Final Report

Consequences of Modification of Photosystem Stoichiometry and Amount in Cyanobacteria

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Willem Vermaas, PI; School of Life Sciences, Arizona State University

This final report focuses on results of the last year of the grant. Earlier results were summarized in previous reports and in a Results of Prior Support section in proposals. The main research question that has been focused on in the past year is what happens to photosynthetic function if the photosystem I (PS I) content is reduced in an organism such as the cyanobacterium *Synechocystis* sp. PCC 6803 that has a high PS I/PS II ratio (approximately 3/1). A second question addressed in this report involves Flv3, which has been interpreted to be a reducing-equivalents-wasting electron release valve at the acceptor side of PS I, transferring electrons to molecular O_2.

As indicated in the abstract of the grant, our working hypothesis was that most of PS I functions in cyclic electron transport, and that reduction in PS I would result primarily in a shortage of ATP rather than reducing power. We tested this hypothesis by reducing the amount of PS I by changing out the promoter region of the *psaAB* operon with a combinatorially modified *psbA2* promoter region in the cyanobacterium *Synechocystis* sp. PCC 6803 and generating about 70 mutants with different PS I content and thereby different PS I/PS II ratios, with some of the mutants having a PS II/PS I ratio closer to that in plants. We analyzed eight of these mutants, representing PS I contents between 8 and 70% of what is present in the wild type (Figure 1). The one with the lowest amount of PS I (8%; strain D1) was unable to grow photoautotrophically, but all others grew photoautotrophically.

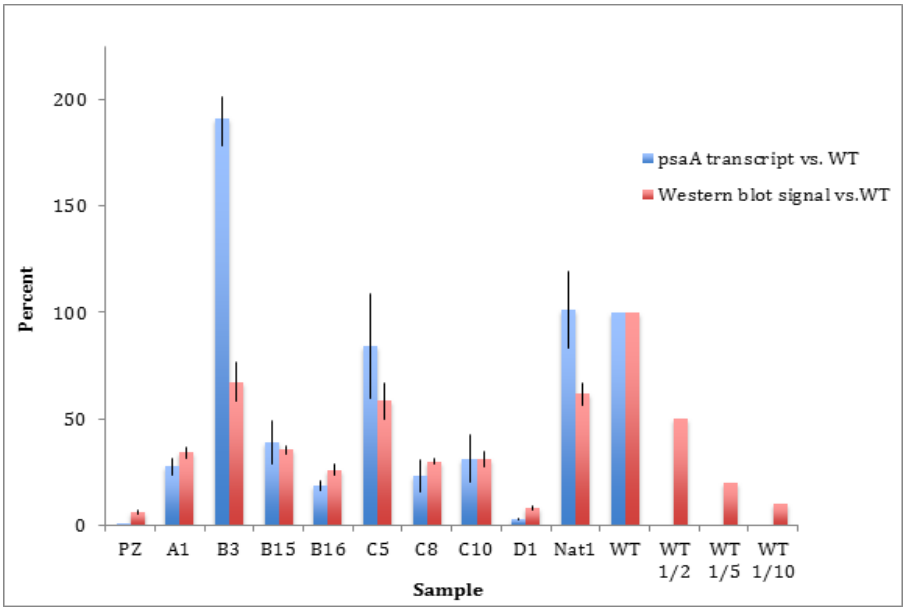

Figure 1. Average transcript levels of *psaA* (blue) and average western blot signals (red) present in each strain relative to wild type (WT). Western blot signals were quantified using ImageJ, and a linear relationship was obtained between the western blot signal and the amount of WT sample loaded (right part of the histogram). Error bars refer to standard error of the mean from three independent experiments.

Interestingly, strains with reduced PS I grew more slowly at low light intensity, but caught up at higher light intensity (100 μmol photons m^{-2} s^{-1}). The reason for this phenomenon is that in strains with less PS I photosynthesis saturates at higher light intensity. As the light intensity needed for saturation is difficult to measure objectively, the light intensity needed for 50% of the light-saturated oxygen evolution rate is plotted in Figure 2. The oxygen evolution data at various light intensities in various

strains (each color plotted is a mutant with a different PS I level; WT is the wild type) are plotted in Figure 3. Indeed, the wild type already is close to saturation at the first light intensity data point, whereas the mutants are much further away from saturation. Note that on a per-cell basis some of the mutants with reduced PS I levels at high light intensity have a higher photosynthesis rate than the wild type, indicating that at high light intensity such mutants will have significant growth benefits compared to the wild-type system.

