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State transitions in cyanobacteria studied with picosecond fluorescence at room temperature

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ABSTRACT

Cyanobacteria can rapidly regulate the relative activity of their photosynthetic complexes photosystem I and II (PSI and PSII) in response to changes in the illumination conditions. This process is known as state transitions. If PSI is preferentially excited, they go to state I whereas state II is induced either after preferential excitation of PSII or after dark adaptation. Different underlying mechanisms have been proposed in literature, in particular i) reversible shuttling of the external antenna complexes, the phycobilisomes, between PSI and PSII, ii) reversible spillover of excitation energy from PSII to PSI, iii) a combination of both and, iv) increased excited-state quenching of the PSII core in state II. Here we investigated wild-type and mutant strains of *Synechococcus* sp. PCC 7942 and *Synechocystis* sp. PCC 6803 using time-resolved fluorescence spectroscopy at room temperature. Our observations support model iv, meaning that increased excited-state quenching of the PSII core occurs in state II thereby balancing the photochemistry of photosystems I and II.

1. Introduction

In photosynthetic organisms harvesting of sunlight is the initial step in the process of converting solar energy to chemical energy [1,2]. Photon absorption in cyanobacteria takes place in membrane-external pigment-protein antenna complexes (phycobilisomes) and membrane-embedded chlorophyll (Chl) *a* antenna complexes [3]. Harvested photon energy is transferred to the reaction centre complexes of photosystems I and II (PSI and PSII) through a series of (down-hill) excitation-energy transfer (EET) steps [4]. The amount of excitation energy arriving at the reaction centre of a photosystem is proportional to the absorption cross section of the associated antenna complexes and the efficiency of the EET process.

Cyanobacteria can rapidly regulate the relative activity of PSI and PSII in response to changes in the illumination conditions [5] including dark-light transitions [6,7]. This regulatory process for optimal photosynthesis under low-light conditions is known as state transitions [2,8–10]. The trigger for state transitions is the redox state of the plastoquinone pool between PSI & PSII [6]. Oxidation of the plastoquinone pool by light which preferentially excites PSI induces a transition to state I whereas reduction of the plastoquinone pool by the preferential excitation of PSII induces a transition to state II. State II can also be induced by respiratory electron flow into the plastoquinone pool in the dark [6]. State transitions are characterized by a relative decrease of PSII fluorescence in state II as compared to state 1 [11].

In green plants, the redistribution of light-harvesting complex II (LHCII) between PSII and PSI is part of the physiological mechanism accompanying state transitions [12–14]. Despite the discovery of state transitions in cyanobacteria long ago [9], a comprehensive understanding of the process is still evasive. The phycobilisome (PBS) antenna in cyanobacteria is the functional equivalent of the LHCII antenna in plants. The (re)distribution of the excitation energy to the photosystems through reversible migration of PBSs between PSI and PSII during state transitions, was proposed in some earlier studies [15–18]. Rapid diffusion of the PBSs along the surface of thylakoid membranes leading to transient interactions (association/disassociation) with the less mobile photosystems is at the basis of the mobile PBS model [19]. However, the PBS mobility model fails to explain the change in ratio of PSI/PSII fluorescence upon Chl *a* excitation [20]. Another model for state transitions in cyanobacteria, the one of direct “spillover” of excitation energy from PSII to PSI was proposed earlier for *Anacystis nidulans* [21] and for *Synechocystis* PCC 6803 [22] to explain the fluorescence changes during state transitions upon Chl *a* excitation. According to the spillover model, the excess excitation energy available

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at PSII in state II (absorbed by the PBS antenna or Chl *a* inner antenna of PSII) spills over from PSII to PSI. The spillover model was supported by the observation that in state I the PSII complexes are arranged in a row while in state II the arrangement of PSII complexes becomes more random, which may result in a decreased distance between PSII and PSI, thereby facilitating direct excitation energy transfer (EET) from PSII to PSI [23]. A functional megacomplex consisting of PBS-PSII-PSI was isolated from *Synechocystis* PCC 6803 [24] and was considered to support this spillover model. Time-resolved fluorescence characterization of the megacomplex revealed that one PBS could transfer the excitation energy to both photosystems and energy transfer to PSI was found to be less efficient than to PSII [24], although direct evidence for this spillover was not provided. In addition, the evidence for the presence of megacomplexes in intact cells and their characterization in state transitions is to date not available [7]. Incompleteness of the spillover model became explicit in a number of studies in which fluorescence changes, characteristic for state transitions, were less pronounced upon Chl *a* excitation as compared to PBS excitation [20]. To account for these differences, a comprehensive model (mixed model), combining the features of PBS mobility and spillover was proposed for *Synechococcus* PCC 7942 [20,25].

In the cyanobacteria *Synechocystis* PCC 6803, *Synechococcus* PCC 7002, and *Anabaena* PCC 7120, mutants lacking the ApcD gene product were reported to be impaired in performing state transitions [26–29]. Emlyn-Jones et al. [30] observed that the *Synechocystis* sp. PCC 6803 rpaC$^-$ mutant did not show any fluorescence changes related to state transitions upon PBS excitation although they were observed upon Chl *a* excitation. This observation clearly revealed that both a spillover as well as a combination model are insufficient to fully describe the presence/absence of fluorescence changes during state transitions. McConnell et al. [20] put forward a modified version of the spillover model to account for this experimental observation. They suggested that excitations created in the PBSs are directly transferred to the PSII reaction centres and cannot be lost via spillover to PSI in state II. In the wild-type cyanobacteria, the rate of EET from PBS to PSI was proposed to increase in state II, whereas it did not increase in the *Synechocystis* rpaC$^-$ and *Synechococcus* 7002 ApcD mutants [20]. On the other hand, it was proposed that excitations in the core antenna complexes (CP43 and CP47) of PSII can spill over to PSI in state II, both in the wild-type and mutants. However, experimental evidence for changes in EET from PSII to PSI and from PBS to PSI in state II during state transitions is lacking so far [7,31]. McConnell et al. [20] concluded that their model, which suggests only slight changes in the association of the PBS-PSII supercomplex with PSI is not compatible with the FRAP data, which indicates a higher lateral mobility of the PBSs as compared to the PSII-associated Chl *a* molecules after photobleaching with a laser flash [19]. In a 77 K time-resolved fluorescence study of state transitions in the cyanobacterium *Synechococcus elongatus* no evidence was found neither for the migration of PBSs from PSII to PSI in state II nor for the spillover of energy from PSII to PSI [31] and both processes were ruled out [31]. Instead the observed decrease in PSII fluorescence in state II was ascribed to direct quenching of PSII complexes [31].

