

UC San Diego

UC San Diego Previously Published Works

Title

Unique attributes of cyanobacterial metabolism revealed by improved genome-scale metabolic modeling and essential gene analysis

Permalink

<https://escholarship.org/uc/item/5tx93216>

Journal

Proceedings of the National Academy of Sciences of the United States of America, 113(51)

ISSN

0027-8424

Authors

Brodrick, Jared T
Rubin, Benjamin E
Welkie, David G
et al.

Publication Date

2016-12-20

DOI

10.1073/pnas.1613446113

Peer reviewed

Unique attributes of cyanobacterial metabolism revealed by improved genome-scale metabolic modeling and essential gene analysis

Jared T. Broddrick^{a,b,1}, Benjamin E. Rubin^{b,c,1}, David G. Welkie^{c,1}, Niu Du^{d,e}, Nathan Mih^f, Spencer Diamond^{b,2}, Jenny J. Lee^b, Susan S. Golden^{b,c,3}, and Bernhard O. Palsson^{a,f}

^aDepartment of Bioengineering, University of California, San Diego, La Jolla, CA 92093; ^bDivision of Biological Sciences, University of California, San Diego, La Jolla, CA 92093; ^cCenter for Circadian Biology, University of California, San Diego, La Jolla, CA 92093; ^dScripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093; ^eMicrobial and Environmental Genomics, J. Craig Venter Institute, La Jolla, CA 92037; and ^fBioinformatics and Systems Biology Graduate Program, University of California, San Diego, La Jolla, CA 92093

Contributed by Susan S. Golden, October 25, 2016 (sent for review August 12, 2016; reviewed by Shota Atsumi and Thomas K. Wood)

The model cyanobacterium, *Synechococcus elongatus* PCC 7942, is a genetically tractable obligate phototroph that is being developed for the bioproduction of high-value chemicals. Genome-scale models (GEMs) have been successfully used to assess and engineer cellular metabolism; however, GEMs of phototrophic metabolism have been limited by the lack of experimental datasets for model validation and the challenges of incorporating photon uptake. Here, we develop a GEM of metabolism in *S. elongatus* using random barcode transposon site sequencing (RB-TnSeq) essential gene and physiological data specific to photoautotrophic metabolism. The model explicitly describes photon absorption and accounts for shading, resulting in the characteristic linear growth curve of photoautotrophs. GEM predictions of gene essentiality were compared with data obtained from recent dense-transposon mutagenesis experiments. This dataset allowed major improvements to the accuracy of the model. Furthermore, discrepancies between GEM predictions and the in vivo dataset revealed biological characteristics, such as the importance of a truncated, linear TCA pathway, low flux toward amino acid synthesis from photorespiration, and knowledge gaps within nucleotide metabolism. Coupling of strong experimental support and photoautotrophic modeling methods thus resulted in a highly accurate model of *S. elongatus* metabolism that highlights previously unknown areas of *S. elongatus* biology.

cyanobacteria | constraint-based modeling | TCA cycle | photosynthesis | *Synechococcus elongatus*

The unicellular cyanobacterium *Synechococcus elongatus* PCC 7942 is being developed as a photosynthetic bioproduction platform for an array of industrial products (1–3). This model strain is attractive for this purpose because of its genetic tractability (4) and its reliance on mainly CO₂, H₂O, and light for metabolism, reducing the environmental and economic costs of cultivation. For low-cost, high-volume products, such as biofuels, however, one of the biggest challenges is attaining profitable product yields (5, 6). Genome-scale models (GEMs) of metabolism provide a valuable tool for increasing product titers by optimizing yield in silico and then, reproducing the changes in vivo (7). For instance, GEMs were used to select the optimal synthetic pathway for 3-hydroxypropanoate biosynthesis in *Saccharomyces cerevisiae* (8). In *Escherichia coli*, GEM optimization was used to realize heterologous production of 1,4-butanediol synthesis and increase titers three orders of magnitude (9). Although there have been numerous modeling efforts in *Synechocystis* sp. PCC 6803 (here in referred to as PCC 6803), this organism is highly divergent from *S. elongatus*, where limited modeling has been done (10).

This deficit can partially be explained by the lack of in vivo validation datasets, such as ¹³C metabolic flux analysis (MFA), for obligate phototrophs (11). Development of metabolic models of *S. elongatus* with strong experimental support is necessary to

exploit the organism as a bioproduction platform and advance models of obligate phototrophic metabolism.

A metabolic network reconstruction is a representation of all metabolic reactions, the enzymes responsible for their catalysis, and the genes that encode them. Genome-scale reconstructions have a proven record of contextualizing organism-specific information and facilitating the characterization and engineering of cellular metabolism (12, 13). When complete, the reconstruction enables quantitative prediction of metabolic phenotypes represented as reaction fluxes. The overall predictive power of a GEM is naturally dependent on its quality (14). Essentiality datasets have been successfully used to increase the accuracy of GEMs (15). We recently determined genome-wide gene essentiality by screening ~250,000 pooled mutants for their survival under standard laboratory conditions with continuous light via random barcode transposon site sequencing (RB-TnSeq) (16). This dataset facilitated the generation and testing of a high-quality genome-scale reconstruction through comparison of the model outputs and in vivo phenotypes at the genome scale. Inconsistencies between model predictions and in vivo data can highlight parts of *S. elongatus* metabolism where current understanding is inadequate (17).

Another key characteristic of an accurate GEM is the application of constraints that place physical, chemical, and biological limitations on a culture and generate biologically relevant phenotypic predictions. Incorporating light, a dominant constraint

Significance

Genome-scale models of metabolism are important tools for metabolic engineering and production strain development. We present an experimentally validated and manually curated model of metabolism in *Synechococcus elongatus* PCC 7942 that (i) leads to discovery of unique metabolic characteristics, such as the importance of a truncated, linear TCA pathway, (ii) highlights poorly understood areas of metabolism as exemplified by knowledge gaps in nucleotide salvage, and (iii) accurately quantifies light input and self-shading. We now have a metabolic model that can be used as a basis for metabolic design in *S. elongatus*.

Author contributions: J.T.B., B.E.R., D.G.W., S.D., S.S.G., and B.O.P. designed research; J.T.B., B.E.R., D.G.W., N.D., N.M., and J.J.L. performed research; J.T.B., B.E.R., D.G.W., N.D., N.M., and J.J.L. analyzed data; and J.T.B., B.E.R., D.G.W., S.S.G., and B.O.P. wrote the paper.

Reviewers: S.A., University of California, Davis; and T.K.W., Pennsylvania State University. The authors declare no conflict of interest.

¹J.T.B., B.E.R., and D.G.W. contributed equally to this work.

²Present address: Department of Earth and Planetary Science, University of California, Berkeley, CA 94704.

³To whom correspondence should be addressed. Email: sgolden@ucsd.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1613446113/-DCSupplemental.

on phototrophic growth, into a GEM remains a challenge (18). Photon uptake is typically fixed based on experimental results, an approach that allows retrospective analysis but not predictive modeling (19). Therefore, no current model inputs light quantity, quality, and shading, resulting in a linear growth curve characteristic of photoautotrophic batch culture.

Here, we present a comprehensive GEM of obligate phototrophic metabolism. We performed a complete reannotation and reconstruction of metabolic genes in the *S. elongatus* genome and developed an approach to incorporate light absorption that factors in the effects of cell shading. In addition, GEM predictions have been compared with and improved by essentiality data (16). These comparisons are also used to reveal unique attributes of the organism's metabolism. The result is a comprehensive metabolic model of *S. elongatus* metabolism.

Results

Genome-Scale Reconstruction of Phototrophic Metabolism. A metabolic reconstruction is a knowledge base that places biochemical, genetic, and genomic information into a structured framework. The reconstruction contains the functional annotation of the genome and defines the organism's metabolic capability: the substrates that it can use and the reactions that it can perform. To properly define the metabolic capability of *S. elongatus*, we curated the genome annotation, leveraged state of the art in silico methods, incorporated comprehensive in vivo essentiality data, and included a detailed reconstruction of light harvesting. The resulting model is an organized collection of the extensive data available for *S. elongatus* in a format that enables accurate predictions of phototrophic metabolism (Fig. S1).

Manual curation of *S. elongatus* genome annotation. Because the metabolic capability reflected in a reconstructed network is dependent on the functional annotation of the organism, we reannotated the metabolic genes using amino acid as well as protein functional domain homology-based methods. This functional reannotation enabled the conversion of an initial draft reconstruction to a completed GEM (20) (*SI Materials and Methods*). Of 2,723 genes in the *S. elongatus* genome, 785 (29%) were included in the final version of the model, and 118 of these genes (15%) had updated functional annotations. This GEM is named *iJB785* (model files are in [Dataset S1](#), and the Excel file is in [Dataset S2](#)) following convention (21).

Protein structure-guided reconstruction. Amino acid and protein domain-based annotations often do not provide sufficient detail to assign enzyme function. Therefore, enzymes are often incorrectly annotated as functionally equivalent (isozymes). In *S. elongatus*, this challenge resulted in instances where multiple genes that are essential in vivo were assigned to the same reaction; however, if the enzymes were truly compensatory, none should be essential. The importance of enzyme structure in catalytic activity suggests that structural homology modeling, which uses in silico-derived 3D analysis of a target protein based on the crystal structure of a similar enzyme, may provide additional insight into protein function.

