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Psb27, a Photosystem II assembly protein, enables quenching of excess light energy during its participation in the PSII lifecycle

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Abstract

Photosystem II (PSII), the enzyme responsible for oxidizing water into molecular oxygen, undergoes a complex lifecycle during which multiple assembly proteins transiently bind to and depart from PSII assembly intermediate complexes. Psb27 is one such protein. It associates with the CP43 chlorophyll-binding subunit of PSII to form a Psb27-PSII sub-complex that constitutes 7-10% of the total PSII pool. Psb27 remains bound to PSII assembly intermediates and dissociates prior to the formation of fully functional PSII. In this study, we compared a series of Psb27 mutant strains in the cyanobacterium *Synechocystis* sp. PCC 6803 with varied expression levels of Psb27: wild type (WT); *psb27* genetic deletion (Del27), genetically complemented *psb27* (Com27); and over-expressed Psb27 (OE27). The Del27 strain demonstrated decreased non-photochemical fluorescence quenching, while the OE27 strain showed increased non-photochemical quenching and tolerance to fluctuating light conditions. Multiple flashes and fluorescence decay analysis indicated that OE27 has the least affected maximum PSII quantum yield of the mutants. OE27 also displayed a minimal impact on the half-life of the fast component of Q_A^- reoxidation over multiple flashes, indicating robust PSII function. We propose that the close association between Psb27 and CP43, and the absence of a fully functional manganese cluster in the Psb27-PSII complex, create a PSII sub-population that dissipates excitation energy prior to its recruitment into the functional PSII pool. Efficient energy dissipation prevents damage to this pre-PSII pool and allows for efficient PSII repair and maturation. Participation of Psb27 in the PSII life cycle ensures high quality PSII assembly.

Keywords: Photosystem II, Photosynthesis, Non-Photochemical Quenching, *Synechocystis* 6803

Statements and Declarations: The authors have no competing interests to declare.

Acknowledgements

We thank Dr. Julian Eaton-Rye for the kind gift of Psb27 antisera. This study was supported by U.S. Department of Energy (DOE), Office of Basic Energy Sciences, grant DE-FG02-99ER20350 to H.B.P. H.L. was partially supported by DOE grant DE-FG02-07ER15902.

Introduction

Photosystem II (PSII) is a unique enzyme, in that it routinely experiences damage caused by one of its substrates, light, and its derivative reactive oxygen species. Additionally, PSII is assembled modularly from chlorophyll-containing subunits, which themselves are sensitive to damage by absorption of light prior to complete PSII assembly. On the organismal level, this photo-damage is detrimental to fitness unless a protective mechanism is adopted to dissipate excess light energy, both during assembly and under photoactive conditions. Cyanobacteria have several known mechanisms of excitation energy quenching and redistribution, collectively called non-photochemical quenching (NPQ). These include quenching via the orange carotenoid protein (OCP), which, in its light-induced active state, uncouples the excitation energy transfer from phycobilisome antennas to PSII, reducing its functional cross section (Wilson, Ajlani et al. 2006, Wilson, Punginelli et al. 2008). Another mechanism is via IsiA, a chlorophyll-containing homolog of CP43, which forms oligomeric ring structures under stress conditions (Chen, Bandyopadhyay et al. 2018) (Ihalainen, D'Haene et al. 2005). These rings are thought to absorb and dissipate excess light energy as a means of photoprotection. Additionally, during assembly, the reaction center proteins of PSII are associated with carotenoid-containing quenching proteins known as HLIPS for protection prior to their assembly into active PSII (Knoppova, Sobotka et al. 2014, Niedzwiedzki, Tronina et al. 2016). This mechanism relies on carotenoids' ability to quench both triplet chlorophyll and singlet oxygen. Cyanobacteria have two other mechanisms of fluorescence quenching and redistribution. These are state transitions, which regulate the distribution of excitation energy between PSII and PSI, and photoinhibition, which results from the high-light-induced inactivation of PSII caused by damage and degradation of the D1 protein.

Elucidation of photoprotective strategies that occur at the molecular level promises to inspire rational redesign of photosynthesis to sustainably meet current and future global food and energy demand (Ort, Merchant et al. 2015). Recently, increasing the responsiveness of photoprotective quenching routes to fluctuating light conditions has been observed to have a significant (15%) positive impact on crop yield under simulated field conditions (Kromdijk, Glowacka et al. 2016). Overall, the increased availability of protective quenchers and accelerated recovery from NPQ in lower light conditions allowed for increased environmental adaptability and thus increased photosynthetic productivity.

Psb27, a small extrinsic membrane protein, is only bound to inactive PSII. It binds either to monomeric PSII during *de novo* assembly and repair, or to dimeric PSII during repair (Nowaczyk, Hebel et al. 2006, Grasse, Mamedov et al. 2011, Liu, Roose et al. 2011, Singh 2017). It is thought to enable the efficient light-driven assembly (photoactivation) of the Mn-cluster (Roose and Pakrasi 2008, Avramov, Hwang et al. 2020). In strains lacking Psb27, PSII can assemble fully, but photoactivation is slower and recovery from photodamage is slower as well (Roose and Pakrasi 2008, Jackson, Hervey et al. 2014, Davinagracia 2021). In the absence of both Psb27 and CP47, unassembled CP43 is degraded at a higher rate (Komenda, Knoppova et al. 2012), which indicates that Psb27 stabilizes CP43 and allows a relatively larger amount of the pre-complex (Psb27-CP43) to form. Psb27 is not found in the structure of active PSII, but recently, structures of PSII assembly complexes bound to Psb27 have been elucidated using cryo-electron microscopy (Zabret, Bohn et al. 2021), (Huang, Xiao et al. 2021). These structures reveal that Psb27 binds to the surface of Loop E of CP43 distal to the Mn binding site, or D1/D2 axis, which is consistent with two previous observations from mass spectrometry crosslinking and protein

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4 foot-printing (Liu, Huang et al. 2011, Liu, Chen et al. 2013). These findings have suggested an
5 allosteric role of Psb27 in maintaining PSII in a nonfunctional state, ready for the final steps of
6 PSII assembly. However, the details of such structural arrangements remain unclear.
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9 Psb27 is conserved across all oxygenic photosynthetic organisms. In *Arabidopsis thaliana*, Psb27
10 is not essential for PSII formation and photoautotrophic growth. Absence of Psb27, however,
11 leads to decreased recovery of the photodamaged PSII complex (Chen, Zhang et al. 2006),
12 especially in fluctuating light (Hou, Fu et al. 2015). We suggest that a similar growth benefit may
13 be conferred by Psb27 in cyanobacteria, and we propose a mechanistic explanation for how
14 Psb27-PSII contributes to optimal PSII function and eco-physiological fitness in cyanobacteria.
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Results

This study analyzed four strains of *Synechocystis* sp. PCC 6803 for growth and photosynthetic parameters: WT, a deletion mutant of *Psb27* (Del27), a strain complementing the deletion (Com27), and a weak over-expression line of *Psb27* (OE27). Figure 1A shows the growth rates of these strains under standard lab conditions: constant, low level ($30 \mu\text{M photons m}^{-2} \text{ s}^{-2}$) light. Figure 1B shows the growth rate under fluctuating light conditions: a 30-minute-high light/30-minute dark cycle. While the growth rates are quite similar, the OE27 strain under continuous light shows slower initial growth before catching up to the WT at about 4-5 days of growth. Under fluctuating light conditions, the growth rate of the strains is similar as well, but the OE27 strain overtakes the WT after several days of growth. Interestingly, under fluctuating light, the Del27 strain and Com27 strains also appear to overtake the WT.

