

**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--SLRLGDTVPNFTQQSSEGEINFDYDWAGDSWVILFSHPADYTPVCTTELGTVAKLKKEE
SynPCC6803    M--ALQLGDVVPDFEQESSQGPISFHEWAGDSWVVLFSHPADYTPVCTTELGTVAKLKPE
SynPCC7002    M--SLRLGDVAPNFTQDSTIGEINFDYDWAGDSWVVLFSHPADFTPVCTTELGEVARLKGE
Hs. PRDX6     MPGGLLLGDVAPNFEANTTVGRIRFHDFLGDWSGILFSHPRDFTPVCTTELGRAAKLAPE
A. thaliana   MP-GITLGDTPVNLEVETTHDKFKLHDYFANSWTVLFSHPGDFTPVCTTELGAMAKYAHE
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CYB_0113      FEKRGVKVIALSVDDVDVSHGVWKDIE----ETQNATLTYPILADADRKVSQLYGMLDQT
CYA_2849      FEKRGVKVLALSVDVDSHLGWVKDIE----ETQNAIVNYPILADVDRKVSQLYGMLDQT
SynPCC7942    FAKRNVKPIALSVDVDESHKGWIGDIN----ETQGTQVNYPILADPDRKVSQLYGMIHPN
SynPCC6803    FDKRNVKPIALSVDVDESHKGWICDID----ETQNTTVNYPILADGDKKVSQLYGMIHPN
SynPCC7002    FEKRNKVIALSVDVDTASHNGWVGDI----ETQGCANVYPILSDADQKVATLYNMIHPN
Hs. PRDX6     FAKRNVKLIALSIDSVEDHLAWSKDINAYNCEPTEKLPFPIIDDRNRELAILLGMLDPA
A. thaliana   FDKRGVKLLGLSCDDVQSHKDWIKDIEAF---NHGSKVNYPILADPNKEIIPQLNMIDPI

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CYB_0113      NLNQEGLPLTVRSVFIIDPNKKIRLILTYPASTGRNFDELLRVIDALQLTDKY--NVVTP
CYA_2849      NLNQEGLPLTVRSVFIIDPNKKIRLILTYPASTGRNFDELLRAIDALQLTDKY--SVVTP
SynPCC7942    ANNT----LTVRSVFIIDPNKKLRLTLTYPASAGRNFDLRLVIDSLQLTDNY--SVATP
SynPCC6803    ALNN----LTVRSVFIIDPAKKLRLTLTYPASTGRNFDEILRVLDLQLTDYH--QVATP
SynPCC7002    ANPK----VTVRIVFVIDTERKVRLTITYPPSTGRNFQEILRVLDLQLTDNY--SVATP
Hs. PRDX6     EKDEKGMPTARVVFVFGPDKKLLKLSILYPATTGRNFDEILRVVLSLQLTAEK--RVATP
A. thaliana   ENG-----PSRALHIVGPDSEIKLSFLYPSTTGRNMDEVLRALDSSLMASKHNNKIATP
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CYB_0113      ADWKDGDVVIIPPSLKDPEVLKEKFPKGYKEI----KPYLRVAPQPKNK
CYA_2849      ADWKDGDVVIIPPSLQDPEVLKEKFPKGYKEL----KPYLRRLTPQPNK
SynPCC7942    ANWWDGDVVVVVPSI-PTEQAREQFPKGVTEV----KPYLRRLTPQPNR
SynPCC6803    ANWQDGDKCVVVVPSI-STEDAKVKFPKGVVEE----KPYLRRLTPQPNK
SynPCC7002    VNWQDGEDVVVSPAI-STEDAKVKFPKGVKEI----KPYLRMTPQPNK
Hs. PRDX6     VDWKDGDVSMVLPTI-PEEEAKKLFKGVFTKELPSGKKYLRYPQP--
A. thaliana   VNWKPDQPVVISPAV-SDEEAKMFPQGFKTADLPSKKGYLRLHTEVS--