Protein structure data have recently been applied in the global analysis of GEMs (22). We set out to apply structural modeling to protein annotation by determining the functional difference between four annotated phosphoglycerate mutases (PGMs; Synpcc7942_2078, Synpcc7942_1516, Synpcc7942_0485, EC 5.4.2.11; and Synpcc7942_0469, EC 5.4.1.12) in the *S. elongatus* genome, three of which are essential in vivo. Previous work in *S. elongatus* suggested that multiple PGMs work in concert to regulate metabolic flux during shifts in CO₂ availability (23). However, it seemed unlikely that three of four PGMs would be essential for regulation in a stable CO₂ environment. To test the hypothesis of divergent functions between the *S. elongatus* PGMs, structural homology models were generated and compared with published control crystal structures (*SI Materials and Methods*, [Table S1](#)).

Based on the structural comparison, it was possible to ascribe a more detailed function to each of the annotated PGMs. The Synpcc7942_0469 protein is structurally distinct from the three other PGMs and was annotated as the primary glycolytic PGM in *S. elongatus* based on its canonical PGM structure and the fact that it is essential. Previous work indicated that two PGMs are required to regulate central carbon flux during a transition from high to low CO₂ (23). The Synpcc7942_2078 protein shares structural features with an *E. coli* PGM control but is nonessential; thus, it was annotated as a PGM performing this regulatory function. Synpcc7942_0485 shares strong structural similarity to a phosphoserine phosphatase (PSP) in *Hydrogenobacter thermophilus* (24) and has sequence homology to the recently characterized PSP in PCC 6803 (25). Thus this gene was confidently annotated as a PSP in *S. elongatus*. Synpcc7942_1516, however, has structural features that could not be classified as a traditional PGM or PSP and is essential in vivo. Based on genomic neighborhood analysis and transcriptome mapping data (26), we hypothesized that Synpcc7942_1516 plays a regulatory role in an uncharacterized signaling network. As a regulatory enzyme, it fell outside the scope of the metabolic model. These results indicate that structural homology modeling is a promising annotation tool to increase the quality of genome-scale reconstructions and hypothesize enzyme function.

Improved reconstruction through incorporation of essential gene data. The essential gene calls for *S. elongatus* determined by RB-TnSeq-enabled refinement of the gene reaction annotations during development of the reconstruction (16). This in vivo dataset provides a gauge of gene importance by identifying genes that cannot sustain insertion mutants, which are interpreted to be essential, and the growth rate of those that can, which are interpreted to be beneficial or nonessential. Alternatively, in silico essentiality calls are made by quantifying the impact on growth when the flux through each enzyme in the model is independently set to zero (*SI Materials and Methods*). Discrepancies between essential gene calls in the in silico draft model and the RB-TnSeq results were investigated, and with sufficient evidence, the gene assignment for the model reaction was updated accordingly. For example, the *S. elongatus* genome encodes two annotated uroporphyrinogen methyltransferases (Synpcc7942_0271 and Synpcc7942_2610, EC 2.1.1.107), catalyzing an early step of both vitamin B12 and siroheme biosynthesis. However, both genes are essential in vivo, suggesting that they are not compensatory. Genomic neighborhood analysis indicated that *synpcc7942_2610* is adjacent to an iron chelataase gene; thus, we proposed that it is dedicated to the biosynthesis of siroheme, not vitamin B12. However, discrepancies between the in silico and in vivo essential gene data were not forced into agreement without additional evidence, and these remaining discrepancies are contained in [Dataset S3](#). For example, *S. elongatus* has two genes annotated for type II NADH oxidoreductases (*synpcc7942_0101* and *synpcc7942_0198*, EC 1.6.5.9); however, the in vivo data indicated that one is essential (*synpcc7942_0101*) and thus, the enzymes are not redundant. Nevertheless, the manual curation process did not reveal any significant difference between the two genes, and they were annotated as isozymes, although the RB-TnSeq data suggest divergent functions.

Discrepancies also led to a more complete representation of cellular biomass. For example, alkanes were not initially included in the biomass, because their function is unknown. However, their synthesis is essential. Recently, it was discovered that alkanes can play a role in cyclic electron flow in PCC 6803 (27). Hypothesizing a homologous function in *S. elongatus*, they were added to the biomass equation. Thus, applying the essential gene data to the curation process increased the quality of the reconstruction.

Explicit modeling of light absorption. The dominant constraints on photoautotrophic growth are light and CO₂ availability. A fundamental barrier to predictive modeling of photoautotrophic growth has been the inability to translate light irradiance into a metabolite. Traditionally, light uptake has been inferred and not

explicitly determined. A two-step optimization method is commonly used, where the CO_2 uptake rate is fixed based on experimental values at the observed growth rate followed by minimization of the photon flux (19, 28). However, the resulting values reflect only the photons that perform metabolic work, not the totality of absorbed light. Excess photon absorption has a significant impact on growth rate and metabolism. Reactive oxygen species, such as singlet oxygen generated in the pigment antenna and photosystem II (29) as well as superoxide at photosystem I (30), damage the photosynthetic apparatus and consume metabolic resources. Thus, the two-step optimization method does not accurately account for total light absorption and is incapable of predictive modeling of phototrophic metabolism.

In a more extensive description of photon capture, a metabolic reconstruction of *Chlamydomonas reinhardtii* accounted for light source quality (31). This approach was more mechanistic than the two-step optimization but still did not consider cellular pigmentation as a factor in photon absorption, and therefore, light uptake could not be quantified into a typical modeling input flux. In another paper, a light distribution function was combined with flux-balance analysis to model cyanobacterial growth in photobioreactors; however, a mechanistic model of light harvesting was not included (32). Our approach goes further by combining light source irradiance with in vivo absorption to define photon use from measurements of incident light. We incorporated the chlorophyll-normalized optical absorption cross-section (33), derived from the in vivo absorption spectrum, to link photon uptake to cellular composition. Because the model's biomass explicitly defines the cellular composition, the biomass-normalized photon absorption rate was calculated from the combination of irradiance, optical absorption cross-section, and the chlorophyll component of the biomass. This approach enabled comparison between the photon absorption capacity of the cell and the photon delivery rate of the light source at a given irradiance (Fig. 1).

Accurate modeling of obligate phototrophic metabolism also required a new level of detail in the reconstruction of the photosystem. We used recent proteomics (34) and fluorescence microscopy data (35) to reconcile membrane localization of electron transport complexes that previous cyanobacterial models had included inaccurately in the cytoplasmic membrane. Additionally, the ferredoxin:plastoquinone oxidoreductase complex gene associations were updated to include additional subunits and the use of ferredoxin as the electron donor (36, 37). We also included the photoinactivation of the D1 subunit of photosystem II. Using the photodamage rates in PCC 6803 (38), we were able to calculate a D1 repair metabolic cost as a proportion of flux through photosystem II. Finally, we generated stoichiometric reactions accounting for the energy transfer efficiencies of each of the photosynthetic pigments. Targeted excitation of *S. elongatus* permaplasts provided relative efficiency metrics for the transfer of energy from a given pigment to the photosystems (39). Reactions including these efficiencies enabled the model to account for light spectrum-specific photosynthetic efficiency. This comprehensive reconstruction of light gathering set the framework for accurate constraint-based modeling of phototrophic metabolism.

The completed reconstruction, *iJB785*, consists of 785 genes, 850 metabolic and transport reactions, and 768 nonunique metabolites distributed over seven cellular compartments (Dataset S2). The reconstruction was completed in the BiGG Models format (40), enabling standardization and cross-referencing to external databases (bigg.ucsd.edu). Combining an updated annotation, whole-genome RB-TnSeq data, and an advanced representation of light harvesting resulted in a comprehensive phototrophic model.

Modeling Phototrophic Growth. Conversion of a reconstruction into a mathematical model and the subsequent application of biologically relevant constraints enable the simulation of cellular

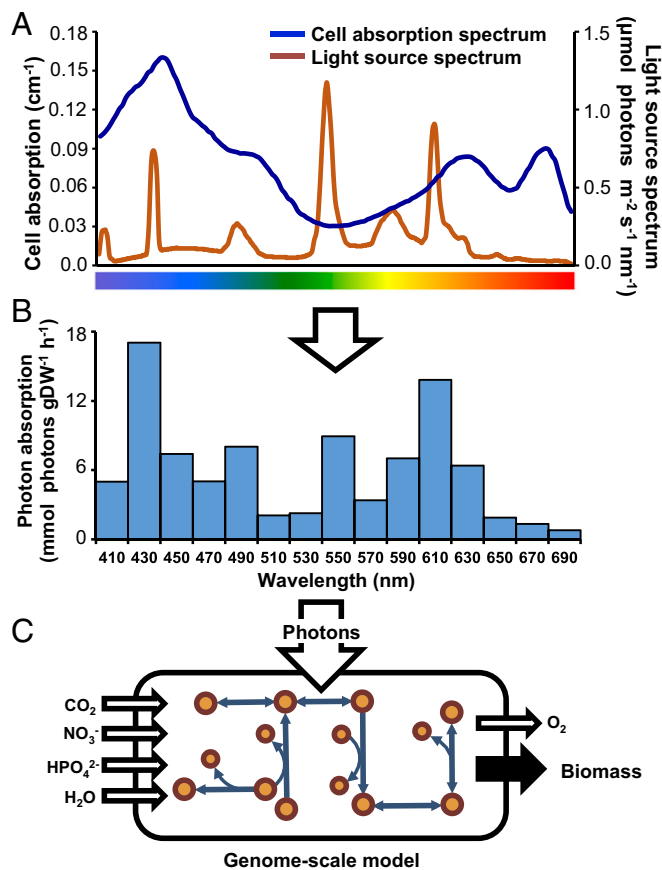


Fig. 1. Deriving light uptake rates for the GEM. (A) The chlorophyll *a*-normalized optical absorption cross-section was calculated from the cellular absorbance in the photosynthetically active range (400–700 nm) and compared with the spectral distribution of the incident light. (B) The photon absorption rate as a function of cell biomass [millimoles photons gram dry weight (DW)⁻¹ hour⁻¹] was determined by combining the optical absorption cross-section and incident light. (C) The GEM was constrained by setting the photon uptake flux to the calculated absorption rate, which was split into 15 20-nm bins across the photosynthetic range.

phenotypes. Modeling cellular growth is typically computed as either yield or specific growth rate, which assumes exponential growth (41). In both cases the inputs are normalized, such that the simulation reports a single value representative of cellular growth as long as there is a constant ratio between the biomass and the input flux. However, as the culture becomes denser, the photon absorption capacity can exceed the amount of light delivered by the light source and in the process, alter the ratio of uptake to biomass. When this phenomenon occurs, it results in a linear growth curve caused by self-shading-induced light limitation. Cells closest to the light source absorb excess photons, preventing cells in the inner culture from achieving their maximum growth rate. We accounted for shading with an unsteady-state growth modeling methodology (42), which resulted in an accurate model of linear photoautotrophic growth over the duration of a batch culture.

