Light Harvesting and Blue-Green Light Induced Non-Photochemical Quenching in Two Different C-Phycocyanin Mutants of *Synechocystis* PCC 6803

Lijin Tian,[†] Ivo H. M. van Stokkum,[‡] Rob B. M. Koehorst,^{†,§} and Herbert van Amerongen^{*,†,§}

[†]Laboratory of Biophysics, Wageningen University, P.O. Box 8128, 6700 ET, Wageningen, The Netherlands

[‡]Biophysics Group, Department of Physics and Astronomy, Faculty of Sciences, VU University, DeBoelelaan1081, 1081 HV Amsterdam, The Netherlands

[§]MicroSpectroscopy Centre, Wageningen University, P.O. Box 8128, 6700 ET, Wageningen, The Netherlands

Supporting Information

ABSTRACT: Cyanobacteria are oxygen-evolving photosynthetic organisms that harvest sunlight and convert excitation energy into chemical energy. Most of the light is absorbed by large light harvesting complexes called phycobilisomes (PBs). In high-light conditions, cyanobacteria switch on a photoprotective mechanism called non-photochemical quenching (NPQ): During this process, absorption of blue-green light transforms the inactive orange form of the orange carotenoid protein OCP (OCP^o) into the red active form OCP^r that subsequently binds to the PB, resulting in a substantial loss of excitation energy and corresponding decrease of the fluorescence. In wild-type cells, the quenching site is a bilin



decrease of the fluorescence. In wild-type cells, the quenching site is a bilin chomophore that fluoresces at 660 nm and which is called APC_Q^{660} . In the present work, we studied NPQ in two different types of mutant cells (CB and CK) that possess significantly truncated PBs, using spectrally resolved picosecond fluorescence spectroscopy. The results are in very good agreement with earlier *in vitro* experiments on quenched and unquenched PBs, although the fraction of quenched PBs is far lower *in vivo*. It is also lower than the fraction of PBs that is quenched in wild-type cells, but the site, rate, and location of quenching appear to be very similar.

1. INTRODUCTION

Cyanobacteria (also known as blue-green algae) are supposedly the earliest and one of the most successful species on earth to perform oxygenic photosynthesis. They play a key role in global carbon cycling and atmospheric oxygen accumulation. Due to their fast growth rate and facile genetic modification, cyanobacteria are thought to possess great potential for hydrogen and biofuel production in the future. Similar to algae and plants, cyanobacteria contain two large photosynthetic complexes in their thylakoid membranes, named photosystem I (PSI) and photosystem II (PSII), and here the light-dependent reactions of photosynthesis are taking place.¹⁻⁴ Besides PSI and PSII, cyanobacteria contain giant light harvesting or antenna systems, the so-called phycobilisomes (PBs). They are located on the stromal side of the thylakoid membrane, and they dramatically increase the cross section for light absorption. PBs have a complicated structure which also depends on species.⁵ PBs of wild-type Synechocystis PCC 6803 (hereafter called Synechocystis) are composed of six Cphycocyanin (C-PC) rods and three allophycocyanin (APC) core cylinders; one C-PC rod contains three hexametric disks, each containing 18 bilin pigments, and in addition a few linker proteins; one core cylinder contains four trimeric APC disks, each containing 6 pigments. One core cylinder is not directly in contact with the thylakoid membrane with the photosystems, and it contains only APC trimers emitting at 660 nm, while

both basal cylinders each contain three chromophores that emit around 680 nm and serve as a terminal energy acceptor of the PB.^{6,7} The whole PB is anchored by the core-membrane linker Lcm to the thylakoid membrane. Detailed PB structures are described by Arteni et al.,^{8,9} whereas further information about the pigments and their spectroscopic properties can be found in refs 5, 10, and 11. It is important to realize that the core contains 66 pigments that fluoresce around 660 nm, whereas only 6 pigments fluoresce around 680 nm.

