**Supplementary Information**

**Metabolic Engineering of *Synechococcus elongatus* 7942 for Enhanced Sucrose Biosynthesis**

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**I. Synthesized DNA fragment *Ptrc-Riboswitch-E\*-gap2-pgk-rrnBT2***

caaGGATCCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCATACGCTCACAATTGGTACCGGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACCCTGCTAAGGAGGCAACAAGAtgattagagtagcgattaatggatttggacgtattggacgcaacttcttgagatgttgggctggtcgcgaaaacagccaactccaagtcgttgggatcaatgcgaccaccgataccaaaagtaatgcccacatgcttcgttatgacacgatgctgggtaaatttgatggcgaaatcgattatgacgccaactctctgactgtcaacggcaacgtaatcaaatgctgttccgacagaaaccccctcaacctcccttggaaagagtggggcgtcgatttggtgatcgaatctaccggtgtattcaacaccgaagaaggatcttctaagcacattacagctggtgcacaaaaagtattgatcacagcccctggtaaaggcggccatatcggcacctatgtagttggtgtaaacgccgatcagtatggccacgacaaacaaaatgtcatcagtaatgccagctgtaccaccaactgcctcgcccccattgttaaagtactcaatgatcgttttgggatcgtcaaagggacgatgaccaccgtccacagttacactggcgaccaacgtatcctcgacaacagccaccgggatctccgtcgggcgcgggcagccgcagaaaatattgtgccgacttctactggcgcagctaaagccgttgccttagttattcccgaaatgaagggtaagctcaacgggatcgccatgcgggtaccgactcccaacgtctccgtagttgacctggttgcccaagtagcgaagcccaccattgctgaggaagttaaccaagttctgaaagaagcttctgaaacctacatgaaagggattttggcttacaccgaagaacccctcgtctcctgtgacttccggggaactgatgtctcttccaccattgacggtagcctcaccctcgccatggacggtgacctaattaaagttgtggcttggtacgacaacgagtggggttattctcaacgggttgttgacctcgcagaaatcgttgcccaaaattggcaagggtaaggaGAATTCagaACGCGTCtgataaaacagaatttgcctggcggcagtagcgcggtggtcccacctgaccccatgccgaactcagaagtgaaacgccgtagcgccgatggtagtgtggggtctccccatgcgagagtagggaactgccaggcatcaaattaagcagaaggccatcctgacggatggcctttttgcgtttctacaaactcttcctgGAGCTCtaag

**II. Determination of chlorophyll *a*, carotenoids and glycogen contents in *S. elongatus* cells**

The cellular chlorophyll *a*, carotenoids, and glycogen contents were determined using a method adapted from previous studies (Zavřel et al., 2015, Pattanayak et al., 2014). Specifically, cell pellets from -80°C were thoroughly resuspended using pipettes in 1 mL of pre-cooled methanol and then covered with aluminum foil. Samples were incubated at 4 °C for 20 min in order to extract the pigments from the cells. Subsequently, the samples were centrifuged at 15,000×g, 4 °C for 5 min. The pellets were visually checked to ensure they appeared bluish or purple with no green color. If the pellet was green, the extraction was repeated and the first and second extracts (supernatants) were pooled together. The extracts were transferred into 1-cm cuvettes and the absorbance of samples and methanol blanks at 470 nm, 665 nm and 720 nm were determined. The concentrations of chlorophyll *a* and carotenoids were calculated using the following equations:

Chla [μg/ml] = 12.9447 (A665 − A720)