To further test the photosynthetic parameters of these strains, cells were grown on BG-11 plates without antibiotics and the Fluorocam 800MF was used to assay for non-photochemical quenching. Figure 2A shows the NPQ trace collected for each of the four strains. Application of a saturating pulse to the dark-adapted plate induced a maximum value of fluorescence, F_m , by closing reaction centers. At that time there was no NPQ because the colonies had been fully dark-adapted. Following a dark relaxation, a sufficiently strong actinic light was applied and an initial rise in fluorescence was observed. This fluorescence was then partially quenched as a result of increasing competition with photochemical and non-photochemical events. Five saturating pulses were applied under actinic illumination that transiently closed all the reaction centers and provided a value of maximal fluorescence in the light-adapted state, termed F_m' . The difference between F_m and F_m' , is due to the contribution of NPQ. Figure 2B plots the plant vitality index, Rfd, derived from peak fluorescence, F_m , attained during the first seconds of the transient, and the steady-state fluorescence, F_s , in the light-adapted phase. In healthy photosynthetic cells, there is a larger value, as shown for WT and Com27, while Del27 has a slower rise than WT. Conversely, OE27 demonstrates an accelerated rise which means that this strain's overall physiological fitness is increased. Figure 2C shows the coefficient of photochemical quenching during actinic light conditions, which reflects the process of photosynthesis itself. Upon light illumination, enzymes in the Calvin-Benson Cycle activate fully, the metabolite pool size increases, and the carbon concentrating mechanism for feeding CO_2 to Rubisco is initiated. These factors lead to an increased electron sink from the photosynthetic electron transport chain and contribute to quenching. To our surprise, Del27 showed effectively no increase in the time range recorded, and WT and Com27 showed a gradual increase, in parallel with that of OE27. The values for WT and Com27, however, are less than that of the OE27. Figure 2D shows the NPQ recorded during the actinic and dark phases, derived from the difference of F_m and F_m' . OE27 has the highest NPQ levels, in contrast to the lowest level in Del27, with WT and Com27 in between.

Figure 3 shows Q_A^- reoxidation over 1000 flashes. Before the measurement, we performed dark-adapted fluorescence induction analysis, known as Kautsky induction (Fig. 3A). The dark-adapted F_v/F_m values of the four strains are comparable. This is the basis for NPQ and the following fluorescence kinetics analysis. The 'multiple flashes' experiment is modified from the S-state program equipped in the FL-200 fluorometer. It is a sequence of 1000 saturating flashes over the course of 500s (see Materials and Methods). We used multiple flashes to compare the charge recombination of the dark-adapted strains after a single flash and its associated S-state, and to compare the first charge recombination with recombination after multiple flashes. Each flash also contributes to the redox state of PSII and thus the thylakoids overall, so the observed values

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4 reflect the redox state changes after actinic light illumination over a longer time scale (>5 sec).
5 Figure 3B compares the fluorescence decay after the first and last (1000) of the flashes for WT
6 and OE27 (data of Del27 and Com27 are not shown for clarity), which is driven by charge transfer
7 from the plastoquinone Q_A to Q_B in the PSII electron transport chain. To model the charge transfer
8 kinetics between Q_A^- and the plastoquinone bound at the Q_B -binding site, we employed a double
9 exponential decay. The fast exponential component describes the charge transfer between Q_A^-
10 and plastoquinone bound at the Q_B site. The second exponential, intermediate, component
11 describes plastoquinone exchange at the Q_B -binding site (Vass, Kirilovsky et al. 1999). Because
12 the fluorescence decay was recorded for approximately 330 ms after the actinic flash (instrument
13 intrinsic function), we did not consider a slow hyperbolic component ($T_{1/2}$ ranging in seconds) that
14 would describe the $S_2Q_A^-$ charge recombination (Biswas and Eaton-Rye 2018). Table 1 and Table
15 2 show the decay kinetics for all strains for the first and last flashes (data is in Table S2). Residuals
16 for the fits are shown in Fig S2.
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21 The fluorescence decay kinetics for the fast and intermediate components after a single saturating
22 flash are nearly the same for all the strains. However, after 1000 saturating flashes, the $T_{1/2}$ and
23 amplitude for the fast component are considerably different for the OE27 strain compared to other
24 strains (Table 2). The $T_{1/2}$ for the intermediate components for all the strains remain similar. The
25 changes in the amplitudes for the intermediate component between the first flash and thousandth
26 flash would suggest increased photochemistry owing to cells transitioning from dark-adapted to
27 steady-state conditions. After multiple saturating flashes, an overall increase in the electron
28 transfer is also evident from a decline in the half-times of both the fast and intermediate
29 components.
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33 Because *Psb27* is not found in active cyanobacterial PSII (Suga, Akita et al. 2015) (Nowaczyk,
34 Hebel et al. 2006, Liu, Huang et al. 2011), deletion of *Psb27* has a minimal impact on the PSII-
35 associated electron transfer processes as compared to WT (Table 1 and 2), as expected. The
36 minimal impact on the $T_{1/2}$ for the fast component in the OE27 strain could indicate the robustness
37 of PSII. However, because *Psb27* is not a component of active PSII, we hypothesize that more of
38 the *Psb27*-bound PSII assembly intermediate is available in the OE27 strain. Increased *Psb27*
39 assures high quality, efficient PSII assembly based on previous functional analysis (Roose 2008,
40 Jackson, Hervey et al. 2014). Under steady-state conditions, an increase in damage to PSII would
41 make PSII maturation in the OE27 strain more efficient than in the other strains and thus a minimal
42 impact on the electron-transfer kinetics is observed, as it is more reflective of new complex
43 formation than adapting PSII centers to light.
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47 Although the fit of the model employed to fit fluorescence decay was satisfactory ($R^2=0.99$), the
48 sinusoidal pattern observed for the residuals (Figure S2) would suggest that a model employing
49 a double exponential does not fully describe the relaxation processes taking place. We also did
50 not consider the slow hyperbolic component for curve fitting, which could be contributing to the
51 structure of the residuals.
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54 Figure 3C shows F_m changes over 1000 saturating flashes. OE27 shows less increase in F_m
55 than other strains, indicative of either a robust non-photochemical quenching, or NPQ, consistent
56 with the results from Figure 2C. Figure 3D shows F_o changes over 1000 flashes. Figure 3E shows
57 F_v over the 1000 flashes, and Figure 3F shows F_v/F_m . Overall, OE27 shows less of a change
58 over the 1000 flashes than the other strains.
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Discussion

In our previous study (Liu, Roose et al. 2011), we demonstrated that within the PSII population, there is always a sub-population (7-10%) of monomeric Psb27-PSII. This population is characterized by loosely bound PsbO and the absence of a functional Mn cluster, and has severely defective Q_A^- reoxidation by forward electron transfer to Q_B . Fluorescence decay profiles in both Psb27-PSII (His27PSII) and Psb27- Δctp PSII (His27 Δctp PSII) samples without DCMU treatment are similar to those treated with DCMU, an electron transport inhibitor that binds to the Q_B site. Compared with functional PSII (HT3-PSII), forward electron transfer from Q_A^- to Q_B in both Psb27-PSII and Psb27- Δctp PSII is blocked, which has been observed previously (Mamedov, Nowaczyk et al. 2007). The results presented in this work demonstrate that Psb27-PSII is not only involved in PSII assembly, but also correlated with a photoprotective energy-dissipation mechanism. It is thought that the removal of the Mn cluster results in a rise of the redox potential of Q_A^- , rendering electron transfer to Q_B less efficient and promoting direct relaxation of Q_A^- without forming the triplet-state of chlorophyll, a precursor of harmful singlet oxygen (Krieger-Liszkay, Fufezan et al. 2008, Rutherford, Osyczka et al. 2012, Kato, Nagao et al. 2016).