The presence of antenna systems on the one hand increases the capability of the photosystems to absorb sunlight, which is important in low-intensity sunlight, but on the other hand, this also increases the probability that photodamage occurs in the presence of strong light. To overcome this problem, different kinds of self-protection mechanisms were developed during the long evolutionary history of the photosynthetic organisms, including cyanobacteria. One important protection mechanism, called blue-green light induced NPQ, was found in all cyanobacteria that contain the so-called orange carotenoid protein (OCP), and the phenomenon has been studied

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extensively during the past 10 years.^{11–29} This protection is realized *in vivo* by interactions between at least three different proteins, including OCP, the fluorescence recovery protein (FRP), and one of the core proteins of the PBs.^{24,30} In strong blue-green light, OCP changes from its inactive orange form (OCP°) into its red active (OCP^r) form, binds to APC, and quenches to a large extent the electronic excitations in the PBs by dissipating the excitation energy as heat. In this way, most of the excitations are prevented from reaching the photosystems, thereby protecting them against photodamage. When the intensity of the blue-green light is significantly decreased again, the binding between OCP^r and APC is released with the help of FRP, and the quenching process is stopped and thus the excitation energy can reach the reaction centers (RCs) again in a "normal" way.²⁴

In a recent study, we have performed time-resolved fluorescence measurements on isolated PBs from wild-type cells and from two different mutants. We found the same quenching site and a similar quenching rate in vitro as was reported before for wild-type cells in vivo.11 The in vitro measurements were performed in the presence of a large excess of OCP as compared to PBs (approximate ratio of 40:1), which led to 85% of quenching for the CK phycobilisomes and even higher values for the CB and wild-type PBs. Earlier measurements on CK cells also showed the presence of NPQ, but the level of quenching was below 10%. This is probably partly due to the occurrence of competing excitation energy transfer (EET) to the photosystems but possibly also due to the presence of FRP and to relatively low concentrations of OCP. In order to investigate whether the rates of EET and the rates and locations of quenching as observed for isolated PBs are also the same for the two types of mutant cells in vivo, the present study was undertaken. Another reason to study these antenna size-truncated mutants is the increasing interest in the question to which extent the antenna size influences the production of biomass.^{31,32} Although this is not the main issue of the present work, a kinetic model including light harvesting and photoprotection as will be proposed here can potentially be useful. One of the two studied mutants, called CK, contains PSI and PSII as well as the APC core, but it completely lacks the C PC rods.^{33,34} The other one (CB) is lacking both the intermediary and the core-distal C_PC hexamers and only contains coreproximal C PC besides the other photosynthetic complexes, PSI, PSII, and APC core.³⁵ Picosecond time-resolved fluorescence measurements were performed using a synchroscan streak-camera system. The obtained data sets were analyzed by using both global analysis and compartmentalbased target analysis in a similar way, as was done before for wild-type cells.²⁶ It turns out that the blue-green light quenching takes place on APC₆₆₀ pigments in both mutants with a slightly smaller quenched fraction than in wild-type cells under similar conditions. The quenching rates cannot be determined very accurately due to the small fraction of quenched PBs for both mutants, but the quenching rates appear to be rather similar in vivo and in vitro.

2. MATERIALS AND METHODS

2.1. Growing Conditions of Synechocystis Cells and Construction of Mutants. For the construction of the CK and CB mutants, we refer to refs 33-37. Two mutants were grown photoautotrophically in a modified BG11 medium,³⁸ with twice the concentration of sodium nitrate and with 10 mM NaHCO₃ added. Cells were grown in 250 mL flasks with 60

mL growing volume in a rotary shaker (45 rpm) at 30 °C and illuminated by white light, giving a total intensity of 40 μ mol·m⁻²·s⁻¹. All the cells were kept in the logarithmic growth phase by refreshing the medium every 2 or 3 days depending on growth rate.

2.2. Steady-State Absorption. Absorption spectra were recorded on a Cary SE spectrophotometer, equipped with an integrating diffuse reflectance sphere (DRA-CA-50, Labsphere) to minimize distortion of the absorption spectra by light scattering. The optical path length of the cell was 1 cm. All measurements were performed at room temperature.