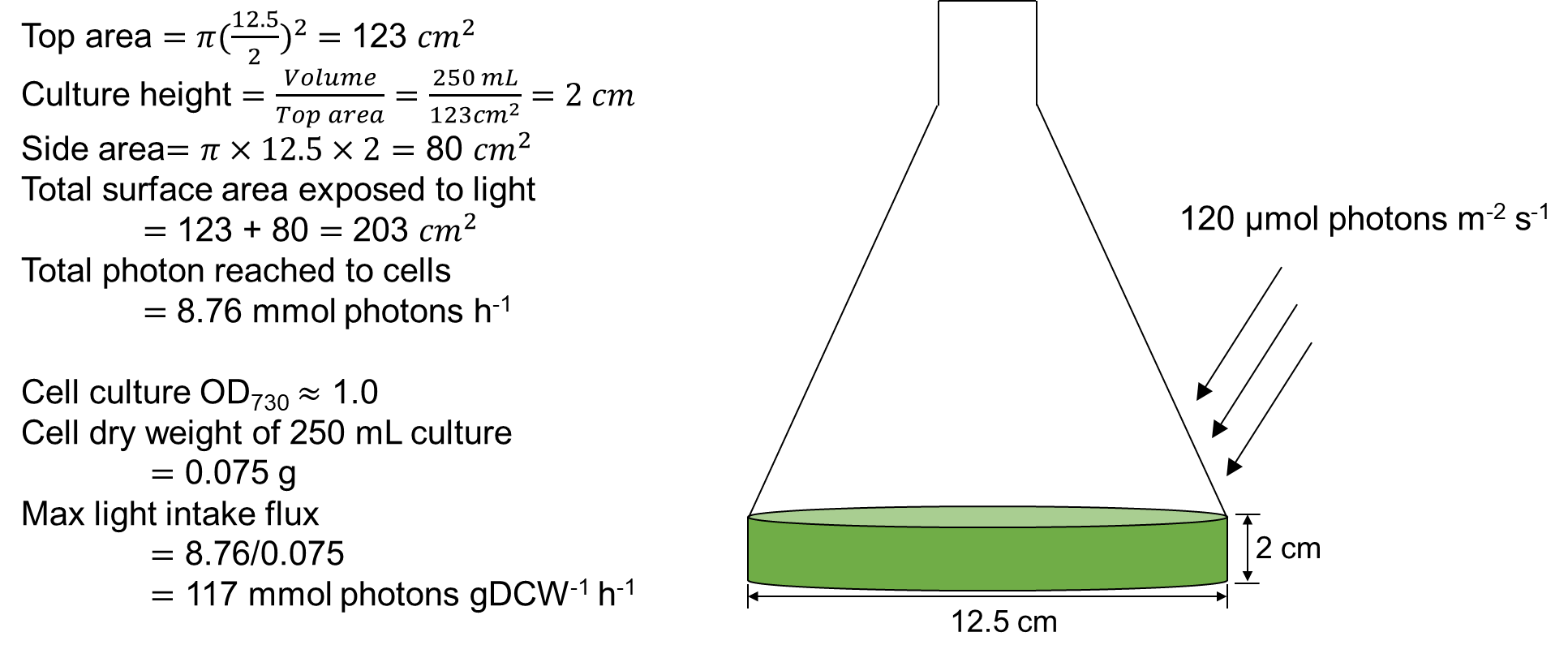
Chla [μM] = 14.4892 (A665 − A720); for Chla molar mass = 893.4890 g/mol

