Supporting Information

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SI Materials and Methods

His-Tagged Strains Construction. The *Synechocystis* PCC 6803 genome region containing the OCP gene (*slr1963*) and the hypothetical gene *slr1964* was amplified by PCR using the XhoIcar7 and the SpeIcar6 oligonucleotides (all of the oligonucleotides used in this work are described in Fig. S1). The PCR product of 2.2 kb was cloned in SK(+) ampicillin-resistant vector. Then, a 1.3-kb kanamycin resistance gene or a 2.2-kb DNA fragment containing the *aadA* gene from Tn7, conferring resistance to spectinomycin and streptomycin (Sp/Sm) were introduced in the unique HindIII restriction site of the *slr1964* gene. The plasmid obtained was used to construct C-terminal or N-terminal His-tagged OCP mutants by site-directed mutagenesis using the Quickchange XL site-directed mutagenesis kit of Stratagene and synthetic mutagenic oligonucleotides. *Synechocystis* 6803 WT cells were transformed by double recombination by these plasmids (Fig. S2*A*). The mutant containing the His-tagged N-terminal OCP presented a low blue-light induced fluorescence quenching (data not shown) and it was not further used. To obtain a strain overexpressing the OCP, the *slr1963* and *slr1964* genes were amplified by PCR using the 5′OCPNdeI and 3′OCPHpaI oligonucleotides. The PCR product of 1.5 kb was cloned in the pPSBA2 ampicillin-resistant vector (31). Nucleotides encoding for 6 His were added in the 3′side of the *slr1963* gene by site-directed mutagenesis. A 1.3-kb kanamycin resistance gene was then inserted in the unique HpaI restriction site of pPSBA2. The plasmid obtained was used to transform by double recombination the WT and the Cter6HSp/Sm mutant (Fig. S2*B*). The point mutation (W to S) was added in the *slr1963* gene in the normally expressing and overexpressing plasmids by site-directed mutagenesis.

Purification of the Orange Carotenoid Protein. In this study, we used OCP isolated from the K_m-resistant and Sp-resistant His-tagged OCP mutants. His-tagged OCP mutant cells grown as described in Wilson *et al.* (9) (≈15 litres) at OD800 = 1 were harvested by centrifugation. The pellets were resuspended in 0.1 M Tris·HCl pH = 8 supplemented with 1-mM benzamidine, 1-mM aminocaproic acid, 5-mM EDTA, 1-mM phenyl-methylsulfonyl fluoride, and 50-μg/ml^{-1} DNase I (bovine pancreas type IV, Sigma) to a final chlorophyll concentration of 1 mg.ml^{-1}. The suspension was broken in mild light with four cycles of French Press at 700 psi. After removing unbroken cells by centrifugation, membranes were pelleted by centrifugation at 180,000 × *g* for 1h30 min at 4°C. The supernatant was loaded on a column of Ni-ProBond resin (Invitrogen), which had been equilibrated with 40-mM Tris·HCl pH8, 150-mM K^+ phosphate buffer, 500-mM NaCl, and 10% glycerol. The column was washed with 4 volumes of the equilibrating buffer supplemented with 30-mM imidazole and the OCP was eluted with 160-mM imidazole in the equilibrating buffer. The eluate was dialysed against 40-mM Tris·HCl pH = 8 at 4°C for 24 h. The protein was further purified on a Whatman DE-52 cellulose column. The OCP was eluted using 80-mM NaCl in 40-mM Tris·HCl pH 8, dialysed against 40-mM Tris·HCl pH = 8 at 4°C for 24 h and concentrated to OD495 = 2. To determine protein molecular weight and purity, the protein was analyzed by SDS-page on a 12% polyacrylamide/2M urea in a Tris/Mes system (32) (Fig. S3*A*). UV and visible absorption spectra of the protein were recorded on Uvikon XL. The A467/A280 absorbance ratio was used as a criterion of purity and in most preparations was 1.97 (Fig. S3*B*). To quantify the OCP present in the different strains, membrane-phycobilisome fractions were isolated (10) and were analyzed by SDS page on 12%/2M urea in a Tris/Mes system (32). The OCP was detected by a polyclonal antibody against OCP (10).