An additional constraint was required to capture the point when light absorption became excessive. As the other dominant constraint on growth, the maximum carbon uptake rate marks the transition between a light- and a carbon-limited culture. It is dependent on the availability of inorganic carbon in the media (43) and the acclimated state of the cell (44). However, an approximation of maximum photosynthetic output, a proxy for carbon uptake, can be captured in a single parameter: the oxygen evolution

rate (45). We used experimentally determined oxygen evolution rates to constrain the maximum photosynthetic output at a given irradiance (Fig. S2). Excess photon absorption was allowed to leave the system in a manner that did not incur a metabolic cost, simulating loss as heat or fluorescence. Reactive oxygen species production in the light-harvesting antenna caused by excess light is currently not modeled.

A growth curve for a typical *S. elongatus* culture was simulated using the oxygen evolution constraint and the calculated photon uptake rate. To account for self-shading, at 1-h intervals, the flask was sectioned into 50 concentric rings, with each ring modeling the biomass production of a 2% fraction of the culture. Light was modeled from the side of the flask, and the photon absorption of an outer ring was made unavailable to the remainder of the inner rings (Fig. S3). For the nonshading simulation, the growth rate matched the in vivo culture until light limitation, at which point the in silico growth remained exponential, whereas the in vivo curve became linear (Fig. 2). Growth simulations factoring in self-shading transitioned into linear phase on light limitation, characteristic of in vivo growth. This more accurate prediction of photoautotrophic growth was made possible by the combination of modeling photon uptake and shading as a function of culture density.

Model parameters are specific to the cellular phenotype: in particular, the chlorophyll-normalized optical absorption cross-section, which depends on the photoacclimation state of the cell. The primary photon-harvesting complex in *S. elongatus*, the phycobilisome, which can efficiently deliver light energy to both photosystems (39), is highly adaptive (46) but devoid of chlorophyll. Photosystem I contains 80–95% of the chlorophyll *a* in *S. elongatus* (39). Because photoacclimation causes fluctuation in the photosystem I to photosystem II ratio and changes to the phycobilisome (47), a given chlorophyll-normalized optical absorption cross-section may no longer be representative. The model can be easily reparameterized to account for this adaptation. Additionally, the oxygen evolution rate can be adjusted to account for phenotypic changes in different carbon environments. The mechanistic nature of this approach is generally applicable to any phototrophic reconstruction and expands genome-scale modeling into new phenotypes, such as photoacclimation and with additional constraints, photoinhibition.

Validation and Refinement of the GEM Through Essential Gene Comparison.

The RB-TnSeq data served as not only a powerful guide during reconstruction but also a validation metric of the resulting model. The accuracy of the model was determined through a comparison of the in silico essential gene calls with the in vivo dataset. The gap between model predictions and in vivo realities highlights the

limitations of the model as well as additional constraints on cellular metabolism. Evaluation of the disagreements enabled development of additional constraints beyond simple network connectivity (nonnetwork constraints), increasing the accuracy of the model predictions.

Essential gene-based model validation. We compared the in silico essential gene calls between our reconstructed model, *iJB785*, and a previous model of *S. elongatus*, *iSyf715* (48) (Table 1). Minimal standardized constraints were applied to both models, allowing a direct comparison of the metabolic flexibility of the two networks. Of 752 genes in *iJB785* with in vivo data, 587 (78%) were correctly assigned as either essential or nonessential (Fig. 3A and Dataset S3). The 165 disagreements were separated into explanatory categories (Fig. 3B). *iSyf715* contained 683 genes with in vivo data, of which 377 (55%) were correctly assigned. Neither model was able to accurately predict the reduced growth rate phenotype indicative of genes categorized as beneficial (growth defect when mutated). The 319 genes essential in vivo that were not included in *iJB785* participate in cellular processes, such as protein synthesis and transcription, that are out of scope for this GEM (Fig. S4). Incorporating the RB-TnSeq data during manual curation prevented the addition of excess metabolic flexibility (i.e., metabolic capabilities that are implied from the genome annotation but not observed in vivo) as evident by the increased accuracy of *iJB785* compared with *iSyf715*.

Although both models incorporate only 25–30% of the ORFs identified in the *S. elongatus* genome, ~50% of the experimentally shown essential genes and 44% of functionally annotated ORFs are represented in *iJB785*. Additionally, of 157 genes labeled beneficial in vivo, 46% are present in *iJB785*. This enrichment of genes that impact cellular fitness underscores the value of GEMs for contextualizing meaningful in vivo genetic perturbations.

Increased model accuracy through nonnetwork constraints. GEMs offer a tool for visualizing the metabolic network use for a given KO genotype. The resulting flux map identifies alternate routes available to the network to respond to genetic perturbations. However, there are instances where the network connectivity indicates a metabolic pathway, but an additional constraint prevents its use in vivo. Such nonnetwork constraints resulted in a disagreement in the essentiality call for the pyruvate dehydrogenase (PDH) complex. Single-gene deletion of PDH in silico indicated that phosphoketolase (Synpcc7942_2080, EC 4.1.2.9) enables bypass of lower glycolysis by generating acetyl phosphate from the Calvin cycle intermediate fructose-6-phosphate or xylose-5-phosphate. Acetyl phosphate is converted to acetyl-CoA by the combined action of reversible acetate kinase and acetyl-CoA synthase, enabling bypass of PDH. This bypass has been investigated in PCC 6803 (49), and flux balance analysis in that organism also bypassed lower glycolysis with this pathway (50). The essential nature of PDH in vivo indicated an additional constraint that prevents this bypass from carrying sufficient flux to satisfy the acetyl-CoA needs of the cell. However, proteomics (51) and transcriptomics (26) datasets for *S. elongatus* indicated phosphoketolase abundance on the same order of magnitude as PDH subunits.

Because enzyme abundance could not explain the essentiality of PDH, we investigated metabolite channeling as a factor. Channeling is the result of spatial aggregation of pathway enzymes that prevents the intermediates from being acted on by enzymes outside of the pathway. MFA in PCC 6803 suggested metabolite channeling of Calvin cycle intermediates (11). This phenomenon can be modeled by coupling the flux between two reactions, forcing a ratio, and analyzing the result on the metabolic network. The analysis in silico of metabolite channeling indicated that, if more than 1% of Calvin cycle intermediates were allowed to enter the phosphoketolase bypass, PDH would be nonessential. These results indicate that either substantial metabolite channeling occurs in the Calvin cycle of

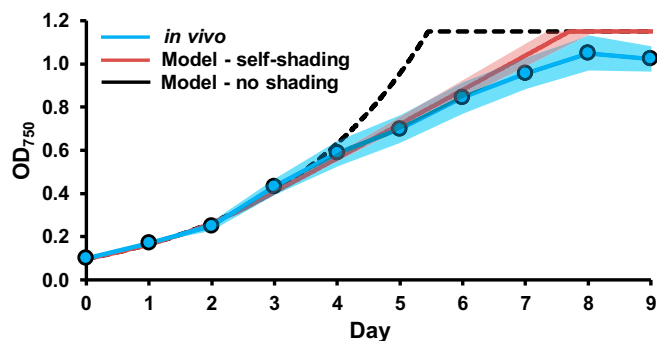


Fig. 2. Modeling the self-shading of cultures increases the accuracy of growth rate prediction. OD₇₅₀ values for the in vivo culture were the mean of three individual cultures. SE is represented by the shaded areas. SE for the in silico growth rate was determined from the SE of the in vivo inputs to the model.

Table 1. Comparison of essentiality results between *iJB785* and *iSyf715*

		<i>S. elongatus</i> PCC 7942					
		<i>iJB785</i> (this study)			<i>iSyf715</i> (previous model)		
Gene category	ORFs	Included in GEM	Essentiality prediction	Correct* (%)	Included in GEM	Essentiality prediction	Correct* (%)
Essential	718	399	457	350 (88)	360	134	118 (33)
Beneficial	157	72	5	0 (0)	56	1	0 (0)
Nonessential	1,748	281	323	237 (85)	266	579	258 (97)
No in vivo data	100	33	0	N/A	32	0	N/A
Total genes	2,723	785	785	587 (78 [†])	714	714	376 (55 [†])

N/A, not applicable due to a lack of in vivo data.

*Equal to the number of genes correctly predicted to be essential in silico.

[†]Total correct genes/(total genes included in GEM – model genes with no in vivo data) × 100%.