2.3. Time-Resolved Fluorescence. Time-resolved fluorescence measurements were performed on a picosecond streak-camera system.^{36,39} Images were corrected for background signals and detector sensitivity, averaged, and sliced up into traces of 4 nm wide. The time window was 800 ps for all measurements. A laser power of 30 μ W was used. The excitation spot size was typically $\sim 100 \ \mu m$ in diameter, and the laser repetition rate was 250 kHz. The instrument response function (IRF) was described with a double Gaussian, consisting of a dominating narrow Gaussian of ~8.0 ps fwhm on top of a minor broad Gaussian of \sim 40–50 ps in the case of 590 nm excitation. For the 400 nm excitation experiments, one Gaussian of ~9.2 ps fwhm was sufficient to describe/fit the data. The sample was stirred with a magnetic stirring bar (stirring rate ~ 10 Hz). All measurements were performed at 21 °C, and one measurement took 20 min. Cells were concentrated 3-5 times by low-speed centrifugation and dark adapted for 5 min before the time-resolved fluorescence measurements were started.

Conditions for measuring time-resolved fluorescence of cells during quenching have been described previously;²⁶ the same actinic blue-green light was used.

2.4. Data Analysis. Data obtained with the streak-camera setup were analyzed with the TIMP package for the "R project for Statistical Computing (R Development Core Team 2008)" and its graphical user interface (GUI) Glotaran; for details, see refs 40 and 41.

With global analysis, the data were fitted globally as a sum of exponential decay functions convolved with an IRF and the amplitudes of each decay component as a function of wavelength constitute the so-called decay-associated spectra (DAS). Apart from providing an objective mathematical description of the data sets, this analysis provides global insight into the underlying excitation energy transfer and charge separation processes.⁴²

For a more detailed analysis of the data, use is made of target analysis where the data sets are fitted with different compartmental schemes (also called target models). The spectrum of each compartment and the energy transfer rates between them are estimated together with various decay rates, for instance, corresponding to charge separation or nonphotochemical quenching. The methodology of global and target analysis is described in detail in ref 43.

3. RESULTS

3.1. Absorption Spectra. The absorption spectra of WT *Synechocystis* and CK and CB mutants are shown in Figure 1. The spectra have been normalized at 680 nm. Although the spectra were recorded with an integrating sphere to minimize distortion due to scattering, the spectra are still deformed to some extent, partly by scattering and partly by sieving, especially on the blue side (i.e., at short wavelengths). The

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Figure 1. Room-temperature absorption spectra of wild-type and mutant *Synechocystis* cells. All spectra are normalized at 680 nm.

CK mutant shows absorption due to the APC core, which contributes substantially to the spectrum between 550 and 670 nm and both photosystems which are mainly responsible for the absorption below 500 nm and the large absorption band around 675 nm. The CB mutant shows extra absorption (mainly between 550 and 650 nm) due to the additional presence of C-PC, but the absorption is lower than that of the WT cells. It should be noted that at 400 nm the absorption is mainly due to Chl's and Car's and the presence of APC and C-PC hardly alters the absorption at this wavelength (the contribution is less than 10%³⁶). Therefore, in the time-resolved measurements with 400 nm excitation, mainly PSI and PSII are excited. Excitation at 590 nm on the other hand, which was used for studying the CK, CB, and WT cells, leads to a large fraction of directly excited PBs.

3.2. Time-Resolved Fluorescence. Time-resolved fluorescence spectra were recorded with a time resolution of several ps, using two different excitation wavelengths: 400 nm (see Figure S1 in the Supporting Information), exciting mainly PSI and PSII, and 590 nm (see Figure S1, Supporting Information), leading to increased excitation of the PBs. A time window of 800 ps was used for all experiments.

3.2.1. Global Analysis. Excitation at 400 nm. In Figure 2, the DAS are shown that result from global analysis of the fluorescence kinetics of the two mutants upon 400 nm excitation. In both cases, a fit with four lifetime components leads to a satisfactory description of the data. The corresponding lifetimes of the DAS are also given in Figure 2. The 5 ps component has a conservative spectrum, whereas the 27/28 ps component is all-positive and dominates the kinetics for both mutants. The 5 and 27/28 ps lifetimes and corresponding spectra are very similar to those observed in previous studies for isolated PSI and for BE cells, that contain PSI but no PSII and PBs.^{36,44} The 5 ps DAS corresponds to fast excitation-energy equilibration between pigments fluorescing below 700 nm and so-called red pigments fluorescing above 700 nm. The 28 ps component mainly represents fluorescence decay due to charge separation from the equilibrated excited state of PSI. These results indicate that the PSI kinetics can be separated rather well from the kinetics of the rest of the system. Note that the 400 nm excitation light leads to some direct excitation of the PBs, about 7%, as shown in Table 1, and this