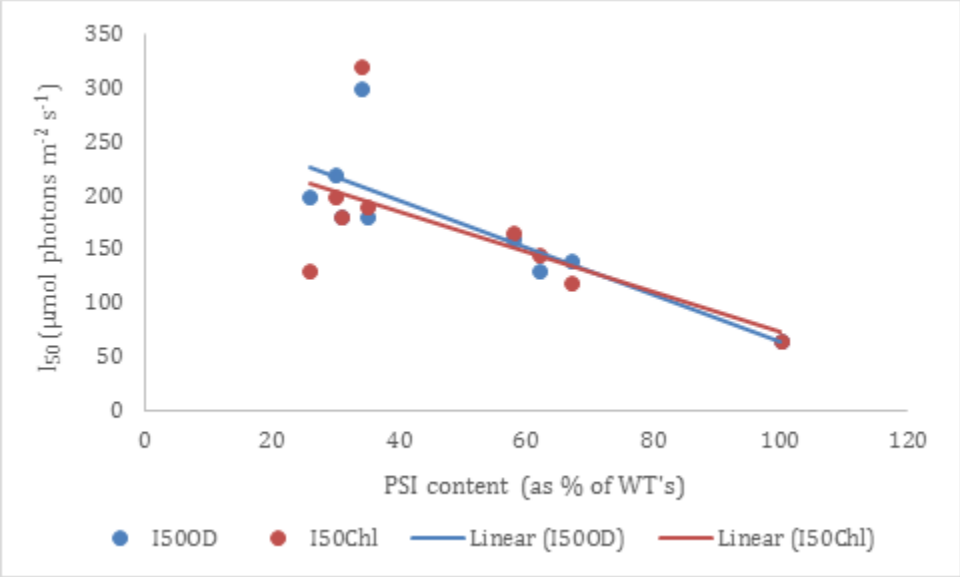

Figure 2. Light intensity required for half-saturation of oxygen evolution (10 mM $NaHCO_3$ as electron acceptor) as a function of the PS I content of specific strains. The error in measurements with strains with low PS I content is relatively high because of the decreased oxygen evolution rates in these strains.

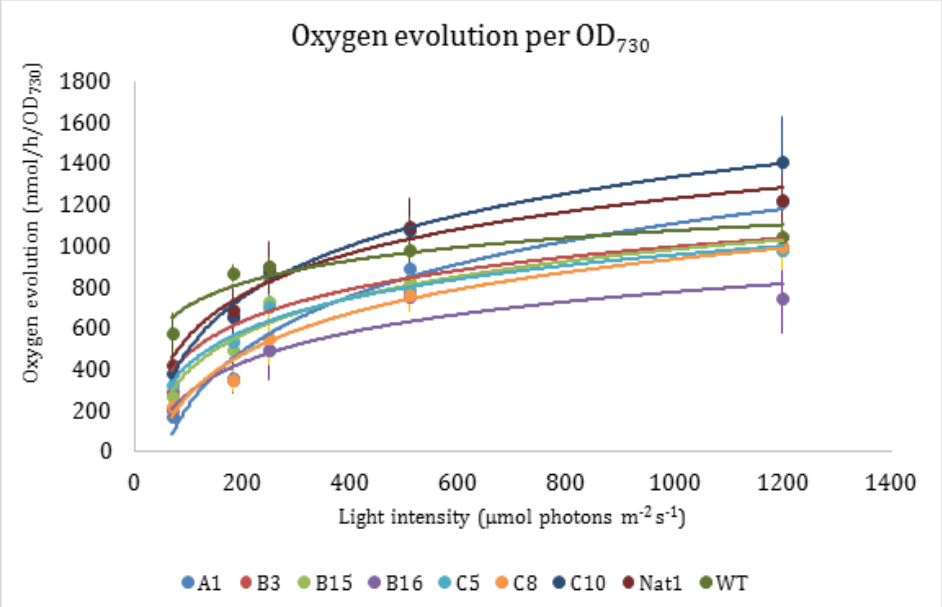

Figure 3. Bicarbonate-mediated oxygen evolution on a OD_{730} (per-cell) basis in wild type (WT) and various photoautotrophic mutants with reduced PS I levels. The mutants shown in this graph had at least 25% of the native amount of PS I as strains with lower PS I levels were not photoautotrophic. Note the early saturation of WT vs. the strains with less PS I.