Which model or combination of models presented above, would best describe the state transitions in cyanobacteria at physiologically relevant conditions? Is there some common explanation for all or most of the experimental observations reported in literature regarding state transitions in cyanobacteria? Do PBSs and PSII complexes have independent roles during state transitions? Is the PBS core involved in the physical mechanism required for the state transitions in cyanobacteria?

To find answers to these questions we investigated strains of the cyanobacteria *Synechococcus* sp. PCC 7942 (hereafter *Synechococcus*) and *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) using time-resolved fluorescence spectroscopy. Light harvesting in cyanobacteria is primarily accomplished by the PBS antenna [32–34]. Structurally, a PBS is composed of fan-like phycocyanin (PC) rods which radiate from a central core. The core attaches to the stromal side of the photosynthetic membrane and is comprised of cylinders (number of cylinders is specific to the organism) which are composed of trimeric discs of allophycocyanin (APC) [34]. In *Synechococcus* and *Synechocystis* the PBS core is comprised of bi-and tri-cylindrical APC trimeric discs, respectively. In the case of *Synechocystis* only two basal cylinders of the core bind to the thylakoid membrane [29]. Three important components of this core are the phycobiliproteins ApcD, ApcE and ApcF. ApcD and ApcE are the final acceptors of PBS-absorbed excitation energy, meaning that their lowest excited states are lower in energy than those of the other pigments in the PBS. ApcF on the other hand influences the optical properties of ApcE and could modify the energy transfer to one or both photosystems depending on the strain [27,28,33]. These three components of the PBS core transfer the PBS-absorbed excitation energy to the photosystems [28,29]. To characterize the role of the PBS core and its low-energy pigments during state transitions, the mutants lacking the ApcD and ApcF components in both strains were also studied. The lack of ApcD and ApcF was concluded to inhibit/decrease state transitions in *Synechocystis* [28,29] but not in *S. elongatus*. A mutant lacking ApcE component was not used in this work, because the PBSs are no longer stably attached; the ApcE subunit, also known as L_{CM}, is essential for the PBS assembly and is also a structural component of the PBS core and involved in thylakoid membrane interaction [29,35–37]. All of the experiments in the present study were performed at room temperature (RT) and states I and II were induced by keeping the cells in blue light and darkness, respectively.

2. Materials and methods

2.1. Strains and growth conditions

Synechocystis and *Synechococcus*, WT, ΔApcD and ΔApcF strains were grown in BG11 medium (20 ml/l) buffered with sodium bicarbonate (0.85 g/l), and sodium nitrate (1.75 g/l) at pH ~8.0. Medium for mutants deficient in ΔApcF was complemented with spectinomycin (25 μg/ml) and streptomycin (10 μg/ml). Media for *Synechocystis* ΔApcD and *Synechococcus* ΔApcD were complemented with chloramphenicol (20 μg/ml) and kanamycin (40 μg/ml), respectively. All strains were grown at 30 °C under white light illumination at 50 μmol photons m^{-2} s^{-1} in 250 ml flasks shaken at 100 rpm, containing culture volumes of 60 ml as described in [31]. The construction of *Synechocystis* ΔApcD and ΔApcF was described in [35] and that of *Synechococcus* ΔApcD and ΔApcF in [29].

2.2. Sample preparation

All the WT and mutant cells were harvested during their logarithmic growth phase at optical density ~0.6–0.7 at 800 nm (OD_{800}) for a path length of 1 cm. Cells were diluted with their respective fresh medium to OD_{630}~0.35 as measured with a Cary 4000 UV–Vis spectrophotometer with integrating sphere. To drive the cells to state I and II, they were kept in blue light (of 452 ± 40 nm at ~40 μmol photons m^{-2} s^{-1} intensity) and darkness, respectively, for about 15 min. Using a pulse-amplitude modulated fluorometer (PAM101; Walz, Effeltrich, Germany) state transitions were monitored. Measurements were performed in a 1 cm × 1 cm stirred quartz cuvette.

2.3. Replicates

All time-resolved fluorescence experiments were performed at least twice on each individual sample on the same day (technical replicates) while biologically independent experiments (biological replicates) were performed on different samples on different days separated by a few weeks up to several months. Representative data from each strain is presented in this work and all changes/differences that are relevant for our conclusions are fully reproducible. Standard deviations are given for the decrease in PSII fluorescence upon State I to State II transition.

2.4. Time-resolved fluorescence spectroscopy

Time-resolved fluorescence measurements were performed at room temperature with a synchro scan streak camera [38] as described in [7,31]. Sub-picosecond pulses of 577 nm and 430 nm wavelength with repetition rate of 3.8 MHz were used to preferentially excite the PBSs and photosystems, respectively. The laser power was ~20 μW for PBS excitation and ~60 μW for Chl *a* excitation. The laser spot size was ~0.1 mm in both cases. Time-resolved emission spectra were recorded with a time window of 800 ps for resolving the fast components and 2000 ps for estimation of the slowest component. Sixty streak images were recorded, where each image was an analogue integration of 20 exposures with exposure time of 1112 ms each. Streak images were corrected for background and for wavelength-dependent sensitivity of the detector. Processed streak images were then globally analysed using the Glotaran [39] and TIMP package for R [40] to determine the fluorescence lifetimes and decay-associated spectra (DAS) as described in [7,31]. For all of our measurements the time-resolved fluorescence is described with five components after 577 nm excitation and four components after 430 nm excitation. For each measurement the obtained DAS were summed, which provided the estimated fluorescence spectrum at $t = 0$, i.e. immediately upon excitation and this t = 0 spectrum was normalized to a value of 1 in the maximum. All the individual DAS were corrected with the same scaling factor. Steady-state fluorescence spectra were subsequently calculated as a sum of the products of amplitudes (DAS) and their respective decay times and below they are referred to as reconstructed (steady-state) spectra. For all streak experiments cells adapted to state I were kept under the same light condition for the duration of the measurement. To verify the reversibility of the state transitions in our samples, cells measured in state I were adapted first to darkness for ~15 min and then again measured in state I. Time-resolved emission of both state I measurements were compared and were found to be virtually identical. Measurements were performed in a 1 cm × 1 cm stirred quartz cuvette.

3. Results

3.1. Pulse-amplitude modulated fluorometry

In cyanobacteria, state transitions balance the relative amount of PSI and PSII excitations, which is reflected in the (relative) amount of PSI and PSII fluorescence. Therefore, state transitions can readily be monitored by measuring the level of PSI and PSII fluorescence at 77 K [41–44]. At room temperature the fluorescence decay of PSI is extremely fast (~20 ps) and therefore has a very small contribution to the steady-state fluorescence which is dominated by PSII emission [45].

