAKYAHE
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CYB_0113      FEKRGVKVIALSVDDVDVSHVGVVKDIE----ETQ NATLTYPILADADRKVSQLYGMLDQT
CYA_2849      FEKRGVKVLALSVDVDSHLGVVKDIE----ETQNAIVNYPILADVDRKVSQLYGMLDQT
SynPCC7942    FAKRNVKPIALSVDVESHKGWIGDIN----ETQGTQVNYPI LADPDRKVS DLYDMIHPN
SynPCC6803    FDKRNVKVIALSVDVESHKGWICDID----ETQNTTVNYPILADGDKKVS DLYGMIHPN
SynPCC7002    FEKRNKVIALSVDVTASHNGWVGDIID----ETQGC AVNYPILSDADQKVATLYNMIHPN
Hs. PRDX6     FAKRNVKLIALSIDSVEDHLAWSKDINAYNCEEPTEKLPFPIIDDRNRELA ILLGMLDPA
A. thaliana   FDKRGVKLLGLSCDDVQSHKDWIKDIEAF---NHGSKVNYPI IADPNKEIIPQLNMIDPI

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CYB_0113      NLNQEGLPLTVRSVFVIDPNKKIRLILTYPASTGRNFDELLRVIDALQLTDKY--NVVTP
CYA_2849      NLNQEGLPLTVRSVFVIDPNKKIRLILTYPASTGRNFDELLRAIDALQLTDKY--SVVTP
SynPCC7942    ANNT----LTVRSVFIIDPNKKLRLLTLYPASAGRNFDELLRVIDSLQLTDNY--SVATP
SynPCC6803    ALNN----LTVRSVFIIDPAKKLRLLTFTYPASTGRNFDEILRVIDSLQLTDYH--QVATP
SynPCC7002    ANPK----VTVRTVFVIDTERKVRLTITYPPSTGRNFQEILRVLDLQLTDNY--SVATP
Hs. PRDX6     EKDEKMPVTARVVFVFGPDKKLLKLSILYPATTGRNFDEILRVVISLQLTAEK--RVATP
A. thaliana   ENG-----PSRALHIVGPD SKIKLSFLYPSTTGRNMDEVLRALD SLLMASHKHNKIATP
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CYB_0113      ADWKDGDDVVIIPPSLKDPEVLKEKFPKGYKEI-----KPYLRVAPQPKNK
CYA_2849      ADWKDGDDVVIIPPSLQDPEVLKEKFPKGYKEL-----KPYLRRLTPQPNK
SynPCC7942    ANWNDGDDVVVPSI-PTEQAREQFPKGVTEV-----KPYLRRLTPQPNR
SynPCC6803    ANWQDGDKCVVPSI-STEDAKVKFPKGVVEEI-----KPYLRRLTPQPNK
SynPCC7002    VNWQDGEDVVVSPAI-STEDAKVKFPKGVKEI-----KPYLRMTTPQPNK
Hs. PRDX6     VDWKDGDSVMVLPTI-PEEEAKKLFKGVFTKELPSGKKYLRYTPQP--
A. thaliana   VNWKPDQPVVISPAV-SDEEAKMFPQGFKTADLPSKKGYL RHTEVS--

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**Supplementary Figure 1B. 2-Cys Prx**

CYB\_2254 MS-----  
 CYA\_0537 MS-----  
 SynPCC7942 MT-----  
 SynPCC6803 MT-----  
 SynPCC7002 MA-----  
 A. thaliana MSMASIASSSSTLLSSSRVLLPSKSSLLSPTVSPFRIIPSSASSSSLCSGFSSLSGLT  
 A. thaliana MA--SVASSTTLISSPSSRV-FPAKSSLSPPSVSFLRTLSSPSA-SASLRSGFARRSSLS  
 Hs. PRDX1 MS-----  
 Hs. PRDX2 MA-----  
 Hs. PRDX3 MAAAVGRLLRASVARHVSIAI-----PWGISATAALRPAACGRSLTNLL  
 Hs. PRDX4 MEALPLLAATTPDHGRHRRLLLLPLLLFLLPAGAVQGWETEERPRTREEECHFYAGGQVY  
 CHLAMY PRX1 MAALQSASRS SAVA FSRQARVAPRVAA-----  
 CHLAMY PRX2 MV-----  
 CHLAMY PRX7 -----

CYB\_2254 -----QEGCLRVGQPAPDFSATAVY-DMEFKTVKLSDYRGKKYVVLFFYP  
 CYA\_0537 -----QEGCLRVGQPAPDFSATAVY-DMEFKTVRLSDYRGKKYVVLFFYP  
 SynPCC7942 -----EGALRVGQLAPDFEATAVV-DQEFQTIKLSNYRG-KYVVLFFYP  
 SynPCC6803 -----EVLRVGQPAPDFTATAIV-DQSFQTVKLSTYRG-KYLVLFFYP  
 SynPCC7002 -----AQVQLAPDFTATAVI-DQEFKTIKLSDYRG-QYVVLFFYP  
 A. thaliana TNRSASRRNFAVKAQADDLPLVGNKAPDFEAEAVF-DQEFIKVKLSEYIGKKYVILFFYP  
 A. thaliana ---STSRRSFAVKAQADDLPLVGNKAPDFEAEAVF-DQEFIKVKLSDYIGKKYVILFFYP  
 Hs. PRDX1 -----SGNAKIGHAPAPNFKATAVMPDGQFKDISLSDYK-KYVVLFFYP  
 Hs. PRDX2 -----SGNARIGKPAPDFKATAVV-DGAFKEVKLSDYK-KYVVLFFYP  
 Hs. PRDX3 CS-GSSQAKLSTSSSCHAPAVTQHAPYFKGTAVV-NGEFKDLSDDFKG-KYLVLFFYP  
 Hs. PRDX4 PG-EASRVSVADHSLHLSKAKISKAPYWEGTAVI-DGEFKELKLTDIRG-KYLVLFFYP  
 CHLAMY PRX1 ---SVARRSLVVRASHAEKPLVGSVAPDFKAQAVF-DQEFQEI TLSKYRG-KYVVLFFYP  
 CHLAMY PRX2 -----AKIGAPAPKFKAQAVV-NGEIKI EISLDDYK-KYVVLFFYP  
 CHLAMY PRX7 -----PMFVSAAVV-DGEITKISLSDYK-KYVCLFFYP

