

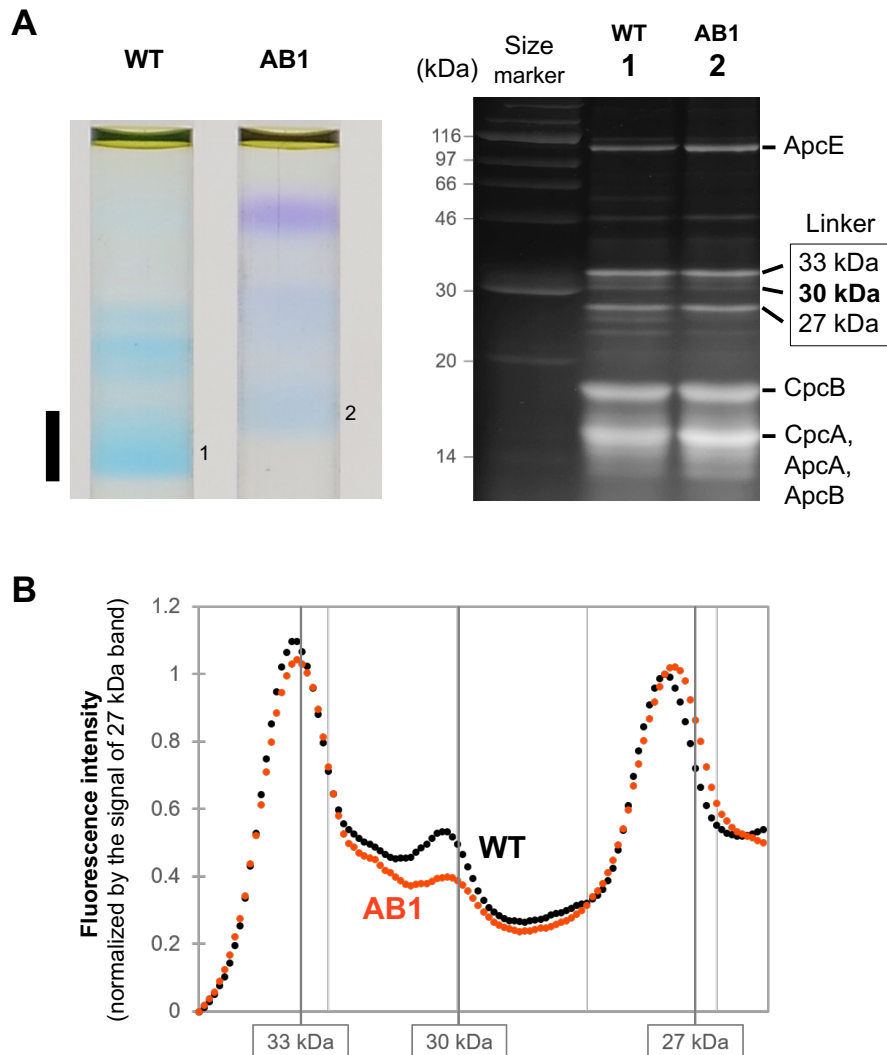
Additional Information

**Comparative Study of Phycoerythrobilin Synthases for Fine-Tuning
Photosynthetic Light-Harvesting Complexes, Phycobilisomes**

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1. Sequence files of pBNS-pcyX, -pebS, and -pebAB