To estimate the time it takes to induce state I and state II in cyanobacteria, use was made of pulse-amplitude modulated (PAM) fluorometry. With this method, state transitions in photosynthetic organisms can be followed at room temperature, since they are characterized by changes in the levels of PSII fluorescence as a function of the intensity and spectrum of the actinic light, which is used to drive photosynthesis [46]. In Fig. 1 a fluorescence time trace is shown for *Synechococcus* WT cells in different illumination conditions as recorded with a PAM fluorometer.

The PAM fluorescence trace contains some contribution from free phycobilisomes (PBS) and PSI but the major contribution stems from PSII and PBS complexes connected to the photosystems [47]. F_0 and F_s' represent the minimum fluorescence for cells kept in darkness and the steady-state fluorescence in the (blue) light, respectively. Minimum fluorescence was measured with non-actinic 1 μsec pulses of measuring light with 1.6 kHz frequency. The minimum fluorescence represents the fluorescence of cells with mainly open reaction centres. Periodic saturating pulses of white light closed the PSII RCs, causing the fluorescence to reach the maximum level, i.e. F_m for cells in darkness and F_m' for cells in the light. The extent of state transitions is proportional to the relative change in the maximum fluorescence.

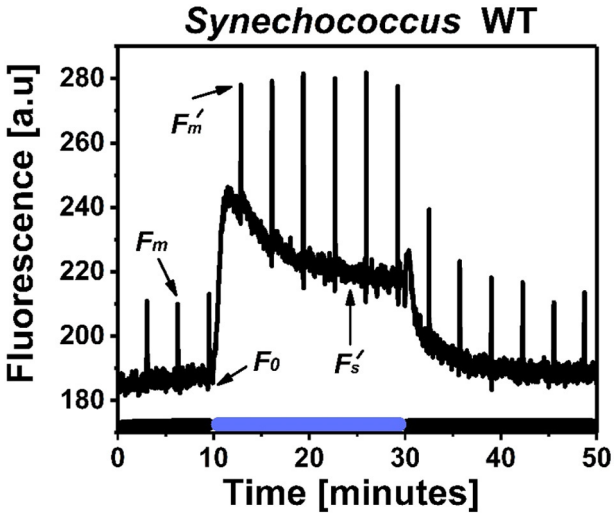


Fig. 1. Time traces of variable fluorescence in wild-type *Synechococcus 7942* measured with the pulse-amplitude modulated fluorescence technique. The fluorescence detection window was > 700 nm. Black and blue thick solid lines along the time-axis represent the time periods of cells in darkness and blue light respectively. Blue light with central wavelength 452 nm and ~40 μmol photons m^{-2} s^{-1} was used to bring the cells to state I. Blue-light-adapted cells were subsequently put in darkness to bring the cells to state II. F_0, F_m represent minimum and maximum fluorescence, respectively, for dark-adapted cells. F_s' and F_m' are steady-state and maximum fluorescence, respectively, for cells in blue light.

A sharp rise in fluorescence for F_0, F_s' and F_m levels was seen when dark-adapted cells were exposed to blue light to bring them into state I. A decrease was observed when light-adapted cells were again put in darkness to drive the cells to state II. Periods of 20 min were found to be sufficient to bring the majority of the cells to state I/II from state II/I, respectively.

3.2. Time-resolved fluorescence spectroscopy results

Use was made of the time-resolved fluorescence spectroscopy to study the state transitions in both wild-type species of *Synechococcus*, *Synechocystis* and their ΔApcD and ΔApcF mutants.

In time-resolved fluorescence spectroscopy an accurate interpretation of the fluorescence decay lifetimes and their associated wavelength-dependent amplitudes is crucial for the understanding of the phenomena under investigation. To be sure that the fluorescence changes observed in our measurements are represantative of state transitions only, all the measurements were done at the F_m (closed PSII reaction centres) level of the cells. Fig. 2 shows the fluorescence decay traces at ~680 nm (PSII and PBS emission wavelengths) for *Synechococcus* cells, both in state I and state II at various powers of the 577 nm excitation pulses. The fluorescence decay traces measured with weak-intensity pulses (average laser output 0.1 μW–0.15 μW (black curves)) correspond to the situation in which the PSII reaction centres were predominantly open.

Raising the power to 3 μW led to fluorescence with closed PSII reaction centres. Increasing the power further (22 μW–25 μW) did not cause an additional slowing down of the kinetics and the kinetics were similar/identical to those with the reaction centres closed by the use of DCMU. The similarity of the fluorescence decay kinetics in the range of 3–25 μW also demonstrates that the fluorescence kinetics were not affected by singlet-singlet or singlet-triplet annihilation which would have shortened the excited-state lifetime at higher excitation powers [46].

In Fig. 3 characteristic fluorescence decay traces at the maximum

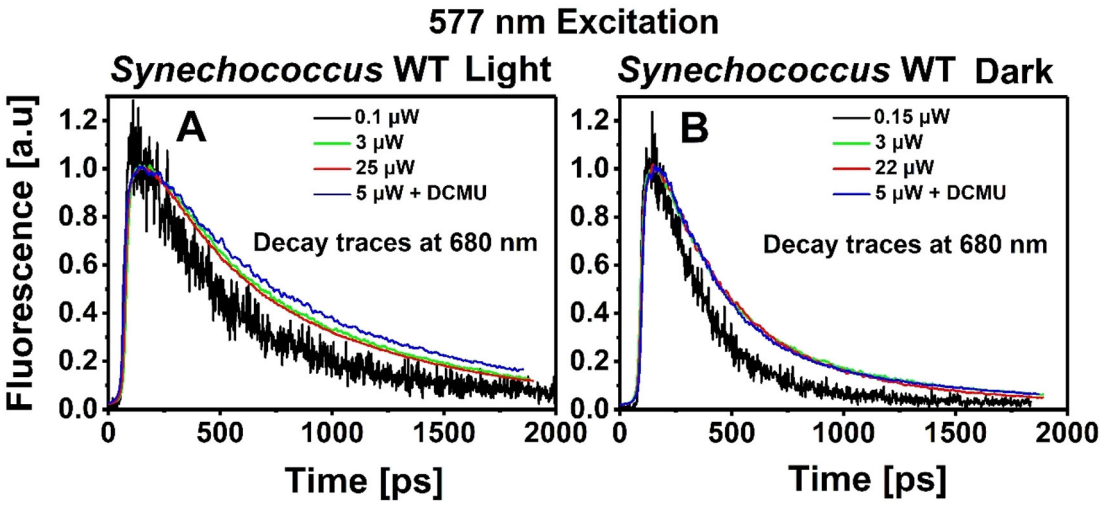


Fig. 2. Fluorescence decay traces of *Synechococcus* cells at 680 nm obtained with various laser powers after 577 nm excitation (left) light-adapted cells (state I) and (right) dark-adapted cells (state II). Black, green and red traces correspond to cells without DCMU and the blue trace to DCMU-treated cells. All traces were normalized to each other at their maximum emission. Measurements were performed at room temperature.

emission (~680 nm) of PBS terminal emitters and PSII, for *Synechococcus* and *Synechocystis* cells in state I and II are shown upon 577 nm excitation. The fluorescence kinetics at 680 nm show a slower decay for the cells in state I as compared to the cells in state II for both strains, which is characteristic for state transitions in cyanocteria. The fluorescence changes are much more pronounced for *Synechococcus* (Fig. 3A) than for *Synechocystis* (Fig. 3B).