S. elongatus or the phosphoketolase pathway is functioning in a yet uncharacterized way.

An additional nonnetwork constraint suggested by discrepancies between the model and the in vivo data is the phototrophic reaction catalyzed by ferredoxin-NADP oxidoreductase (FNOR; Synpcc7942_0978, EC 1.18.1.2), an essential reaction in vivo. The model indicated that FNOR was bypassed by NADPH:NAD⁺ transhydrogenase (Synpcc7942_1610, Synpcc7942_1611, and Synpcc7942_1612, EC 1.6.1.2), resulting in a discrepancy between the model and in vivo data. The canonical function of transhydrogenase is to provide metabolic flexibility by interconverting the two primary redox carriers. However, previous work in PCC 6803 had called into question the presence of an active transhydrogenase in cyanobacteria (52, 53). Previous modeling of PCC 6803 also observed dramatic changes in flux predictions depending on the activity of the transhydrogenase reaction (50), with the authors retaining the canonical transhydrogenase function. However, when we repeated the in silico essential gene assessment setting the trans-

hydrogenase reaction bounds to zero; along with additional non-network constraints, such as routing flux through PDH, 13 genes that had previously been discrepancies fell into alignment with in vivo data. These additional constraints, suggested for modeling in constant light, are provided in *SI Results*.

Central carbon metabolism flux predictions. The intracellular flux distribution maps the metabolic reaction use in a given condition. This visualization provides insight into highly used pathways that can be drawn on for product synthesis. The photoautotrophic flux distribution for central carbon metabolism in *S. elongatus* predicted by our GEM is shown in Fig. 4. Our flux values were in close alignment with PCC 6803 ¹³C MFA data (11), the in vivo equivalent of these in silico data. In PCC 6803, the flux ratio between carbon uptake and fixation was 1:1.27, whereas our model prediction for *S. elongatus* was 1:1.14. The flux ratio between the Calvin cycle and lower glycolysis, indicative of the biosynthetic carbon requirements of the cell, was 7.9:1 in our model prediction, in good agreement with the ratio of 9.8:1 observed in PCC 6803. The differences may be attributed to variations in the biomass composition for the two species or the fact that the model predicts optimality, whereas the in vivo data reflect inefficiencies naturally present in a living organism. Overall, the high accuracy of the essential gene assessments and consistency with published photoautotrophic flux data underscore the quality of the GEM.

Unusual Attributes of *S. elongatus* Metabolism. Taking into account the essential gene dataset (16) during the modeling process greatly improved the consistency of the model and the in vivo data. Still, reactions for which no evidence existed to bring the in silico prediction into agreement with the in vivo data deserved additional attention. The network reconstruction represents a repository of current knowledge; thus, discrepancies between in silico and in vivo results highlighted potential gaps in understanding of *S. elongatus* metabolism. Disagreements between *iJB785* and the RB-TnSeq essential gene calls were separated into categories reflecting the hypothesized source of the discrepancies (Fig. 3B and Dataset S3). Some categories, such as the Network Bypass and Annotated Isozymes, have already been discussed. Only 7% of discrepancies were categorized as Out of Scope, and these reactions were included in *iJB785* for completeness but operate in cellular processes not necessary for in silico growth. For example, the tRNA modification queuosine (54) is beneficial according to the RB-TnSeq data but was not explicitly modeled in silico, resulting in a discrepancy for all genes in the pathway.

The Nonessential Biomass discrepancies include enzymes that synthesize biomass components of the WT cellular composition but do not result in a significant in vivo growth defect when missing. Because every component of the defined biomass is required for in silico growth, this category represents in vivo flexibility not present in *iJB785*. Two such examples of known nonessential biomass components are sulfoquinovosyl diacylglycerol,

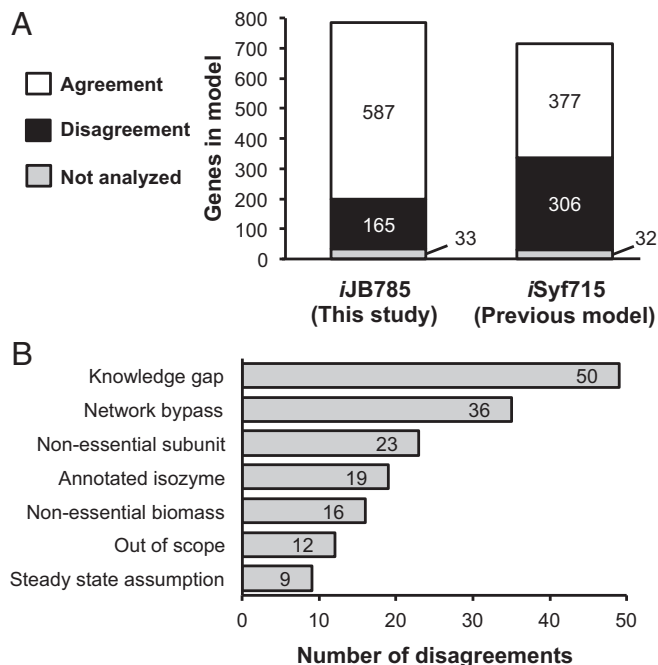


Fig. 3. Comparison of in vivo vs. in silico gene essentiality. (A) Comparison of in silico gene essentiality results for the model *iJB785* and the previous model of *S. elongatus* *iSyf715*. The agreements/disagreements are based on the comparison with in vivo RB-TnSeq results. (B) Sources of disagreements between in silico *iJB785* and in vivo gene essentiality.

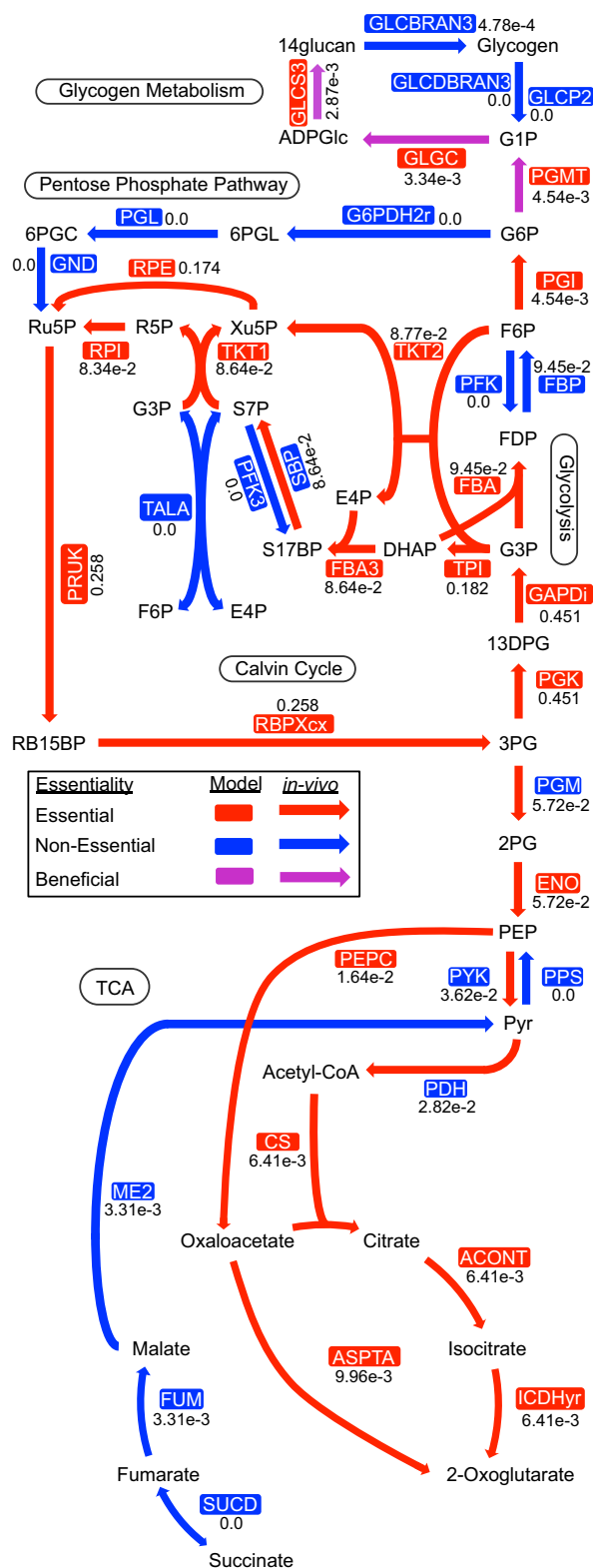


Fig. 4. Central carbon metabolism. In silico flux values are given numerically next to each reaction. The model essentiality calls are given by the color of the enzyme name, whereas arrow color indicates in vivo gene essentiality. Beneficial indicates a growth defect phenotype when the gene is mutated. Reaction and metabolite abbreviations are given in BiGG format (bigg.ucsd.edu) and found in [Dataset S2](#).

a component of the photosynthetic membranes (55), and the *O*-antigen polysaccharide, which even confers a fitness advantage against predators when mutated (56).

We also identified Nonessential Subunits of multiprotein complexes. If one gene in the complex is essential in silico, every subunit associated with that reaction is considered essential, even if the loss is tolerated in vivo. These disagreements included known nonessential subunits of the PCC 6803 photosynthetic electron transport chain (57) and ferredoxin: plastoquinone oxidoreductase complex (36).

Nucleotide salvage metabolism. Other discrepancies were placed into the categories Steady-State Assumption and Knowledge Gaps. Examples of both can be found in nucleotide salvage metabolism. Although *S. elongatus* encodes a complete set of enzymes for de novo biosynthesis of both purine and pyrimidine nucleotides, salvage reactions are mostly absent (Fig. S5). One exception is adenine phosphoribosyltransferase (Synpcc7942_2454, EC 2.4.2.7), which recycles adenine into AMP and is nonessential in vivo but essential in silico. Without this reaction, adenine produced during biosynthesis of polyamines would accumulate and violate the Steady-State Assumption in the model. However, the in vivo data suggest that adenine accumulation is not lethal to the organism, likely because of the small predicted flux through this pathway. Conversely, the catabolism of uracil into UMP by the enzyme uracil phosphoribosyltransferase (Synpcc7942_1715, EC 2.4.2.9) is essential in vivo but not in silico. The in vivo source of uracil and the metabolic requirement to salvage it to UMP represent a Knowledge Gap in our understanding of *S. elongatus*. Therefore, overlaying in vivo essentiality information over the model's predictions reveals multiple classes of unknowns in nucleotide salvage alone.