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

CYB\_2254 MS-----  
 CYA\_0537 MS-----  
 SynPCC7942 MT-----  
 SynPCC6803 MT-----  
 SynPCC7002 MA-----  
 A. thaliana MSMASIASSSSTTLSSSRVLLPSKSSLLSPTVSPRIIPSSASSSSLCSGFSSLSGLT  
 A. thaliana MA--SVASSTTLISSPSSRV-FPAKSSLSPPSVSFLRTLSSPSA-SASLRSGFARRSSLS  
 Hs. PRDX1 MS-----  
 Hs. PRDX2 MA-----  
 Hs. PRDX3 MAAAVGRLLRASVARHVSAI-----PWGISATAALRPAACGRSLTNLL  
 Hs. PRDX4 MEALPLLAATTPDHGRHRRLLLLPLLLFLLPAGAVQGWETEERPRTREEECHFYAGGQVY  
 CHLAMY PRX1 MAALQSASRSSAVAFSRQARVAPRVAA-----  
 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 ---STRRSFVAVKAQADDLPLVGNKAPDFEAEAVF-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-DQEFQEIITLSKYRG-KYVVLFFYP  
 CHLAMY PRX2 -----AKIGAPAPKFKAQAVV-NGEIKIISLDDYK-KYVVLFFYP  
 CHLAMY PRX7 -----PMFVSAAVV-DGEITKISLSDYK-KYVCLFFYP

CYB\_2254 LDFTFVCPTTEITAFSDRYDDFAKLDTEILGVSVDSEYSHLAWIQTRKAGGVGELRYPLV  
 CYA\_0537 LDFTFVCPTTEITAFSDRYDEFKLDTEILGVSVDSEYSHLAWIQTRKAGGVGELRYPLV  
 SynPCC7942 LDFTFVCPTTEITAFSDRYADFSALNTEILGVSVDSQFSLAWIQTSRKEGGLGDLAYPLV  
 SynPCC6803 LDFTFVCPTTEIIAFSDRHSEFTALDTEVVGISVDSEFSLAWIQTERKMGGININYPLV  
 SynPCC7002 LDFTFVCPTTEVGAFSDRHGEFQKLNTEVLGVSVDSEFAHLAWIQTRKMGVGDLAGPLV  
 A. thaliana LDFTFVCPTTEITAFSDRYEEFEKLNTEVLGVSVDVSVFSLAWVQTRKSGGLGDLNYPLV  
 A. thaliana LDFTFVCPTTEITAFSDRHSEFEKLNTEVLGVSVDVSVFSLAWVQTRKSGGLGDLNYPLI  
 Hs. PRDX1 LDFTFVCPTTEIIAFSDRAEEFKLNCQVIGASVDSHFCHLAWVNTPKKQGGGLPMNIPLV  
 Hs. PRDX2 LDFTFVCPTTEIIAFSNRAEDFRKLGCEVLGVSVDVSVFSLAWINTPRKEGGLGPLNIPLL  
 Hs. PRDX3 LDFTFVCPTTEIVAFSDKANEFHDVNCEVVAVSVDSHFSLAWINTPRKNGGLGHMNIALL  
 Hs. PRDX4 LDFTFVCPTTEIIAFGDRLEEFRSINTEVVACSVDSQFTHLAWINTPRRQGGGLPIRIPLL  
 CHLAMY PRX1 LDFTFVCPTTEITAFSDRYKEFKDINTEVLGVSVDVSVFSLAWIQTRKEGGLGDLAYPLV  
 CHLAMY PRX2 LDFTFVCPTTEIVAFSDRVEEFRAINTEVIGASIDSQFTHLAFSNTPRTKGGLGCKYPLV  
 CHLAMY PRX7 KDFTFVCPTTEIIAFSDRAKEFAAANCQLIAASTDTEETHLAWIRTPRNRGGLGYMQIPIL  
 # #