9 **2. Figures**

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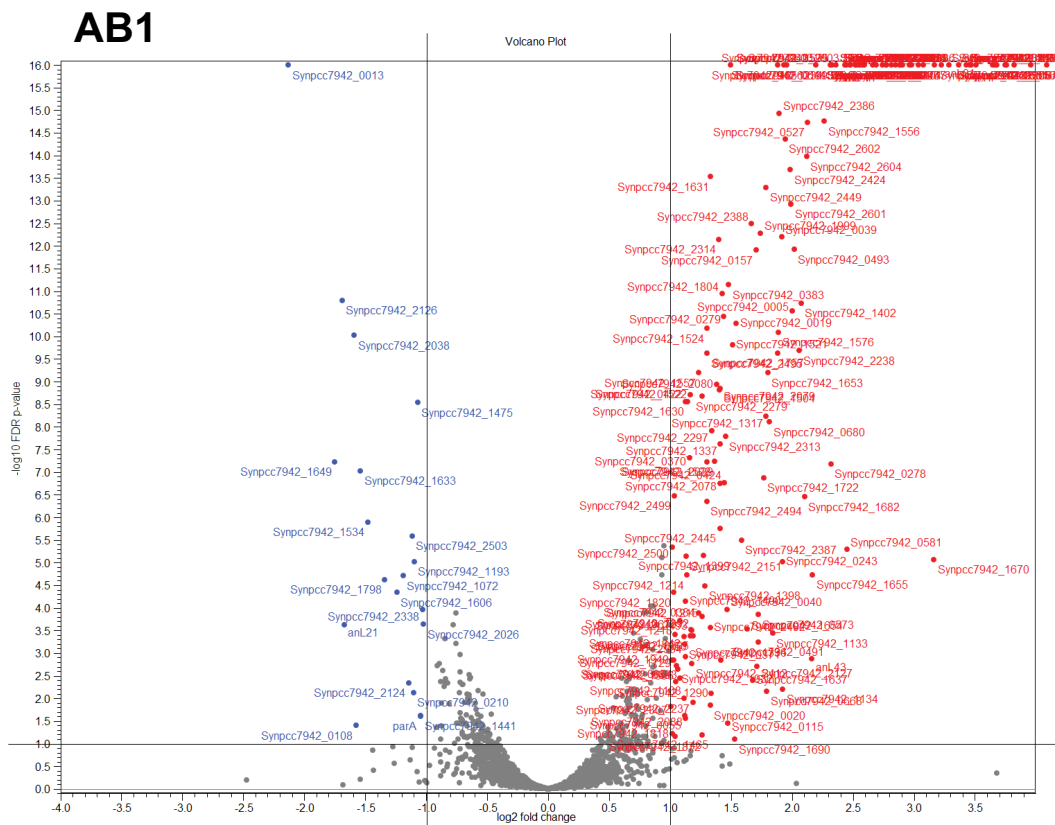
11 **Supplementary Figure S1. Comparison of the linker protein content in PBS complexes**

12 Phycobilisome complexes from wild-type (WT) and AB1, fractionated by SDG, were
 13 fluorescently labelled and separated by SDS-PAGE to compare the quantities of linker proteins
 14 (33, 30, and 27 kDa). (A) PBS fraction image after SDG centrifugation (part of Fig. 3A) and
 15 SDS-PAGE image of PBS complex proteins stained with fluorescent reagent (EzLabel
 16 FluoroNeo, ATTO Co., WSE-7010)). (B) Comparison of the fluorescence intensity of the
 17 linker protein around 30 kDa as shown in gel image A.