CYB\_2254 LDFTFVCPT EITAFSDRYDDFAKLDTEILGVSVDSEYSHLAWIQ TDRKAGGVGELRYPLV  
 CYA\_0537 LDFTFVCPT EITAFSDRYDEFKLDTEILGVSVDSEYSHLAWIQ TDRKAGGVGELRYPLV  
 SynPCC7942 LDFTFVCPT EITAFSDRYADFSALNTEILGVSVD SQFSLAWIQ TDRKAGGVGELRYPLV  
 SynPCC6803 LDFTFVCPT EIIAFSDRHSEFTALDTEVVGISVDSEFSLAWIQ TERKMGGININYPLV  
 SynPCC7002 LDFTFVCPT EIVAFSDRHSEFEKLNTEVLGVSVDSEFAHLAWIQ TDRKMGVGD LAFPLV  
 A. thaliana LDFTFVCPT EITAFSDRYEEFEKLNTEVLGVSVD SVFSLAWVQTDRKSGGLD LNYPLV  
 A. thaliana LDFTFVCPT EITAFSDRHSEFEKLNTEVLGVSVD SVFSLAWVQTDRKSGGLD LNYPLI  
 Hs. PRDX1 LDFTFVCPT EIIAFSDRAEEFKLNCQVIGASVDSHFCHLAWVNTPKKQGG LGPMNIPLV  
 Hs. PRDX2 LDFTFVCPT EIIAFSNRAEDFRKLGCEVLGVSVD SQFTHLAWINTPRKEGGLG PLNIPLL  
 Hs. PRDX3 LDFTFVCPT EIVAFSDKANEFDVNCEVVAVSVDSHFSLAWINTPRKNGGLGH MNIALL  
 Hs. PRDX4 LDFTFVCPT EIIAFGDRLEEFRSINTEVVACSVDSQFTHLAWINTPRRQGG LGPIRIPLL  
 CHLAMY PRX1 LDFTFVCPT EITAFSDRYKEFKDINTEVLGVSVD SQFTHLAWIQ TDRKEGGLD LAYPLV  
 CHLAMY PRX2 LDFTFVCPT EIVAFSDRVEEFRAINTEVIGASIDSQFTHLAFSNTPRTKGGLGCKY PLV  
 CHLAMY PRX7 KDFTFVCPT EIIAFSDRAKEFAAANCQLIAASTDTEETHLAWIRTPRNRGGLGYM QIPIL  
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CYB\_2254 SDLKKEISAAYNVLDP---EAGVALRGLFIIDKEGIIQHATINNLA FGRSVD ETLR TLQA  
 CYA\_0537 SDLKKEISAAYNVLDP---AAGVALRGLFIIDKEGIIQHATINNLA FGRSVD ETLR TLQA  
 SynPCC7942 ADLKKEISTAYNVLDP---AEGIALRGLFIIDKEGVIQHATINNLA FGRSVD ETLR VLQA  
 SynPCC6803 SDLKKEISQAYNVLEP---DAGIALRGLFIIDREGILQYATVNNLS FGRSVD ETLR VLKA  
 SynPCC7002 SDLNKTISTAYGVLEP---EAGISLRGLFIIDPEGMIQHITVNNLS FGRSLD ETLR VLQA  
 A. thaliana SDITKSISKSFVGLIP---DQGIALRGLFIIDKEGVIQHSTINN LGIGRSVD ETLR TLQA  
 A. thaliana SDVTKSISKSFVGLIH---DQGIALRGLFIIDKEGVIQHSTINN LGIGRSVD ETLR TLQA  
 Hs. PRDX1 SDPKRTIAQDYGV LKA---DEGISFRGLFIIDDKGILRQITVNDLPVGRSVD ETLR LVQA  
 Hs. PRDX2 ADVTRRLSE DYGV LKT---DEGIAYRGLFIIDGKGVLRQITVNDLPVGRSVD ETLR LVQA  
 Hs. PRDX3 SDLTKQISR DYGV LLE---GSGIALRGLFIIDPNGVIKHL SVNDLPVGRSVD ETLR LVKA  
 Hs. PRDX4 SDLTHQISK DYGV LLE---DSGHTLRGLFIIDDKGILRQITLNDLPVGRSVD ETLR LVQA  
 CHLAMY PRX1 ADLKKEISKAYGV LT---EDGISLRGLFIIDKEGVVQHATINNLA FGRSVD ETLR VLQA  
 CHLAMY PRX2 ADLTKQIAK DYGV LIEDGPDAGVTLRGLFISPTGVLRQITINDLPVGRSVD ETLR VLKA  
 CHLAMY PRX7 ADTTKDISARYGV LIE---KLGVALRGLFIINPQGVVQHV TINDLP IGRSVD ETLR TLQA  
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CYB\_2254 IQYVQSHPDEVCPANWQPGQKTMHPDPVKSKEFFAAIAK-  
 CYA\_0537 IQYVQAHPDEVCPANWQPGQRTLNPDPVKSKEFFAAVAK-  
 SynPCC7942 IQYVQSHPDEVCPANWQPGAATMNPDPVKSKEFFAAV---  
 SynPCC6803 IRHVQSHPNEVCPVDWQEGDKTMIIPDPEKAKTYFETVAEP  
 SynPCC7002 IQHVQTNHNEVCPVDWQVGDRTMVPNPSEAQAYFSTL---  
 A. thaliana LQYVQENPDEVCPAGWKPGEKSMKPDPKLSKEYFSAI---  
 A. thaliana LQYIQENPDEVCPAGWKPGEKSMKPDPKLSKEYFSAI---  
 Hs. PRDX1 FQFTDKH-GEVCPAGWKPGSDTIKPDVQKSKEYFSKQK--  
 Hs. PRDX2 FQYTDEH-GEVCPAGWKPGSDTIKPNVDDSKEYFSKHN--  
 Hs. PRDX3 FQYVETH-GEVCPANWTPDSPTIKPSPAASKEYFQKVNQ-  
 Hs. PRDX4 FQYTDKH-GEVCPAGWKPGSETIIPDPAGLKLYFDKLN--  
 CHLAMY PRX1 IQYVQSNPDEVCPAGWKPGDKTMKPDPKGSKEYFSAV---  
 CHLAMY PRX2 FQFTDEH-GEVCPANWNPAGAKTMKADPTKSLEYFSTLS--  
 CHLAMY PRX7 IQYHAEH-GEVCPANWKPGSKTMVADAEKSLLEYFSEV---

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**Supplementary Figure 1C. Prx-BCP / PrxQ**