Photorespiration. The key carbon-fixing enzyme of the Calvin cycle in photosynthetic metabolism is ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO; Synpcc7942_1426 and Synpcc7942_1427, EC 4.1.1.39). This enzyme can fix not only CO₂ but O₂ as well, generating 2-phosphoglycolate. Buildup of this molecule is toxic and needs to be recycled through the process of photorespiration (58). To represent photorespiration in silico, a basal level of oxygenase activity needed to be added to the model's RuBisCO reaction. Based on extrapolation from ¹³C flux analysis in PCC 6803 (11) and metabolite concentrations in low- vs. high-carbon experiments in *S. elongatus* (59), we set the model RuBisCO oxygenase flux at 1% of total RuBisCO activity. Interestingly, the first step of the photorespiratory pathway is nonessential in vivo, although the model predicts it to be essential. In this step, phosphoglycolate phosphatase catalyzes the conversion of 2-phosphoglycolate to glycolate (Fig. S6). This enzyme, present upstream of the branching of photorespiration, has no high-confidence isozymes in *S. elongatus*. The nonessentiality of phosphoglycolate phosphatase suggests either an unknown enzyme for this function or dispensability of the pathway.

It is improbable that the photorespiratory pathway is non-essential. In PCC 6803, three pathways of photorespiration exist and have been included in previous GEMs of this species (50): the plant-like C₂ cycle, full decarboxylation, and the glycerate pathway—which when disrupted in concert, cause a high-CO₂ dependency (60). *S. elongatus* may be missing the last of these pathways (Fig. S6). The glycerate pathway begins with glyoxylate carboxylase (GCL), an enzyme that combines two molecules of glyoxylate and ends with the central carbon metabolite 3-phosphoglycerate after the investment of ATP, NAD(P)H, and the release of CO₂. The gene found in PCC 6803 (*sll1981*) that is most similar to GCL in *E. coli* does not have a homolog in *S. elongatus*. Therefore, it is possible that only the plant-like C₂ cycle and full decarboxylation via formate occur in *S. elongatus*.

The potential to generate glycine through photorespiration raised the possibility that the process could compensate for de novo glycine/serine biosynthesis. When RuBisCO oxygenase activity was set at 1%, the model predicted that sufficient glycine

would be created to support growth, similar to predictions in previous cyanobacterial GEMs (50, 61). Therefore, even when the de novo synthesis pathway of glycine from 3-phosphoglycerate via serine was broken in the model, it still predicted that cells grow at 72% of their normal rate. This finding runs counter to the in vivo data, which show that the entire biosynthesis pathway of serine from 3-phosphoglycerate is essential. The experimental data suggest low flux to amino acid biosynthesis through photorespiration, which could be explained by lower than expected photorespiration activity. Along these lines, in silico essential gene results became consistent with the in vivo data only when RuBisCO's oxygenase activity was lowered to 0.15% of its carbon-fixing activity. Another possibility is decreased flux specifically toward the amino acid biosynthesis pathway of photorespiration. Glycine hydroxymethyltransferase (*Synpcc7942_0282*, EC 2.1.2.1) in PCC 6803 is a choke point for the conversion of glycine from photorespiration into serine (62); it is possible that the same limitation exists in *S. elongatus*. There is also evidence for essentiality of the de novo serine biosynthetic pathway beyond simple metabolic requirements in PCC 6803 (25). Therefore, because of lower than expected photorespiration, limited flux toward glycine synthesis, or undiscovered requirements of de novo serine synthesis, photorespiration is not able to replace de novo amino acid biosynthesis from 3-phosphoglycerate.

A truncated TCA cycle. The completeness of the cyanobacterial TCA cycle has been an oft-debated subject (63). Since the discovery that 2-oxoglutarate dehydrogenase is missing in cyanobacteria, it was accepted for many years that the TCA cycle is incomplete (64). However, a complete TCA cycle is responsible for the majority of energy intermediates created by oxygenic metabolism and nearly ubiquitous throughout nature (65). For this reason, extensive effort has been applied to uncover routes that complete cyanobacterial TCA cycles. A number of bypasses of the missing 2-oxoglutarate dehydrogenase have been discovered, such as the 2-oxoglutarate decarboxylase pathway (66), the GABA shunt (67), and the glyoxylate shunt (68). These bypasses revealed that cyanobacteria harbor complete, albeit noncanonical TCA cycles (63). More recently, however, the necessity of the newly circularized TCA cycles of cyanobacteria has been called into question by experimental (16) and modeling studies (50). Therefore, the structure and biological relevance of a TCA cycle remains an open question in cyanobacteria.

For these reasons, we were particularly interested in discrepancies between the draft model simulations and the in vivo essentiality for two enzymes of the TCA cycle: fumarase (*Synpcc7942_1007*, EC 4.2.1.2) and malic enzyme (*Synpcc7942_1297*, EC 1.1.1.40). These enzymes are required by the model's steady-state assumption for the recycling of fumarate, a by-product of both purine and arginine synthesis. However, the overlay of the in vivo data on the model not only suggests that this recycling is not an essential function but more broadly, led us to evaluate the importance of a complete TCA cycle in *S. elongatus*.

We began by examining the bypasses that complete TCA cycles in other model cyanobacteria (63) for their potential presence in *S. elongatus*. Our reconstruction, however, revealed none of the known cyanobacterial bypasses (Fig. 5A). Additionally, we were unable to find evidence of the core TCA-cycle enzymes malate dehydrogenase (EC 1.1.1.37), malate:quinone oxidoreductase (EC 1.1.5.4), and succinyl-CoA synthetase (EC 6.2.1.5). Furthermore, the succinate dehydrogenase genes (*synpcc7942_0314*, *synpcc7942_0641*, and *synpcc7942_1533*, EC 1.3.5.1) are nonessential in vivo in addition to genes for fumarase and malic enzyme. Together, these data provide evidence that the metabolically important portion of the TCA cycle in *S. elongatus* is highly abridged. We call this oxidative, noncyclic portion of the TCA cycle that is essential in *S. elongatus* the TCA pathway (Fig. 5B). To explain the feasibility of this TCA pathway, we examined whether it would be sufficient to accomplish

the central functions of the TCA cycle: precursor metabolite production, by-product recycling, and energy generation.

The TCA pathway preserves the enzymes necessary for the synthesis of oxaloacetate and 2-oxoglutarate, which are precursors for many required biomass components; additionally, 2-oxoglutarate serves as the gateway to nitrogen assimilation. Therefore, functionality in producing precursor metabolites and nitrogen assimilation can be provided by the TCA pathway.

The TCA pathway does not include functionality for the recycling of fumarate. Fumarate is created as a by-product of nucleotide and arginine synthesis, the salvage of which is posited by previous models to be essential (69). However, loss of function mutants for fumarase and malic enzyme show that this recycling function is not required for viability on solid media or in liquid culture (Fig. 5C and Fig. S7 A and B). The dispensability of fumarate salvage could be explained by the ability of *S. elongatus* to excrete fumarate into the media (70), and when this possibility was added into the model, the in silico predictions for fumarase and malic enzyme become nonessential in agreement with the in vivo data. In addition, *iJB785* shows a minor cost of excreting useable carbon backbones (Fig. S7C). This cost is commensurate with the in vivo data that show a significant decrease in colony size in the fumarase and malic enzyme mutants (Fig. 5C). Therefore, fumarate recycling is a dispensable function of the TCA cycle, despite a slight fitness cost.

The final core function of the TCA cycle is energy production. During photosynthetic metabolism in *S. elongatus*, however, full oxidation of pyruvate by the TCA cycle would amount to "metabolic suicide," in which the cell is fixing and degrading the same carbon compounds concurrently (71). The wastefulness of a complete TCA cycle for energy generation in *S. elongatus* is supported by viability of mutants defective for succinate dehydrogenase subunit B (*Synpcc7942_1533*, EC 1.3.5.1) (Fig. 5C), which in addition to its importance for cycle flux, is an electron donor to the electron transport chain in PCC 6803 (53). At nighttime, however, when the cell switches from photosynthesis to glycogen as its energy source (72), we might expect that a cyclic TCA process would become essential, because it would enable further energy generation from glycogen. In fact, expression of TCA cycle enzymes has been shown to occur in the dark period during light–dark cultivation (73), and modeling has shown cyclic flux through the cycle in PCC6803 under these conditions (50). Therefore, we repeated the viability assay under day–night conditions but found that fumarase, malic enzyme, and succinate dehydrogenase remain nonessential (Fig. 5D). Furthermore, *iJB785* simulations of dark metabolism indicated that full oxidation of glycogen through the oxidative pentose phosphate (OPP) pathway could generate equivalent ATP compared with a complete TCA cycle in *S. elongatus* (24.4 mol ATP/mol glucose in OPP vs. 24.7 mol ATP/mol glucose via TCA bypass). This prediction is supported by previous experimental evidence that the OPP pathway is important for diurnal survival in *S. elongatus* (74–76). Taken together, the model, the essential gene dataset, and our loss of function mutants support the hypothesis that an abridged TCA pathway, focused on generation of precursor metabolites, and not the traditional TCA cycle is the physiologically relevant TCA process for *S. elongatus*. This finding diverges from the current paradigm of complete TCA cycles in cyanobacteria (63).