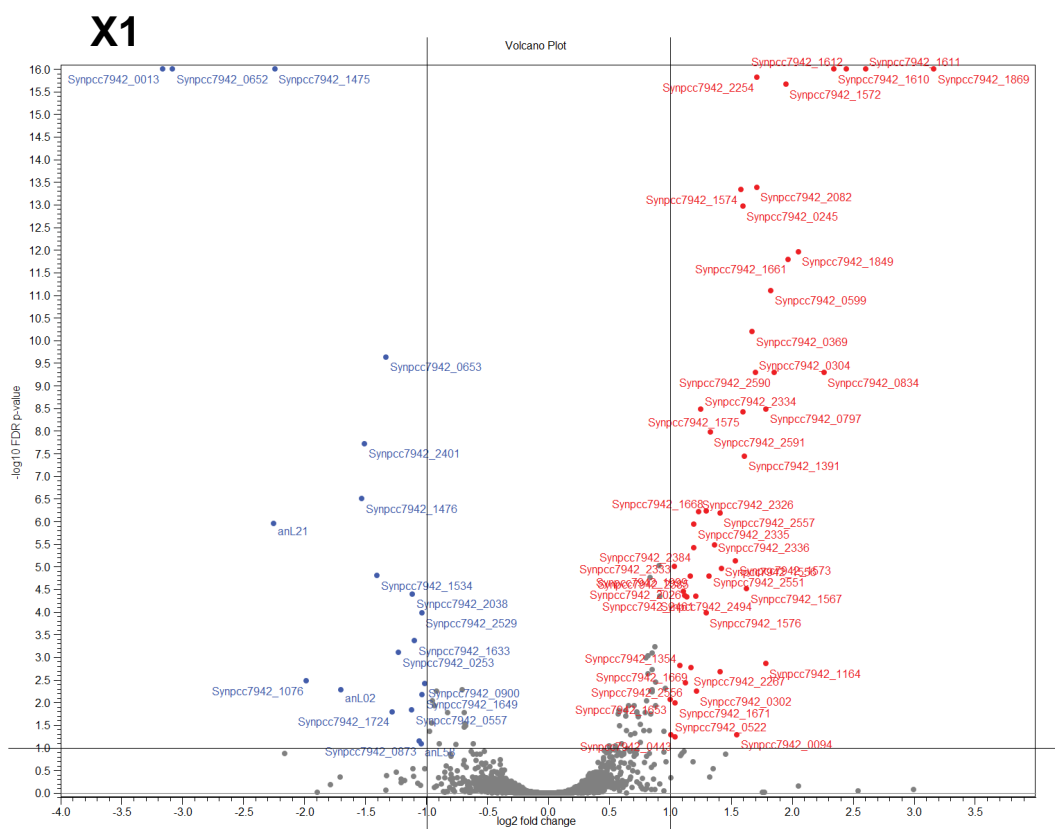
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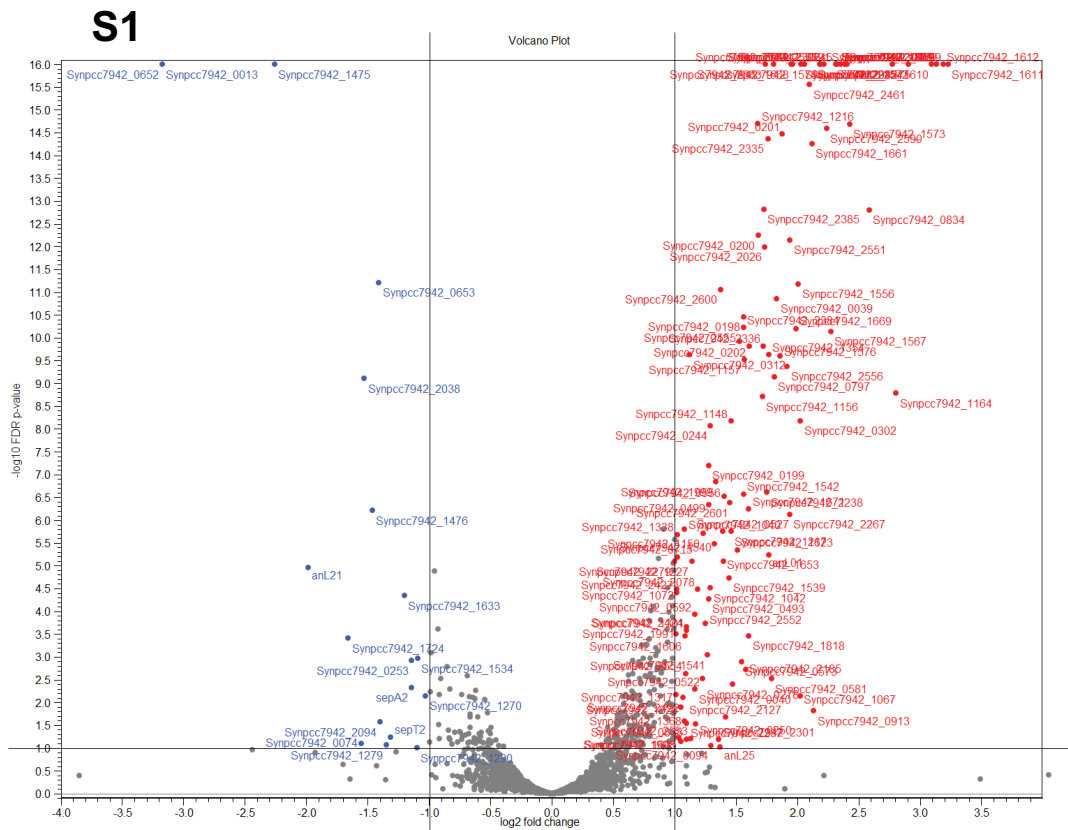
Comparative Study of Phycoerythrobilin Synthases in Cyanobacteria