In the absence of Psb27-PSII in Del27 cells of *Synechocystis* sp. PCC 6803, the NPQ analysis demonstrated the absence of a fluorescence quenching component. This phenotype is fully complemented in Com27 (Fig. 2A), which indicates that the Psb27-PSII protein complexes present in WT comprise a PSII quenching pool. In the overexpression strain of Psb27, non-photochemical quenching parameters are even higher than in WT *Synechocystis* sp. PCC 6803 (Fig. 2A and 2D). This finding supports the hypothesis that the Psb27-PSII subpopulation is involved in energy quenching and that the pool size can be modified by genetically controlling levels of Psb27. We propose that the altered conformation of Loop E of CP43 that is conferred by Psb27 binding contributes to the rearrangement of cofactors (carotenoids, chlorophylls, plastoquinone) that enables the quenching capability of Psb27-PSII (Mamedov, Nowaczyk et al. 2007, Liu, Chen et al. 2013, Huang, Xiao et al. 2021, Zabret, Bohn et al. 2021). Psb27-bound PSII lacks a functional Mn cluster and has an altered low temperature chlorophyll fluorescence profile at 77K (Nowaczyk, Hebel et al. 2006, Liu, Roose et al. 2011), but the relation of these phenomena to enhanced non-photochemical quenching in Psb27-PSII requires further research.

In Figure 3, we observed the behavior of PSII fluorescence induction and relaxation over the course of 1000 saturating flashes. We showed that OE27 shows less of a change over time as compared with the WT strain, indicating a higher resilience to light-induced change, and larger pool of PSII assembly complexes in this strain.

We propose a model for Psb27 as an agent of non-photochemical quenching of excitation in PSII assembly and repair intermediates in cyanobacteria. Increased Psb27 levels lead to an increased pool of Psb27-PSII, ready to mature into active PSII as damage takes place or more PSII is needed, such as in a denser growth condition. This larger pool of non-photosynthetically active PSII can absorb light energy, dissipates it so as not to damage the protein subunits before PSII can function. Interestingly, (Bentley, Luo et al. 2008) found that the Δ Psb27 strain had a higher rate of oxygen evolution following high light stress, indicating that quenching species are not present, raising the apparent rate compared to chlorophyll content. Our data are consistent with a model of Psb27 expression as the determining factor controlling the amount of Psb27-PSII intermediate complexes, which can quench excitation. It has been shown in previous work that

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4 PSII decouples from phycobilisome excitation energy transfer prior to manganese cluster
5 assembly (Hwang, Nagarajan et al. 2008). It was also shown that, in the absence of PsbU, an
6 extrinsic PSII subunit which binds following manganese (Veerman, Bentley et al. 2005). Our
7 findings contribute another analogous strategy of the phenomenon decoupling excitation energy
8 prior to full activation of PSII in order to prevent damage.
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11 Cyanobacteria, in particular *Synechocystis* sp. PCC 6803, are widely used to study
12 photosynthesis. However, unlike plants, cyanobacteria used for photosynthesis research are
13 usually grown under continuous-light conditions in a constant-temperature growth chamber. In
14 nature, these conditions are nonexistent. Not only are there night/day cycles of light and dark, but
15 during the day light fluctuates due to variable shading caused by wind blowing tree branches, and
16 current movement in a water column. An adaptive photoprotective mechanism under such
17 conditions would have a more significant impact on eco-physiological fitness. We suggest that
18 this is why the quenching function of Psb27 in cyanobacteria has been overlooked until now. A
19 more pronounced phenotype may be evident in a stronger overexpression strain, or with different
20 varying light schemes other than the 30 min on/off cycle tested in this work. It is also likely that
21 there are conditions under which over-expressing Psb27 is not advantageous, and the current
22 level is a balance between a beneficial quenching state, and an over-protective mechanism.
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Materials and Methods

Culture and Growth of *Synechocystis* sp. PCC 6803 strains

Wild type (WT), Psb27 deletion (Del27) (Roose and Pakrasi 2008), Psb27 complemented (Com27), and Psb27 Over-expression (OE27) of *Synechocystis* 6803 were grown in BG11 medium (Allen 1968) at 30 °C under 30 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$. The genetically modified strains were grown in BG11 supplemented with antibiotics as follows: 5 $\mu\text{g/mL}$ chloramphenicol (Del27), 5 $\mu\text{g/mL}$ chloramphenicol, 2 $\mu\text{g/mL}$ gentamicin (Com27), 2 $\mu\text{g/mL}$ gentamicin (OE27). For growth assays in liquid medium, cells were grown to mid log phase, harvested by centrifugation, washed in fresh BG11, and again centrifuged to pellet. The cell pellets were resuspended and diluted to $\text{OD}_{730} = 0.05$ in BG11 without antibiotics and grown with shaking (200 rpm). The OD_{730} was measured every 24 h on a μQuant Microplate spectrophotometer (Biotek Instruments).

Mutant Construction and Psb27 quantification

A complemented strain of the Δpsb27 (Com27) was generated by inserting a copy of the *psb27* locus (*slr1645*) containing 200 bp of upstream and downstream DNA and the coding region of the *slr1645* gene into the *psbA1* locus, a gene that is not expressed under lab growth conditions (Fig. S1A) (Mohamed and Jansson 1989) in the Del27 mutant background (Roose 2008, Roose and Pakrasi 2008). Briefly, *psbA1* upstream and downstream DNA fragments were amplified using primers listed respectively in Table S1. The *psb27* DNA fragment was amplified using primer pair Psb27Comp5 and Psb27Comp3. Segregation of the modified *psbA1* locus was verified by PCR analysis using A1segF and A1segR primers (Fig. S1B). The OE27 was generated by using the same construct as in Com27, with *psb27* under the native promoter, but introduced into the WT background. All primer sequences are listed in Table S1.

Cyanobacterial cells were harvested and broken by bead beating as described previously (Kashino, Lauber et al. 2002) with minor modifications. Cells were re-suspended in RB buffer (25% glycerol (wt/vol), 10mM MgCl_2 , 5mM CaCl_2 , 50 mM MES buffer pH 6.0) and broken by vortexing with 0.17 mm glass beads. Membrane fraction was isolated by centrifugation, re-suspended in RB, and solubilized by addition of β -D-dodecyl maltoside (DDM) to a final concentration of 0.8%. After incubation on ice in dark for 30 min, the solubilized membranes were separated from the insoluble material by centrifugation at gradually increasing speed from 120 \times g to 27,000 \times g at 4 °C for 20 min.

SDS-PAGE was performed by loading isolated membrane proteins on a same-chlorophyll basis on a 12.5% acrylamide resolving gel. After electrophoresis, proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore-Sigma), blocked using 5% bovine serum albumin (Thermo-Fisher) for 2 h at room temperature, and then separately incubated with the primary rabbit antibody raised against Psb27 (Eaton-Rye lab) overnight at 4°C. The horseradish peroxidase (HRP)-conjugated secondary antibody goat anti-rabbit IgG (H+L)-HRP conjugate (Bio-Rad) was diluted at 1:5,000 in 1.5% BSA. Bands were visualized and quantified using chemiluminescence reagents (Millipore-Sigma) with a LI-COR Odyssey Fc (LI-COR Biotechnology) imager. Psb27 protein levels, on a per chlorophyll basis, in WT and OE27 are shown in Fig. S1C. OE27 has close to two times the Psb27 content of WT.