Therefore, PS I levels in the cell can be reduced without much ill effect if the light intensity is high. This is consistent with the idea that excess PS I in cyanobacteria has a role in cyclic electron flow around PS I, producing extra ATP. To test this further, we monitored the rates of $P700^+$ reduction under conditions where linear electron flow was blocked, so that all electrons re-reducing $P700^+$ need to come from either cyclic electron flow around PS I or respiration. Thus, reduction of $P700^+$, after P700 oxidation by a

light flash, was monitored in the presence of DCMU, a PS II inhibitor. For this experiment, $P700^+$ reduction was measured after a series of 10 flashes delivered at 2 Hz in order to partially oxidize the PQ pool. The expectation is that under these conditions the PQ pool will be more oxidized if there is less electron influx into the pool from cyclic electron flow, and that the PQ pool will be less depleted of electrons when there is less PS I per cell. Figure 4 tracks the changes in P700 redox state with this light treatment. An increase in voltage represents P700 oxidation.

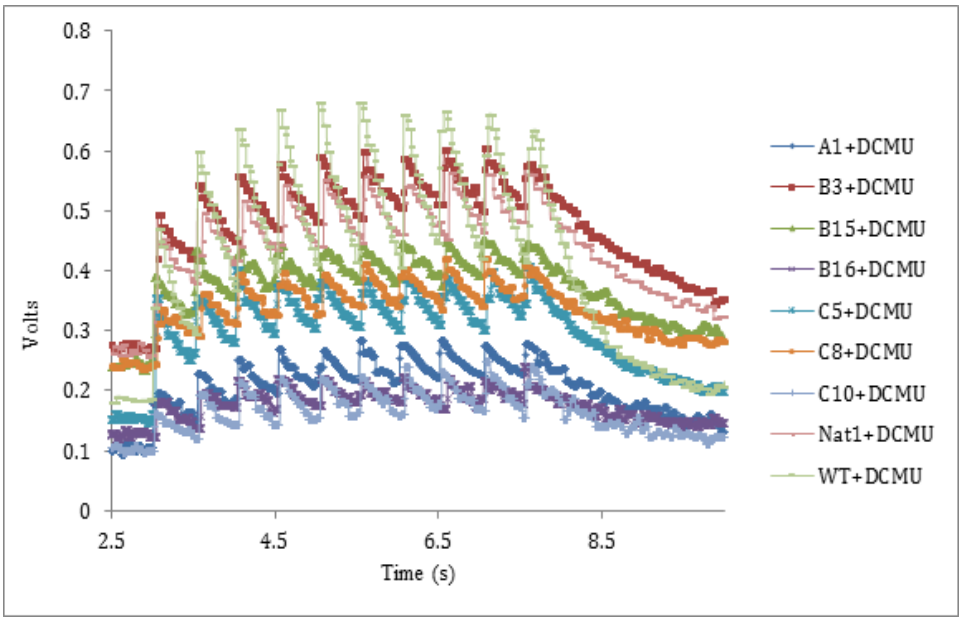

Figure 4. P700 oxidation and reduction curves measured using a Dual-PAM-100 fluorometer with 20 ms pulses of actinic light approximately every 500 ms in the presence of DCMU. Half-times of $P700^+$ reduction were determined following the tenth actinic light pulse. P700 was fully reduced 25 s after the last actinic light pulse.

As indicated in Figure 4, an increasing amount of oxidized P700 accumulates in subsequent 20-ms flashes, until about five flashes when a steady state $P700^+$ concentration is formed during a flash. $P700^+$ becomes (partially) re-reduced in the dark period after each flash, and the $P700^+$ amplitude and the half-time of its re-reduction was measured. As expected, the $P700^+$ amplitude corresponded with PSI abundance. Moreover, strains with relatively high levels of PS I showed $P700^+$ re-reduction kinetics after the last flash that were about two-fold slower compared to wild type, suggesting that electron supply to the PQ pool (for example, by cyclic electron flow) has slowed down in these strains. However, interestingly, at least some of the strains with lower levels of PSI (B16 and C10) showed $P700^+$ re-reduction kinetics that were similar to those of wild type even though their $P700^+$ amplitude was much reduced. As in such strains the amount of electron flux per cell to $P700^+$ has been reduced because of less PS I, other sources of electron donation to the PQ pool (such as respiration) may have become relatively more significant.