CYB\_0523 -----  
 CYB\_2186 -----MGLVVGLLVGFGMTQPAW  
 CYA\_0907 -----MGLVGLFTALLVSWAGVQPAL  
 CYB\_1376 -----  
 CYB\_1057 -----  
 CYA\_2145 -----  
 CYA\_2305 -----  
 CYA\_0672 -----  
 SynPCC7942 -M-----PVSRRQLLSLLALP-ALVLAPRS  
 SynPCC7942 -----  
 SynPCC7942 -----  
 SynPCC7942 -----  
 SynPCC6803 -MTSK-----KFSWPKTIIALLTLGLWGLADLP  
 SynPCC6803 -----  
 SynPCC7002 -M-----LQFFRTILITVVAIA-FMWFPGEA  
 SynPCC7002 -----  
 A. thaliana MAASSSFTLCNHTTLRLTLPKRKTLVTKTQFSVPTKSSSESNFFGSTLTHSSYISPVSSSS  
 CHLAMY PRX6 MQTIR-----APARAPVASSRRVATFRAAPRVSR

CYB\_0523 -----MPLAVGDPAPEF-----TLPD-----AEGN-----PISLSQLRG-QRVVLYFYPR  
 CYB\_2186 AQWGTTPLPPIGSPAPEF-----ALPD-----QSGQ-----IRRLADFRG-EWVLYFYPR  
 CYA\_0907 AQWGTTPLPQIGAPAPEF-----ELPD-----QSGQ-----VRRLADFRG-KWVLYFYPR  
 CYB\_1376 -----MGIVIGAVAPDF-----SLPAVGKGIQKGA-----QISLAAYRGKSKVLLAFYPA  
 CYB\_1057 -----MVLAVGTPAPGF-----NTTD-----TQGN-----QVSLADFAG-KTVVLYFYPK  
 CYA\_2145 -----MALAVGDPAPEF-----TLPD-----AEGN-----LVLSQLRG-RRVLYFYPR  
 CYA\_2305 -----MGIVIGTAAPDF-----NLPAVGKGIKGA-----TISLGAYRGKSKVLLAFYPA  
 CYA\_0672 -----MVLAVGTVAPGF-----NTTD-----THGN-----QVSLADFTG-KTVVLYFYPK  
 SynPCC7942 AQALGGPQPVPDEPAPDF-----SLPT-----DDGRE-----RLSLADFRG-QWLVLVLYFYPK  
 SynPCC7942 -----MAIavgdvapdf-----SLPA-----QDGT-----TVSLDFRGQKPVVLYFYPK  
 SynPCC7942 -----MALTVGTAAPDF-----TALD-----DAGQ-----SIQLSQFRG-KTIVLYFYPK  
 SynPCC7942 -----MPLQVGDRAAPDF-----TLPD-----QQGN-----PVSLTDLRG-QRVVLYFYPK  
 SynPCC6803 TYALGGIQPELDQAPLDF-----TLPS-----TTGEG-----EVNLTDIRG-QWVLYFYPK  
 SynPCC6803 ----MATALETNQAPTF-----SAPN-----AEGK-----TISDDFLG-QWLVLVLYFYPK  
 SynPCC7002 AIALGGPQPELNQLAPEF-----TLLG-----NDGEGKIQEFSLQDYRG-QWVLYFYPK  
 SynPCC7002 -----MTTLTIGQAAPDF-----ALTN-----AQGE-----ARTLADYSG-KWLVLYFYPK  
 A. thaliana LKGLIFAKVNGQAAPDF-----TLKD-----QNGK-----PVSLKYYKG-KPVVLYFYPA  
 CHLAMY PRX6 PVVVVRAELKVGKLEDPNYKVLKT-----SEGK-----TISLSSYKQKQPIVLYFYPK

CYB\_0523 DNTPGCTQEACGFRDAYADYQAHGIAILGVSADDARSHQKFAQKLQLPFLLVDEGAKVA  
 CYB\_2186 DFTSGCTIEARRFQODLPKFRAMGAQVVGVSADSVDSHRRFCSAEGQLQFPLLSDPDGTVS  
 CYA\_0907 DFTAGCTIEARRFQODLPKFRTLGAEIVGVSADSVDSHRRFCSAEGQLQFPLLSDPEGTVS  
 CYB\_1376 DFSPVCTSEMRCFREDWGAFAAGCEIILGISSDPLSRHQAFAEQLKLEFPLLSDVDRTVS  
 CYB\_1057 DDTPGCTKEACGFRDAYADYQSKDVVVLGVSMDDQASHQKFAEKYNLPPFLLVDSSGVIT  
 CYA\_2145 DNTPGCTREACGFRDAYAEYQAHGIHVLGVSADDARSHQKFAQKLQLPFLLVDEGAKVA  
 CYA\_2305 DFSPVCTSEMRCFREDWGAFAAGCEIILGISSDPLSRHEAFAEQLKLEFPLLSDVDKVS  
 CYA\_0672 DDTPGCTKEACGFRDAYADYQSKNVVVLGVSMDDQASHQKFAEKYNLPPFLLVDTSGVIA  
 SynPCC7942 DGTGCTLEAQRFFQODLAAAYAERNAQIVGVSADDLSSHSRFRENEGLSYPLLADVKGEVS  
 SynPCC7942 DDTPGCTIEACFRDSYTAQEVGAVVLGVSSDSIDSHQRFQKYNLPPFLLSDAGDRLR  
 SynPCC7942 DDTPGCTKEACFRDSYSAYQGKDIIVLGVSTDDSSSHEQFKTKFSLPFPLVADPDRSIT  
 SynPCC7942 DDTPGCTKEACFRDDFSLFEQAGIVVLGVSKDPASKHQKFIKYLFPFLLTDADAAVA  
 SynPCC6803 DFTPGCTLEAQRFRDLTKYQALNAQVIGVSDVLDLSHEAFCDAGLQFPLLADSDGAVI  
 SynPCC6803 DNTPGCTTEAIDFSEKLPEFTDLNAVVGVSVDSEKSHGKFIKHNLTQVLLSDPEHELA  
 SynPCC7002 DFTPGCTLEARRFQODLPKYLERNVQVLGVSDVDDVDSHEAFCDAGLQFPLLADSTGDVVS  
 SynPCC7002 DNTPGCTTEALDFTALVPEFTALNAVIGVSDSEKSHGRFIEKELAIELLSDPDHQTA  
 A. thaliana DFTPGCTKQACAFRDSYKFKKAGAEVIGISSDSSASHKAFASKYKLPYTLSDGKNVR  
 CHLAMY PRX6 AATPGCTKEACFRDEYSRFTAAGAVVFGISSDSPADNAAFGKANNLPYPLVTDENSILR  
 # #