Fluorescence Analysis

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4 For non-photochemical quenching (NPQ) analysis using the Fluorocam 800MF (Photon System
5 Instruments, Brno, Czech Republic), cells were grown to mid log phase, harvested by
6 centrifugation, washed in fresh BG11, and again centrifuged to pellet. The cell pellets were
7 resuspended and diluted to $OD_{730} = 0.05$ in BG11 without antibiotics and spotted (2 μ L) to BG11
8 plates without antibiotics. WT and mutant cells were allowed to grow under the same temperature
9 and light conditions as liquid culture for 3 weeks before NPQ analysis.

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12 Fluorescence emission over many flashes was recorded on a double-modulation fluorometer
13 (Photon System Instruments, Brno, Czech Republic) with a built-in analyzing program, FluorWin
14 using a modified S-state program. 1000 flashes were delivered at 500 ms intervals over a period
15 of 500 seconds. The sample concentration was adjusted to 5 μ g/ml of Chla in BG11. All samples
16 were dark adapted for 5 min at room temperature before the measurements. Instead of using 10
17 actinic flashes that advance S-state by each flash, 1000 flashes (200 second duration) were used
18 to test the Q_A^- reoxidation in three mutants versus WT. The charge recombination after first flash
19 and last flash were numerically deconvoluted. The fluctuation of F_o , F_m , F_v was plotted against
20 flash number.

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23 To determine the charge transfer kinetics between Q_A^- and the plastoquinone bound at the Q_B -
24 binding site, we employed a double exponential decay to fit the data in Table S2, where A_1 and
25 A_2 are amplitudes, and T_1 and T_2 are time constants for the fast and intermediate components,
26 respectively. To calculate respective decay $T_{1/2}$, the time constants were multiplied by $\ln(2)$. $F(t)$
27 is the fluorescence level, and F_o is the base fluorescence.

$$F(t) - F_o = A_1 \exp\left(-\frac{t}{T_1}\right) + A_2 \exp\left(-\frac{t}{T_2}\right)$$

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30 The first exponential (fast component) describes the charge transfer between the Q_A and
31 plastoquinone bound at the Q_B site. The second exponential (intermediate component)
32 describes plastoquinone exchange at the Q_B -binding site (Vass, Kirilovsky et al. 1999). Because
33 the fluorescence decay was recorded for approximately 330 ms after the actinic flash, we did
34 not consider a slow hyperbolic component ($T_{1/2}$ ranging in seconds) that would describe the
35 $S_2Q_A^-$ charge recombination (Biswas and Eaton-Rye 2018). Residuals for the fits are shown in
36 Fig S2.
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References

- Allen, M. M. (1968). "Simple Conditions for Growth of Unicellular Blue-Green Algae on Plates(1, 2)." *J Phycol* **4**(1): 1-4.
- Avramov, A. P., H. J. Hwang and R. L. Burnap (2020). "The role of Ca(2+) and protein scaffolding in the formation of nature's water oxidizing complex." *Proc Natl Acad Sci U S A* **117**(45): 28036-28045.
- Bentley, F. K., H. Luo, P. Dilbeck, R. L. Burnap and J. J. Eaton-Rye (2008). "Effects of inactivating psbM and psbT on photodamage and assembly of photosystem II in *Synechocystis* sp. PCC 6803." *Biochemistry* **47**(44): 11637-11646.
- Biswas, S. and J. J. Eaton-Rye (2018). "PsbY is required for prevention of photodamage to photosystem II in a PsbM-lacking mutant of *Synechocystis* sp. PCC 6803." *Photosynthetica* **56**: 200-209.
- Chen, H., D. Zhang, J. Guo, H. Wu, M. Jin, Q. Lu, C. Lu and L. Zhang (2006). "A Psb27 homologue in *Arabidopsis thaliana* is required for efficient repair of photodamaged photosystem II." *Plant Mol Biol* **61**(4-5): 567-575.
- Chen, H. Y. S., A. Bandyopadhyay and H. B. Pakrasi (2018). "Function, regulation and distribution of IsiA, a membrane-bound chlorophyll a-antenna protein in cyanobacteria." *Photosynthetica* **56**(1): 322-333.
- Davinagracia, J. (2021). *The Role of Lipoprotein Psb27 in Photosystem II Biogenesis*. Master of Science, University of Otago.
- Grasse, N., F. Mamedov, K. Becker, S. Styring, M. Rogner and M. M. Nowaczyk (2011). "Role of novel dimeric Photosystem II (PSII)-Psb27 protein complex in PSII repair." *J Biol Chem* **286**(34): 29548-29555.
- Hou, X., A. Fu, V. J. Garcia, B. B. Buchanan and S. Luan (2015). "PSB27: A thylakoid protein enabling *Arabidopsis* to adapt to changing light intensity." *Proc Natl Acad Sci U S A* **112**(5): 1613-1618.
- Huang, G., Y. Xiao, X. Pi, L. Zhao, Q. Zhu, W. Wang, T. Kuang, G. Han, S.-F. Sui and J.-R. Shen (2021). "Structural insights into a dimeric Psb27-photosystem II complex from a cyanobacterium *Thermosynechococcus vulcanus*." *Proc Natl Acad Sci U S A* **In Press**.
- Hwang, H. J., A. Nagarajan, A. McLain and R. L. Burnap (2008). "Assembly and disassembly of the photosystem II manganese cluster reversibly alters the coupling of the reaction center with the light-harvesting phycobilisome." *Biochemistry* **47**(37): 9747-9755.
- Ihalainen, J. A., S. D'Haene, N. Yeremenko, H. van Roon, A. A. Arteni, E. J. Boekema, R. van Grondelle, H. C. Matthijs and J. P. Dekker (2005). "Aggregates of the chlorophyll-binding protein IsiA (CP43) dissipate energy in cyanobacteria." *Biochemistry* **44**(32): 10846-10853.
- Jackson, S. A., J. R. Hervey, A. J. Dale and J. J. Eaton-Rye (2014). "Removal of both Ycf48 and Psb27 in *Synechocystis* sp. PCC 6803 disrupts Photosystem II assembly and alters Q(A)(-) oxidation in the mature complex." *FEBS Lett* **588**(20): 3751-3760.
- Kashino, Y., W. M. Lauber, J. A. Carroll, Q. Wang, J. Whitmarsh, K. Satoh and H. B. Pakrasi (2002). "Proteomic analysis of a highly active photosystem II preparation from the cyanobacterium *Synechocystis* sp. PCC 6803 reveals the presence of novel polypeptides." *Biochemistry* **41**(25): 8004-8012.
- Kato, Y., R. Nagao and T. Noguchi (2016). "Redox potential of the terminal quinone electron acceptor QB in photosystem II reveals the mechanism of electron transfer regulation." *Proc Natl Acad Sci U S A* **113**(3): 620-625.
- Knoppova, J., R. Sobotka, M. Tichy, J. Yu, P. Konik, P. Halada, P. J. Nixon and J. Komenda (2014). "Discovery of a chlorophyll binding protein complex involved in the early steps of photosystem II assembly in *Synechocystis*." *Plant Cell* **26**(3): 1200-1212.
- Komenda, J., J. Knoppova, J. Kopečna, R. Sobotka, P. Halada, J. Yu, J. Nickelsen, M. Boehm and P. J. Nixon (2012). "The Psb27 assembly factor binds to the CP43 complex of photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803." *Plant Physiol* **158**(1): 476-486.