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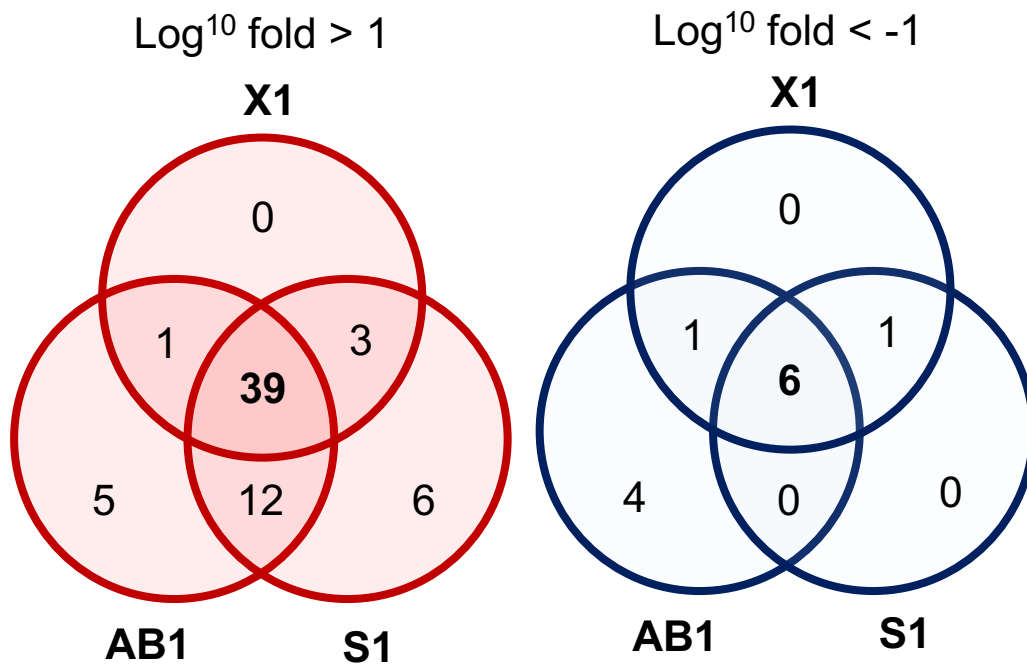
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Supplementary Figure S2. Genes exhibiting expression changes in FDBR-expressing strains

Enlarged image of volcano plot shown in Figure 6A. Results of the comparison between WT and FDBR-expressing strains (AB1, X1, and S1). Upregulated and downregulated genes are depicted in red and blue, respectively ($\log_2 \text{FC} \geq |1|$, $\text{FDR } p \leq 0.1$). The X-axis corresponds to $\log_2$ fold change, and the Y-axis displays $-\log_{10}$ of an adjusted p -value of FDR. ORF IDs showing significant variation were indicated.

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34 **Supplementary Figure S3. Comparative transcriptomic analysis of *Synechococcus***
 35 ***elongatus* PCC 7942 expressing different bilin reductases.**

36 Venn diagram showing the overlap of differentially expressed genes (CDS) between wild-type
 37 (WT) cells and strains expressing *pebA-pebB* (AB1), *pcyX* (X1), or *pebS* (S1). CDSs were
 38 defined as differentially expressed when the absolute log² fold change exceeded 1 (i.e., log²
 39 fold change > 1 for up-regulated genes shown in red or < -1 for down-regulated genes in blue)
 40 and the p-values of false discovery rate (FDR) was < 0.1. The numbers in each section indicate
 41 the count of genes that are uniquely or commonly up- or down-regulated among the respective
 42 comparisons. The shared DEGs represent genes whose expression consistently responded to
 43 altered bilin biosynthesis across different enzyme introductions.

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3. Table**Table S1. Comparison of transcriptome between WT and FDBRs expressing strains**
(additional Excel file)

Compared to WT, genes that increased by more than two-fold were highlighted in red, while those that decreased to less than half were highlighted in blue. 39 upregulated and 9 downregulated genes common to all three lineages ($\log_2 \text{FC} \geq |1|$, $\text{FDR } p \leq 0.1$) were extracted from the entire list.

Table S2. Oligonucleotide primers used for strain construction.