CYB\_2254 SDLKKEISAAYNVLDP---EAGVALRGLFIIDKEGIIQHATINNLAFGRSVDETLRVLQA  
 CYA\_0537 SDLKKEISAAYNVLDP---AAGVALRGLFIIDKEGIIQHATINNLAFGRSVDETLRVLQA  
 SynPCC7942 ADLKKEISTAYNVLDP---AEGIALRGLFIIDKEGVIQHATINNLAFGRSVDETLRVLQA  
 SynPCC6803 SDLKKEISQAYNVLEP---DAGIALRGLFIIDREGILQYATVNNLSFGRSDETLRVLKA  
 SynPCC7002 SDLNKTISTAYGVLEP---EAGISLRGLFIIDPEGMIQHITVNNLSFGRSDETLRVLQA  
 A. thaliana SDITKSISKSFGVLIP---DQGIALRGLFIIDKEGVIQHSTINNLSGIGRSVDETMRTLQA  
 A. thaliana SDVTKSISKSFGVLIH---DQGIALRGLFIIDKEGVIQHSTINNLSGIGRSVDETMRTLQA  
 Hs. PRDX1 SDPKRTIAQDYGVLKA---DEGISFRGLFIIDDKGILRQITVNDLPVGRSDETLRLVQA  
 Hs. PRDX2 ADVTRRLSEDYGVLKT---DEGIAYRGLFIIDGKGVLRQITVNDLPVGRSDEALRLVQA  
 Hs. PRDX3 SDLTKQISRDYGVLE---GSGLALRGLFIIDPNGVIKHLVNDLPVGRSVEETLRLVKA  
 Hs. PRDX4 SDLTHQISKDYGVYLE---DSGHTLRGLFIIDDKGILRQITLNDLPVGRSDETLRLVQA  
 CHLAMY PRX1 ADLKKEISKAYGVL---EDGISLRGLFIIDKEGVVQHATINNLAFGRSVDETKRVLQA  
 CHLAMY PRX2 ADLTKQIAKDYGVLIEDGPDAGVTLRGLFISPTGVLRQITINDLPVGRSDETLRLVKA  
 CHLAMY PRX7 ADTTKDISARYGVLE---KLGVALRGLFIINPQGVVQHVITNDLPVGRSDEALRTLQA  
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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-GEVCPAGWKPGSETIIPDPAGKLYFDKLN--  
 CHLAMY PRX1 IQYVQSNPDEVCPAGWKPGDKTMKPDPKGSKEYFSAV---  
 CHLAMY PRX2 FQFTDEH-GEVCPANWNPAGAKTMKADPTKSLEYFSTLS--  
 CHLAMY PRX7 IQYHAEH-GEVCPANWKPGSKTMVADA EKSL EYFSEV---

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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-----KFSWPKTIALLLTLGLWLGLADLP  
 SynPCC6803 -----  
 SynPCC7002 -M-----LQFFRTILITVVAIA-FMWFPGEA  
 SynPCC7002 -----  
 A. thaliana MAASSSFTLCNHTTLRLTLPKRKTLVTKTQFSVPTKSSSESNFFGSTLTHSSYISPVSSSS  
 CHLAMY PRX6 MQTIR-----APARAPVASSRRVATFRAAPRVSR

CYB\_0523 -----MPLAVGDPAPEF-----TLPD-----AEGN----PISLSQLRG-QRVVLYFYPR  
 CYB\_2186 AQWGTTPPLPIGSPAPEF-----ALPD-----QSGQ----IRRLADFRG-EWVLYFYPR  
 CYA\_0907 AQWGTTPLPQIGAPAPEF-----ELPD-----QSGQ----VRLADFRG-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 -----MPLQVGDrapdf-----TLPD-----QQGN----PVSLTDLRG-QRVVIYFYPK  
 SynPCC6803 TYALGGIQPELDQPAPLF-----TLPS-----TTGEG---EVNLTdyrg-QWVLYFYPK  
 SynPCC6803 ----MATALETNQPAPTF-----SAPN-----AEGK----TISDDFLG-QWLVLVLYFYPK  
 SynPCC7002 AIALGGPQPELNQLAPEF-----TLLG-----NDGEGKIQEFSLQDYRG-QWVLYFYPK  
 SynPCC7002 -----MTTLTIGQAAPDF-----ALTN-----AQGE---ARTLADYSG-KWLVFYFYPK  
 A. thaliana LKGLIFAKVNGQAAPDF-----TLKD-----QNGK----PVSLKYYKG-KPVVLYFYPA  
 CHLAMY PRX6 PVVVVRAELKVGKLEDYPNYKVLKT-----SEGK----TISLSSYKKGQPIVLFYFYPK