Figure 2. Fitting results (DAS) of the time-resolved fluorescence data obtained with the streak-camera setup using 400 nm excitation and a time window of 800 ps: (a) CK and (b) CB mutant cells. The corresponding lifetimes are listed in the figure. The solid lines correspond to unquenched cells, and the dashed spectra correspond to quenched cells.

has a small effect on the shape of the DAS of these two fastest components, especially for the CB mutant.

Besides these two DAS, two extra lifetime components are observed, with values of ~ 100 and ~ 600 ps, respectively. Although for the CK and CB cells the amplitudes of the third and fourth DAS are rather small, these components are responsible for a relatively large amount of the steady-state fluorescence below 680 nm due to the long fluorescence lifetimes. For the CK mutant, the two slowest DAS components (137 and 629 ps) have similar shapes peaking at 680 nm, representing the emission of mainly Chl's in PSII. The slowest DAS components (106 and 588 ps) of the CB cells show additional fluorescence due to the presence of C-PC. The additional fluorescence of APC with a peak around 660 nm in the 106 ps component is due to extra excitations that have arrived on APC from C-PC. The fluorescence around 640 nm in the slowest 588 ps DAS is due to part of the C-PC that apparently is not able to transfer its excitation energy. It is not due to Boltzmann-equilibrated excitations in intact PBs because it can easily be estimated that only $\sim 1\%$ of the excitations should reside on C-PC in that case, which should hardly be visible in this 588 ps DAS.

When the cells are brought into the quenched state by illumination by strong blue-green light, only a minor change in the DAS is obtained after 400 nm excitation, reflecting the fact

Table 1. Initial, Relative Absorbed Energies	: (%) Estimated from the Target Analysis ^a
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mutant	λ_{ex} (nm)	PSI_U	PSII_U	PSI_C	PSII_C	total PSI	total PSII	PSI/ II_Chl	PSI/ II_RC	PB_I	PB_II	C_PC_U	total PB	total chi	total exc
СК	400	69	11	10	3	79	14	6	2	3	4	0	7	93	100
	590	31	5	4	1	35	6	6	2	28	31	0	58	42	100
CB	400	67	6	15	5	82	11	7	3	3	3	1	7	93	100
	590	12	1	3	1	15	2	7	3	29	33	20	83	17	100

"Energy is considered to be distributed over PSI and PSII coupled (PSI_C and PSII_C) and not coupled to PBs (PSI_U and PSII_U), PBs coupled to PSI and PSII (PB_I and PB_II), and unattached C-PC (C-PC_U). The ratios PSI/II_Chl and PSI/II_RC are based on the number of chlorophylls and reaction centers, respectively. The total excitation was normalized to 1.

that the Chl's are not quenched directly but quenching takes place in the PBs.^{14,15,22,26}

590 nm Excitation. Time-resolved measurements were also performed on CK and CB cells with 590 nm excitation in order to "selectively" excite the outer antenna (APC/C-PC) and study excitation energy transfer (EET) to the RCs. The DAS and corresponding lifetimes for CK and CB cells are shown in Figure 3a and b, respectively. For the CK mutant, the 30 ps DAS has a complicated shape. On the one hand, it shows some EET characteristics reflecting transfer from APC (~660 nm) to PSII/PSI, and at the same time, there seems to be some contribution of PSI decay with fluorescence extending to the red. The 75 ps DAS, peaking at 660 nm, reflects mainly the fluorescence decay of APC. It is not accompanied by a negative



Figure 3. DAS of CK (a) and CB (b) mutants upon excitation of 590 nm. The corresponding lifetimes are listed in each figure. The solid lines correspond to unquenched cells, and the dashed spectra correspond to quenched cells.