Figure 5 shows a model explaining $P700^+$ re-reduction kinetics based on PSI abundance. With the most PS I, wild type shows the greatest capacity for re-reduction of $P700^+$ while mutant strains with an intermediate amount of PS I are less able to do so rapidly. On the other hand, strains with the lowest PS I content show rapid re-reduction because available P700 reaction centers are quickly re-reduced by respiratory electrons. It should be pointed out that we measured the concentration of ATP vs. ADP and AMP, and the energy charge in all mutants is similar to that of the wild type (~0.8), indicating that the reduction in cyclic electron flow as a consequence of less PS I does not lead to a loss in ATP production, suggesting that ATP production is not limiting for growth under the experimental conditions.

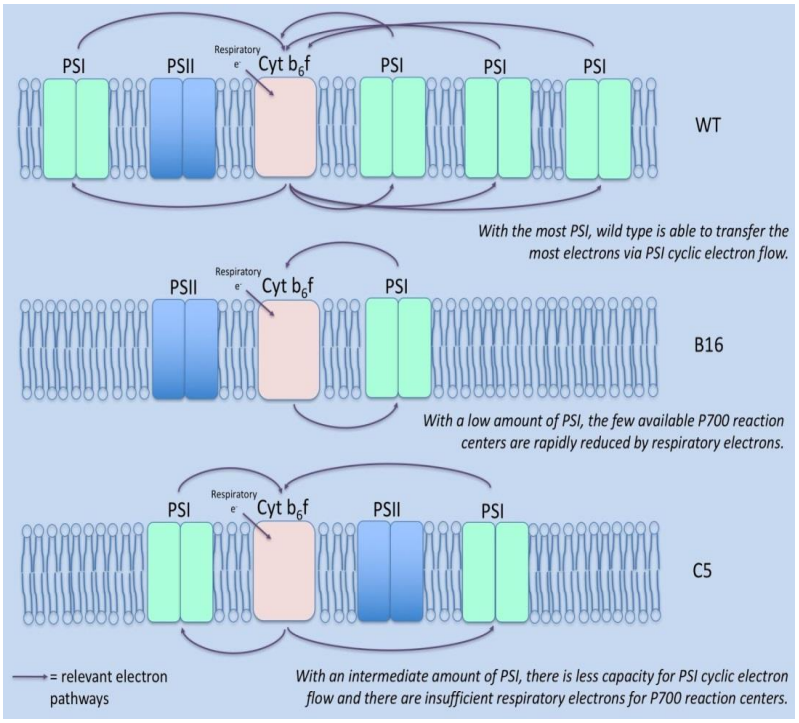

Figure 5. Model of how electron flow to PS I may vary by the abundance of PS I. With the highest amount of PS I reaction centers, wild type (WT) can transfer the most electrons through cyclic electron flow. With the lowest amount of reaction centers among mutants here, B16 has the least potential to perform cyclic electron flow, but this strain's $P700^+$ may be quickly reduced by respiratory electrons. With an intermediate amount of PS I reaction centers, a strain like C5 does not demonstrate as much cyclic electron flow as wild type does, nor does its $P700^+$ get reduced by respiratory electrons to the degree B16's do as less respiratory electrons are available per reaction center.

Flv3, a flavoprotein thought to be involved in a Mehler-type reaction

The second question explored in this funding period relates to a Mehler-type reaction catalyzed by two flavoproteins, Flv1 and Flv3, which form a heterodimer that accepts electrons from PS I and that are typically believed to funnel these electrons to molecular O_2. Therefore, according to this way of thinking Flv1 and Flv3 potentially function as an electron safety valve leading to no useful purpose of the photosynthesis-generated electrons. According to some reports in the literature, up to 40% of the electrons generated by the linear electron transport chain may be consumed by the Flv1/Flv3 complex. However, we observed that in strains lacking Flv3 (which have been shown to no longer have an active Flv1/Flv3 complex) the amount of linear electron flow was no different than in the wild type at any measured light intensity (not shown), thus making it hard to reconcile that Flv3 would be involved in catalyzing a Mehler reaction. Therefore, we are studying strains lacking Flv3 in more detail, in comparison with the wild type.