3.3. State transitions in Synechococcus WT, ΔApcD and ΔApcF

Measurements were performed both with selective excitation of the PBSs (577 nm excitation pulses) and the photosystems (430 nm excitation pulses) to determine the individual roles of the phycobilisomes and photosystems during state transitions. The recorded time-resolved fluorescence spectra in each case were analysed with the global-analysis method to obtain the DAS.

Five-components DAS for WT, ΔApcD, and ΔApcF *Synechococcus* cells are shown in Fig. 4 for cells in state I (dotted lines) and II (solid lines). The ≤10 ps DAS with negative peak at 650 nm reflect EET within phycocyanin (PC) discs [31,48,49]. EET occurs predominantly from higher-energy to lower-energy pigments. The ~20 ps DAS with positive peak at 630 nm and negative peak at 660 nm describe downhill EET from PC rods to the APC_{660} core. The ~80–90 ps DAS describe EET from higher-energy pigments in the PC rods (~655 nm peak) to the lower-energy pigments in APC_{680} (~680 nm) and Chls. The first three DAS are very similar for each type of cells in state I and II and demonstrate that EET within PBSs is not substantially affected during state transitions.

Transitioning of the WT and mutant cells from state I to state II (light to dark) was accompanied with: (i) a decrease in the amplitude of the (slowest) τ = ~1.0 ns component and (ii) a concomitant rise of the amplitude of the τ = ~0.17–0.2 ns component at 680 nm. In an earlier study of *Synechococcus* 6301 the ~0.15 ns component with the emission band around ~680 nm (corresponding to ~0.17–0.2 ns component in the present work) was assigned to the PBS terminal emitters [44].

However, in our present work we also observe this ~0.2 ns component with emission band around 680 nm upon selective excitation of Chl *a* (Figs. 5 and 7), strongly suggesting that it originates from PSII. Tian et al. in [45] probed the picosecond fluorescence kinetics of the PSII complexes (with closed reaction centres) using the PAL (PBS-less) mutant of *Synechocystis* 6803. Similar kinetic components (~0.2 ns and ~1.0 ns) were resolved in their work and show that the PSII fluorescence decay in cyanobacteria is inherently heterogenous (biphasic/multiphasic) and is not the result of a mixed status of the reaction

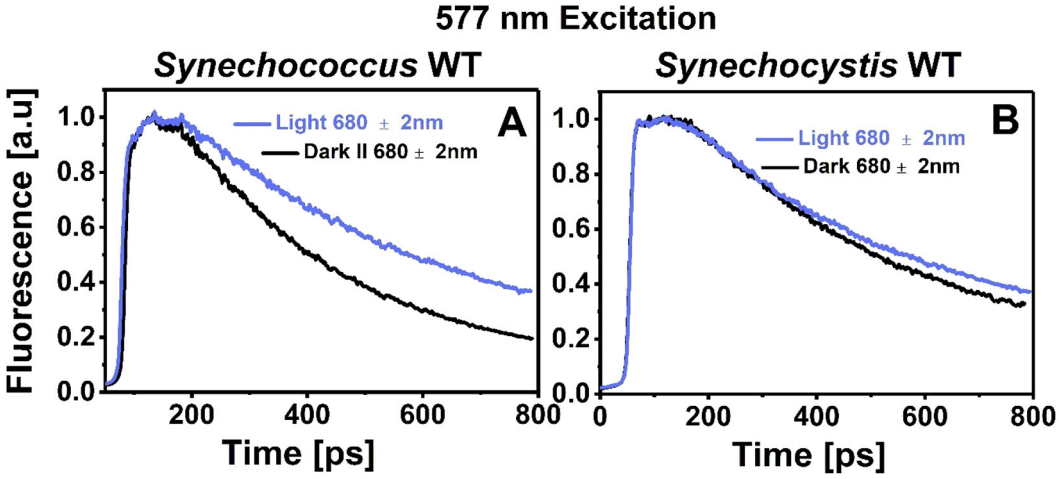


Fig. 3. Normalized decay traces at 680 nm (PBS terminal emitter (APC_{680}) and PSII) for dark-adapted (black lines) and light-adapted cells (blue lines) of (A) *Synechococcus* and (B) *Synechocystis*. Excitation wavelength was 577 nm and measurements were done at room temperature.