CYB\_0523 RAYGVYGPKKFMGKEYNGIHRITTFVIDPEGKIEAVITK-VKV--ETHAAELLKQLTG---  
 CYB\_2186 RAYGSWMGDV-----ALRNTFLIDPEGILREIDPI-VNP--SRHSAEVLALQQLIR

CYA\_0907 RAYGSWTGEM-----ALRNTFLIDPEGILRAIDPI-VNP--SRHSAEVLALQLELAQ  
 CYB\_1376 QQYGVDSLGL-----TRRAYFLVDSQGILRYQHVE-WLPLFKRDNRELEAVRAI--  
 CYB\_1057 RAYDVDGGGY-----AKRVTYVIDGEGKIAKVYQN-INT--ETHARDILADLGL---  
 CYA\_2145 SAYGVYGPKKFMGKVYNGIHRRTTFVIDPEGKIEAIITK-VKV--EAHAAELKQLTG---  
 CYA\_2305 QLYGVDSFLG-----TRRAYFVDSQGILRYQHVE-WLPIFKRDNRELEAVRAV--  
 CYA\_0672 RAYDVDGGGY-----AKRVTYVIDAEGKIAKVYQN-INT--ETHARDILADLGL---  
 SynPCC7942 KRYGSWLAPF-----SLRHTYIIDPEGVLRANFTA-VRP--VIHSKEVLAKLDELQA  
 SynPCC7942 QTYGVPKTLF-----VIPGRVTYVIDKEGKVRHIFDSSLNA--QAHIQESLNILRSL--  
 SynPCC7942 QAYDVDGGGY-----AKRVTYVIDGEGQIRVYDS-VKT--DTHAGDILADLGL---  
 SynPCC7942 SAYDSYGLKKFMGREYMGMMRHTYVIDVEGKIEQIYTK-VKP--ETHARQILTDLGVAVA  
 SynPCC6803 KTYGSWLSGM-----ALRHTYIIDPEGILRERFLG-VRP--ATHSEEVLARLAEQA  
 SynPCC6803 AAYGAWGPKKFMGKECEGILRSTFLINPQGNIAHIWPN-VRV--KGHAEKVLEKLQQLNS  
 SynPCC7002 KQYGSYLTGY-----SLRHTYLIDPEGILRKIYLG-VNP--AIHSQEVLTDLDSLMA  
 SynPCC7002 GVVGVWQLKKFMGKEYMGIVRSTFLIDPQGQVAIWSK-VKV--KGHAQTVLETLOT---  
 A. thaliana KDWGVPGLDF-----GALPGRQTYVLDKNGVVQLIYNNQFQP--EKHIDETLKFLKA---  
 CHLAMY PRX6 KTFGIKGFDFL-----GLLPGRQTYVIDVNGKCVMAFNDQLNV--EQHVDEALKVVASVKV

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CYB\_0523 -----S  
 CYB\_2186 -----N  
 CYA\_0907 -----AG  
 CYB\_1376 -----S  
 CYB\_1057 -----  
 CYA\_2145 -----S  
 CYA\_2305 -----G  
 CYA\_0672 -----  
 SynPCC7942 -----G  
 SynPCC7942 -----  
 SynPCC7942 -----  
 SynPCC7942 -----E  
 SynPCC6803 -----  
 SynPCC6803 -----AD  
 SynPCC7002 NSSAQLPPMG  
 SynPCC7002 -----  
 A. thaliana -----A  
 CHLAMY PRX6 -----AA

**Supplementary Figure 2.** Alignments of PsbA1 of *Synechococcus* OS-B' (CYB) to selected homologs; *Synechococcus* OS-A (CYA), *Cyanotheca* sp. PCC 7424 (CyanPCC7424), *Anabaena variabilis* ATCC 29513 (AvaATCC29413), *Acarychloris marina* MBIC11017 (AmrMBIC11017), *Synechococcus* sp. PCC 7002 (SynPCC7002), *Synechococcus* sp. PCC 7942 (SynPCC7942), *Synechocystis* sp. PCC 6803 (SynPCC6803), *Arabidopsis thaliana* (A. thaliana) and *Chlamydomonas reinhardtii* (Chlamy). Light grey shadowing indicates the non-conserved 3 amino acid insertion in the N-terminal region of the protein, dark grey shadowing indicates the single amino acid insertion at position 256 (*Synechococcus* OS-B' position), yellow and asterisk indicates the position of the otherwise conserved DLA sequence prior to the predicted cleavage site, which is marked with a space and an arrow.

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CYB_0216      MSTVIRRSLAARQLWSWNGFCQWITSTENRLYIGWFGVLMIPTLLAAAFcfvIAfIAAAPP
CYA_1748      MSVVVRRSAAARRLWSWESFCQWITSTENRLYIGWFGVLMIPTLLAAATFCFVIAfIAAAPP
CyanPCC7424   MSNVILGRRELDLGTWEKFCQWITSTENRIYIGWFGVLMIPTLFAAAMCFIAfVcCAPP
AvaATCC29413 MSTIVQRQKEFNFFDLWDSFCAWITSTENRIYIGWFGVLSIPTLLAAATTCFVLAfIAAAPS
AmrMBIC11017 MSTTFQTPSRLPTVSAWDQFCEWITSTHNRLYVGFGLLMIpSLFVSAITfMLAWVAAPS
SynPCC7002    MTTTLQQRGSAS---LWEKFCQWITSTENRIYVGFVLMIPTLLTATTCFIIAfIAAAPP
SynPCC7942    MTTALQRRESAS---LWQQFCEWVTSDNRLYVGFVLMIPTLLTATICFIVAfIAAAPP
SynPCC6803    MTTTLQQRRESAS---LWEQFCQWVTSTNNRIYVGFGLMIpTLTATTCFIIAfIAAAPP
A. thaliana   MTAILERRESSES---LWGRFCNWITSTENRLYIGWFGVLMIPTLLTATSVFIIAfIAAAPP
Chlamy        MTAILERRENSS---LWARFCEWITSTENRLYIGWFGVIMIPCLLTATSVFIIAfIAAAPP