Carotenoid Characterization. Carotenoids were extracted as previously described (20). Mass analysis was conducted on aliquots of the dried extract dissolved in 400 μl of acetonitrile/water (90:10, v:v) using a Quattro LC instrument with an electrospray ionization Z-spray interface (Micromass), MassLynx software, an Alliance 2695 separation module (Waters), and a Waters 2487 dual UV detector set at 450 nm. Separation was achieved on two 100 × 4.6 mm and 150 × 4.6 mm, reverse-phase Adsorbosphere HS C18 3-mm columns in series () using a linear 20-min gradient of ethyl acetate (0 to 95%) in acetonitrile/water/triethylamine (9:1:0.01, v:v:v) at a flow rate of 0.5 ml/min and at a temperature of 30°C.

Ultrafast Spectroscopy. Ultrafast spectroscopy was carried out on a transient absorption setup based on an amplifier Ti:sapphire laser system operating at 1 kHz (Coherent Legend-USP seeded by a broadband Vitesse oscillator) equipped with optical parametric amplifiers (Coherent OperA). A 475 nm 50 fs pump pulse at 200-nJ energy was used excite the sample. A white light continuum was used for probing. The polarization between pump and probe beams was set at the magic angle 54.7°. The sample was placed in a quartz flow cell of 2-mm optical path and circulated by means of a peristaltic pump. The probe beam was spectrally dispersed by a spectrograph and projected on a 256-element diode array (Hamamatsu). The time resolved raw data, acquired by automatized measurement routine, were globally analyzed in term of interconverting evolution-associated difference spectra: that is, 1→ 2→ 3→ and so forth. Global analysis (33) was applied to estimate the lifetime and relative concentration of each component at each wavelength. TIMP, an R package for modeling multiway spectroscopic measurements, was used.

A

Oligonucleotides used to amplify the *slr1963* and *slr1964* genes

XhoI creating primer car 7

5’-CGGCCGCTCGAGTGACTATTGTCGCGACTAGGGA-3’

SpeI creating primer car 6

5’CACCGGACTAGTCAAAAACTATCTGCTGGCGATCG-3’

NdeI creating primer 5’OCPNdeI

5’-GCATTCCATATGCCATTCACCATTGACTCTGCCC-3’

HpaI creating primer 3’OCPHpaI

5’-GCATCGGTTAACGGTGTACACCTACAGCAAGCAA-3’

B

Oligonucleotides to introduce a His-tag in the OCP:

OCP Nter6H a : containing the *PmlI* restriction site

5’-CACCACCACCACCACCACGTGACCATTGACTCTGCCCGCGGAATTTTCC-3’

OCP Nter6H b :

5’-CACGTGGTGGTGGTGGTGTGGCATAGGTTTTATACGTGAATCAACT-3’

OCP Cter6H a : containing the *ApaLI* restriction site.

5’-GTGCACCACCACCACCACCACTAGAATAACTCCCTTCAGAGTTTTGTCT-3’

OCP Cter6H b :

5’-GTGGTGGTGGTGGTGGTGCACAGCAAAGTTGAGTAATTCTTTGGGGGAA-3’

C

Oligonucleotides used to introduce the W110S mutation (TGG to TCG)

5'-CATTAAACTTGGCTTCTCGTACCGTTTAGGCG-3'

5'-CGCCTAAACGGTACGAGAAGCCAAGTTTAATG-3'

Fig. S1. Oligonucleotides used as primers in the construction of His-tagged mutants.