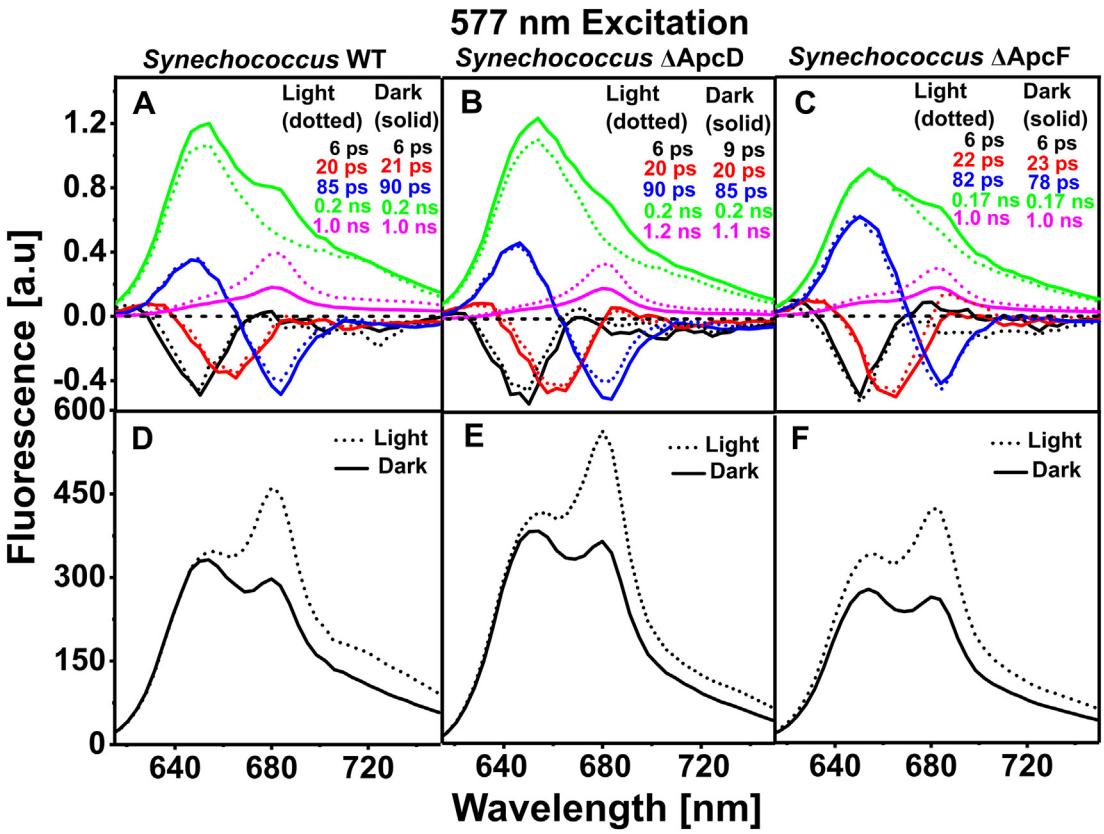


Fig. 4. Decay-associated spectra obtained from global fitting of time-resolved fluorescence results after 577 nm excitation. (A, B, C) five-components DAS of *Synechococcus* WT, *Synechococcus* ΔApcD and *Synechococcus* ΔApcF, in state I (dotted lines) and state II (solid lines). DAS-associated lifetimes are presented in the insets with corresponding colours. In A and C the slowest lifetime is fixed to 1.0 ns. (D, E, F) reconstructed steady-state spectra for *Synechococcus* WT, *Synechococcus* ΔApcD and *Synechococcus* ΔApcF, respectively, in state I (dotted line) and state II (solid line). All spectra are scaled as described in Materials and Methods.

centres (partially closed). However, upon PBS excitation, the presence of this particular ~0.2 ns decay component with its maximum emission at 680 nm is not readily observable in the five component global analysis of the time-resolved fluorescence of light-adapted (state I) cells (Fig. 4). It overlaps with the ~0.17–0.2 ns DAS in state I (Fig. 4A) with a broad positive peak at ~655 nm and a depression around ~680 nm which is due to EET from phycocyanins and allophycocyanins to the photosystems. This makes it difficult to reliably separate them in our global analysis. The ~1.0 ns DAS with the maximum emission around ~680 nm is assigned to the PSII complexes with closed reaction centres and PBS terminal emitters (free and partially connected PBSs). From now on we will mainly discuss the ~0.17–0.2 ns and ~ 1.0 ns components with their emission maxima at ~680 nm and refer to them as the slow (~0.17–0.2 ns) and the slowest (~1.0 ns) component. The value of the lifetime of the slowest component is not extremely accurate and was found to be in the range of ~0.8–1.0 ns. Cells in state II had consistently slightly higher values for the slowest component (Fig. S2) as observed in [44].

Reconstructed steady-state fluorescence spectra of *Synechococcus* WT, ΔApcD and, ΔApcF mutants in Fig. 4 show a ~35.8 ± 2.5%, 35.0 ± 0.1% and, 39.4 ± 4.9% decrease of PSII fluorescence in state II, respectively. The ApcD and ApcF components are therefore concluded not to be required for the state transitions in *Synechococcus,* in agreement with the conclusions in [29].

Fig. 5 presents the four DAS components for *Synechococcus* WT and the ΔApcD and, ΔApcF mutants, obtained upon selective Chl *a* excitation at 430 nm. Due to the higher Chl *a* content in PSI, the fluorescence kinetics are dominated by the ~20 ps component (λ = 690 nm), representing the kinetics of PSI excitation trapping. The fast component (~6–8 ps) with a broad positive peak around 680–690 nm corresponds to excitation-energy equilibration in the bulk chlorophylls of PSI and PSII. The negative peaks in the same DAS around ~645 nm and above 700 nm indicate EET from the bulk Chls to the PBSs and to the lower-energy Chls, respectively.

Excitation at 430 nm is at the absorption maximum of Chl *a*, where only negligible absorption is due to PBSs [31]. Therefore, the slow component (~0.2 ns) with a band around 680 nm is assigned to the PSII complexes. Similar to the 577 nm excitation case (Fig. 4), reciprocal differences in amplitudes of the slow and the slowest components are observed for cells in state I and state II, both for the WT and mutant cells (Fig. 5A–C). The value of the slowest component upon 430 nm excitation was typically found between ~0.7 ns–1.0 ns. Reconstructed steady-state emission spectra for *Synechococcus* WT, ΔApcD, and ΔApcF (Fig. 5D–F) show a pronounced decrease of the room-temperature PSII fluorescence in state II, as was observed before at 77 K [29,31]. From the reconstructed steady-state fluorescence spectra, the fluorescence decrease in state II was found to be 39.6 ± 1.1%, 42.7 ± 1.2% and 47.3 ± 1.1% for *Synechococcus* WT, ΔApcD, and ΔApcF, respectively.

3.4. State transitions in Synechocystis WT, ΔApcD and ΔApcF

In this section, state transitions in *Synechocystis* WT and its ΔApcD and ΔApcF mutants are compared to those of *Synechococcus* as described above, regarding their fluorescence properties. It turns out that the overall kinetics are rather similar for *Synechococcus* and *Synechocystis*.

Upon 577 nm excitation, state II is again characterized by a decrease in amplitude of the slowest component and a concomitant rise of the amplitude of the slow component (Fig. 6A). However, these characteristic changes are completely absent in *Synechocystis* ΔApcD