CYB\_0523 DNTPGCTQEACGFRDAYADYQAHGIAILGVSADDARSHQKFAQKLQLPFLLVDEGAKVA  
 CYB\_2186 DFTSGCTIEARRFQODLPKFRAMGAQVVGVSADSVDSHRRFCSAEGQLFPPLSDPDGTVS  
 CYA\_0907 DFTAGCTIEARRFQODLPKFRTLGAEIVGVSADSVDSHRRFCSAEGQLFPPLSDPEGTVS  
 CYB\_1376 DFSPVCTSEMRCFREDWEAFRAAGCEILGISDPLSRHQAFAEQLKLEFPPLSDVDRKVS  
 CYB\_1057 DDTPGCTKEACGFRDAYADYQSKDVVVLGVSMDQASHQKFKTEKYNLPPFLLVDSSGVIT  
 CYA\_2145 DNTPGCTREACGFRDAYAEYQAHGIHVLGVSADDARSHQKFAQKLQLPFLLVDEGAKVA  
 CYA\_2305 DFSPVCTSEMRCFREDWGAFAAGCEILGISDPLSRHEAFAEQLKLEFPPLSDVDRKVS  
 CYA\_0672 DDTPGCTKEACGFRDAYADYQSKNVVVLGVSMDQASHQKFAEKYNLPPFLLVDTSGVIA  
 SynPCC7942 DGTPGCTLEAQRFFQDLAAAYAERNAQIVGVSADDLSSHSRFRENEGLSYPLLADVKGEVS  
 SynPCC7942 DDTPGCTIEACFRDSYTAQEVGAVVLGVSSDSIDSHQRFQKYNLPPFLLSDAGDRLR  
 SynPCC7942 DDTPGCTKEACFRDSYSAYQGDIIIVLGVSTDDSSHEQFKTKFSLPFPLVADPDRSIT  
 SynPCC7942 DDTPGCTKEACFRDDFSLFEQAGIVVLGVS KDPASKHQKFI AKYELPFTLLTDADAAVA  
 SynPCC6803 DFTPGCTLEAQRFFQODLTKYQALNAQVIGVSVDLDSHEAFCD AEGKLFPLLADSDGAVI  
 SynPCC6803 DNTPGCTTEAIDFSEKLPFTDLNAVVGVS PDSEKSHGKFI DKHNLTVQLLSDPEHELA  
 SynPCC7002 DFTPGCTLEARRFQODLPKYLERNVQVLGVSVDVDSHEAFCD AEGKLFPLLADSTGDVVS  
 SynPCC7002 DNTPGCTTEALDFTALVPEFTALNAVIGVS PDSEKSHGRFIEKKELAI ELLSDPDHQTA  
 A. thaliana DETPGCTKQACAFRDSYEFKFKAGAEVIGISGDD SASHKAFASKYKLPYTLTSDGKVR  
 CHLAMY PRX6 AATPGCTKEACFRDEYSRFTAAGAVVFGISSD SPADNAAF GKANNLPYPLVTDENSILR  
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CYB\_0523 RAYGVYGPKKFMGKEYNGIHRITTFVIDPEGKIEAVITK-VKV--ETHAAELLKQLTG---  
 CYB\_2186 RAYGSWMGDV-----ALRNTFLIDPEGILREIDPI-VNP--SRHSAEVL AQLQLLR