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CYB_0216      VDVDGIREPVIgSLIGGNNLISAavVPTSAAIghLFYPIWEAAslDEWLYnggPYQLIVL
CYA_1748      VDVDGIREPVIgSLIGGNNLISAavVPTSAAIghLFYPIWEAAslEELWLYnggPYQLIVF
CyanPCC7424   VDMdGIREPVLgSLLSGNNFISAavIPTSAAIghLHYPIWDAASlDEWLYnggPYQLIIF
AvaATCC29413 VDMdGIREPIMgSLMDGNNLITAavVPTSAAIghLFYPIWEAAsmDEWLYnggPYQLIVL
AmrMBIC11017 VDMEGIREPIISSLGGSNVITAavIPTSAAIghLHLYPLWEATsmDEWLYnggPYQLIIL
SynPCC7002    VDIDGIREPVAgSLLYGNNIISgAVVPSSNAIghLFYPIWEAAslDEWLYnggPYQLVIF
SynPCC7942    VDIDGIREPVAgSLMYGNNIISgAVVPSSNAIghLFYPIWEAAslDEWLYnggPYQLVVF
SynPCC6803    VDIDGIREPVAgSLLYGNNIISgAVVPSSNAIghLFYPIWEAAslDEWLYnggPYQLVVF
A. thaliana   VDIDGIREPVSGSLLYGNNIISgAIIPTSAAIghLFYPIWEAAsvDEWLYnggPYELIVL
Chlamy        VDIDGIREPVSGSLLYGNNIITGAVIPtSNAIghLFYPIWEAAslDEWLYnggPYQLIVC

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CYB_0216      HFLIGVWCYLGRQWELSYRLGMRPWIaVAFsAPAAAAATAVLLVYPIgQGSfSEGLPLGIA
CYA_1748      HFLIGVWCYLGRQWELSYRLGMRPWIaVAFsAPAAAAATAVLLVYPIgQGSfSEGLPLGIA
CyanPCC7424   HFLIGIWCYLGRLWELSYRLGMRPWIaVAYSAPVAAASAVFLVYPIgQGSfSEGmPLGIS
AvaATCC29413 HFLIGIWCLLGRFWElsYRLGMRPWIaVAYSAPVIAATSVLLVYPIgQGSfSDGLPLGIA
AmrMBIC11017 HFLIAIWTYLGRQWELSYRLGMRPWIAMAFsAPVAAATAVLLVYPMGQGSfSEGLPLGIS
SynPCC7002    HFLIGVFCYMGREWELSYRLGMRPWICVAFsAPVAAATAVFLIYPIgQGSfSDGMPLGIS
SynPCC7942    HFLIGVFCYMGREWELSYRLGMRPWICVAYSAPVAAATAVFLIYPIgQGSfSDGMPLGIS
SynPCC6803    HFLIGIFCYMGRQWELSYRLGMRPWICVAYSAPVSAATAVFLIYPIgQGSfSDGMPLGIS
A. thaliana   HFLLGVACYMGREWELSFRLGMRPWIaVAYSAPVAAATAVFLIYPIgQGSfSDGMPLGIS
Chlamy        HFLLGVYCYMGREWELSFRLGMRPWIaVAYSAPVAAASAVFLVYPIgQGSfSDGMPLGIS

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CYB_0216      GTFYFMLAFQAEHNI LMHPASWLGvAGVfGGALLASlhGSLVISSlIREtSEEEsQNAGY
CYA_1748      GTFYFMLAFQAEHNI LMHPASWLGvAGVfGGALLASlhGSLVISSlIREtSEEEsQNAGY
CyanPCC7424   GTFYFMLGFQADHNI LMHPPLHMLAVAGIffGALLSSlhGSLVTSSlVQETTEEEsINQGY
AvaATCC29413 GTFHFMLAFQGDHNI LMHPFHMLGVAGVfGGALLSSlhGSLVASTLIRNTDENESINGGY
AmrMBIC11017 GTFHFMMAVQAEHNI LMHPFHMLGVVGFfGGAFLsAMHGSLVTSSlVQETSSlKSVNTGY
SynPCC7002    GTFNFMIvFQAEHNI LMHPFHMLGVAGVfGGSFLsAMHGSLVTSSlVRETTETESQNYGY
SynPCC7942    GTFNFMFVfQAEHNI LMHPFHMLGVAGVfGGSFLsAMHGSLVTSSlVRETTETESQNYGY
SynPCC6803    GTFNFMIvFQAEHNI LMHPFHMLGVAGVfGGSFLsAMHGSLVTSSlIRETTENESANEGY
A. thaliana   GTFNFMIvFQAEHNI LMHPFHMLGVAGVfGGSFLsAMHGSLVTSSlIRETTENESANEGY
Chlamy        GTFNFMIvFQAEHNI LMHPFHMLGVAGVfGGSFLsAMHGSLVTSSlIRETTENESANEGY

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CYB_0216	RFGQEEVTYNFLAGHVAFLGRLGFPGLGLRNSRSVHFWMALPTVGIWAAAIGIGIMAFN
CYA_1748	RFGQEEVTYNFLAGHYAFLGRLGIPSLGWRNSRSVHFWMALPTLGIWAAAIGIGLMAFN
CyanPCC7424	RFGQEEVTYNLLAGHGGYLGRLLVPLGLRNSRSLHFI IAAVPVIGIWCATLAVGTMAFN
AvaATCC29413	KLGGQQVQTYKYLAGHNSFLGRLLIPTFASRNHRAFHFLLAALPTIGIWFAAMGVCSMAFN
AmrMBIC11017	KFGQEEATYNLLAGHAGYLGRLLFIPDIAFRNSRSIHFLAVLPTIGIWFAALGIGTMAFN
SynPCC7002	KFGQEEETYNIVAAGH-GYFGRLIFQYASFNNRSRSLHFFLGAWPVVGIWFTALGVSTMAFN
SynPCC7942	KFGQEEETYNIVAAGH-GYFGRLIFQYASFNNRSRSLHFFLAAWPVVGIWFTSLGISTMAFN
SynPCC6803	KFGQEEETYNIVAAGH-GYFGRLIFQYASFNNRSRSLHFFLGAWPVVGIWFTAMGVSTMAFN
A. thaliana	RFGQEEETYNIVAAGH-GYFGRLIFQYASFNNRSRSLHFFLAAWPVVGIWFTALGISTMAFN
Chlamy	RFGQEEETYNIVAAGH-GYFGRLIFQYASFNNRSRSLHFFLAAWPVVGIWFTALGLSTMAFN