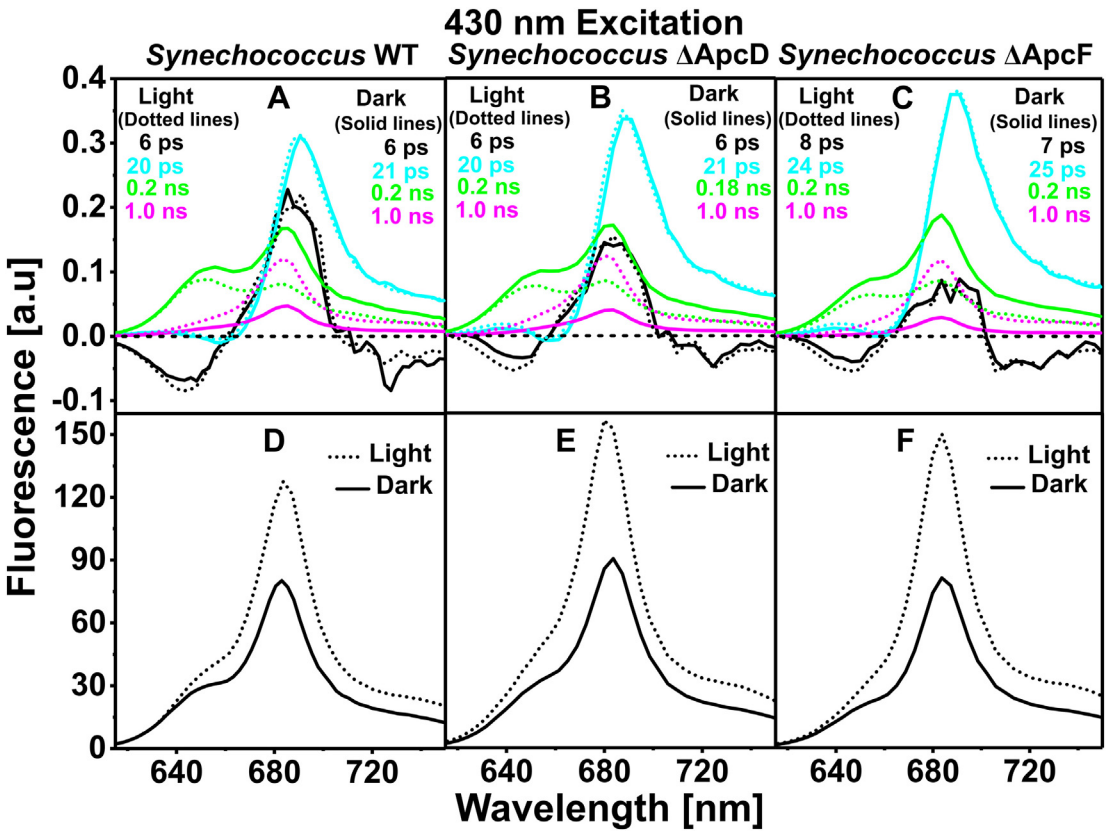


Fig. 5. Decay-associated spectra (DAS) obtained from global fitting of time-resolved fluorescence results upon 430 nm excitation. (A, B, C) four-components DAS of *Synechococcus* WT, *Synechococcus* ΔApcD and *Synechococcus* ΔApcF, respectively in state I (dotted lines) and state II (solid lines). The slowest lifetime in each case was fixed to 1.0 ns. The ~20 ps DAS in each case is multiplied with 0.5 to make the two slowest DAS better visible. DAS-associated lifetimes are shown in the legend with corresponding colours. (D, E, F) reconstructed steady-state spectra for *Synechococcus* WT, *Synechococcus* ΔApcD and *Synechococcus* ΔApcF, respectively in state I (dotted line) and state II (solid line). All spectra are scaled as described in Materials and Methods.

(Fig. 6B) and are largely diminished in *Synechocystis* ΔApcF (Fig. 6C). The slowest component in the case of *Synechocystis* ΔApcD and *Synechocystis* ΔApcF has considerable emission at 650–660 nm, indicating that EET from the corresponding pigments is rather inefficient, possibly due to disconnection of some PBSs as was concluded for *Synechocystis* ΔapcF in [29].

In Fig. 6D–F reconstructed steady-state emission spectra of dark-and light-adapted *Synechocystis* WT, ΔApcD and ΔApcF cells are presented. From the reconstructed steady-state fluorescence spectra, the fluorescence decrease in state II was found to be 19.2 ± 1.0%, −3.3 ± 1.0% and 10.9 ± 2.9% for *Synechocystis* WT, ΔApcD, and ΔApcF, respectively. From a comparison of the time-resolved and reconstructed steady-state emission spectra of the *Synechocystis* WT and its ΔApcD mutant, it seems that the ΔApcD cells are fixed in state I as was observed before [28]. The fluorescence ratio F_{660}/F_{680} in the ΔApcD mutant is higher as compared to the one observed for state I in WT cells, indicating that energy transfer from the PBSs to one or both photosystems is less efficient. In the absence of PSII some loss of energy transfer to PSI was found at room temperature in *Synechocystis* 6803 ΔApcD [28]. However, in [29] it was indicated that the absence of ΔApcD does not affect the energy transfer from PBSs to the photosystems in *Synechocystis* 6803.

The higher F_{660}/F_{680} ratio in the ΔApcF mutant signifies inefficient EET from high-energy pigments in the PBSs to either low-energy pigments in the PBSs or the photosystems. ApcF has been found essential for energy transfer to both photosystems in *Synechocystis* [28,29]. Due to the inefficient EET to the photosystems in *Synechocystis* ΔApcF, the slowest component in Fig. 6C might contain a considerable contribution from the PBS terminal emitters. The diminished characteristic fluorescence changes in the dark-and light-adapted *Synechocystis* ΔApcF cells as compared to WT cells are concluded to be due to less efficient EET from the PBS core to either PSII or to both photosystems.

Upon 430 nm excitation, the resulting four DAS components for *Synechocystis* WT, ΔApcD and ΔApcF are shown in Fig. 7A–C and the reconstructed steady-state fluorescence spectra in Fig. 7D–F. From the reconstructed steady-state fluorescence spectra, the fluorescence decrease in state II was found to be 20.9 ± 1.6%, 9.4 ± 1.1% and 12.7 ± 3.9% for *Synechocystis* WT, ΔApcD, and ΔApcF, respectively. The fluorescence changes, characteristic for state transitions are observable both for WT and mutant cells but they are far less outspoken than for *Synechococcus*. However, for mutant cells (especially for ΔApcD) the differences are smaller than for WT cells.

4. Discussion

In this work we applied time-resolved fluorescence spectroscopy to study state transitions in the cyanobacteria *Synechococcus* sp. PCC 7942, *Synechocystis* sp. PCC 6803 and their ΔApcD and ΔApcF mutants at room temperature. The most important observation is the decrease in amplitude of the slowest decay component of ~1 ns with a peak around 680 nm in state II, and the concomitant increase of the amplitude of the ~0.2-ns component, upon Chl *a* excitation. This implies that there is a significant shortening of the average lifetime of the PSII fluorescence, which corresponds to a decrease of PSII fluorescence in the (reconstructed) steady-state spectra. Such an increase of the ~0.2 ns component was also observed in an earlier study performed at room temperature but in that case Chl *a* excitation was not selective and the increase was ascribed to PBS fluorescence [44]. However, when we