TCA cycles focused on biosynthesis instead of energy generation have precedents. Green sulfur bacteria run their TCA cycle in reverse to fix CO₂ in a process called the reductive TCA cycle (77). It also is common for obligate autotrophs to lack the enzyme 2-oxoglutarate dehydrogenase (71). The hypothesized TCA mechanism in the absence of 2-oxoglutarate is a bifurcated process, in which a reductive branch leads to succinyl-CoA and an oxidative branch leads to 2-oxoglutarate (71). According to *iJB785*, however, *S. elongatus* does not require succinyl-CoA or any other metabolites of the reductive branch of this TCA process. Thus, it is likely that just the

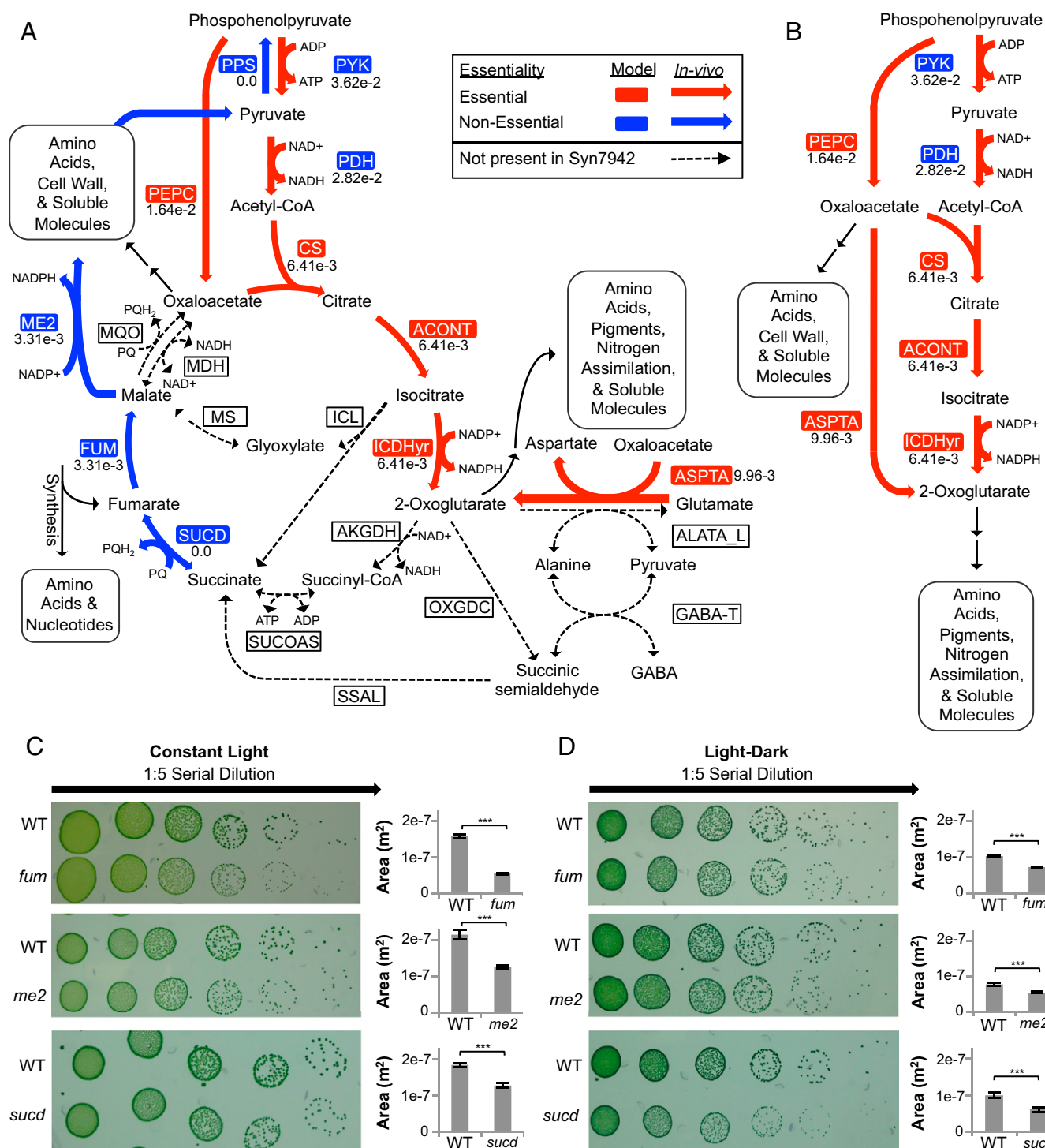


Fig. 5. The TCA pathway. (A) The canonical TCA cycle is shown, with bypasses and alternative reactions present in other cyanobacterial species indicated by dotted lines. (B) The TCA pathway, the proposed biologically relevant TCA process. Insertion loss of function mutants in *fum* (8542-O6; *synpcc7942_1007*), *me2* (8529-J6; *synpcc7942_1297*), and *sucd* (851-JJ4; *synpcc7942_1533*) were made (*SI Materials and Methods, Table S2*), and growth was compared with the WT in both (C) continuous light and (D) cycles of alternating light–dark (12–12 h). Colony area was measured using ImageJ (80). Reaction and metabolite abbreviations are given in BiGG format (bigg.ucsd.edu) and found in *Dataset S2*. ***Significance level of 0.001 (t test).

oxidative wing of the TCA cycle, represented as the TCA pathway, is important largely as a biosynthetic pathway in *S. elongatus*.

The truncated TCA pathway of *S. elongatus* probably generalizes to other members of the phylum. Even if we artificially model the complete TCA cycle in *S. elongatus* by adding malate dehydrogenase and the 2-oxoglutarate dehydrogenase bypass present

in *Synechococcus* sp. PCC 7002 (66), the model still predicts no cyclic flux through the completed TCA cycle. In PCC 6803, which contains a complete TCA cycle, both flux balance analysis (50) and ¹³C MFA (78) show negligible flux from 2-oxoglutarate to the rest of the TCA cycle. Furthermore, when the complete TCA cycle of PCC 6803 is blocked, only minor decreases in growth rate are

observed (67). Finally, a bifurcated TCA cycle with a reductive branch for succinyl-CoA synthesis is likely to be nonessential in many cyanobacteria because of the presence of a heme biosynthesis pathway that begins with 2-oxoglutarate instead of succinyl-CoA (79). Based on these data, the abridged TCA pathway as opposed to a complete or bifurcated TCA process is likely relevant in other cyanobacteria, even those with genes making a complete TCA cycle.

Conclusions

The *iJB785* GEM of metabolism in *S. elongatus* presented here is a comprehensive representation of obligate phototrophic metabolism. Our mechanistic modeling of photon absorption and self-shading addresses the persistent challenge of accurately modeling light as a metabolite. This approach can be applied to any phototrophic GEM, enabling modeling of core aspects of light-driven metabolism. The predictive nature of the method also enables tailored light regimes for bioprocess optimization of photosynthetic platforms.

In addition to *iJB785*'s value for metabolic engineering and its technical improvement to phototrophic modeling, it serves as a platform for biological discovery. In synthesizing much of the physiological understanding available for *S. elongatus*, *iJB785* reveals the holes in this knowledge. Some of these holes include missing elements of the nucleotide salvage system, the reason that phosphoketolase is unable to bypass lower glycolysis, and the apparent noncanonical activity of the transhydrogenase. Furthermore, the incorporation of essential gene data both improved the model's accuracy and highlighted disagreements, which could not

be explained by published data for *S. elongatus*. Many of these inconsistencies represent new biology for *S. elongatus*, such as the importance of a linear, noncyclic TCA pathway. Finally, as a representation of our current best understanding of *S. elongatus*, *iJB785* is an ideal surface on which to overlay whole-genome datasets. The future addition of omic datasets to the model will both greatly improve in silico representation of *S. elongatus* metabolism and identify additional biological unknowns.

Materials and Methods

Methods used to generate the genome-scale reconstruction, derive constraints, and generate in silico results are presented in *SI Materials and Methods*. Briefly, the metabolic reconstruction was assembled using an established protocol (20), and biomass-normalized photon absorption rate for a given wavelength range was calculated from the combination of irradiance, optical absorption cross-section, and the chlorophyll *a* component of the biomass equation. Growth rates and reaction fluxes were simulated by maximizing the biomass objective function. All additional experimental protocols can be found in *SI Materials and Methods*.

ACKNOWLEDGMENTS. A draft reconstruction of *S. elongatus* metabolism was provided by Henning Knoop and Ralf Steuer. This research was supported by NIH Cell and Molecular Genetics Training Grant T32GM00724 (to B.E.R.), University of California, San Diego Frontiers of Innovation Scholars Program 2-P3071 (to D.G.W.), National Science Foundation Grant MCB1244108 (to S.S.G.), and US Department of Energy, Office of Science, Office of Biological and Environmental Research Award DE-SC0008593 (to B.O.P.).