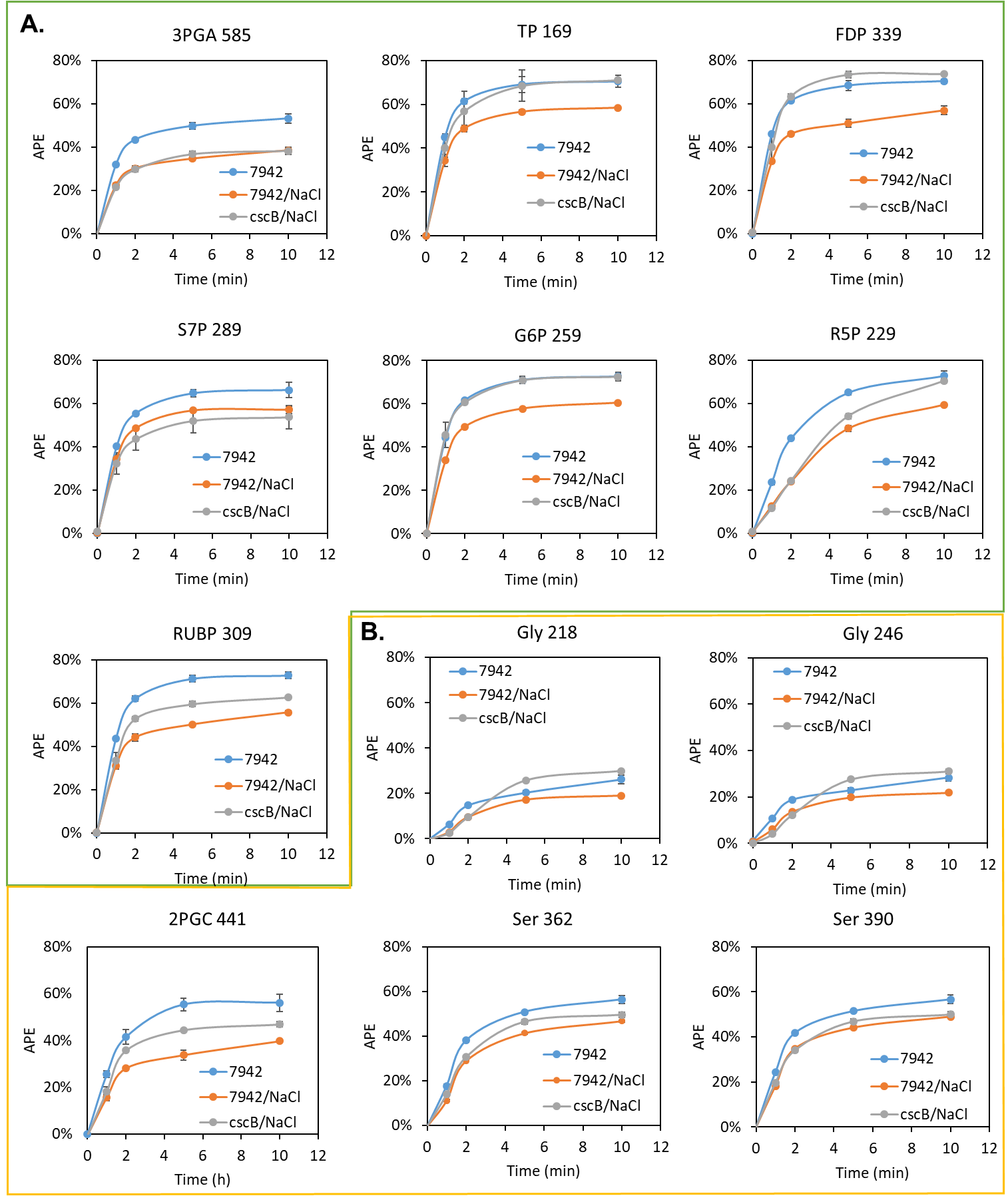
Carotenoids [μg/ml] = [1,000 (A470 − A720) − 2.86 (Chla [μg/ml])] / 221

To determine the cellular glycogen contents, the pellets after pigment extraction were resuspended in 300 μL of 40% KOH followed by incubation at 95°C for 1.5 h. Then, 600 μL of 100% ethanol was added to each extract and the samples were incubated at -20°C for 16 h to precipitate the glycogen. Subsequently, samples were centrifuged for 1 h at 4°C, and pellets were washed twice with cold ethanol (900 μL, 450 μL, respectively). Following that, the pellets were resuspended in 200 μL of 2 N HCl and incubated at 95°C for 30 min to hydrolyze the glycogen to glucose. 200 μl of 2 N NaOH and 100 μL 1 M phosphate buffer (pH 7), were used to neutralize the sample. The samples were centrifuged at 17000 x g, for 2 min, and 200 μl of supernatant of each sample was analyzed on YSI 2300 Stat installed with a glucose membrane (YSI, Yellow Springs, OH, USA).