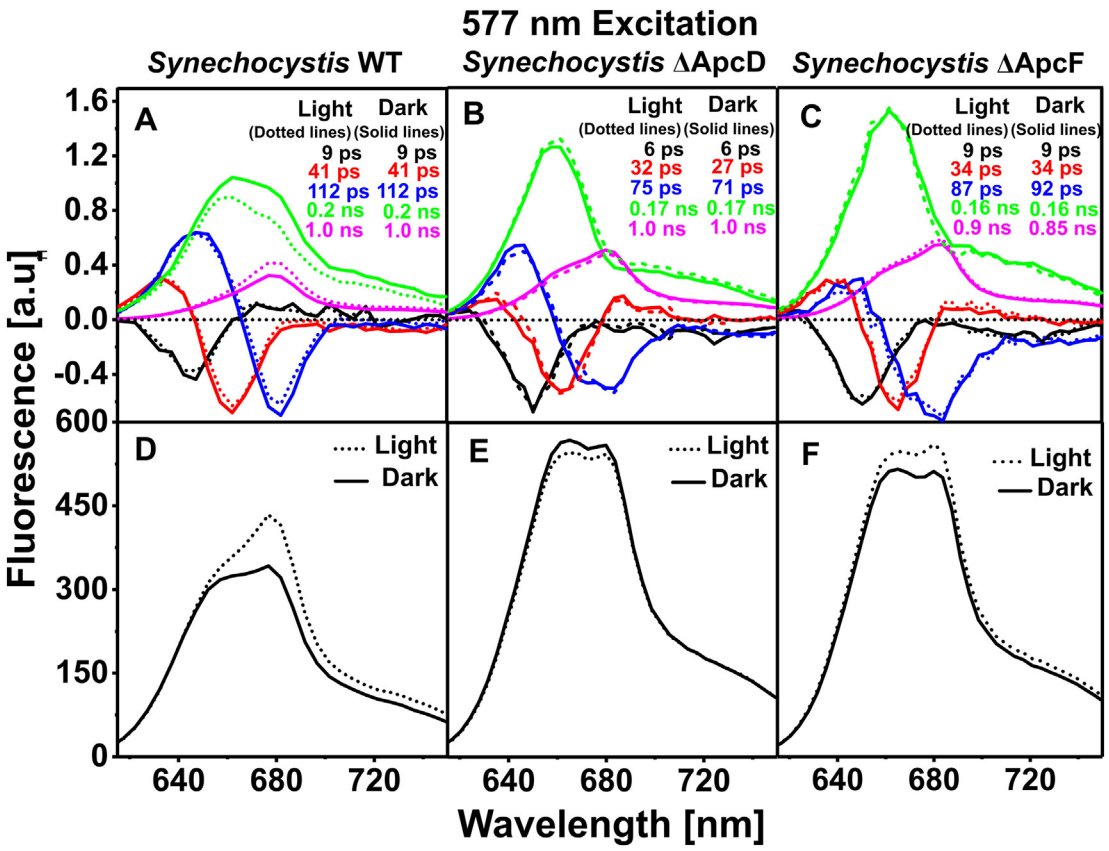


Fig. 6. (A, B, C) five-components DAS of *Synechocystis* WT, *Synechocystis* ΔApcD and *Synechocystis* ΔApcF, respectively in state I (dotted lines) and state II (solid lines). DAS-associated lifetimes are presented at the top with corresponding colours. In A and B the slowest lifetime was fixed to 1.0 ns. (D, E, F) reconstructed steady-state spectra for *Synechococcus* WT, *Synechococcus* ΔApcD and *Synechococcus* ΔApcF, respectively in state I (dotted lines) and state II (solid lines). Excitation was at 577 nm.

selectively excite PBSs we observe a similar increase of the ~0.2 ns component as observed for selective Chl *a* excitation. Therefore, in agreement with the 77 K results reported in [31], we conclude that reversible quenching of PSII is the main mechanism to balance the photochemistry of the two photosystems during state transitions. This quenching mechanism is apparently not affected by a change from room temperature to 77 K.

In the case of PBS excitation, the decrease of PSII fluorescence in state II (Figs. 4 and 6) can in principle be due to (i) a decrease of the absorption cross-section/functional antenna size of PSII and/or (ii) quenching of (a fraction of) of the PSII complexes. A decrease of the absorption cross-section/functional antenna size of PSII in state II was ascribed earlier to migration of PBSs from PSII to PSI in the mobile antenna model [15–18]. The spillover model [20,50–52] on the other hand, ascribed quenching of PSII in state II to EET to PSI, whereas also a combination of both models was proposed [20,25]. All these models predict an increase of EET to PSI in state II at the expense of excitations in PSII. In principle this should lead to an increase of PSI fluorescence in state II. At room temperature such an increase in PSI fluorescence might be difficult to observe due to the relatively slow EET from PBS to PSI (~0.15–0.17 ns) followed by fast excitation trapping in PSI (~20 ps). However, also at 77 K where the PSI fluorescence kinetics is far slower (~1.0 ns) no increase could be observed for state II, both for wild-type *Synechococcus* and *Synechocystis* [7,31]. Therefore, Ranjbar Choubeh et al. concluded that neither migration of PBSs from PSII to PSI, nor spillover of excitation energy from PSII to PSI is involved in the transition from state I to state II and fluorescence changes were ascribed to reversible direct quenching of the PSII core [31]. In our room-temperature measurements on WT cells upon PBS excitation a fluorescence decrease of 35.8 ± 2.5% and 19.2 ± 1.0% in state II was observed, for *S. elongatus* and *Synechocystis,* respectively. Upon Chl *a* excitation, the decrease in PSII fluorescence was 39.6 ± 1.1% for *S. elongatus* and 20.9 ± 1.6% for *Synechocystis*. The similar decrease in the amount of PSII fluorescence in state II, both upon PBS and Chl *a* excitation, supports the conclusion in [31] that the PSII core is partially quenched. Although such a difference is also observed for dark-and light-adapted *Synechocystis* ΔApcD (Fig. 7(B, E)) upon Chl *a* excitation at 430 nm, albeit to a lesser extent, it is totally absent upon PBS excitation at 577 nm (Fig. 6(B, E)). This observation may cast some doubt on the implicit conclusion in [31] that PBSs play no role in state II. Instead PBSs might show reduced EET to PSII in state II in WT cells, either by disconnecting from PSII or by quenching excitations. This role appears to be disabled with the deletion of ApcD in Synechocystis. The disabled role of PBSs alone, however, does not explain why PBS-absorbed energy is not quenched at the PSII core after PBS excitation as is the case for Chl *a* absorbed excitations in this mutant. A solution to this problem was suggested by McConnell et al. [20]. They proposed that small scale membrane changes in state II bring PSII and PSI closer together, such that the rate of excitation transfer from PBSs (shared by both photosystems) to PSI increases, whereas also spillover of excitation energy from PSII to PSI occurs. At the same time it was proposed that the PBS-absorbed excitations are transferred directly to the PSII reaction centres and cannot spill over to PSI via CP43 and CP47 antennas, whereas excitations created in CP43 or CP47 upon Chl *a* excitation can spill over to PSI.