1
2
3
4 Krieger-Liszkay, A., C. Fufezan and A. Trebst (2008). "Singlet oxygen production in photosystem II and
5 related protection mechanism." Photosynth Res **98**(1-3): 551-564.
6 Kromdijk, J., K. Glowacka, L. Leonelli, S. T. Gabilly, M. Iwai, K. K. Niyogi and S. P. Long (2016). "Improving
7 photosynthesis and crop productivity by accelerating recovery from photoprotection." Science
8 **354**(6314): 857-861.
9 Liu, H., J. Chen, R. Y. Huang, D. Weisz, M. L. Gross and H. B. Pakrasi (2013). "Mass spectrometry-based
10 footprinting reveals structural dynamics of loop E of the chlorophyll-binding protein CP43 during
11 photosystem II assembly in the cyanobacterium *Synechocystis* 6803." J Biol Chem **288**(20): 14212-14220.
12 Liu, H., R. Y. Huang, J. Chen, M. L. Gross and H. B. Pakrasi (2011). "Psb27, a transiently associated
13 protein, binds to the chlorophyll binding protein CP43 in photosystem II assembly intermediates." Proc
14 Natl Acad Sci U S A **108**(45): 18536-18541.
15 Liu, H., R. Y. C. Huang, J. Chen, M. L. Gross and H. B. Pakrasi (2011). "Psb27, a transiently associated
16 protein, binds to the chlorophyll binding protein CP43 in photosystem II assembly intermediates."
17 Proceedings of the National Academy of Sciences of the United States of America **108**(45): 18536-18541.
18 Liu, H., J. L. Roose, J. C. Cameron and H. B. Pakrasi (2011). "A genetically tagged Psb27 protein allows
19 purification of two consecutive photosystem II (PSII) assembly intermediates in *Synechocystis* 6803, a
20 cyanobacterium." J Biol Chem **286**(28): 24865-24871.
21 Mamedov, F., M. M. Nowaczyk, A. Thapper, M. Rogner and S. Styring (2007). "Functional
22 characterization of monomeric photosystem II core preparations from *Thermosynechococcus elongatus*
23 with or without the Psb27 protein." Biochemistry **46**(18): 5542-5551.
24 Mohamed, A. and C. Jansson (1989). "Influence of light on accumulation of photosynthesis-specific
25 transcripts in the cyanobacterium *Synechocystis* 6803." Plant Mol Biol **13**(6): 693-700.
26 Niedzwiedzki, D. M., T. Tronina, H. Liu, H. Staleva, J. Komenda, R. Sobotka, R. E. Blankenship and T.
27 Polivka (2016). "Carotenoid-induced non-photochemical quenching in the cyanobacterial chlorophyll
28 synthase-HliC/D complex." Biochim Biophys Acta **1857**(9): 1430-1439.
29 Nowaczyk, M. M., R. Hebel, E. Schlodder, H. E. Meyer, B. Warscheid and M. Rogner (2006). "Psb27, a
30 cyanobacterial lipoprotein, is involved in the repair cycle of photosystem II." Plant Cell **18**(11): 3121-
31 3131.
32 Ort, D. R., S. S. Merchant, J. Alric, A. Barkan, R. E. Blankenship, R. Bock, R. Croce, M. R. Hanson, J. M.
33 Hibberd, S. P. Long, T. A. Moore, J. Moroney, K. K. Niyogi, M. A. Parry, P. P. Peralta-Yahya, R. C. Prince, K.
34 E. Redding, M. H. Spalding, K. J. van Wijk, W. F. Vermaas, S. von Caemmerer, A. P. Weber, T. O. Yeates, J.
35 S. Yuan and X. G. Zhu (2015). "Redesigning photosynthesis to sustainably meet global food and
36 bioenergy demand." Proc Natl Acad Sci U S A **112**(28): 8529-8536.
37 Roose, J. L. (2008). "Assembly and function of cyanobacterial photosystem II, Ph.D thesis. Washington
38 University, St. Louis, MO."
39 Roose, J. L. and H. B. Pakrasi (2008). "The Psb27 protein facilitates manganese cluster assembly in
40 photosystem II." J Biol Chem **283**(7): 4044-4050.
41 Rutherford, A. W., A. Osyczka and F. Rappaport (2012). "Back-reactions, short-circuits, leaks and other
42 energy wasteful reactions in biological electron transfer: redox tuning to survive life in O(2)." FEBS Lett
43 **586**(5): 603-616.
44 Singh, H. (2017). The role of the low-molecular-weight proteins of the CP43 pre-assembly complex of
45 Photosystem II. Doctor of Philosophy, University of Otago.
46 Suga, M., F. Akita, K. Hirata, G. Ueno, H. Murakami, Y. Nakajima, T. Shimizu, K. Yamashita, M. Yamamoto,
47 H. Ago and J. R. Shen (2015). "Native structure of photosystem II at 1.95 Å resolution viewed by
48 femtosecond X-ray pulses." Nature **517**(7532): 99-103.
49 Vass, I., D. Kirilovsky and A. L. Etienne (1999). "UV-B radiation-induced donor- and acceptor-side
50 modifications of photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803." Biochemistry
51 **38**(39): 12786-12794.
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Veerman, J., F. K. Bentley, J. J. Eaton-Rye, C. W. Mullineaux, S. Vasil'ev and D. Bruce (2005). "The PsbU subunit of photosystem II stabilizes energy transfer and primary photochemistry in the phycobilisome-photosystem II assembly of *Synechocystis* sp. PCC 6803." *Biochemistry* **44**(51): 16939-16948.

Wilson, A., G. Ajlani, J. M. Verbavatz, I. Vass, C. A. Kerfeld and D. Kirilovsky (2006). "A soluble carotenoid protein involved in phycobilisome-related energy dissipation in cyanobacteria." *Plant Cell* **18**(4): 992-1007.

Wilson, A., C. Punginelli, A. Gall, C. Bonetti, M. Alexandre, J. M. Routaboul, C. A. Kerfeld, R. van Grondelle, B. Robert, J. T. Kennis and D. Kirilovsky (2008). "A photoactive carotenoid protein acting as light intensity sensor." *Proc Natl Acad Sci U S A* **105**(33): 12075-12080.

Zabret, J., S. Bohn, S. K. Schuller, O. Arnolds, M. Moller, J. Meier-Credo, P. Liauw, A. Chan, E. Tajkhorshid, J. D. Langer, R. Stoll, A. Krieger-Liszkay, B. D. Engel, T. Rudack, J. M. Schuller and M. M. Nowaczyk (2021). "Structural insights into photosystem II assembly." *Nat Plants* **7**(4): 524-538.

