Figures and figure supplements

Quantitative insights into the cyanobacterial cell economy

Tomáš Zavřel et al
Figure 1. Experimental setup and evaluation of Synechocystis sp. PCC 6803 (substrain GT-L) phenotype stability. Panel A: Photobioreactor setup. Cultures were cultivated in a flat-panel photobioreactor vessel (400 mL) in a turbidostat regime according to Zavrčel et al. (2009). All other parameters were set as described in Nedbal et al. (2008) and Červený et al., 2009. Panel B: Representative measurement of the OD$_{680}$ signal (black lines) within a turbidostat cultivation under increasing red light intensity (supplemented with low intensity of blue light). Calculation of specific growth rates (blue circles) is detailed in Materials and methods. Calculation of uptake and refilling rates of selected nutrients (including Na, (N, S, Ca, Mg, P and Fe)) during the turbidostat cultivation is detailed in Material and methods. Panel C: Calculation of growth rates from the OD$_{680}$ data from this study according to Platt et al. (1980). Source data are available in Figure 1—source data 1. Panel D: Comparison of specific growth rates using an identical experimental setup throughout four successive years 2013–2017 (source data are available in Figure 1—source data 2). Panel E: Rates of gross photosynthesis and dark respiration, measured as O$_2$ evolution and consumption rates directly within the photobioreactor vessel throughout 5 min of light and dark periods in 2016–2017 (this study) and in 2015–2017 (Zavrčel et al., 2017). The dashed line represents a P-I curve fit of data from this study according to Platt et al. (1980). Source data are available in Figure 1—source data 3. Figure 1C: n = 3–11, Figure 1D: n = 6–11, Figure 1E: n = 4–6. Error bars (Figure 1C–1E) represent standard deviations.

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Figure 2. Variations in morphology and composition of Synechocystis cells with changing growth rate. Under increasing light intensity and changing growth rate, the following parameters were estimated: cellular volume (A) and dry weight (B), gross photosynthesis (C, D) and dark respiration (E, F), and content of glycogen (G, H), proteins, DNA (I, J), phycobiliproteins (K, L), chlorophyll a and carotenoids (M, N). The data are plotted relative to cellular dry weight (C, E, G, I, K, M) as well as per cell (D, F, H, J, L, N). DNA content was normalized to its initial value after standardization per dry weight and per cell, the measurement was only semi-quantitative. All values represent averages from 3 – 11 independent biological replicates, error bars represent standard deviations. If error bars are not visible (panel A), the standard deviation was too small for visualization. Within each figure, data points are displayed in three different color shades to reflect (from bright to dark) light-limited, light-saturated and light-inhibited growth. Data plotted as a function of light intensity are available in Figure 2—source data 1. Comparison with data available in the literature is summarized in Figure 2—figure supplement 1. DOI: https://doi.org/10.7554/eLife.42508.007
Figure 2—figure supplement 1. Allocation of key cellular resources as a function of light intensity.

DOI: https://doi.org/10.7554/eLife.42508.008
Figure 3. Synechocystis proteome allocation as a function of growth rate. Panel A: The workflow. Samples were harvested and analyzed by mass spectrometry (the proteomics dataset is available in Figure 3—source data 1). A Kruskal-Wallis test was used to distinguish between growth-rate groups.
Figure 3 continued

dependent and growth-independent proteins. 779 growth-dependent and 577 growth-independent proteins were identified. Panel B: Clustering analysis. Based on k-means clustering analysis (Figure 3—figure supplement 1), the 779 growth-dependent proteins were separated into seven clusters. Gray dashed lines represent protein abundances as medians of 5 biological replicates, normalized by the respective means. Blue dashed lines represent centroids of the respective clusters. Panel C: Proteins were annotated using the GO classes, the matrix represents the annotation mapped to GO slim categories. Proteins can be associated to several GO slim categories. The highest ranking annotation per cluster is highlighted in dark blue.

DOI: https://doi.org/10.7554/eLife.42508.011
Figure 3—figure supplement 1. Elbow method for the identification of an appropriate number of clusters (grey dashed line at seven clusters).

DOI: https://doi.org/10.7554/eLife.42508.012
Figure 4. Proteomaps of proteome reallocation in *Synechocystis* under light-limited (27.5 μmol(photons) m⁻² s⁻¹), light-saturated (440 μmol(photons) m⁻² s⁻¹) and photoinhibited growth (1100 μmol(photons) m⁻² s⁻¹).