Primer name	Sequence (5' to 3') ^a
F1	GGAAACAGACCATGGAATTCATGATATGGGAACGCCTGATCA
R2	CCGCCAAAACAGCCAAGCTTTTACTTAATGGTGGGAAACAAGTTATC
F3	GGAAACAGACCATGGAATTCATGACAAAGAATCCTAGGAACAATAAACC
R4	CCGCCAAAACAGCCAAGCTTTTATTTGTAGCTGAATAAGAAGTC
F5	CATGGTCTGTTTCCTGTGTGAAATT
R6	AAGCTTGGCTGTTTTGGCGGATGAGAGAA
F7	CAGACCATGGAATTCTTCGACTCTTTCCTCAAC
R8	AAAACAGCCAAGCTTCTACGACAAGGAAGTTGAG
F9	CATCGCTATCTCTTAGGACTTCGCAG
R10	CAAACAGGTGCAGCAGCAACT

67 **Table S3. Sequences of codon-optimized *pcyX* and *pebS* gene.**

68	<i>pcyX</i>
69	ATGATATGGGAACGCCTGATCAAACCTCGAGAAAGAGATCATTGAGGTTCTCGACAAACA
70	CCTGATTGAGTACAATGAGCCAGGAATGGATCGCTTCAACAAACCAGGATGGACGAATC
71	GGACATGGAGCAACATGTCCATACGTCGAGCACATGTGGATGTAGTCGATGCTCGAGAA
72	ACGAAAGGGTTATGGATGGCGCATATCTGCCTTTTCCCATGAAGAAAAATGGTGGACC
73	GATTTATGGCTTTGACATAATTGCAGGCAAGAAGAAGGTTACTGGTGC GTTTCACGACTT
74	TTCTCCTCTGTTGAAAAAGGAGCATCCGTTAACTCGTTGGTTCATCGAAGAGAACAAGTG
75	GTACAAACCGTCAAAAGTACGCGAATTACCTGATTGGGCTAAAGCCATTTTCAGCGAAG
76	GCATGATTGCTGCAGGGAATGTGCAAGAGGAGAGGGAACTCAACCAAATCTGTACCATG
77	GCAGTCTCGAATCTTAACGCGTATATCGACAAAATCGGTCACTTCAATTCGGATTCCAAT
78	GAAGAGGATGTGATTTCGTGCGCAAACTTCTATTGCGAAAACCAGCAGAAAAATCCACA
79	TACACCTAGAACGATGAAGAGTCTAGGTTTGCCAGAAGAAGACATCAAACCTGTTTTGTG
80	CCGATAACTTGTTTTCCCAACCATTAAGTAA
81	<i>pebS</i>
82	ATGACAAAGAATCCTAGGAACAATAAACCCAAAAAGATCCTTGATAGCTCGTATAAGTC
83	TAAGACGATTTGGCAGAACTACATTGATGCCCTATTTGAGACTTTTCCGCAACTTGAGAT
84	TTCAGAGGTTTGGGCTAAATGGGATGGTGGCAATGTCACGAAAGATGGTGGAGATGCGA
85	AATTGACAGCGAATATACGCACTGGCGAACACTTCTTGAAGGCTCGTGAAGCACATATC
86	GTGGACCCAAATTCCGACATCTACAACACGATTCTGTATCCGAAAACCTGGTGCAGATCTC
87	CCCTGTTTTGGGATGGATCTCATGAAATTCTCAGACAAGAAAGTGATCATTGTCTTTGAC
88	TTTCAACATCCACGAGAGAAATACCTGTTTTTCGGTAGACGGTTTACCGGAAGATGACGGC
89	AAATATCGCTTCTTCGAAATGGGGAATCACTTCTCTAAGAACATCTTTGTTCGGTATTGC
90	AAACCTGATGAAGTGGATCAGTATCTCGATACATTCAAACCTGTATTTGACCAAGTACAAA
91	GAGATGATAGACAACAACAAACCTGTAGGAGAAGACACCACAGTTTACTCCGATTTTGA
92	CACCTATATGACGGAGTTAGATCCAGTAAGAGGTTATATGAAAAACAAGTTTGGCGAAG
93	GACGTAGTGAGGCATTCGTCAATGACTTCTTATTCAGCTACAAATAA

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