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4 **Figure Legends**
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7 **Figure 1 OE27 growth outpaces WT *Synechocystis* 6803 in fluctuating light.** A) Growth
8 under continuous light. This figure shows the growth curve of WT, Del27, Com27, OE27 under
9 normal growth continuous light ($30 \mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$). B) Growth under $1200 \mu\text{mol photons}$
10 $\text{m}^{-2}\cdot\text{s}^{-1}$, 30 minute on-off cycles.
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12 **Figure 2: NPQ is increased in the Psb27 overexpression strain** (A) NPQ traces from
13 Fluorocam 800MF of four cell lines. Light and dark scheme are indicated with saturating pulses
14 applied. F_m , F_d , and F_s are marked for WT trace. Deconvolution of the traces followed
15 manufacturer's protocol (Photo System Instruments, Brno, Czech Republic). (B) Plant vitality
16 index R_{fd} (F_d/F_s) at intervals during light exposure. (C) Coefficient of quenching ($F_m'-F$)/(F_m' -
17 F_o'). (D) NPQ ($(F_m-F_m')/F_m'$). Data are representative of 3 replicates.
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20 **Figure 3: OE27 is resilient to light adaptation over 1000 flashes** (A) Fluorescence induction
21 (Kautsky effect); (B) Fluorescence decay over 500ms for WT and OE27 strains after first (F) and
22 last (L, 1000) flashes. (C) F_m fluctuation over 1000 flashes. (D) F_o fluctuation over 1000 flashes.
23 (E) \bar{F}_v derived from (C) and (D). \bar{F}_v value shows periodic change which is the opposite of flash
24 induced oxygen yield. Some studies use these values to derive S-state distribution analysis. (F)
25 \bar{F}_v/F_m maximum quantum yield of PSII chemistry.
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28 **Figure 4: Schematic of the role of Psb27 NPQ.** Psb27 is the determining factor in the pool size
29 of Psb27-PSII from both the synthesis and repair pathways. A larger pool of the quenching
30 species Psb27-PSII forms when more Psb27 is present in the cell, which enables higher NPQ.
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Table 1: Fluorescence decay kinetics after a single actinic flash

Strains and treatment	Fast component		Intermediate component	
	Amplitude (%)	T _{1/2} (μs)	Amplitude (%)	T _{1/2} (ms)
WTF	59.1	252.7	40.9	2.1
Del27F	60.5	273.2	39.5	2.2
Com27F	65.6	229.7	34.4	2.2
OE27F	68.0	237.9	32.0	2.6

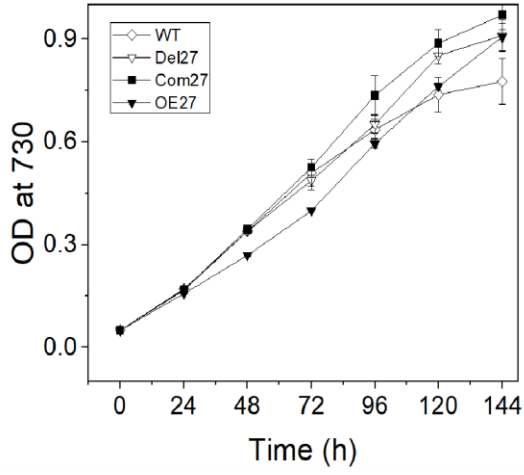
Table 2: Fluorescence decay kinetics after 1000 actinic flashes

Strains and treatment	Fast component		Intermediate component	
	Amplitude (%)	T _{1/2} (μs)	Amplitude (%)	T _{1/2} (ms)
WTL	39.2	178.8	60.8	1.3
Del27L	42.4	195.1	57.6	1.3
Com27L	43.3	179.8	56.7	1.2
OE27L	50.2	243.9	49.8	1.6