component with similar area at longer wavelength, which would have been indicative of EET. However, this component is possibly due to EET to PSII followed by immediate charge separation in PSII with open RCs in which case no negative component is expected. Also, EET to PSI would lead to a similar spectrum. The DAS of the slowest component 535 ps is peaking at 678 nm, which is slightly blue-shifted from the DAS of the two slowest components upon 400 nm excitation (see Figure S2 in the Supporting Information) and shows a shoulder around 660 nm. These differences are mainly caused by the different excitation distributions over PBs and photosystems upon 590 and 400 nm excitation. At 590 nm, mainly PBs are excited and the excitations equilibrate over the chromophores in the PBs and the Chl's in the photosystems, while, at 400 nm, also many Chl's are excited in uncoupled photosystems, that do not equilibrate with the blue-shifted chromophores of the PBs (for more details, see target analysis below). Although the presence of unconnected APC cannot be excluded here, the fraction of unconnected APC must be rather small, if not negligible.

For the CB mutant, five components are needed to get a satisfactory fit of the data at all wavelengths. From the DAS, several clear EET steps can be observed (Figure 3b). The fastest component of 7 ps is almost entirely negative with a peak around 645 nm. It probably reflects EET within C-PC from blue pigments with relatively low dipole strength to somewhat more red-shifted pigments with higher dipole strength.²⁶ The 25 ps component shows a positive peak at 640 nm and a negative one at 663 nm and is indicative of EET from C-PC to APC. The 77 ps component has a positive peak around 655 nm and a negative one at 680 nm, reflecting EET to both the lower energy pigments of the APC and Chl's in PSII.²⁶ The 135 ps component is rather similar to the 106 ps component that is obtained upon excitation at 400 nm, as shown in Figure S2 of the Supporting Information. The 765 ps DAS has its main peak at 680 nm (PSII), but there is a substantial shoulder around 640 nm which is ascribed to some disconnected C-PC. The presence of some disconnected or badly connected C-PC was also seen upon excitation at 400 nm.

3.2.2. Target Analysis. To analyze in more detail EET between the different "pools" of pigments (compartments), target analysis was performed, leading to species associated spectra (SAS) for the different compartments. A "pool" represents a group of pigments that shows fast internal excitation equilibration.

In our previous work, a detailed model was built that was used to determine the quenching site of blue-green light induced NPQ in *Synechocystis*.²⁶

It should be realized that, under the measuring conditions used, some PSII reaction centers are open and others are



Figure 4. Compartmental schemes for target analysis. Compartments (colored boxes) represent pools of chromophores that are spectrally indistinguishable, and within which excitation energy is equilibrated on a time scale that cannot be resolved with the setup used. Forward and backward energy transfer processes between these compartments are indicated by the arrows, while rate constants of the individual transfer steps are indicated by the numbers. The orange and black dashed lines enclose compartments belonging to the CK and CB mutants, respectively. Insets a and b show species associated spectra of the various chromophores contained in compartments of the same color. In case the rates of particular energy or electron transfer steps are different in both mutants, the rates for the CK mutant are shown in orange.

closed. During the fitting, their relative contributions cannot be estimated and therefore only average electron-transfer and recombination rates for open and closed RCs were considered. We observe some variation in the obtained fitting parameters for PSII charge combination and recombination, but the shapes of the fitted DAS and the rates of EET and NPQ in the PBs are not affected very much.

The compartment scheme contains the following branches:

- Branch i: PBs transferring energy to PSI.⁴⁵
- Branch ii: PBs transferring energy to PSII.⁴⁶
- Branch iii: Part of C_PC that is not coupled to APC in the CB mutants.³⁵
- Branch iv: PSI, not coupled to PBs.⁴⁷
- Branch v: PSII, not coupled to PBs.⁴⁷

The relative numbers of excitations in the various branches were estimated in the target analysis and are listed in Table 1. To enable the fitting, a number of constraints on the input parameters and the spectra are required, and they are listed and discussed in the Supporting Information.