- Angermayr SA, Gorchs Rovira A, Hellingwerf KJ (2015) Metabolic engineering of cyanobacteria for the synthesis of commodity products. *Trends Biotechnol* 33(6):352–361.
- Atsumi S, Higashide W, Liao JC (2009) Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. *Nat Biotechnol* 27(12):1177–1180.
- Oliver JW, Machado IM, Yoneda H, Atsumi S (2013) Cyanobacterial conversion of carbon dioxide to 2,3-butanediol. *Proc Natl Acad Sci USA* 110(4):1249–1254.
- Clerico EM, Ditty JL, Golden SS (2007) Specialized techniques for site-directed mutagenesis in cyanobacteria. *Methods Mol Biol* 362:155–171.
- Gudmundsson S, Nogales J (2015) Cyanobacteria as photosynthetic biocatalysts: A systems biology perspective. *Mol Biosyst* 11(1):60–70.
- Savakis P, Hellingwerf KJ (2015) Engineering cyanobacteria for direct biofuel production from CO₂. *Curr Opin Biotechnol* 33:8–14.
- Bordbar A, Monk JM, King ZA, Palsson BO (2014) Constraint-based models predict metabolic and associated cellular functions. *Nat Rev Genet* 15(2):107–120.
- Borodina I, et al. (2015) Establishing a synthetic pathway for high-level production of 3-hydroxypropionic acid in *Saccharomyces cerevisiae* via β -alanine. *Metab Eng* 27:57–64.
- Yim H, et al. (2011) Metabolic engineering of *Escherichia coli* for direct production of 1,4-butanediol. *Nat Chem Biol* 7(7):445–452.
- Shih PM, et al. (2013) Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. *Proc Natl Acad Sci USA* 110(3):1053–1058.
- Young JD, Shastri AA, Stephanopoulos G, Morgan JA (2011) Mapping photoautotrophic metabolism with isotopically nonstationary (¹³C) flux analysis. *Metab Eng* 13(6):656–665.
- Kim TY, Sohn SB, Kim YB, Kim WJ, Lee SY (2012) Recent advances in reconstruction and applications of genome-scale metabolic models. *Curr Opin Biotechnol* 23(4):617–623.
- Lewis NE, Nagarajan H, Palsson BO (2012) Constraining the metabolic genotype-phenotype relationship using a phylogeny of in silico methods. *Nat Rev Microbiol* 10(4):291–305.
- Monk J, Nogales J, Palsson BO (2014) Optimizing genome-scale network reconstructions. *Nat Biotechnol* 32(5):447–452.
- Chowdhury R, Chowdhury A, Maranas CD (2015) Using gene essentiality and synthetic lethality information to correct yeast and CHO cell genome-scale models. *Metabolites* 5(4):536–570.
- Rubin BE, et al. (2015) The essential gene set of a photosynthetic organism. *Proc Natl Acad Sci USA* 112(48):E6634–E6643.
- Reed JL, et al. (2006) Systems approach to refining genome annotation. *Proc Natl Acad Sci USA* 103(46):17480–17484.
- Baroukh C, Muñoz-Tamayo R, Steyer JP, Bernard O (2015) A state of the art of metabolic networks of unicellular microalgae and cyanobacteria for biofuel production. *Metab Eng* 30:49–60.
- Shastri AA, Morgan JA (2005) Flux balance analysis of photoautotrophic metabolism. *Biotechnol Prog* 21(6):1617–1626.
- Thiele I, Palsson BO (2010) A protocol for generating a high-quality genome-scale metabolic reconstruction. *Nat Protoc* 5(1):93–121.
- Reed JL, Vo TD, Schilling CH, Palsson BO (2003) An expanded genome-scale model of *Escherichia coli* K-12 (iJR904 GSM/GPR). *Genome Biol* 4(9):R54.
- Brunk E, et al. (2016) Systems biology of the structural proteome. *BMC Syst Biol* 10:26.
- Jablonsky J, Hagemann M, Schwarz D, Wolkenhauer O (2013) Phosphoglycerate mutases function as reverse regulated isoenzymes in *Synechococcus elongatus* PCC 7942. *PLoS One* 8(3):e58281.
- Chiba Y, et al. (2013) Structural units important for activity of a novel-type phosphoserine phosphatase from *Hydrogenobacter thermophilus* TK-6 revealed by crystal structure analysis. *J Biol Chem* 288(16):11448–11458.
- Klemke F, et al. (2015) Identification of the light-independent phosphoserine pathway as an additional source of serine in the cyanobacterium *Synechocystis* sp. PCC 6803. *Microbiology* 161(Pt 5):1050–1060.
- Vijayan V, Jain IH, O'Shea EK (2011) A high resolution map of a cyanobacterial transcriptome. *Genome Biol* 12(5):R47.
- Berla BM, Saha R, Maranas CD, Pakrasi HB (2015) Cyanobacterial alkanes modulate photosynthetic cyclic electron flow to assist growth under cold stress. *Sci Rep* 5:14894.
- Hendry JI, Prasannan CB, Joshi A, Dasgupta S, Wangikar PP (2016) Metabolic model of *Synechococcus* sp. PCC 7002: Prediction of flux distribution and network modification for enhanced biofuel production. *Bioresour Technol* 213:190–197.
- Krieger-Liszak A (2005) Singlet oxygen production in photosynthesis. *J Exp Bot* 56(411):337–346.
- Patterson CO, Myers J (1973) Photosynthetic production of hydrogen peroxide by *Anacystis nidulans*. *Plant Physiol* 51(1):104–109.
- Chang RL, et al. (2011) Metabolic network reconstruction of *Chlamydomonas* offers insight into light-driven algal metabolism. *Mol Syst Biol* 7:518.
- He L, Wu SG, Wan N, Reding AC, Tang YJ (2015) Simulating cyanobacterial phenotypes by integrating flux balance analysis, kinetics, and a light distribution function. *Microb Cell Fact* 14:206.
- Dubinsky Z, Berman T, Schanz F (1984) Field experiments for in situ measurement of photosynthetic efficiency and quantum yield. *J Plankton Res* 6(2):339–349.
- Liberton M, et al. (2016) Global proteomic analysis reveals an exclusive role of thylakoid membranes in bioenergetics of a model cyanobacterium. *Mol Cell Proteomics* 15(6):2021–2032.
- Liu LN, et al. (2012) Control of electron transport routes through redox-regulated redistribution of respiratory complexes. *Proc Natl Acad Sci USA* 109(28):11431–11436.
- Gao F, et al. (2016) NdhV is a subunit of NADPH dehydrogenase essential for cyclic electron transport in *Synechocystis* sp. strain PCC 6803. *Plant Physiol* 170(2):752–760.
- Battchikova N, et al. (2011) Identification of novel Ssl0352 protein (NdhS), essential for efficient operation of cyclic electron transport around photosystem I, in NADPH: plastoquinone oxidoreductase (NDH-1) complexes of *Synechocystis* sp. PCC 6803. *J Biol Chem* 286(42):36992–37001.
- Allakhverdiev SI, Murata N (2004) Environmental stress inhibits the synthesis de novo of proteins involved in the photodamage-repair cycle of Photosystem II in *Synechocystis* sp. PCC 6803. *Biochim Biophys Acta* 1657(1):23–32.
- Stamatakis K, Tsimilli-Michael M, Papageorgiou GC (2014) On the question of the light-harvesting role of β -carotene in photosystem II and photosystem I core complexes. *Plant Physiol Biochem* 81:121–127.
- King ZA, et al. (2016) BiGG Models: A platform for integrating, standardizing and sharing genome-scale models. *Nucleic Acids Res* 44(D1):D515–D522.
- Feist AM, Palsson BO (2010) The biomass objective function. *Curr Opin Microbiol* 13(3):344–349.