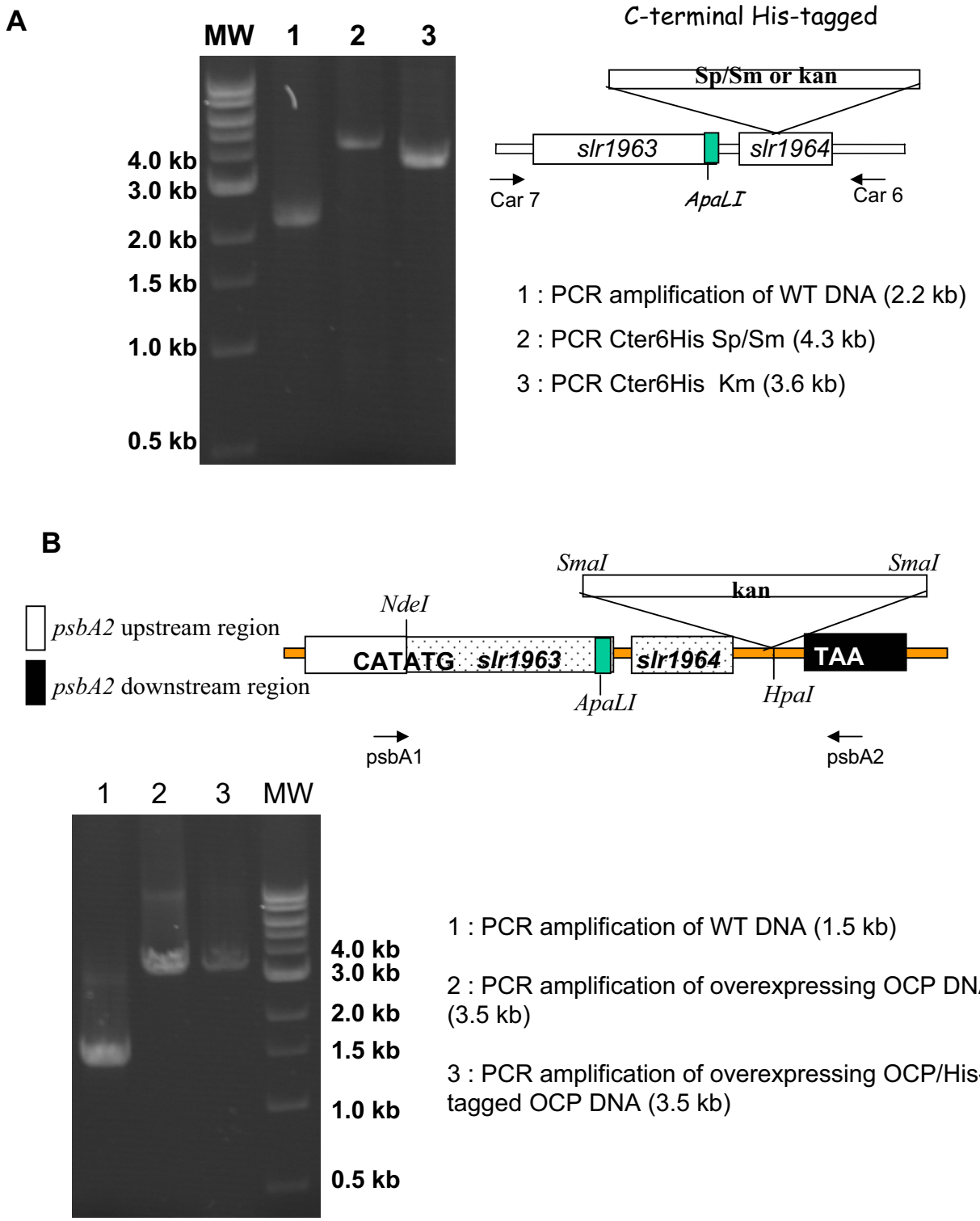

Fig. S2. Construction of OCP mutants. (*A*) Gene arrangement of the *slr1963* and *slr1964* genes and amplification of genomic *Synechocystis* DNA from His-tagged mutants using *car6* and *car7* oligonucleotides as primers. The amplification gave a unique fragment of 4.3 kb (including the Sp/Sm resistance cassette) or of 3.6 kb (including the K_m resistance cassette) instead of 2.2 kb observed with WT DNA, indicating that in the mutants a complete segregation was obtained. The presence of the nucleotides coding for the 6 His was confirmed by cutting with the restriction enzyme ApaLI and by DNA sequencing. (*B*) Gene arrangement in the *psbA2* region of the mutant overexpressing the OCP and amplification of genomic *Synechocystis* DNA from the WT and the overexpressing OCP mutants using *psbA1* and *psbA2* as primers. The amplification gave a unique fragment of 3.5 kb in the mutants and a fragment of 1.5 kb in the WT. *psbA1* : 5′-ACGCCCTCTGTTTACCCATGGAA-3′ *psbA2* : 5′-CCAGGCCTCAACCCGGTACAGAG-3′