In order to take into account the fact that the amount of open and closed PSII RCs differs for the different samples and also can vary for different excitation wavelengths, the charge recombination rate was not globally fixed but was allowed to vary. On the other hand, the PSI kinetics hardly depends on the fact of whether PSI is open or closed (see, for instance, Wientjes et al., 2011).⁴⁸ Compared to the kinetics of PSII, the PSI kinetics is relatively straightforward to model (see, for instance, also the 400 nm excitation results in Figure 2), and the same parameters were used as in our previous work.²⁶

For the CK mutant, the charge separation rate was determined to be 8.2 ns⁻¹, and it was forced to be the same for the two different excitation wavelengths. For 400 nm excitation, the charge-recombination rate was ~9.4 ns⁻¹, while it becomes ~5.5 ns⁻¹ for 590 nm excitation, and the slower charge recombination rate in the second case is probably due to

the presence of more open RCs. In addition to both photosystems, the CK mutant also contains the APC core complex. Its spectrum, as obtained from the target analysis, peaks around 660 nm, as shown in green in Figure 4. Note that the APC core also contains a small fraction of pigments that fluoresce around 680 nm;^{6,9} this fraction is not included here as a separate pool, since equilibration between APC₆₈₀ and Chl's is probably reached within ps and their spectra are rather similar.²⁶

For the CB mutant, we observe two additional pools of pigments, i.e., PC_S and PC_L, which lead to fluorescence peaking at 643 nm (orange spectrum) and 655 nm (magenta spectrum), respectively. Table 1 shows that \sim 7% of the excitations at 400 nm and \sim 83% at 590 nm excitation are on the PBs. It can be concluded from the results at 400 nm that the PSI/PSII ratio (relative number of RCs) is \sim 2.6, which is higher than for the CK mutant where it is \sim 2.0.

In the CB mutant, ~25% of the C-PC appears to be disconnected, giving rise to a long-lived fluorescence component peaking at 643 nm. To simplify the model, the corresponding spectrum was forced to be the same as that of PC_s (the first pool in blue). The presence of some long-lived C-PC fluorescence is in agreement with previous sucrose-gradient results,³⁵ that show free PC bands at the top of the sucrose gradient.

With regard to the blue-green light induced quenching, the location of the quenching site was tested by adding an extra decay channel on one of the pools for the quenched samples. Each time, only one pool is allowed to be quenched. It turns out that $APC_{660 \text{ nm}}$ is the species that is directly quenched in both mutants but the fraction of quenched PBs differs and the same is true for the quenching rates. In the case of the CK mutant, only 11% of the excited PBs was quenched with an overall quenching rate of ~14 ns⁻¹, while, in the CB mutant, ~17% of the excited PBs was quenched with a quenching rate

of ~26 ns⁻¹. Note that, probably somewhat fortuitously, for both mutants the *in vivo* quenching rates are nearly the same as the corresponding rates *in vitro*, namely, ~26 and ~26 ns⁻¹, respectively, for CB and ~15 and ~14 ns⁻¹ for CK. However, as we have tested, different quenching rates combined with different quenching fractions of PBs can also fit the data sets equally well; thus, each quenching rate can range from 12 to 50 ns⁻¹, which is the same for both mutants.

All the SAS that are obtained during the fitting procedure are shown in Figure 4 as insets. All of them show very reasonable spectral shapes and the correct positions of their maxima.

In summary, with the model shown in Figure 4 in combination with the input parameters listed in Table 1, all data sets can be described satisfactorily. The target analysis of the time-resolved fluorescence measurements of the two studied mutants provides a systematic and globally consistent description of the EET and charge-separation rates and pathways in *Synechocystis* and of the fluorescence spectra of the most prominent pigment pools. Moreover, also the quenching site, the quenched fraction of PBs, and the quenching rate could be determined for both mutants.

4. DISCUSSION

4.1. Energy Harvesting Kinetics of PBs in the CB and CK Mutants in Vivo. Energy absorbed by the PBs is transferred to either PSI or PSII. The DAS of the CK mutant reveals a 30 ps transfer process from the 660 nm pigments in APC to the pool of Chl's (including the terminal emitter pigments in the APC core) fluorescing around 675-680 nm. The combination of this transfer process and the trapping process in PSI/PSII explains the strong positive peak in the DAS around 660 nm and the dip around 675-680 nm. The 75 ps DAS also shows emission around 660 nm, but there is no negative peak present that reflects the emission of the acceptor pigments. The most likely explanation for the absence of such a negative peak is "immediate" charge separation after energy transfer, which is indeed expected to occur in PSI but not in PSII where charge separation occurs on a much slower time scale.