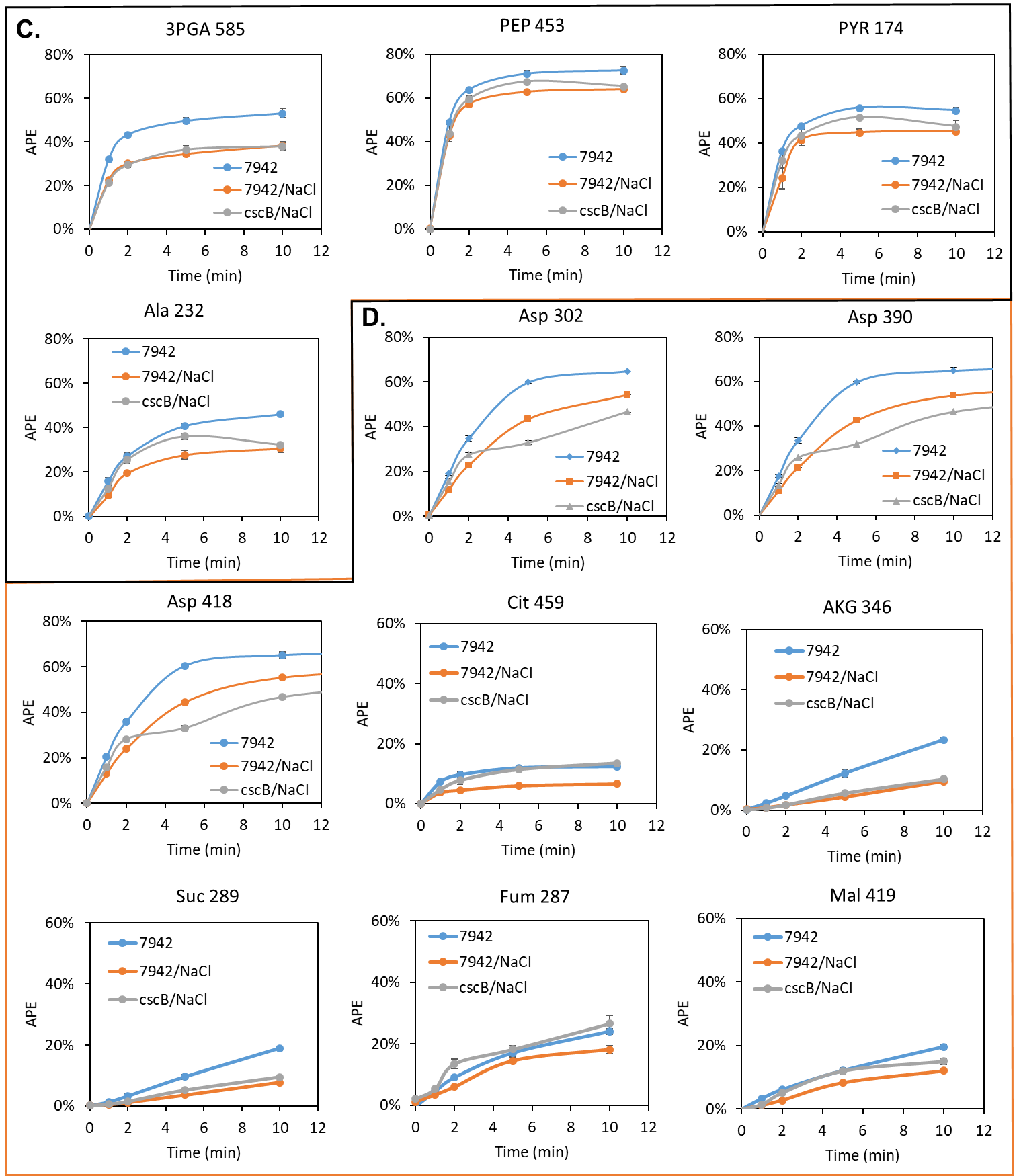
**III.** **Constraint of the photon intake fluxes**

The light-exposed surface area of the 250 mL culture in a 1-L flask is simplified as the top area plus the side surface area of a cylinder. Therefore, the total surface area equals 203 cm2, and the maximum photon intake flux was 117 mmol photons gDCW-1 h-1. The photon intake flux upper bounds in the genome-scale models were set as 150% of the calculated maximum photon intake flux, *i.e.*, 175 mmol photons gDCW-1 h-1.