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Figure 1

A



B

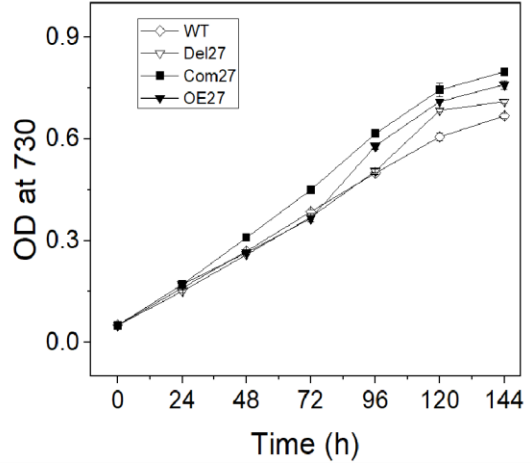


Figure 2

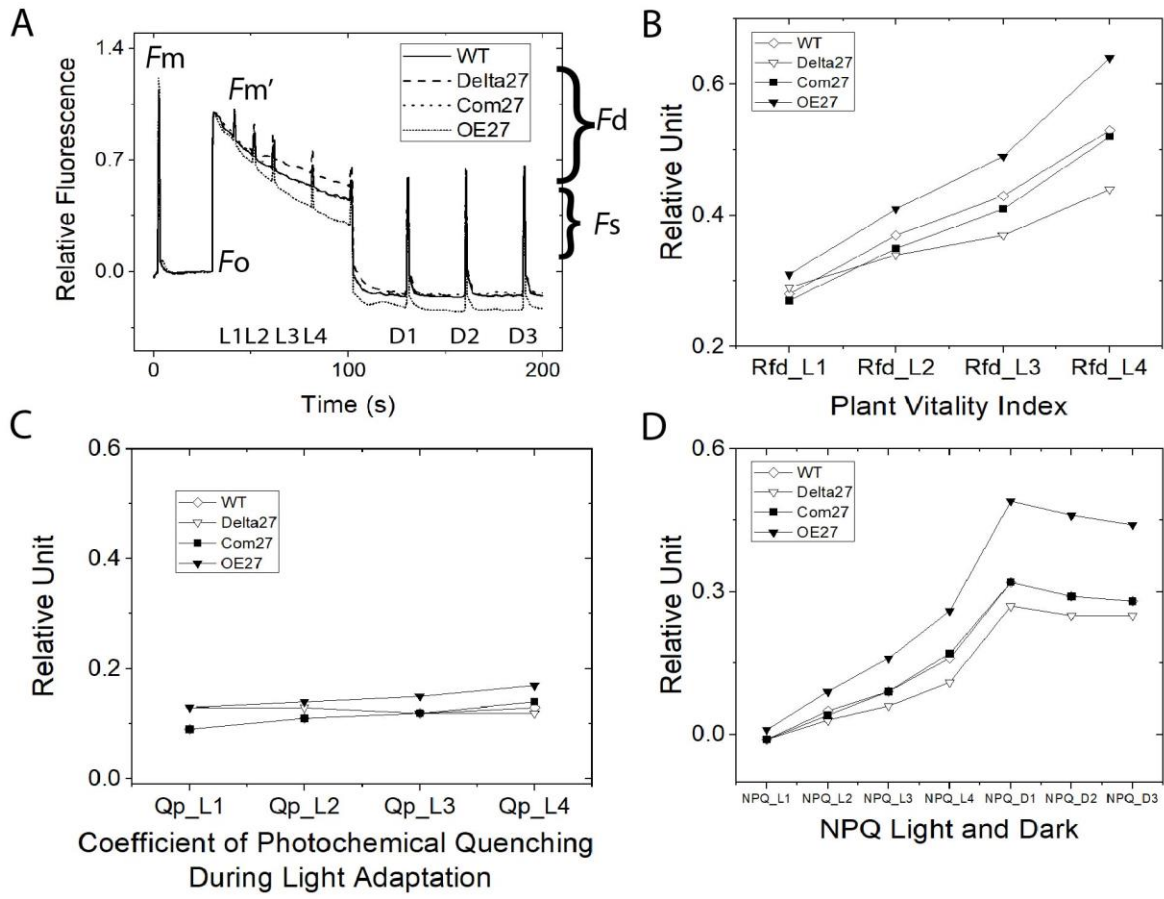


Figure 3

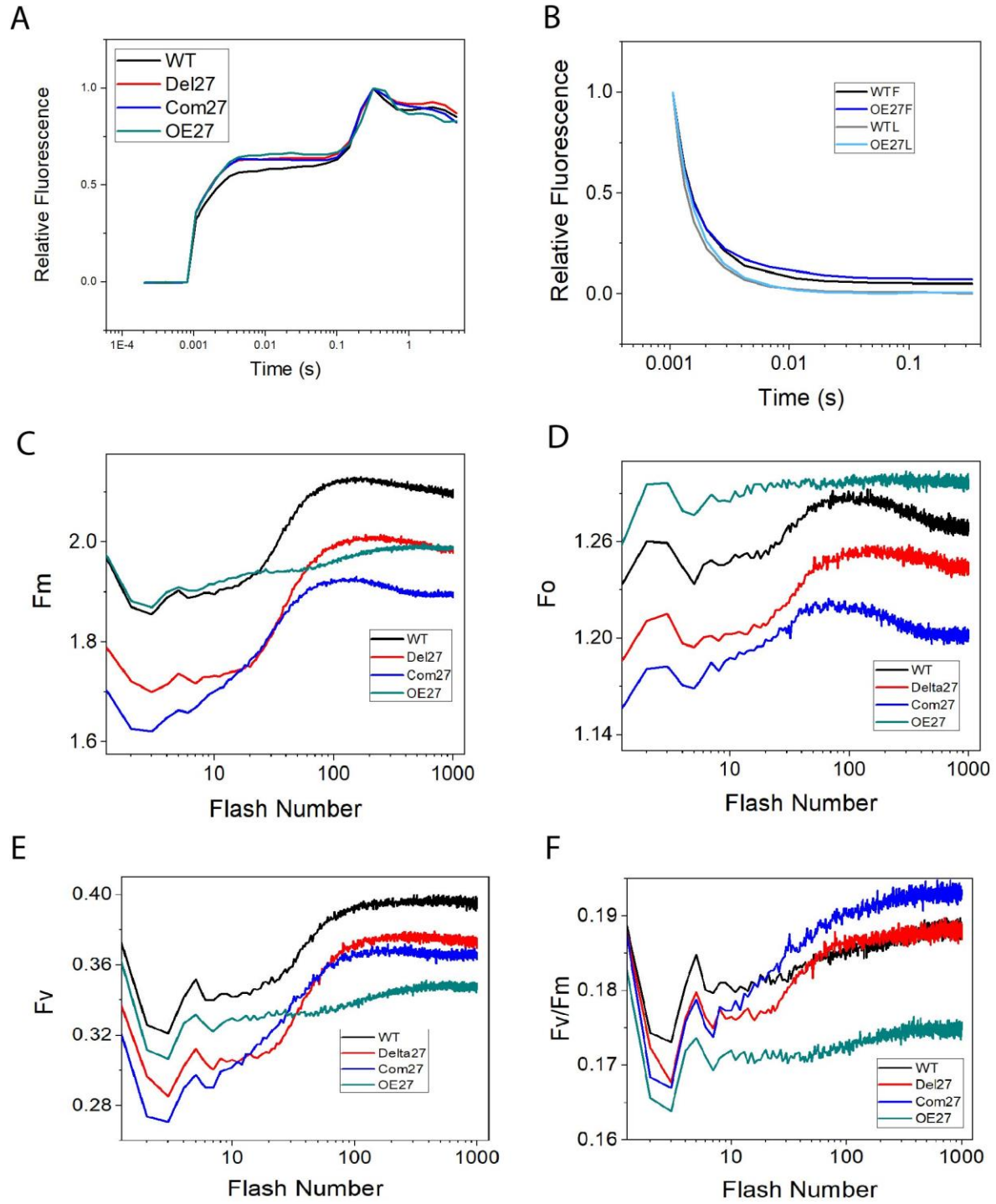
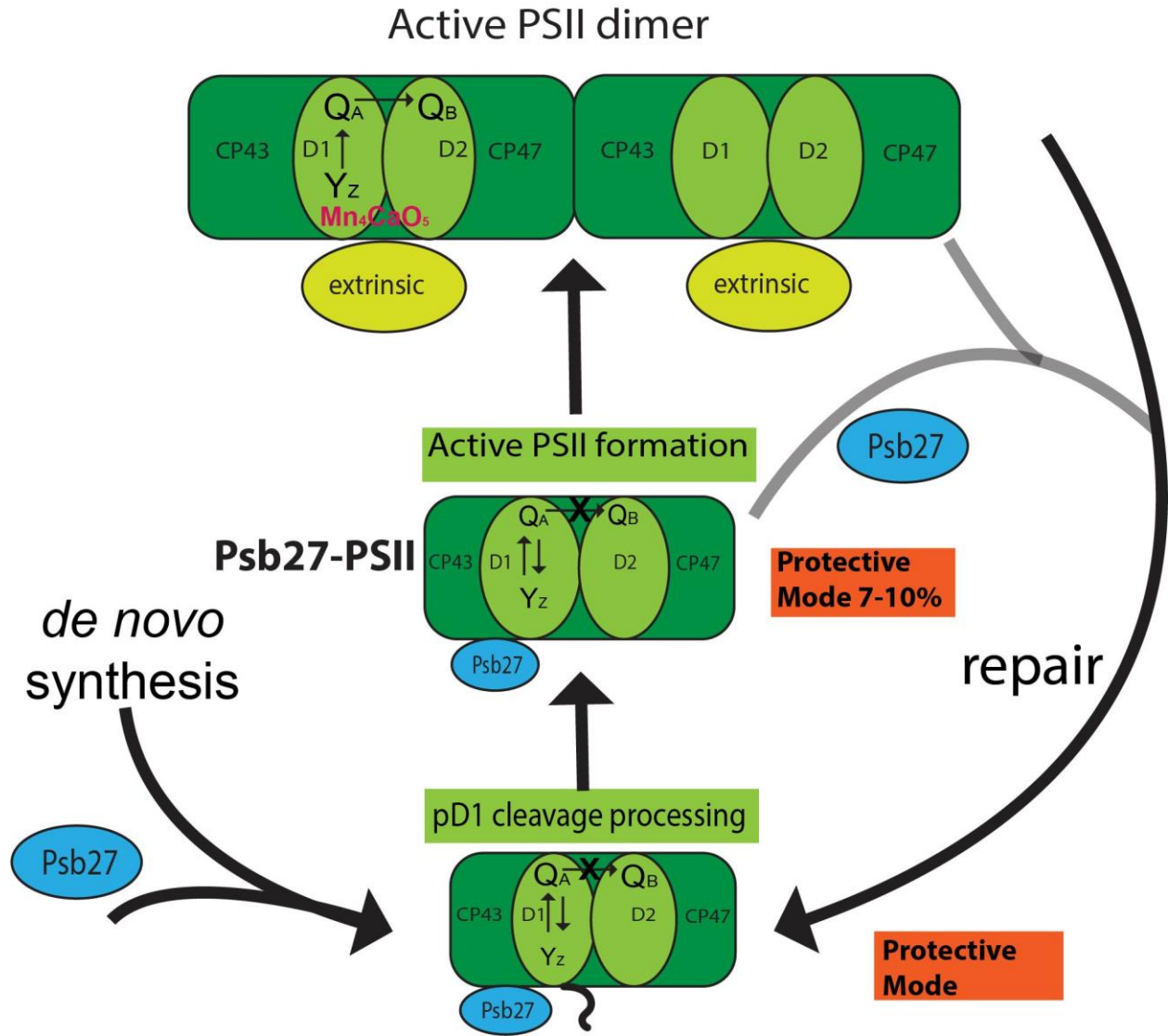


Figure 4



Pakrasi: PRES-D-21-00111- response to reviewers

We thank the reviewers for their helpful comments to improve our manuscript. Please find below our point-by-point responses (reviewer comments in red, responses in black text).

Reviewer #1: The manuscript provides data to support the hypothesis that photoprotection of the Psb27-PSII precomplex plays a role in enhancing the capacity of PS II to enhance PS II repair thereby provides an eco-physiological advantage.

Some comment of the following points would be useful.