42. Varma A, Palsson BO (1994) Stoichiometric flux balance models quantitatively predict growth and metabolic by-product secretion in wild-type *Escherichia coli* W3110. *Appl Environ Microbiol* 60(10):3724–3731.
43. Yu J, et al. (2015) *Synechococcus elongatus* UTEX 2973, a fast growing cyanobacterial chassis for biosynthesis using light and CO₂. *Sci Rep* 5:8132.
44. Shibata M, et al. (2001) Distinct constitutive and low-CO₂-induced CO₂ uptake systems in cyanobacteria: Genes involved and their phylogenetic relationship with homologous genes in other organisms. *Proc Natl Acad Sci USA* 98(20):11789–11794.
45. Woodger FJ, Badger MR, Price GD (2005) Sensing of inorganic carbon limitation in *Synechococcus* PCC7942 is correlated with the size of the internal inorganic carbon pool and involves oxygen. *Plant Physiol* 139(4):1959–1969.
46. McConnell MD, Koop R, Vasil'ev S, Bruce D (2002) Regulation of the distribution of chlorophyll and phycobilin-absorbed excitation energy in cyanobacteria. A structure-based model for the light state transition. *Plant Physiol* 130(3):1201–1212.
47. Muramatsu M, Hihara Y (2012) Acclimation to high-light conditions in cyanobacteria: From gene expression to physiological responses. *J Plant Res* 125(1):11–39.
48. Triana J, et al. (2014) Generation and evaluation of a genome-scale metabolic network model of *Synechococcus elongatus* PCC7942. *Metabolites* 4(3):680–698.
49. Xiong W, et al. (2015) Phosphoketolase pathway contributes to carbon metabolism in cyanobacteria. *Nat Plants* 2:15187.
50. Knoop H, et al. (2013) Flux balance analysis of cyanobacterial metabolism: The metabolic network of *Synechocystis* sp. PCC 6803. *PLoS Comput Biol* 9(6):e1003081.
51. Guerreiro AC, et al. (2014) Daily rhythms in the cyanobacterium *synechococcus elongatus* probed by high-resolution mass spectrometry-based proteomics reveals a small defined set of cyclic proteins. *Mol Cell Proteomics* 13(8):2042–2055.
52. Angermayr SA, Paszota M, Hellingwerf KJ (2012) Engineering a cyanobacterial cell factory for production of lactic acid. *Appl Environ Microbiol* 78(19):7098–7106.
53. Cooley JW, Vermaas WF (2001) Succinate dehydrogenase and other respiratory pathways in thylakoid membranes of *Synechocystis* sp. strain PCC 6803: Capacity comparisons and physiological function. *J Bacteriol* 183(14):4251–4258.
54. Reader JS, Metzgar D, Schimmel P, de Crécy-Lagard V (2004) Identification of four genes necessary for biosynthesis of the modified nucleoside queuosine. *J Biol Chem* 279(8):6280–6285.
55. Aoki M, Sato N, Meguro A, Tsuzuki M (2004) Differing involvement of sulfoquinovosyl diacylglycerol in photosystem II in two species of unicellular cyanobacteria. *Eur J Biochem* 271(4):685–693.
56. Simkovsky R, et al. (2012) Impairment of O-antigen production confers resistance to grazing in a model amoeba-cyanobacterium predator-prey system. *Proc Natl Acad Sci USA* 109(41):16678–16683.
57. Schneider D, Volkmer T, Rögner M (2007) PetG and PetN, but not PetL, are essential subunits of the cytochrome b₆f complex from *Synechocystis* PCC 6803. *Res Microbiol* 158(1):45–50.
58. Husic DW, Tolbert NE (1987) Inhibition of glycolate and D-lactate metabolism in a *Chlamydomonas reinhardtii* mutant deficient in mitochondrial respiration. *Proc Natl Acad Sci USA* 84(6):1555–1559.
59. Schwarz Z, et al. (2011) Metabolic and transcriptomic phenotyping of inorganic carbon acclimation in the Cyanobacterium *Synechococcus elongatus* PCC 7942. *Plant Physiol* 155(4):1640–1655.
60. Eisenhut M, et al. (2008) Metabolome phenotyping of inorganic carbon limitation in cells of the wild type and photorespiratory mutants of the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Plant Physiol* 148(4):2109–2120.
61. Nogales J, Gudmundsson S, Knight EM, Palsson BO, Thiele I (2012) Detailing the optimality of photosynthesis in cyanobacteria through systems biology analysis. *Proc Natl Acad Sci USA* 109(7):2678–2683.
62. Eisenhut M, et al. (2006) The plant-like C₂ glycolate cycle and the bacterial-like glycerate pathway cooperate in phosphoglycolate metabolism in cyanobacteria. *Plant Physiol* 142(1):333–342.
63. Steinhäuser D, Fernie AR, Araújo WL (2012) Unusual cyanobacterial TCA cycles: Not broken just different. *Trends Plant Sci* 17(9):503–509.
64. Pearce J, Leach CK, Carr NG (1969) The incomplete tricarboxylic acid cycle in the blue-green alga *Anabaena variabilis*. *J Gen Microbiol* 55(3):371–378.
65. Fernie AR, Carrari F, Sweetlove LJ (2004) Respiratory metabolism: Glycolysis, the TCA cycle and mitochondrial electron transport. *Curr Opin Plant Biol* 7(3):254–261.
66. Zhang S, Bryant DA (2011) The tricarboxylic acid cycle in cyanobacteria. *Science* 334(6062):1551–1553.
67. Xiong W, Brune D, Vermaas WF (2014) The γ -aminobutyric acid shunt contributes to closing the tricarboxylic acid cycle in *Synechocystis* sp. PCC 6803. *Mol Microbiol* 93(4):786–796.
68. Zhang S, Bryant DA (2015) Biochemical validation of the glyoxylate cycle in the cyanobacterium *Chlorogloeopsis fritschii* strain PCC 9212. *J Biol Chem* 290(22):14019–14030.
69. Beck C, Knoop H, Axmann IM, Steuer R (2012) The diversity of cyanobacterial metabolism: Genome analysis of multiple phototrophic microorganisms. *BMC Genomics* 13:56.
70. Hickman JW, et al. (2013) Glycogen synthesis is a required component of the nitrogen stress response in *Synechococcus elongatus* PCC 7942. *Algal Res* 2(2):98–106.
71. Wood AP, Aurikko JP, Kelly DP (2004) A challenge for 21st century molecular biology and biochemistry: What are the causes of obligate autotrophy and methanotrophy? *FEMS Microbiol Rev* 28(3):335–352.
72. Diamond S, Jun D, Rubin BE, Golden SS (2015) The circadian oscillator in *Synechococcus elongatus* controls metabolite partitioning during diurnal growth. *Proc Natl Acad Sci USA* 112(15):E1916–E1925.
73. Welkie D, et al. (2014) Transcriptomic and proteomic dynamics in the metabolism of a diazotrophic cyanobacterium, *Cyanothece* sp. PCC 7822 during a diurnal light-dark cycle. *BMC Genomics* 15:1185.
74. Doolittle WF, Singer RA (1974) Mutational analysis of dark endogenous metabolism in the blue-green bacterium *Anacystis nidulans*. *J Bacteriol* 119(3):677–683.
75. Broedel SE, Jr, Wolf RE, Jr (1990) Genetic tagging, cloning, and DNA sequence of the *zwf* mutant of *Synechococcus* sp. strain PCC 7942. *J Bacteriol* 177(9):2550–2553.
76. Scanlan DJ, Sundaram S, Newman J, Mann NH, Carr NG (1995) Characterization of a *zwf* mutant of *Synechococcus* sp. strain PCC 7942 gene (gnd) encoding 6-phosphogluconate dehydrogenase. *J Bacteriol* 172(7):4023–4031.
77. Atomi H (2002) Microbial enzymes involved in carbon dioxide fixation. *J Biosci Biotech* 94(6):497–505.
78. You L, Berla B, He L, Pakrasi HB, Tang YJ (2014) 13C-MFA delineates the photo-mixotrophic metabolism of *Synechocystis* sp. PCC 6803 under light- and carbon-sufficient conditions. *Biotechnol J* 9(5):684–692.
79. Obornik M, Green BR (2005) Mosaic origin of the heme biosynthesis pathway in photosynthetic eukaryotes. *Mol Biol Evol* 22(12):2343–2353.
80. Schindelin J, Rueden CT, Hiner MC, Eliceiri KW (2015) The ImageJ ecosystem: An open platform for biomedical image analysis. *Mol Reprod Dev* 82(7-8):518–529.
81. Camacho C, et al. (2009) BLAST+: Architecture and applications. *BMC Bioinformatics* 10:421.
82. UniProt Consortium (2015) UniProt: A hub for protein information. *Nucleic Acids Res* 43(Database issue):D204–D212.
83. Marchler-Bauer A, et al. (2015) CDD: NCBI's conserved domain database. *Nucleic Acids Res* 43(Database issue):D222–D226.
84. Ebrahim A, Lerman JA, Palsson BO, Hyduke DR (2013) COBRApy: COnstraints-Based Reconstruction and Analysis for Python. *BMC Syst Biol* 7:74.
85. Perez F, Granger BE (2007) IPython: A system for interactive scientific computing. *Comput Sci Eng* 9(3):21–29.
86. Bond CS, White MF, Hunter WN (2001) High resolution structure of the phosphohistidine-activated form of *Escherichia coli* cofactor-dependent phosphoglycerate mutase. *J Biol Chem* 276(5):3247–3253.
87. Rigden DJ, Littlejohn JE, Henderson K, Jedrzejak MJ (2003) Structures of phosphate and triphosphate complexes of *Bacillus stearothermophilus* phosphatase PhoE: Structural and functional analysis in the cofactor-dependent phosphoglycerate mutase superfamily. *J Mol Biol* 325(3):411–420.
88. Roy A, Kucukural A, Zhang Y (2010) I-TASSER: A unified platform for automated protein structure and function prediction. *Nat Protoc* 5(4):725–738.
89. Yang J, Roy A, Zhang Y (2013) Protein-ligand binding site recognition using complementary binding-specific substructure comparison and sequence profile alignment. *Bioinformatics* 29(20):2588–2595.
90. Humphrey W, Dalke A, Schulten K (1996) VMD: Visual molecular dynamics. *J Mol Graph* 14(1):27–38.
91. Roberts E, Eargle J, Wright D, Luthy-Schulten Z (2006) MultiSeq: Unifying sequence and structure data for evolutionary analysis. *BMC Bioinformatics* 7:382.
92. Russell RB, Barton GJ (1992) Multiple protein sequence alignment from tertiary structure comparison: Assignment of global and residue confidence levels. *Proteins* 14(2):309–323.
93. Ritchie RJ (2006) Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynth Res* 89(1):27–41.
94. Falkowski PG, Raven JA (2007) *Aquatic Photosynthesis* (Princeton Univ Press, Princeton), 2nd Ed.
95. Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine-phytoplankton. *J Mar Res* 38(4):687–701.
96. Schellenberger J, et al. (2011) Quantitative prediction of cellular metabolism with constraint-based models: The COBRA Toolbox v2.0. *Nat Protoc* 6(9):1290–1307.
97. Lewis NE, et al. (2010) Omic data from evolved *E. coli* are consistent with computed optimal growth from genome-scale models. *Mol Syst Biol* 6:390.
98. Orth JD, et al. (2011) A comprehensive genome-scale reconstruction of *Escherichia coli* metabolism—2011. *Mol Syst Biol* 7:535.
99. Chen Y, Holtman CK, Taton A, Golden SS (2012) Functional analysis of the *Synechococcus elongatus* PCC 7942 genome. *Functional Genomics and Evolution of Photosynthetic Systems*, eds Burnap R, Vermaas W (Springer, Dordrecht, The Netherlands), pp 119–137.
100. Holtman CK, et al. (2005) High-throughput functional analysis of the *Synechococcus elongatus* PCC 7942 genome. *DNA Res* 12(2):103–115.
101. Ashby MK, Houmard J (2006) Cyanobacterial two-component proteins: Structure, diversity, distribution, and evolution. *Microbiol Mol Biol Rev* 70(2):472–509.