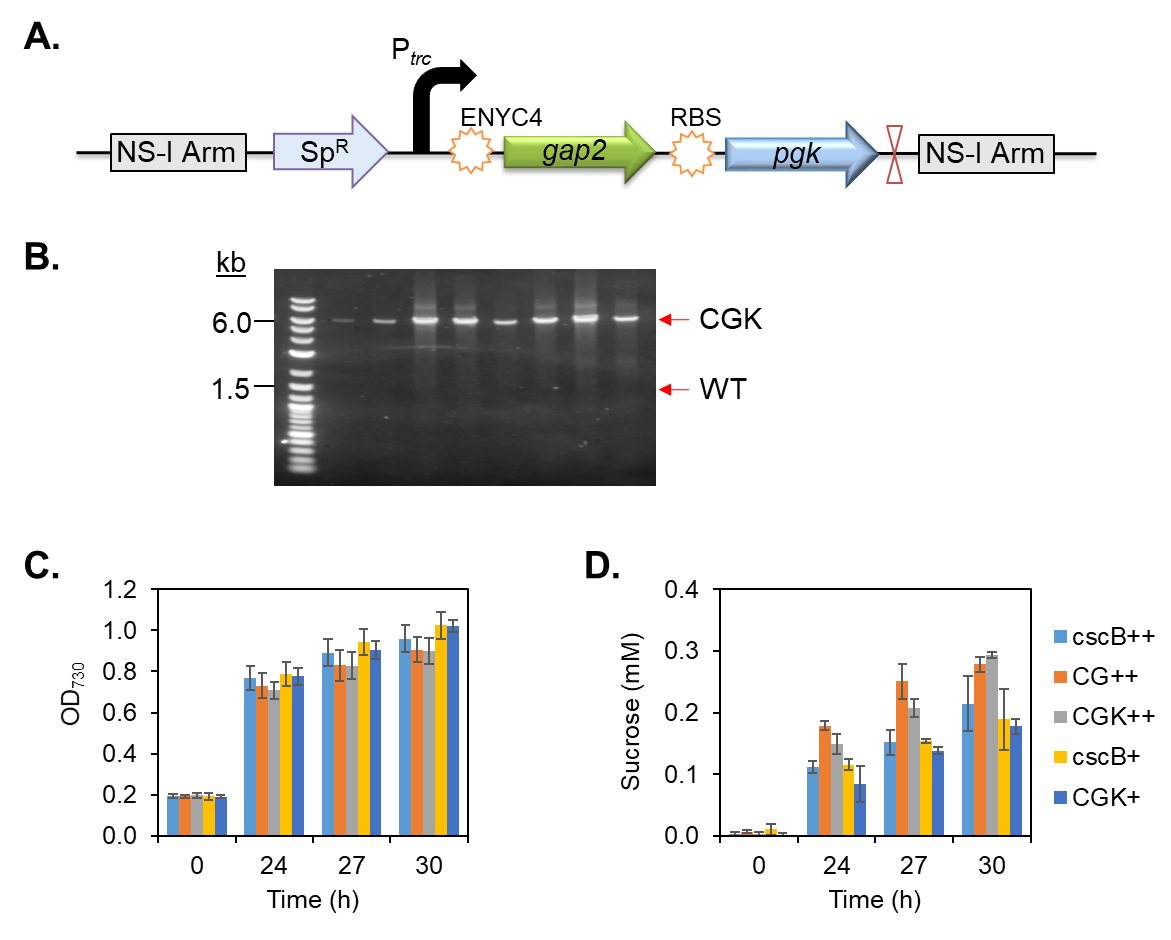




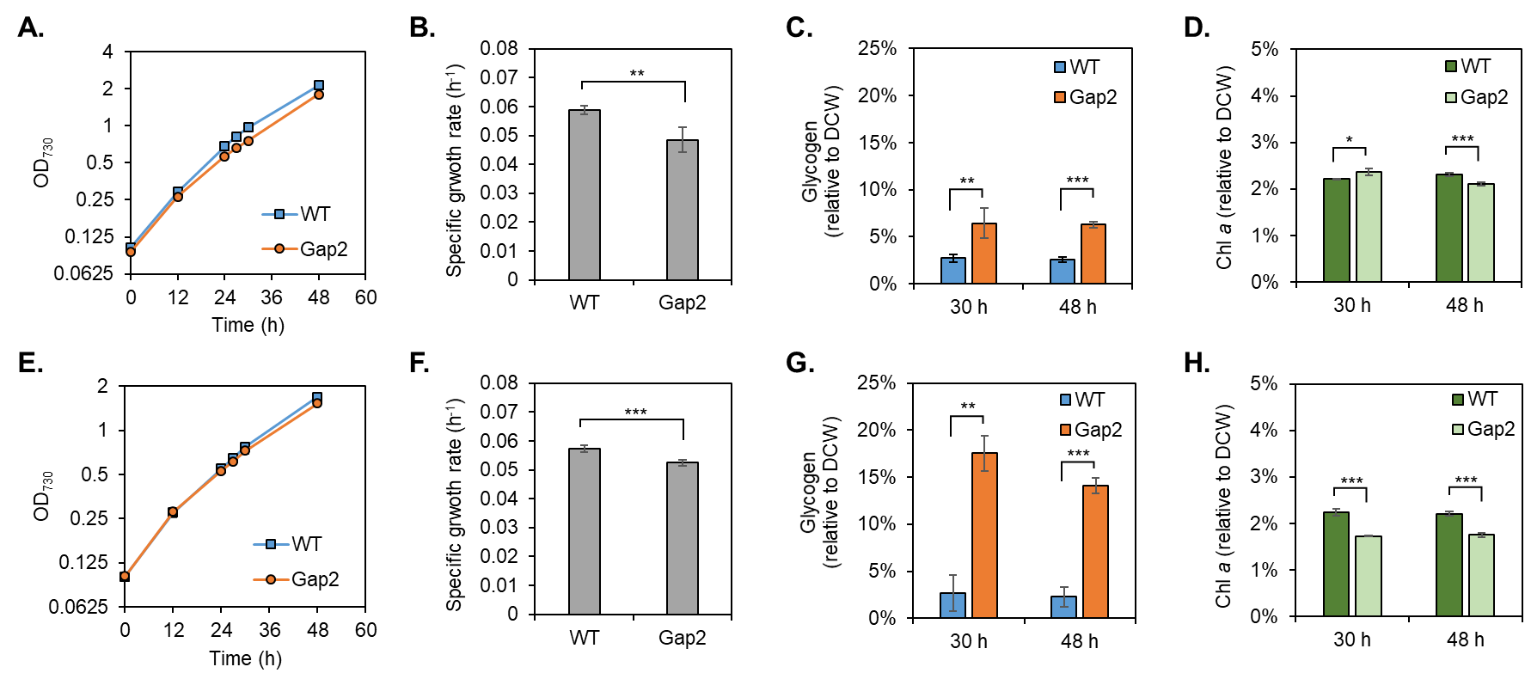
**Fig S1**Average percent enrichment (APE) of metabolites in the core metabolic network. (A) Calvin-Benson-Bassham (CBB) cycle. (B) Photorespiration pathway. (C) Glycolytic pathway. (D) Tricarboxylic acid (TCA) cycle.