DOI: https://doi.org/10.7554/eLife.42508.018
Proteomaps level 2: cellular processes

Proteomaps level 3: cellular processes

Proteomaps level 4: individual proteins

Figure 4—figure supplement 1. Proteomaps of levels 2, 3 and 4.
DOI: https://doi.org/10.7554/eLife.42508.019
Figure 5. A model of phototrophic growth and reproduction of experimental growth curves. Panel A: A coarse-grained model of phototrophic growth, adopted from Faizi et al. (2018). The model describes optimal proteome allocation under conditions of (i) light-limited, (ii) light-saturated and (iii) light-inhibited growth. Coarse-grained cellular processes include passive ($v_d$ and $v_m$) and active import ($v_t$) of external inorganic carbon $c_i$, conversion of inorganic carbon $c_i$ into amino acids $aa$ ($v_m$), light harvesting and provision of cellular energy by photosynthesis ($v_1$ and $v_2$), as well as maintenance and photodamage ($m_v$ and $v_i$). Amino acids are translated into coarse-grained protein fractions for transport ($T$), metabolism ($M$), ribosomes ($R$), photosynthetic electron transport ($P$), as well as a growth-independent proteome fraction $Q$. Translation is limited by the amount of available ribosomes $R$. Panel B: The model reproduces the measured growth curve (Figure 1C–D) as a function of light intensity. Shown are the specific growth rate $\mu$, as well as the main proteome fractions predicted by the model: ribosome ($R$) fraction, photosynthetic electron transport ($P$) fraction, and metabolism ($M$) fraction, as a function of light intensity.

DOI: https://doi.org/10.7554/eLife.42508.020
Figure 6. Changes in protein abundance as a function of specific growth rate compared to the predictions obtained from a computational model of proteome allocation. Panel A: Schematic representation of ribosome, photosynthetic units and metabolic enzyme classes considered in the proteome allocation. Panel B: Relative proteome quantification. Panel C: Relative quantification of selected proteins by immunoblotting.
Figure 6 continued

allocation model. Panel B: Relative proteomics data (LFQ, label-free quantification intensities, left axes, mean fold change ± SD) of protein classes in comparison with the model predictions (grey lines, right axes). Panel C: Relative protein abundances obtained by immunoblotting analysis for selected proteins (left axes, median fold change ± SD) in comparison with coarse-grained model predictions (grey lines, right axes). Experimental values represent averages from 5 independent experiments, the error bars represent standard deviations. Panels B-C: The experimental data points are displayed in three different color shading to reflect (from bright to dark) light-limited, light-saturated and light-inhibited growth. The full dataset of the immunoblotting analysis is provided in Figure 6—source data 1 and Figure 6—figure supplement 1. The list of proteins considered for ribosome, photosynthetic unit and metabolic enzyme classes is listed in Figure 6—source data 2. The influence of constant ribosomal, photosynthetic unit and metabolic enzyme classes on cellular growth rate is simulated in Figure 6—figure supplement 2.

DOI: https://doi.org/10.7554/eLife.42508.021
Table 1. List of antibodies used in this study.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Agrisera catalogue number</th>
<th>Dilution</th>
<th>Protein apparent MW</th>
</tr>
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<tbody>
<tr>
<td>Rabbit Anti-RbcL (Rubisco large subunit, form I and form II)</td>
<td>AS03 037</td>
<td>1:5000</td>
<td>52.5 kDa</td>
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<tr>
<td>Rabbit Anti-PscC (Psc-C core subunit of photosystem I)</td>
<td>AS10 939</td>
<td>1:1000</td>
<td>9 kDa</td>
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<tr>
<td>Rabbit Anti-PsbA (D1 protein of PSII, C-terminal)</td>
<td>AS05 084</td>
<td>1:10000</td>
<td>28-30 kDa</td>
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<tr>
<td>Rabbit Anti-S1 (30S ribosomal protein S1)</td>
<td>AS08 309</td>
<td>1:2000</td>
<td>35 kDa</td>
</tr>
<tr>
<td>Rabbit Anti-L1 (50S ribosomal protein L1)</td>
<td>AS11 1738</td>
<td>1:1000</td>
<td>25 kDa</td>
</tr>
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Table 2. List of protein standards used in this study.

<table>
<thead>
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<th>Standard</th>
<th>Agrisera catalogue number</th>
<th>Protein apparent MW</th>
<th>Concentrations</th>
</tr>
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<tbody>
<tr>
<td>Purified spinach RbcL</td>
<td>AS01 017S</td>
<td>52.7 kDa</td>
<td>0.375, 0.75, 1.5 pmol</td>
</tr>
<tr>
<td>Recombinant PscC from Synechocystis PCC 6803</td>
<td>AS04 042S</td>
<td>11.5 kDa</td>
<td>0.075, 0.3, 0.6 pmol</td>
</tr>
<tr>
<td>Recombinant PsbA from Synechocystis PCC 6803</td>
<td>AS01 016S</td>
<td>41.5 kDa</td>
<td>0.125, 0.5, 1 pmol</td>
</tr>
</tbody>
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Figure 6—figure supplement 1. Immunoblots and a list of antibodies used for the immunoblotting analysis.

DOI: https://doi.org/10.7554/eLife.42508.022
Figure 6—figure supplement 2. Model simulations for investigating the influence of constant enzyme fractions on the cellular growth rate.

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