As indicated in Figure 6, $P700^+$ does not readily accumulate in a strain lacking Flv3 until about 20 s after the start of illumination (presumably when the Calvin Cycle starts to operate, thus utilizing accumulated NADPH), indicating that Flv3 indeed serves as an electron acceptor at the donor side of PS I: Early after the start of accumulation, additional reducing equivalents cannot be accommodated at the acceptor side of PS I and P700 cannot be oxidized.

We are currently working on determining whether in the absence of Flv3 cyclic electron flow back to $P700^+$ is impaired. This is measured in the presence of DCMU, thus blocking linear electron flow. Preliminary data indicate a slow-down of cyclic electron flow around PS I in the absence of Flv3. If cyclic electron flow indeed is impaired in the strains lacking Flv3, this implies that *in vivo* the Flv1/Flv3 complex

may not transfer electrons to O_2 but rather funnel them back to the plastoquinone pool to participate in cyclic electron flow around PS I.

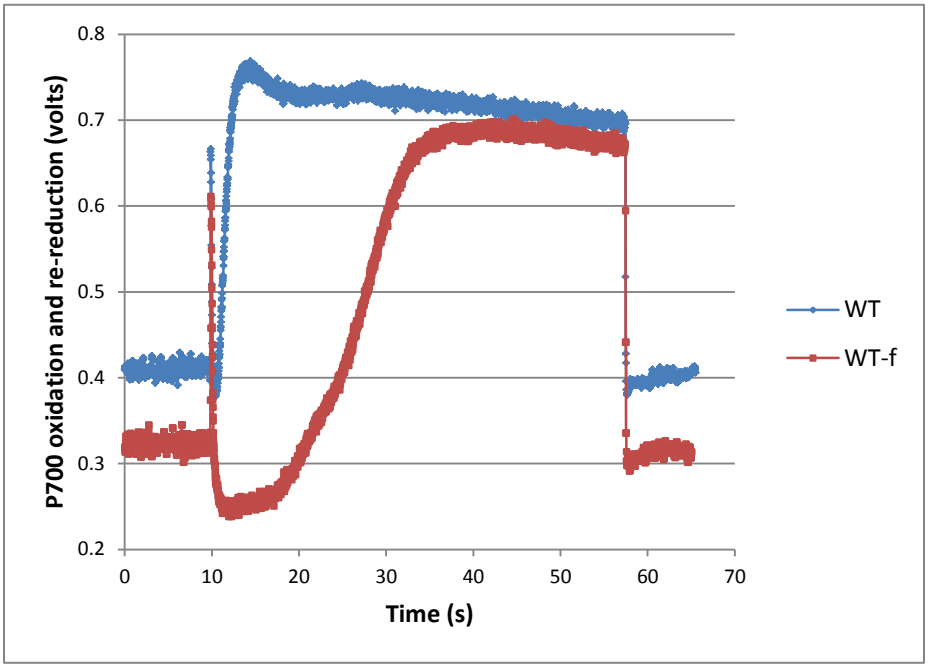

Figure 6. P700 oxidation and $P700^+$ re-reduction in wild type (blue) and a strain lacking Flv3 (red). Actinic light was turned on at around 10 s and turned off at around 57 s. Upon P700 oxidation, the voltage rises. Therefore, $P700^+$ accumulation leads to a high signal and reduced P700 leads to as low voltage signal.

Based on this research, we have established that a reduction in PS I levels in cyanobacteria may very well lead to better growth and biomass production when grown at higher light intensity. This is of obvious interest to those who intend growing strains at larger scale under natural light, and in the longer term may help guide the design of biohybrid devices for solar energy conversion. The results of our work on the role of Flv3 appears to suggest its involvement in cyclic electron flow around PS I, thus changing the Flv1/Flv3 pathway from the concept of a reducing-equivalents-wasting one to one where energy is partially conserved. Together, this research shows that significant advances in overall photosynthetic efficiency are possible by modification of PS I levels or PS I electron transport pathways.

We are very grateful to the Photosynthetic Systems program at DOE-BES for long-standing support of our research.