CYB_0216	LNGLFNFQSIILDSQGRFIPTYADLLNRANLGIQVMHAPNAHHFPLLLA	AQP-----
CYA_1748	LNGLFNFQSIILDSQGRFIPTYADLLNRANLGIQAMHAPNAHHFPLLLA	AKAD-----
CyanPCC7424	LNGLFNFQSVVDSQGHPIYTDADLLNRANLGIQAMHAPNAHHFPLTLA	GGEALPIS----
AvaATCC29413	LNGLFNFNHSILDSRGNVIRSDADILNRANIGLSVMHAPNVHNFPLVLS	SGQPIPVS----
AmrMBIC11017	LNGLFNFNHSLLDSSGRPIRTEADLLNRATMGLQVMHAPNAHHFSLTLA	STESKEIPTIPI
SynPCC7002	LNGLFNFQSIILDSQGRVINTWADILNRANLGMFVMHERNAHNFPLDLA	AGEQAPVALQAP
SynPCC7942	LNGLFNFQSVLDSQGRVINTWADVLNRANLGMFVMHERNAHNFPLDLA	AGEATPVALTAP
SynPCC6803	LNGLFNFQSIILDSQGRVIGTWADVLNRANIGFVVMHERNAHNFPLDLA	SGEQAPVALTAP
A. thaliana	LNGLFNFQSVVDSQGRVINTWADIINRANLGMFVMHERNAHNFPLDLA	AVEAPSTNG---
Chlamy	LNGLFNFQSVVDSQGRVINTWADIINRANLGMFVMHERNAHNFPLDLA	STNSSNN----

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CYB_0216	----
CYA_1748	----
CyanPCC7424	----
AvaATCC29413	----
AmrMBIC11017	MTS-
SynPCC7002	AING
SynPCC7942	AING
SynPCC6803	AVNG
A. thaliana	----
Chlamy	----

**Supplementary Table 1**

Gene	Locus tag	<i>Synechococcus</i> OS-A	<i>Synechococcus</i> sp. PCC 7942	<i>Synechococcus</i> sp. PCC 7002	<i>Synechocystis</i> sp. PCC 6803
<b>CCM</b>					
<i>ccmM</i>	CYB_1794	0.85	0.51	0.45	0.50
<i>ccmN</i>	CYB_1793	0.76	0.44	0.30	0.35
<i>rbcL</i>	CYB_2579	0.98	0.87	0.88	0.88
<i>chpX</i>	CYB_1810	0.93	0.64	0.60	0.59
<i>chpY</i>	CYB_2877	0.97	0.76	0.75	0.76
<i>cmpA</i>	CYB_0604	0.98	0.59	ND	0.68
<i>cmpB</i>	CYB_0603	0.97	0.65	ND	0.66
<i>cmpC</i>	CYB_0602	0.97	0.71	ND	0.73
<i>cmpD</i>	CYB_0601	0.92	0.69	ND	0.65
<i>bicA</i>	CYB_1611	0.93	ND	0.63	0.61
<i>ccmR</i>	CYB_1948	0.88	0.58	0.55	0.57
<b>ROS</b>					
<i>sodB</i>	CYB_2514	0.96	0.66	0.69	0.70
2-Cys-Prx	CYB_2254	0.96	0.84	0.70	0.74
<i>crtP</i>	CYB_1694	0.93	0.69	0.66	0.68
<i>crtO*</i>	CYB_0017	ND	ND	ND	0.37
CYB_0556	CYB_0556	0.94	0.62	0.62	0.60
<b>Photosynthesis</b>					
<i>psbA1</i>	CYB_0216	0.94	0.72	0.75	0.71
<i>psbA2</i>	CYB_0371	0.99	0.90	0.88	0.88
<i>psbA3</i>	CYB_0433	Aminoacids identical to CYB_0371			

\*Highest homology to an acidobacterial sequence

**Supplementary Table 1.** Amino acid identities of proteins encoded by specific genes (left most column) on the genome of *Synechococcus* OS-B' to potential orthologs in selected cyanobacterial genomes, including *Synechococcus* OS-A, *Synechococcus* sp. PCC 7942, *Synechococcus* sp. PCC 7002 and *Synechocystis* sp. PCC 6803. Identities are based on values obtained from the Kegg database. <http://www.genome.jp/kegg/>.



## Supplementary Information 1. Description of genes selected for expression analysis and gene annotation.

### *Genes encoding proteins involved in the CCM*

Five different  $C_i$  uptake systems have been identified in cyanobacteria, including three for  $HCO_3^-$  and two for  $CO_2$  (Price *et al.*, 2008). A cyanobacterial mutant in which all five of the  $C_i$  transport systems are disrupted ( $\Delta 5$  mutant) is unable to actively take up either  $CO_2$  or  $HCO_3^-$ , and will not grow in an aqueous environment that is saturated with atmospheric levels of  $CO_2$  (Xu *et al.*, 2008). Furthermore, the growth of the  $\Delta 5$  mutant in high  $CO_2$  was strongly inhibited under high light, suggesting that even in the presence of high  $CO_2$  this strain has a diminished ability to fix  $CO_2$  and sequester organic carbon; this strain is less effective in productively using the absorbed light energy (photochemical quenching of excitation energy). These findings demonstrate the integral relationship between efficient utilization of excitation energy and the ability of the cells to concentrate  $C_i$ .

*CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> uptake.* Both  $CO_2$ -uptake systems, previously identified in various cyanobacteria, are encoded on the *Syn* OS-B' genome [gene designations *chpX*, *chpY*; the latter has also been called *cupA* (Shibata *et al.*, 2001)], while the *Syn* OS-B' genome encodes only two of the three identified  $HCO_3^-$  transporters (BCT1 and BicA). The BCT1 transporter is encoded by the *cmpABCD* putative operon (CYB\_0601-0604) (see Figure 1); this system belongs to the traffic ATPase family. All genes in the *Syn* OS-B' *cmpABCD* putative operon exhibit high identity (59-73%) to analogous genes on the genome of *Synechococcus* sp. PCC 7942 (Omata *et al.*, 1999) (Supplementary Table 1). CYB\_1611 was identified as encoding a putative BicA transporter, with relatively high identity (63%) to the BicA polypeptide of *Synechococcus* sp. PCC 7002 (Price *et al.*, 2004) (Supplementary Table 1).

*Carboxysome and carbonic anhydrase.* Another set of cyanobacterial CCM genes encode components of the carboxysome, a microcompartment in which CO<sub>2</sub> is concentrated in the immediate vicinity of RuBisCo, allowing the enzyme to more efficiently fix CO<sub>2</sub>. Many polypeptides integral to the carboxysome, including a carbonic anhydrase, have been identified (So *et al.*, 2002; Badger, 2003; So *et al.*, 2004; Cot *et al.*, 2008). Carbonic anhydrase facilitates the conversion between CO<sub>2</sub> and bicarbonate and plays a critical role in delivering CO<sub>2</sub> to RuBisCo. Furthermore, some structural components of the carboxysome appear to impede diffusion of CO<sub>2</sub> away from the site of fixation (Dou *et al.*, 2008). We found that the operon encoding the carboxysome polypeptides in *Syn* OS-B' likely consists of *ccmK2*, *ccmK1*, *ccmL*, *ccmM*, *ccmN* and *ccmO* (Figure 1).