**Fig S1**Average percent enrichment (APE) of metabolites in the core metabolic network. (Continued)



**Fig S2** Effects of overexpression of 3-phosphoglycerate kinase on cell growth and sucrose production. (A) Schematic diagram showing *gap2-pgk* expression cassette inserted at the genomic neutral site I (NS-I) of the *S. elongatus* strain CGK. (B) Agarose gel electrophoresis following colony PCR to confirm the genomic insertion of the *gap2-pgk* expression cassette and the complete genome segregation of *S. elongatus* strain CGK. (C) Cell growth of *S. elongatus* strains cscB, CG and CGK as indicated by the optical density (OD730). (D) Sucrose excreted to the culture broth. ‘+’ indicates IPTG (which induces expression of genes *cscB* and *pgk*) was added into the culture; ‘++’ indicates both IPTG and theophylline (which together induce expression of *gap*2) were added into the culture. Data represent means and standard deviations of three biological replicates.

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**Fig S3** Effects of overexpressing GAPDH in *S. elongatus* under normal (A-D) and salt-stressed (E-H) growth conditions. (A & E) Cell growth curves. (B & F) Calculated specific growth rates. (C & G) Glycogen contents. (D & H) Chlorophyll *a* contents. All cultures were grown in 250-mL flasks containing 60 mL of the specified medium. Data represent means and standard errors from at least three biological replicates. Asterisk (\*) indicates *p*-value < 0.05. \*\* indicates *p*-value < 0.01. \*\*\* indicates *p*-value < 0.001.

**Table S1** Plasmids used in this study.

|  |  |  |
| --- | --- | --- |
| **Plasmid name** | **Expression cassette** | **Source** |
| pAM1414 | -- | Golden Lab (Andersson et al., 2000) |
| pNSI-Gap2 | *SpR-Ptrc-Riboswitch-E\*-gap2-rrnBT2* | This work |
| pNSI-Gap2-Pgk | *SpR-Ptrc-Riboswitch-E\*-gap2-pgk-rrnBT2* | This work |