CYA\_0907 RAYGSWTGEM-----ALRNTFLIDPEGILRAIDPI-VNP--SRHSAEVLAQLQELAQ  
 CYB\_1376 QQYGVDSLGL-----TRRAYFLVDSQGILRYQHVE-WLPLFKRDNRELEAVRAI--  
 CYB\_1057 RAYDVDGGGY-----AKRVTYVIDGEGKIAKVYQN-INT--ETHARDILADLGL---  
 CYA\_2145 SAYGVYGPKKFMGKVYNGIHRITTFVIDPEGKIEAIITK-VKV--EAHAAELLKQLTG---  
 CYA\_2305 QLYGVDSFLG-----TRRAYFVVDSSQGILRYQHVE-WLPIFKRDNRELEAVRAV--  
 CYA\_0672 RAYDVDGGGY-----AKRVTYVIDAEGKIAKVYQN-INT--ETHARDILADLGL---  
 SynPCC7942 KRYGSWLAPF-----SLRHTYIIDPEGVLRANFTA-VRP--VIHSKEVLAKLDELQA  
 SynPCC7942 QTYGVPKTLF-----VIPGRVTYVIDKEGKVRHIFDSSLNA--QAHIQESLNILRSL--  
 SynPCC7942 QAYDVDGGGY-----AKRVTYVIDGEGQIIRVYDS-VKT--DTHAGDILADLGL---  
 SynPCC7942 SAYDSYGLKKFMGREYMGMMRHTYVIDVEGKIEQIYTK-VKP--ETHARQILTDLGVAVA  
 SynPCC6803 KTYGSWLSGM-----ALRHTYVIDPEGILRERFLG-VRP--ATHSEEVLARLAEQA  
 SynPCC6803 AAYGAWGPKKFMGKECEGILRSTFLINPQGNIAHIWPN-VRV--KGHAEKVLEKLQQLNS  
 SynPCC7002 KQYGSYLTYG-----SLRHTYLIDPEGILRKIYLG-VNP--AIHSQEVLTDLDSLMA  
 SynPCC7002 GVYGVWQLKKFMGKEYMGIVRSTFLIDPQGQVAIWSK-VKV--KGHAQTVLETLOT---  
 A. thaliana KDWGVPGLDF-----GALPGRQTYVLDKNGVVQLIYNNQFQP--EKHIDETLKFLKA---  
 CHLAMY PRX6 KTFGIKGFDF-----GLLPGRQTYVIDVNGKCVMAFNDQLNV--EQHVDEALKVVASVKV  
 #

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 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 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      MSTVIRRSLAARQLWSWNGFCQWITSTENRLYIGWFGVLMIPTLLAAAFCFVIAFIAAPP
CYA_1748      MSVVVRRSAAARRLWSWESFCQWITSTENRLYIGWFGVLMIPTLLAAATFCFVIAFIAAPP
CyanPCC7424   MSNVILGRRELDLGTTEWKFQWITSTENRIYIGWFGVLMIPTLFAAAMCFIAFVFCAPP
AvaATCC29413 MSTIVQRQKEFNFFDLWDSFCAWITSTENRIYIGWFGVLSIPTLLAATTCFVLAFAIAAPS
AmrMBIC11017 MSTTFQTPSRLPTVSAWDQFCEWITSTHNRLYVWGFLLMIPSLFVSAITFMLAWVAAPS
SynPCC7002    MTTTLQQRGSAS---LWEKFCQWITSTENRIYVWGFVLMIPTLLTATTCFIIAFIAAPP
SynPCC7942    MTTALQRRESAS---LWQQFCQWITSTDNRLYVWGFVLMIPTLLTATTCFIVAFIAAPP
SynPCC6803    MTTTLQQRRESAS---LWEQFCQWITSTNNRIYVWGFGLMIPSTLLTATTCFIIAFIAAPP
A. thaliana   MTAILERRESSES---LWGRFCNWITSTENRLYIGWFGVLMIPTLLTATSVFIIAFIAAPP
Chlamy        MTAILERRENS---LWARFCQWITSTENRLYIGWFGVIMIPCLLTATSVFIIAFIAAPP