In *Synechocystis* sp. PCC 6803 the *psb27* gene is in an operon with a gene for ribonuclease III (*slr1646*). How was the expression of *slr1646* impacted in the strains constructed for this study?

Although the c-terminus of Psb27 (*slr1645*) overlaps with the n-terminus of *slr1646*, the deletion construct preserves the start codon of *slr1645*. However, the chloramphenicol cassette does contain a transcription terminator. We would also like to point out previous studies involving Psb27 deletion in *Synechocystis* sp. PCC 6803 (Komenda, Knoppova et al. 2012, Jackson, Hervey et al. 2014). In both studies, deletion of Psb27 negligibly affected either the mutant's photoautotrophic growth or its PSII-specific oxygen-evolving activity under normal growth conditions. It has also been found that deletion of Psb27 does not significantly affect the expression levels of the PSII or PSI proteins. (Singh 2017, Davinagracia 2021). As our current manuscript deals with the role(s) of Psb27 in photoprotection and quenching, we did not look at the expression of *slr1646* (ribonuclease III) in this study, but we do not expect that a change in its expression would strongly affect the photosynthetic parameters tested.

The doubling times of the cultures in the plate assay appear to be quite long (in panel A it appears to take close to 48 h to go from OD 0.3 to OD 0.6). Typically, I'd expect *Synechocystis* sp. PCC 6803 to double every 12 hours or so?

While the optimal doubling time of *Synechocystis* is 12 hours during logarithmic growth, the growth curves obtained are consistent with *Synechocystis* cells grown in our growth chamber, shaking in flasks with ambient air.

Under fluctuating light, the Del27 strain also appears to overtake the wild type and the Com 27 is doing better than wild type; however, in the text our attention is only drawn to the OE27 strain. Further comment on these growth curves would be helpful.

The text has been modified to further discuss the growth curves.

- In Figure 3B it appears the legend in the figure panel is incorrect (you could also remove some of the numbers on the y axes in panels E and F).

The legend in Figure 3B is correct. Panels E and F have been modified accordingly.

-When the protocol for delivering the 1000 flashes was described in the methods it was not clear to me exactly how this was done. Were 1000 actinic flashes given over a period of 200 seconds?

Yes, the 1000 flashes were delivered at 500 ms intervals, so the total flashes were given over a period of 500 seconds. We have added clarifying text to explain the procedure in more detail.

Reviewer #2: The authors provide a physiological analysis regarding the function of an assembly protein, Psb27, that is involved in the maturation of the PS2 complex. The authors make some interesting observations on mutants that either lack the protein or overexpress the protein regarding chlorophyll fluorescence quenching characteristics. They are able to trace the differences to the presence of a sub-population of PS2 existing as an assembly intermediate influenced by the abundance of the assembly protein. They arrive at the conclusion that Psb27 is involved in the regulation of excitation energy delivery to photosystem two during the assembly process to avoid damage in the precursor forms of the complex. This is an interesting and significant contribution to our understanding of the assembly process.

Comments:

Overall, the writing is very nice although a couple of references to similar phenomena in the literature will improve as noted below. The analysis of the fluorescence appears to be excellent, but some details need attending. Generally the figures are OK, but there are some issues.

Figure 1 and associated discussion: The growth curves of the different strains gives surprising results. What type of replication was performed for the growth experiment? The differences are significant, but not necessarily expected. As one example, it appears that the Del27 actually grows to higher densities compared to the other strains. Here the details of the statistics are important for validating this interesting finding.

We have 3 replicates associated with the figure presented in our manuscript in either continuous light or fluctuating light conditions. Under longer time scale growth condition, without the extra process (assembly phase) of Psb27 attaching to the PSII subcomplex, the Del27 strain get adapted to the light scheme (constant and even fluctuating light) and thus overtake the WT.

Fig. 2:

The time axis is more appropriately scaled in seconds rather than milliseconds.

The axis has been rescaled appropriately.

Is the label F_0' correctly placed in Panel A? Wouldn't this more correctly be steady state max. fluorescence under illumination?

We agree this label was placed confusingly. We have modified the figure appropriately.

Methods section:

For the fluorescence analysis, it's important to describe which growth phase that cells were obtained from. Also, minor issue regarding the description of the program used in the software: it's the S-state

programming software, but it's not being used for that purpose, but is instead being modified for a many flash experiment.

Yes, the multiple flashes experiment was done with the S-state programming customized to multiple single-turnover flashes. We used multiple flashes to compare charge recombination of the dark-adapted cells after a single flash and its associated S-state, and to compare the first charge recombination with that of cells after multiple flashes. Each flash also contributes the redox state of PSII and thus thylakoid membrane overall, so the observed F_0 , F_m , etc. values reflect the redox state changes after actinic light illumination at a longer time scale (>5 sec). We have added clarifying text to the methods section and to the results section.

The construction to obtain each of the strains well documented except for the overexpression strain, OE27. This appears to be a strain that was previously constructed and described in the literature by Avramov et al (?)--in any event the construction of this strain, particularly with regard to the promoter, needs description.

While similar to the strain constructed by Avramov *et al.* (Avramov, Hwang et al. 2020), this was a strain constructed in the Pakrasi Lab 10 years ago and was reported at a conference in 2011. It introduces the *psb27* gene under its native promoter at the *psbA1* neutral site, with Gm resistance cassette. We have modified the methods section to describe this strain more clearly, and added a gel showing the PCR product of the A1 locus and a western blot of Psb27 in the overexpression strain to Figure S1 to clarify.

Discussion section:

The authors should discuss why increased expression of Psb27 has not already been fixed in natural populations if it can be experimentally shown, as they do here, that higher levels of Psb27 increase fitness. It's probably worth noting that the concept of fitness is complex.

We agree that the concept of fitness is complex, and hard to make definitive statements about. By suggesting that Psb27 increases fitness, we mean that the reason for its presence rather than absence is to contribute to PSII function, enhancing overall fitness. It is quite possible that its overexpression is detrimental under certain conditions as well.

We have added further comment on this point to improve the discussion section.

The authors may wish to consider whether or not the phenomena being observed for fluorescence quenching and F_0 levels, relates to some previous observations. For example, it was shown that the loss of PsbU changes the fluorescence characteristics of cyanobacteria (Veerman et al *Biochemistry* 44:1693). It was also showing that the removal of the manganese cluster decreases the coupling between phycobilisomes and PSII (Hwang et al *Biochemistry* 47:9747).

These are relevant and important previous observations. We have added discussion of these references to improve the discussion section.

Avramov, A. P., H. J. Hwang and R. L. Burnap (2020). "The role of Ca(2+) and protein scaffolding in the formation of nature's water oxidizing complex." *Proc Natl Acad Sci U S A* **117**(45): 28036-28045.


Davinagracia, J. (2021). The Role of Lipoprotein Psb27 in Photosystem II Biogenesis. Master of Science, University of Otago.

Jackson, S. A., J. R. Hervey, A. J. Dale and J. J. Eaton-Rye (2014). "Removal of both Ycf48 and Psb27 in *Synechocystis* sp. PCC 6803 disrupts Photosystem II assembly and alters Q(A)(-) oxidation in the mature complex." *FEBS Lett* **588**(20): 3751-3760.

Komenda, J., J. Knoppova, J. Kopečna, R. Sobotka, P. Halada, J. Yu, J. Nickelsen, M. Boehm and P. J. Nixon (2012). "The Psb27 assembly factor binds to the CP43 complex of photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803." *Plant Physiol* **158**(1): 476-486.

Singh, H. (2017). The role of the low-molecular-weight proteins of the CP43 pre-assembly complex of Photosystem II. Doctor of Philosophy, University of Otago.





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