**Table S2** Primers used in this study.

|  |  |  |
| --- | --- | --- |
| **Primer name** | **DNA sequence (5’ to 3’)** | **Target** |
| TB-Fw | CAATCCGCCCTCACTACAACCG | DNA fragment *Ptrc-Riboswitch-E\*-gap2-rrnBT2* synthesized by Twist Bioscience |
| TB-Rev | TCCCTCATCGACGCCAGAGTAG | DNA fragment *Ptrc-Riboswitch-E\*-gap2-rrnBT2* synthesized by Twist Bioscience |
| PGK1 | ggaGAATTCagAtggctaagaaatcagttgc | gene *pgk* on the genome of *Synechococcus* PCC7002 |
| PGK2 | caGACGCGTttaggcatcatcgagg | gene *pgk* on the genome of *Synechococcus* PCC7002 |
| NS15 | GGCTGCTTGGCAAAAAC | NSI homologous arm flanking the inserted DNA of interest |
| NS16 | CCTGTTGTGCTGTTTCGATTG | NSI homologous arm flanking the inserted DNA of interest |
| gap21 | ggtgtattcaacaccgaagaag | gene *gap2* for DNA sequencing |
| gap22 | ggtcgccagtgtaactg | gene *gap2* for DNA sequencing |
| pgk1 | ccgaatttgccaagcaactc | gene *pgk* for DNA sequencing |
| pgk2 | tcaagagcttgtcgcacttttc | gene *pgk* for DNA sequencing |

**Table S3** Update of iJB785 for constructing genome-scale models *i*BW792, *i*BW792\_WT/NaCl and *i*BW792\_cscB/NaCl in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Reaction ID** | **Reaction name** | **Notes** |  |
| ORNTA | Ornithine transaminase | Removed. The gene apparently encodes a truncated inactive enzyme. |  |
| MDH | Malate dehydrogenase | Added. Based on fluxomics data (Broddrick et al., 2019). Gene reaction rule unknown. |  |
| MPTSS | Molybdopterin synthase sulfurylase | Added. Molybdopterin cofactor biosynthesis. |  |
| MOADSUx | MoaD sulfuration (nadh, assumed) | Added. Molybdopterin cofactor biosynthesis. |  |
| GTPC | GTP 3,8-cyclase | Added. Molybdopterin cofactor biosynthesis. |  |
| CPMPS | Cyclic pyranopterin monophosphate synthase | Added. Molybdopterin cofactor biosynthesis. |  |
| MPTS | Molybdopterin synthase | Added. Molybdopterin cofactor biosynthesis. |  |
| MPTAT | Molybdopterin adenylyltransferase | Added. Molybdopterin cofactor biosynthesis. |  |
| MOCOS | Molybdenum cofactor synthase | Added. Molybdopterin cofactor biosynthesis. |  |
| CPMPS\_1 | Cyclic pyranopterin monophosphate synthase, MoaCB | Added. Molybdopterin cofactor biosynthesis. |  |
| SK\_colipacy\_e | Modeling: sink reaction | Added. Sink needed to let colipacy leave system since its components have been included in bm\_carbs and bm\_lipid already |  |
| CDPDAGS\_HDE\_PALM | CDP-diacylglycerol synthase (16:1(9Z)/16:0) | Added. PG1619Z160 synthesis |  |
| PGPS\_HDE\_PALM | Phosphatidylglycerol phosphate synthetase (16:1(9Z)/16:0) | Added. PG1619Z160 synthesis |  |
| PGPP\_HDE\_PALM | Phosphatidylglycerol phosphate phosphatase (16:1(9Z)/16:0) | Added. PG1619Z160 synthesis |  |
| CDPDAGS\_PALM\_PALM | CDP-diacylglycerol synthase (16:0/16:0) | Added. PG160 synthesis |  |
| PGPS\_PALM\_PALM | Phosphatidylglycerol phosphate synthetase (16:0/16:0) | Added. PG160 synthesis |  |
| PGS\_PALM\_PALM | Phosphatidylglycerol synthase (16:0/16:0) | Added. PG160 synthesis |  |
| OXGDC | 2 oxoglutarate decarboxylase; acetolactate synthase | Added. Based on 13C-labeling data. |  |
| SSALy | Succinate-semialdehyde dehydrogenase, NAD(P)-dependent | Added. Based on 13C-labeling data. |  |
| EX\_sucr\_e | Sucrose exchange | Added. |  |
| SK\_Sucrose | Modeling: sink reaction (sucr.e) | Added. |  |
| bm\_glucan | Unknown 14glucan sink in biomass | Added. Based on biomass composition analysis data. |  |
|  |  |  |  |
| BIOMASS\_CELL\_WALL | Biomass: Cell wall (LPS and peptidoglycan) | Removed. |  |
| Biomass\_acgam\_acmum | Biomass: Cell wall (peptidoglycan) | Added. |  |
| BIOMASS\_PIGMENTS | Biomass: pigments and xanthophylls | Removed. |  |
| BIOMASS\_CROTENOIDS | Biomass: crotenoids | Added. |  |
| BIOMASS\_\_1 | Biomass objective function | Updated the biomass objective function to reflect biomass compositions of each sample. |  |
| BIOMASS\_DNA | Biomass: DNA | Updated. |  |
| BIOMASS\_RNA | Biomass: RNA | Updated. |  |
| BIOMASS\_LIPIDS | BioLip | Updated biomass\_lipid to replace dgdg161 and mgdg161 with dgdg160 and mgdg160, respectively, and to include pg1619Z160 and pg160, and to include C17 (component in the photosystems), to better reflect the measured total lipid content in *Synechococcus elongatus*. |  |
| BIOMASS\_PROTEIN | Biomass: protein | Updated. |  |
| BIOMASS\_CARB | Biomass: carbohydrates (storage and misc.) | Updated the biomass\_carb reaction to reflect measured monomer sugar and aminosugar contents and to exclude glycogen, the synthesis rate of which is experimentally measured, and constrained separately for modeling. |  |
| GLUDGE\_PALM\_PALM | Monoglucosyldiacylglycerol epimerase(16:1(9Z)/16:1(9Z)) | Updated. DGDG161 changed to DGDG160 to more accurately reflect the lipid composition. |  |
| GLUDGS\_PALM\_PALM | Monoglucosyldiacylglycerol synthase(16:1(9Z)/16:1(9Z)) | Updated. DGDG161 changed to DGDG160 to more accurately reflect the lipid composition. |  |
| DGDGS\_PALM\_PALM | UDP-galactose-dependent DGDG synthase(16:1(9Z)/16:1(9Z)) | Updated. DGDG161 changed to DGDG160 to more accurately reflect the lipid composition. |  |