*Regulators.* Recently, cyanobacterial genes encoding LysR-type transcription factors were implicated in the regulation of C<sub>i</sub>-responsive genes (Omata *et al.*, 2001; Wang *et al.*, 2004; Woodger *et al.*, 2007). CYB\_1948 (designated *ccmR* in Figure 1) shows the highest identity (55-58% in the model organisms and 88% in *Syn* OS-A) to the *ccmR/cmpR* group of CbbR-like regulators (Woodger *et al.*, 2007) (Supplementary Table 1). This gene in *Syn* OS-B' is located downstream of another gene encoding a putative regulator (CYB\_1950; designated *nrdR* in Figure 1), although the genes are separated by two additional genes, one encoding a protein with TPR repeats and the other encoding a protein in the deoxyribodipyrimidine photolyase family (Figure 1).

#### *Genes encoding proteins associated with ROS detoxification and high light acclimation*

Several genes are involved in detoxification of ROS. Although a full annotation and description of these genes and their encoded proteins is beyond the scope of this paper, the reader is referred to recent reviews on the subject (Lesser, 2006; Latifi *et al.*, 2009). In this study we only performed expression analyses of genes for which we have high confidence concerning the function of the

encoded protein; these genes include those encoding proteins involved in both enzymatic and non-enzymatic scavenging of ROS.

*Superoxide dismutase and peroxidases.* The *Syn* OS-B' genome encodes one putative iron superoxide dismutase (*sodB*), which functions to disproportionate  $O_2^-$  to  $H_2O_2$ ;  $O_2^-$  is often formed on the acceptor side of PSI (Nishiyama *et al.*, 2001). We were not able to identify a gene encoding a catalase-peroxidase (*katG*) on either the *Syn* OS-B' or *Syn* OS-A genomes, although there are a number of genes encoding putative peroxidases with unknown physiological functions. The *katG* gene also appears to be absent from the genome of the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1 (Nakamura *et al.*, 2002). One such peroxidase protein family encoded on the *Syn* OS-A and *Syn* OS-B' genomes is that of the peroxiredoxins (Prx), which are thiol-specific peroxidases that use thioredoxins (Trx) as the hydrogen donor for reducing  $H_2O_2$  and lipid hydroperoxides (Chae *et al.*, 1994; Chae *et al.*, 1999). In eukaryotes, some Prx are specific to mitochondria (Watabe *et al.*, 1997), and their expression can be induced in response to oxidant treatments (Araki *et al.*, 1999). Four different sub-classes of Prx have been identified in plants, with the encoded proteins localized to both mitochondria and chloroplasts; they are likely critical for ameliorating the effects of ROS, which are synthesized as a byproduct of photosynthesis, respiration and stress-related processes (Tripathi *et al.*, 2009). We identified six genes with homology to Prx on the genome of *Syn* OS-B': one putative 1-Cys Prx (CYB\_0113), one putative 2-Cys Prx (CYB\_2254) and four putative Prx-BCP (Bacterioferritin Comigratory Protein), which are homologs of plant PrxQ (CYB\_0523, CYB\_1057, CYB\_1376, CYB\_2186) (Jacobson *et al.*, 1989; Chae *et al.*, 1994). No atypical 2-Cys Prx was identified, which is similar to the situation for *Synechococcus* sp. PCC 7942, whereas *Synechocystis* sp. PCC 6803 seems to have one atypical 2-Cys Prx, but only 2 Prx-BCPs (Stork *et al.*, 2005). Alignments of these putative Prx proteins in *Syn* OS-B' to selected sequences are presented in (Supplementary Figure 1). We only analyzed the

levels of transcripts from the putative 2-Cys Prx (CYB\_2254), which has previously been shown to be important for conferring high light and ROS tolerance to *Synechocystis* sp. PCC 6803 (Klughammer *et al.*, 1998) and *Synechococcus* sp. PCC 7942 (Perelman *et al.*, 2003).

*Ligases and carotenoids.* There are several other proteins that help photosynthetic organisms cope with excess excitation. The CYB\_0556 gene encodes a putative glutamate-cysteine ligase, which catalyzes the rate-limiting step of glutathione synthesis; glutathione is an important antioxidant that accumulates in a number of different photosynthetic organisms in response to high light (Latifi *et al.*, 2009). The accumulation of the transcript from this gene was previously investigated in pure cultures of *Syn OS-B'* following different light treatments (Kilian *et al.*, 2007).

Carotenoids are also known to exhibit antioxidant activity, and their synthesis is often elevated in photosynthetic organisms following exposure to high irradiance levels (Steiger *et al.*, 1999). Lycopene as well as a number of other carotenoids serve to effectively scavenge singlet oxygen and hydroxyl radicals (Rousseau *et al.*, 1992). The biosynthesis of lycopene from phytoene involves a four step desaturation. Two different pathways for this conversion are known in cyanobacteria; the bacterial pathway uses one enzyme, while the plant pathway requires three enzymes (Takaichi & Mochimaru, 2007). Our analysis indicates that *Syn OS-B'* uses the latter pathway with CrtP (CYB\_1694) catalyzing the first two desaturation steps that convert phytoene to  $\zeta$ -carotene via a phytofluene intermediate, CrtQ (CYB\_1060) catalyzing desaturation of  $\zeta$ -carotene to lycopene in the poly-cis transfiguration via a neurosporene intermediate, and CrtH (CYB\_1298) catalyzing the isomerization of lycopene to the all trans forms. The closest homolog of *Syn OS-B'* CrtP is in *Thermosynechococcus elongatus* BP-1, which appears to lack a CrtH protein and thus likely depends on light for photoisomerization of lycopene to the all trans forms (Takaichi & Mochimaru, 2007). *Syn OS-B'* also contains a putative  $\beta$ -carotene ketolase encoded by *crtO* (CYB\_0017) (Kilian *et al.*, 2007), potentially catalyzing the synthesis of echinenone and canthaxanthin; both of

these carotenoids are effective scavengers of singlet oxygen and hydroxyl radicals (Rousseau *et al.*, 1992). While the CrtO protein of *Syn OS-B'* is most identical (48%) to an acidobacterium sequence (YP\_828532), no *crtO* gene has been identified on the *Syn OS-A* genome.