In the CB mutant, the 7 ps component is attributed to fast spectral equilibration within the PC rods of the cyanobacteria, which is also supported by previous work, where a 10 ps component was attributed to transfer within the hexameric subunits of the C_PC rods.^{26,49} Phycocyanobilins are covalently linked at three different positions in the phycobiliproteins, and the pigments are called β 155, α 84, and β 84. The DAS reflects energy transfer from β 155 to the pigment pair consisting of α 84 and β 84 which are likely to be excitonically coupled.^{50,51}

Energy transfer from the rods to the core was reported to occur with a time constant of 90 ps for wild-type *Synechococcus* 6301,^{52,53} whereas it was found to take ~20 ps in wild-type *Synechocystis* 6803, as was also reported in another study on *Synechocystis*.^{36,54} Using picosecond fluorescence in combination with target analysis of wild type cells,²⁶ it was possible to disentangle several processes that gave rise to an apparent 20 ps transfer process in previous studies and it was concluded that the energy transfer from PC_L to APC occurs with a time constant of ~75 ps. In the present study, using the same procedure, the time constant is found to be 25 ps for the CB mutant. This is due to the fact that the pool size of C-PC in the CB mutant is only one-third of that in WT cells. Assuming that the transfer time roughly scales with the number of pigments

(see, e.g., Broess et al., 2006),⁵⁵ indeed we find that the rate of this transfer step is approximately tripled in the CB mutant as compared to WT cells.

For isolated PBs of the CK mutant, energy transfer from APC660 to APC680 takes place with a time constant of 43 ps, while *in vivo* it seems to become somewhat faster, namely, 30 ps (see DAS in Figure 3), but it should be realized that also the trapping process in PSI with a time constant of 27/28 ps contributes to the 30 ps DAS. A comparison between results on isolated CB_PBs and *in vivo* results is less straightforward, because different models were used: *in vitro*, an overall transfer component with a lifetime of 34 ps was found, whereas in the present study two subsequent steps of 6 and 25 ps are observed, relatively close to the 34 ps transfer time.

4.2. Quenching Sites and Quenching Rates in Vivo. In order to determine the rate of quenching and the type of chromophore that is being quenched, target analysis was performed. The analysis shows nearly identical spectra for the photosystems of the CB and CK mutants and those of the WT cells (Figure S5, Supporting Information), and also the spectra of the APC660 pools are very similar in all cell types. There is more variation in the spectra of the various chromophores in the C-PC rods. It is found in the present study for both mutants that the species that is directly quenched is APC₆₆₀ in agreement with the in vitro results on isolated PBs from these mutants. Only the quenched fraction of PBs is totally different. In vivo, ~11% of the PBs was quenched for the CK mutant and ~17% for the CB mutant for our growing and measuring conditions. On the contrary, 20 min of blue-green light illumination with similar intensity leads to 85% quenched PBs of isolated CK_PB and a full quenching of isolated CB_PB.¹¹ However, two factors dramatically reduce the quenched fraction in vivo: first, less OCP is present in the intact cells than for isolated complexes, where the OCP concentration was 40 times as high as the PB concentration. Second, the cells contain FRP which enables the recovery process (disappearance of quenching) to take place in the cell. The presence of FRP reduces the amount of OCPr bound to the PB, even though an equilibrium is achieved after the cells have been illuminated for a sufficiently long time. A smaller fraction of PBs was guenched in the CK mutant than in the CB mutant under the same light stress conditions. It cannot be concluded whether this is due to a difference in the relative amounts of OCP/FRP/PB or due to a difference in binding constants due to the lack of C-PC. The quenching rates cannot be determined with very high accuracy due to the relatively low amount of quenching for these in vivo systems, but they are rather similar to those observed for isolated PBs and for intact WT cells. Therefore, our previous conclusion that the quenching is most likely caused by charge transfer between APC₆₆₀^Q and the OCP carotenoid hECN in its activated form extends to the quenching in the CB and CK mutants.

ASSOCIATED CONTENT

Supporting Information

Streak camera images, DAS of the slow components for the CK and CB mutants, detailed description of the target analysis, and a comparison of the SAS obtained. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: Herbert.vanAmerongen@wur.nl.

Notes

The authors declare no competing financial interest.

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