However, the proposal of McConnell et al. cannot explain our data. The excited-state lifetime of the PSII core with closed RCs is of the order of 1 ns and there is plenty of time for excitations to equilibrate over the entire core, irrespective whether they originate from the PBSs or from the core itself. Therefore, if the core would be quenched due to spillover this should also be observed after PBS excitation, followed by transfer to the PSII core. We therefore believe that a more likely explanation is

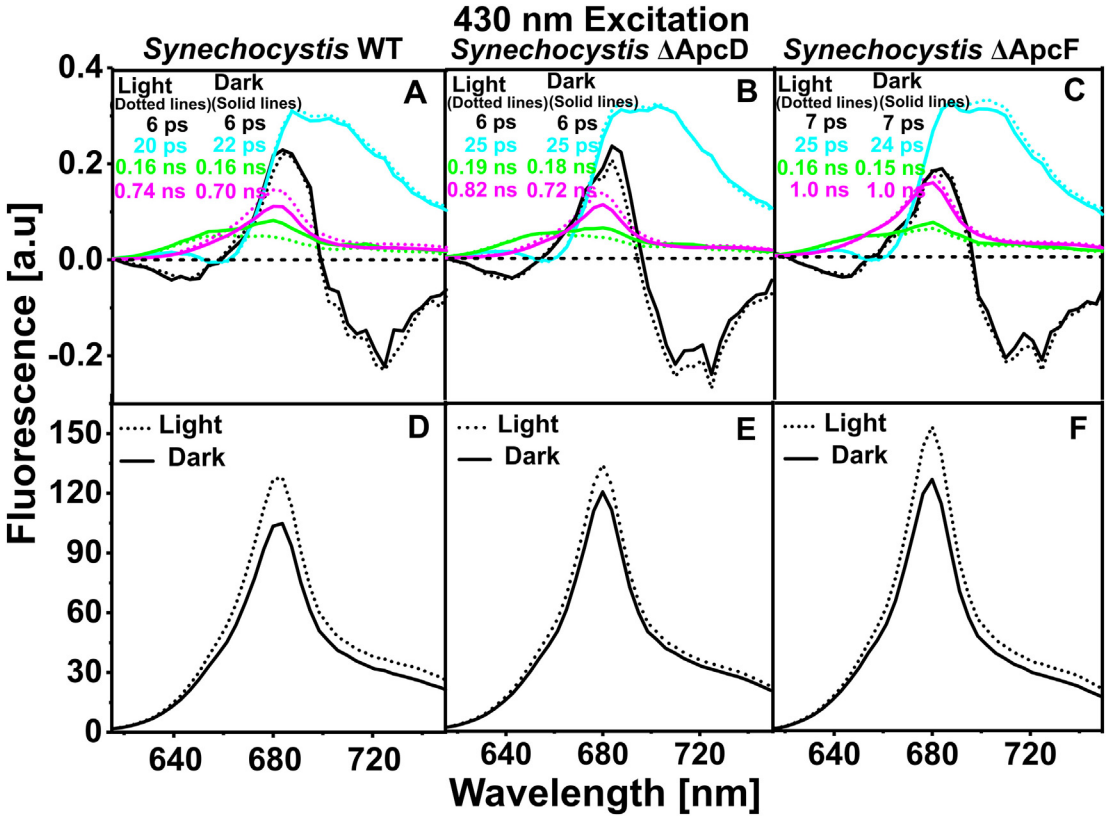


Fig. 7. (A, B, C) four-components DAS of *Synechocystis* WT, *Synechocystis* ΔApcD and *Synechocystis* ΔApcF, respectively in state I (dotted lines) and state II (solid lines). The 20 ps DAS in each case is multiplied by 0.5 to make the two slowest DAS better visible. DAS-associated lifetimes are presented at the top with corresponding colours. In Figure C the lifetime of the slowest component was fixed to 1.0 ns. (D, E, F) reconstructed steady-state spectra for *Synechococcus* WT, *Synechococcus* ΔApcD and *Synechococcus* ΔApcF, respectively in state I (dotted lines) and state II (solid lines).

that PSII is directly quenched in state II leading to less PSII fluorescence while in the absence of ApcD EET from the PBS to PSII is less efficient and therefore leads to more PBS fluorescence, thereby compensating for the loss of PSII fluorescence.

4.1. Possible mechanisms of PSII quenching in state II

Because the PSII core is a delicate piece of equipment that can easily be damaged in case of overexcitation, oxygenic photosynthetic organisms have developed photoprotective mechanisms that lead to excitation quenching such that excess excitations are safely dissipated as heat. A well-known example is the occurrence of non-photochemical quenching in e.g. plants, green algae, cyanobacteria and diatoms, where the organisms can rapidly switch on and off antenna quenching mechanisms that protect PSII from overexcitation [53]. In many cases this requires the involvement of carotenoids in the outer antenna system that function as excitation quenchers in NPQ conditions (see e.g. [54–56]). This heat dissipation occurs very rapidly (< 1 ns after excitation) and with the use of optoacoustic experiment extra heat dissipation was indeed observed for thylakoid membranes in NPQ conditions within 1.4 μs after excitation with a laser flash [57].

On the other hand, quenching can also occur in the PSII RC. This has been suggested as an effective protective mechanism against photodamage in several cyanobacteria [58,59] and other organisms [60,61]. Also in a number of studies on desiccated lichens it was concluded that (auxiliary) quenching occurs within the PSII reaction centre [62–66] and this mechanism was recently also proposed for the desert cyanobacterium *Leptolyngbya ohadii* [67]. The underlying molecular mechanism of RC quenching might be due to radiationless charge recombination in the RC, a process that can occur on a time scale of hundreds of microseconds or longer [57]. Such a mechanism might also be responsible for the PSII quenching in state II, rather than energy dissipation via one of the carotenoids in the PSII core. Such an explanation might be supported by the fact that independent optoacoustic studies of state transitions in the cyanobacteria *Synechococcus* 6301 [68,69] were not able to detect extra heat release in state II within 1.4–2.0 μs after excitation. However, we cannot rule out that other mechanisms might be responsible, for instance the formation of quenchers in CP43 or CP47.

5. Conclusions

In this work we show that at physiological relevant temperatures the average excited-state lifetime of PSII in state II becomes shorter as compared to state I due to partial quenching of the PSII excitations, balancing the photochemistry of the two photosystems. This was proposed before based on fluorescence measurements at 77 K but now we obtained for the first time room-temperature picosecond fluorescence data that are fully consistent with that proposal for *Synechocystis* and *Synechococcus*, WT, ΔApcD and ΔApcF strains. One possible explanation for this quenching might be radiationless charge recombination in the RC of PSII in state II but we can certainly not rule out other quenching mechanisms, for instance in CP43 or CP47. *Synechocystis* ΔApcD spectra in state I and II differ upon Chl *a* excitation but show hardly any difference upon PBS excitation. We demonstrate that an earlier model [20] is insufficient to explain the phenomenon and we provide an alternative explanation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbabio.2020.148255.

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