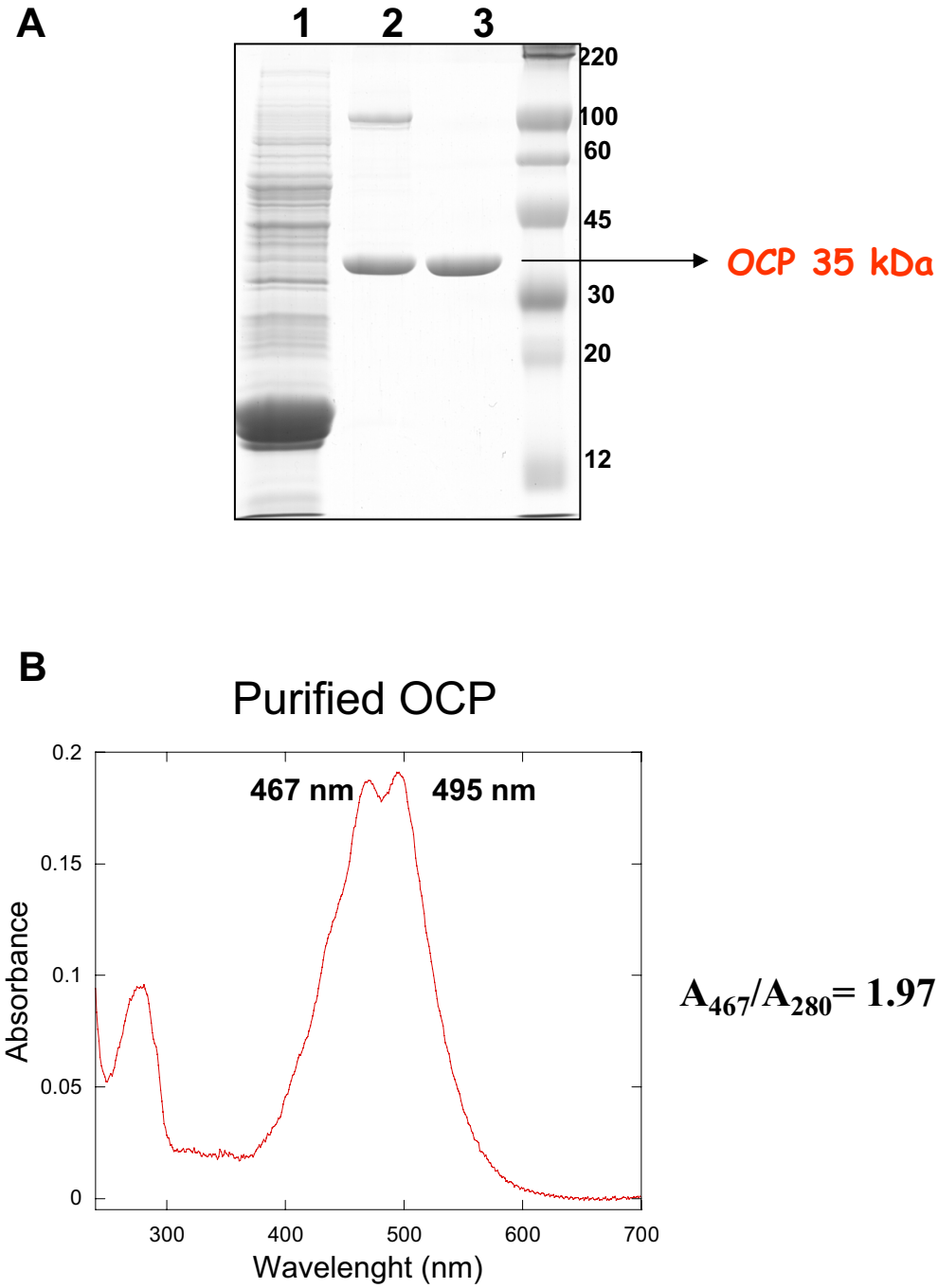

Fig. S3. Isolation of the OCP protein. (*A*) Coomassie blue-stained gel electrophoresis of the soluble phase of His-tagged OCP *Synechocystis* 6803 mutant cells (*A1*), after elution of the Ni-column (*A2*), and after elution of the DE-52 column (*A3*). (*B*) Absorbance spectrum of the isolated OCP. The high ratio A467/A280 indicates a high purity of the protein.