*D1 protein.* A key protein in the core of PSII is the D1 protein; the turnover of the D1 is severely impacted by high light and ROS. There are two different isoforms of D1 encoded on the *Syn OS-B'* genome (Kilian *et al.*, 2007). The transcript for one of the isoforms increases in high light; this isoform is encoded by two *Syn OS-B'* genes (CYB\_0371 and CYB\_0433 are *psbA2* and *psbA3*, respectively), and the encoded proteins likely contain a 10 amino acid (aa) extension (after the DLA sequence) at their carboxy terminus which is removed during assembly of the PSII complex (Marder *et al.*, 1984; Diner *et al.*, 1988). The transcript from a second D1 isoform of *Syn OS-B'* (encoded by CYB\_0216 or *psbA1*) is elevated in low light and does not contain the conserved –DLA– sequence (Satoh & Yamamoto, 2007) characteristic of the carboxy terminal cleavage site, but instead has a –LLA– sequence. This low light *Syn OS-B'* D1 isoform is most similar to (apart from the *Syn OS-A* homolog, which also contains a –LLA–sequence) the analogous protein in *Cyanothece* sp. which, together with analogous proteins in *Anabaena variabilis* ATCC 29413 and the Chl *d*-containing cyanobacterium *Acaryochloris marina* MBIC11017, also do not contain the –DLA– sequence, but instead have –VLS– (*Anabaena variabilis* ATCC 29413) and –TLA– (*Cyanothece* sp. and *Acaryochloris marina* MBIC11017) at the putative cleavage site. Furthermore, the *Syn OS-B'* low-light D1 isoform has a carboxy terminal extension of only 3 aa, while that of *Syn OS-A* has a 4 aa extension. Finally, the low light D1 polypeptides of *Syn OS-B'*, *Syn OS-A*, *Cyanothece* sp., *Anabaena variabilis* ATCC 29413 and *Acaryochloris marina* MBIC11017 all have a 3 aa insertion in their N-terminal regions as well as a 1 aa insertion at position 256 relative to the 45 other D1 proteins that are most similar to D1 of *Syn OS-B'*. An alignment of selected *PsbA1* sequences are shown in (Supplementary Figure 2).

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## Supplementary Methods 1.

RNA isolation protocol. Modified from (Steunou *et al.*, 2006).

### DNA/RNA extraction

- 1 Suspension of frozen mat pieces (0.5 cm<sup>2</sup>, upper 2 mm) in 250 µl of 10 mM sodium acetate (pH 4.5) and 0.3 g of glass beads (Sigma, 150-212 µm). Bead-beating was for 15-30 sec.
- 2 Addition of 37.5 µl of 500 mM Na<sub>2</sub>-EDTA (pH 8.0) to the resuspended mat material, which was then incubated on ice for 5 min.
- 3 Addition of 375 µl Lysis buffer (10 mM sodium acetate, pH 4.5, and 2 % SDS) followed by mixing and bead-beating for 15-30 sec.
- 4 Centrifugation for 10 sec to disperse foam in the solution.
- 5 Vortexing the sample and incubation at 65°C for 3 min.
- 6 Addition of 700 µl hot (65°C) water equilibrated, acidic phenol (pH 4.5) followed by mixing and incubation of the suspension at 65°C for 3 min.
- 7 Rapid cooling of sample tubes on ice followed by separation of the phases by a 2 min centrifugation at 21460xg.
- 8 Transfer of the aqueous phase (top-part) to a new Eppendorf tube containing 700 µl of room temperature phenol:chloroform (1:1). The suspension was mixed and centrifuged for 2 min at 21460xg.
- 9 Repetition of step 8.
- 10 Transfer of the aqueous phase to a new Eppendorf tube followed by the addition of 1 vol chloroform. The suspension was mixed and centrifuged for 2 min at 21460xg.
- 11 Transfer of the aqueous phase to a new Eppendorf tube followed by the addition of 0.1 vol of 10 M LiCl + 2.5 vol of ethanol (100%). The sample was incubated at -20°C for 30-60 min.
- 12 Centrifugation of sample for 30 min at 4°C for 30 min and 21460xg.
- 13 Washing of pellet with 80% ethanol (10 min at 21460xg) followed by drying at room temperature.
- 14 Resuspension of the dried pellet in 97.5 µl DNase/RNase-free H<sub>2</sub>O.

- 15 Transfer of 10  $\mu$ l to a separate Eppendorf tube containing 10  $\mu$ l DNase/RNase-free H<sub>2</sub>O. Storage at -80 °C (no further use in this experiment).

### **DNase treatment**

- 16 Treatment of remaining RNA solution with RNase-free turbo DNase (Ambion Inc., USA): 87.5  $\mu$ l RNA solution + 10  $\mu$ l RDD buffer + 2.5  $\mu$ l DNase. Incubation at 37°C for 30 min.
- 17 Addition of 2.5-5  $\mu$ l TurboDNase. Incubation at 37°C for 30 min.
- 18 Addition of 200  $\mu$ l of DNase/RNase-free water and 300  $\mu$ l phenol:chloroform (1:1). Vortex solution and centrifugation for 2 min at 21460xg.
- 19 Transfer the aqueous phase to a new Eppendorf tube. Addition of 1 vol of chloroform. Vortex solution and centrifugation for 2 min at 21460xg.
- 20 Transfer the upper phase to a new Eppendorf tube followed by addition of 0.1 vol of 10 M LiCl + 2.5 vol of ice cold ethanol (100%). Incubation at -20°C for 30 min.
- 21 Centrifugation at 4°C for 30 min at 21460xg.
- 22 Washing pellet with 80% ethanol (10-15 min at 4°C) and then drying it at room temperature.
- 21 Resuspension of dried pellet in 20-50  $\mu$ l DNase/RNase-free H<sub>2</sub>O.

DNase-treated RNA solutions were monitored for DNA contamination using PCR. In the event of contamination, the DNase treatment was repeated.

The concentration of RNA in the samples was measured by absorption at 260 nm (Nanodrop Thermo Scientific, USA). RNA samples were stored at -80°C.

All solutions used for RNA isolation were treated with (diethyl pyrocarbonate) DEPC 0.1 % v/v (Sigma) by incubating the solutions at 37°C for 1h with gentle agitation; the solutions were then autoclaved for 20 min at 120°C.

### **References**

- Steunou A-S, Bhaya D, Bateson MM, Melendrez MC, Ward DM, Brecht E *et al.* (2006). *In situ* analysis of nitrogen fixation and metabolic switching in unicellular thermophilic cyanobacteria inhabiting hot spring microbial mats. *Proc Natl Acad Sci USA* **103**: 2398-2403.