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CYB_0216      VDVDGIREPVIIGSLIGGNNLISA AVVPTSAAIGLHFYPIWEAASLDEWLYNGGPPYQLIVL
CYA_1748      VDVDGIREPVIIGSLIGGNNLISA AVVPTSAAIALHFYPIWEAASLEEWLYNGGPPYQLIVF
CyanPCC7424   VDMDGIREPVLGSLLSGNNFISA AVIPTSAAGLHYPIWDAASLDEWLYNGGPPYQLIIF
AvaATCC29413 VDMDGIREPIMGSLMDGNNLITA AVVPTSAAIGLHFYPIWEAASLDEWLYNGGPPYQLIVL
AmrMBIC11017 VDMEGIREPIISSLLGGSNVITA AVIPTSAAGLHLYPLWEATSMDEWLYNGGPPYQLIIL
SynPCC7002    VDIDGIREPVAGSLLYGNNIISG AVVPSNAIGLHFYPIWEAASLDEWLYNGGPPYQLVIF
SynPCC7942    VDIDGIREPVAGSLLYGNNIISG AVVPSNAIGLHFYPIWEAASLDEWLYNGGPPYQLVVF
SynPCC6803    VDIDGIREPVAGSLLYGNNIISG AVVPSNAIGLHFYPIWEAASLDEWLYNGGPPYQLVVF
A. thaliana   VDIDGIREPVSGSLLYGNNIISG AIPTSAAGLHFYPIWEAASVDEWLYNGGPPYELIVL
Chlamy        VDIDGIREPVSGSLLYGNNIITG AVIPTSAAGLHFYPIWEAASLDEWLYNGGPPYQLIVC

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CYB_0216      HFLIGVWCYLGRQWELSYRLGMRPWI AVAFSAPAAAAATAVLLVYPIGQGSFSEGLPLGIA
CYA_1748      HFLIGVWCYLGRQWELSYRLGMRPWI AVAFSAPAAAAATAVLLVYPIGQGSFSEGLPLGIA
CyanPCC7424   HFLIGIWCYLGRWLWELSYRLGMRPWI AVAYSAPVAAAASAVFLVYPIGQGSFSEGMPLGIS
AvaATCC29413 HFLIGIWCYLGRQWELSYRLGMRPWI AVAYSAPVIAATSVLLVYPIGQGSFSDGLPLGIA
AmrMBIC11017 HFLIAIWTYLGRQWELSYRLGMRPWI AMAFSAPVAAAATAVLLVYPMGQGSFSEGLPLGIS
SynPCC7002    HFLIGVFCYMGREWELSYRLGMRPWI CVAFSAPVAAAATAVFLIYPIGQGSFSDGMPLGIS
SynPCC7942    HFLIGVFCYMGREWELSYRLGMRPWI CVAYSAPVAAAATAVFLIYPIGQGSFSDGMPLGIS
SynPCC6803    HFLIGIFCYMGRQWELSYRLGMRPWI CVAYSAPVSAATAVFLIYPIGQGSFSDGMPLGIS
A. thaliana   HFLLGVACYMGREWELSFRLGMRPWI AVAYSAPVAAAATAVFLIYPIGQGSFSDGMPLGIS
Chlamy        HFLLGVCYMGREWELSFRLGMRPWI AVAYSAPVAAAASAVFLVYPIGQGSFSDGMPLGIS

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CYB_0216      GTFYFMLAFQAEHNILMHPASWLG VAGVFGGALLASLHGSLVISSLIRETSEESQNYGY
CYA_1748      GTFYFMLAFQAEHNILMHPASWLG VAGVFGGALLASLHGSLVISSLIRETSEESQNYGY
CyanPCC7424   GTFYFMLGFQADHNILMHPHMLAVAG IFFGALLSSLHGSLVTSSLVQETTEESINQGY
AvaATCC29413 GTFHFMLAFQGDHNILMHPFHMLGVAG VFGGALLSSLHGSLVASTLIRNTDENESINGGY
AmrMBIC11017 GTFHFMMAVQAEHNILMHPFHMLGVAG VFGGAFLSAMHGSLVTSSLVRETTEESQNYGY
SynPCC7002    GTFNFMIVFQAEHNILMHPFHMLGVAG VFGGSLFSAMHGSLVTSSLVRETTEESQNYGY
SynPCC7942    GTFNFMIVFQAEHNILMHPFHMLGVAG VFGGSLFSAMHGSLVTSSLVRETTEESQNYGY
SynPCC6803    GTFNFMIVFQAEHNILMHPFHMLGVAG VFGGSLFSAMHGSLVTSSLVRETTEESQNYGY
A. thaliana   GTFNFMIVFQAEHNILMHPFHMLGVAG VFGGSLFSAMHGSLVTSSLIRETTENESANEQY
Chlamy        GTFNFMIVFQAEHNILMHPFHMLGVAG VFGGSLFSAMHGSLVTSSLIRETTENESANEQY