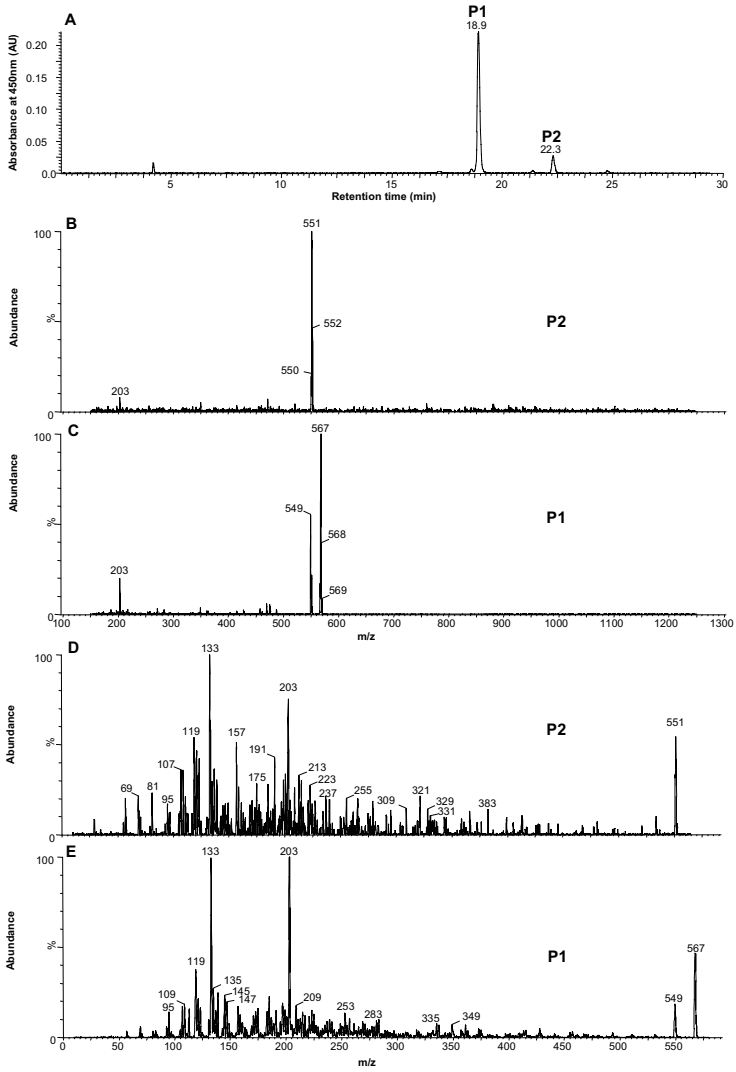

Fig. S4. Liquid Chromatography-Mass Spectrometry (LC–MS) analysis of products extracted from the OCP protein. (*A*) HPLC analysis shows the two major products P1 and P2 (AU, absorption units). (*B*) and (*C*) show the mass spectra (positive mode) of P2 (molecular mass: 550) and P1 (molecular mass: 566) products, respectively. (*D*) and (*E*) show the collisionally induced dissociation (CID) spectra of the MH+ ions of P2 (at 30 eV) and P1 (at 25 eV) products, respectively. HPLC-MS analysis provides molecular mass data supporting the assignment of the structure of the P2 product as echinenone (based on retention time compared with native cyanobacteria echinenone [data not shown] and molecular mass) and of P1 product as 3′ hydroxyechinenone (16 mass units heavier than echinenone and similar CID spectra than echinenone with prior loss of 18 mass units corresponding to the 3-hydroxyl group, 549 M-H_2O).