**Table S4** Reaction lower and upper bounds from 13C-MFA results used for GSM.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **WT** | | **WT/NaCl** | | **cscB/NaCl** | |
| **Reaction name** | LB | UB | LB | UB | LB | UB |
| ‘RBPCcx’ | 2.2861 | 2.9685 | 2.3201 | 2.7705 | 2.719 | 2.9708 |
| ‘PGK’ | 4.0326 | 5.2212 | 4.0825 | 4.9323 | 4.9826 | 5.4561 |
| ‘ENO’ | 0.5467 | 0.7307 | 0.562 | 0.676 | 0.4521 | 0.4969 |
| ‘CS’ | 0.0763 | 0.1633 | 0.0783 | 0.1568 | 0.0622 | 0.0869 |
| ‘FBA3’ | -1.0008 | -0.7704 | -0.9451 | -0.7818 | -0.9835 | -0.9125 |
| ‘PGI’ | -0.1219 | -0.0432 | -0.1226 | -0.0327 | -0.1987 | -0.0904 |
| ‘ME2’ | 0.0108 | 0.0999 | 0 | 0.066 | 0 | 0.0525 |
| ‘PEPC’ | 0.1727 | 0.2754 | 0.138 | 0.2169 | 0.1188 | 0.1757 |
| ‘GLYCK’ | 0 | 0.0712 | 0 | 0.1262 | 0 | 0.0649 |
| ‘EX\_hco3\_e’ + ‘EX\_co2\_e’ \*\* | -2.5441 | -2.0157 | -2.2835 | -1.9433 | -2.6438 | -2.4711 |

\*\* Equals to *f*(*Rubisco*) + *f*(*PPC*) – *f*(*G6PDH*) – *f*(*GLYDC*) – *f*(*ICD*) – *f*(*OXGDC*) – *f*(*ME*) – *k*\**f*(*Biomass*), in which *k* equals to the CO2 coefficients in the Biomass equations in the 13C-MFA models.

**References**

Andersson, C. R., Tsinoremas, N. F., Shelton, J., Lebedeva, N. V., Yarrow, J., Min, H. & Golden, S. S. (2000). Application of bioluminescence to the study of circadian rhythms in cyanobacteria. *Methods Enzymol.,* 305, 527-42. doi: 10.1016/s0076-6879(00)05511-7.

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Zavřel, T., Sinetova, M. A. & Červený, J. (2015). Measurement of Chlorophyll a and Carotenoids Concentration in Cyanobacteria. *Bio-protocol,* 5, e1467. doi: 10.21769/BioProtoc.1467.