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CYB_0216	RFGQEEVTYNFLAGHYAFLGRLGFPGLGLRNSRSVHFWMALPTVGIWAAAIGIGIMAFN
CYA_1748	RFGQEEVTYNFLAGHYAFLGRLGIPSLGWRNSRSVHFWMALPTLGIWAAAIGIGLMAFN
CyanPCC7424	RFGQEEVTYNFLAGHGYLGRLLVPLGLRNSRSLHFIIAAVPVIGIWCATLAVGTMAFN
AvaATCC29413	KLGGQQVVTYKYLAGHNSFLGRLLIPTFASRNHRAFHFLLAALPTIGIWFAMGVCSMAFN
AmrMBIC11017	KFGQEEATYNLLAGHAGYLGRLLFIPDIAFRNSRSIHFLAVLPTIGIWFALGIGTMAFN
SynPCC7002	KFGQEEETYNIVAHAH-GYFGRLIFQYASFNNRSRSLHFFLGAWPVVGIWFTALGVSTMAFN
SynPCC7942	KFGQEEETYNIVAHAH-GYFGRLIFQYASFNNRSRSLHFFLAAWPVVGIWFTSLGISTMAFN
SynPCC6803	KFGQEEETYNIVAHAH-GYFGRLIFQYASFNNRSRSLHFFLGAWPVVGIWFTAMGVSTMAFN
A. thaliana	RFGQEEETYNIVAHAH-GYFGRLIFQYASFNNRSRSLHFFLAAWPVVGIWFTALGISTMAFN
Chlamy	RFGQEEETYNIVAHAH-GYFGRLIFQYASFNNRSRSLHFFLAAWPVVGIWFTALGLSTMAFN

CYB_0216	LNGLNFNQSILDSQGRFIPTYADLLNRANLGIQVMHAPNAHHFPLLLA	AQP-----
CYA_1748	LNGFNFNQSILDSQGRFIPTYADLLNRANLGIQAMHAPNAHHFPLLLA	AKAD-----
CyanPCC7424	LNGFNFNQSVVDSQGHPIYTDADLLNRANLGIQAMHAPNAHHFPLTLA	GGEALPIS----
AvaATCC29413	LNGLNFNHNSILDSRGNVIRSDADILNRANIGLSVMHAPNVHNFPLVLS	SGQPPIVS----
AmrMBIC11017	LNGFNFNHNSLLDSSGRPIRTEADLLNRATMGLQVMHNSVNAHHFSLTLA	STESKEIPTIPI
SynPCC7002	LNGFNFNQSILDSQGRVINTWADILNRANLGMFVMHERNAHNFPLDLA	AGEQAPVALQAP
SynPCC7942	LNGFNFNQSVLDSQGRVINTWADVLNRANLGMFVMHERNAHNFPLDLA	AGEATPVALTAP
SynPCC6803	LNGFNFNQSILDSQGRVIGTWADVLNRANIGFVMHERNAHNFPLDLA	SGEQAPVALTAP
A. thaliana	LNGFNFNQSVVDSQGRVINTWADIINRANLGMFVMHERNAHNFPLDLA	AVEAPSTNG---
Chlamy	LNGFNFNQSVVDSQGRVINTWADIINRANLGMFVMHERNAHNFPLDLA	STNSSNN----

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



## 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.