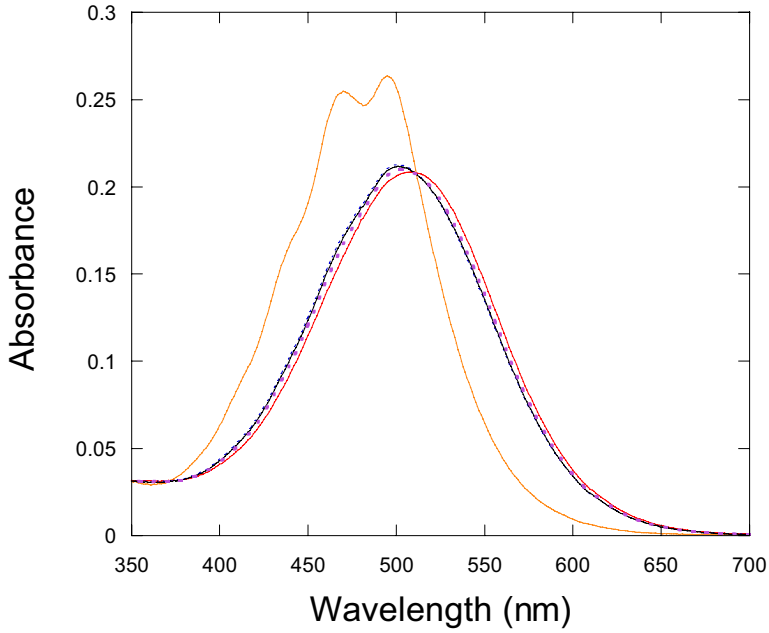

Fig. S5. Absorbance spectra of the isolated OCP^{o} before (*orange*) and after illumination. We took advantage of the slow reconversion at low temperature to test if once formed, the red OCP^{r} can be photoconverted to OCP^{o}. The OCP^{r} (*filled red*) was illuminated for 3 min (12°C) with orange-red light preferentially absorbed by the red form: 600–650 nm (*dashed blue*) and 530–650 nm (*dashed violet*) at 1,200-μmol photons m^{-2} s^{-1}. During orange illumination, a very slight blue shift of the spectrum was observed identical to that induced by 3 min of darkness (*filled black*). Thus, there is no photoreaction converting the red form into the orange form. This suggests that the OCP does not detect different quality of light; it detects only light intensity. The spectrum of the stable OCP^{o} dark form is also shown (*filled orange*).

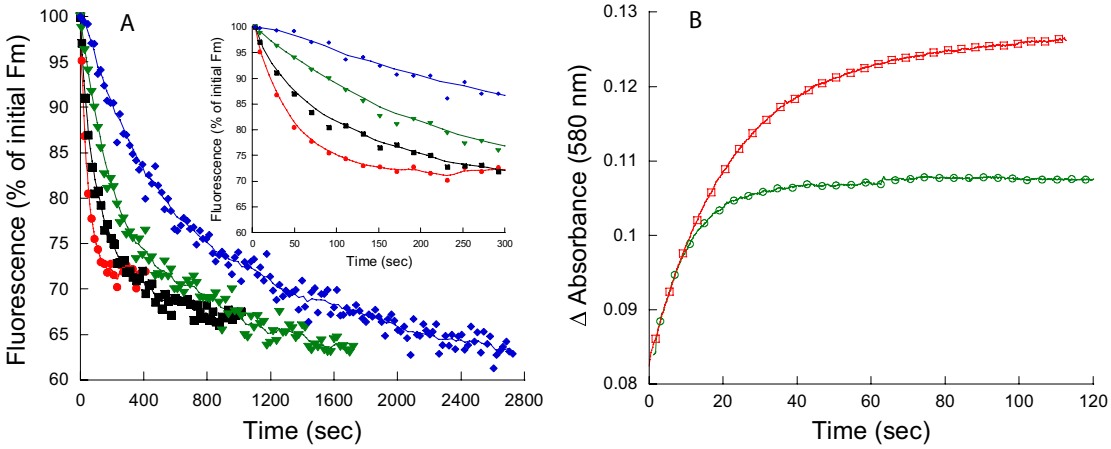

Fig. S6. Blue-green light-induced quenching in whole *Synechocystis* 6803 cells at different temperatures. (*A*) Decrease of Fm′ during exposure of whole WT cells to 740-μmol photons m^{-2} s^{-1} of blue-green light (400–550 nm) at 33 (*red*), 24 (*black*), 16 (*green*), and 7°C (*blue*). (*B*) Photoconversion from the OCP^o to the OCP^r form using a 740-μmol photons m^{-2} s^{-1} blue-green light intensity at 16 (*green*) and 33°C (*red*). These results show that the maximum fluorescence quenching was larger at lower temperatures: 30% of initial Fm′ at 33°C (reached in 2 min) and >43% at 7°C (after one hour illumination). The curves also show that *in vivo*, the kinetics of the induction of the fluorescence quenching decreased by lowering the temperature [see also (14)] and that even at 33°C the induction is slower than the photoconversion of OCP^o to OCP^r, suggesting that this conversion is not the rate-limiting step of the NPQ process. However, although cells and isolated OCP were illuminated with the same light intensity, we do not know the real light intensity detected by the OCP in the interior of the cell. Thus, we can also suggest that only at temperatures lower than the growth temperature (34°C) is the photoconversion not the rate-limiting-step.