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DOI
10.1016/j.bbabio.2017.11.002

Publication date
2018

Document Version
Final published version

Published in
Biochimica et Biophysica Acta - Bioenergetics

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Spectrally decomposed dark-to-light transitions in a PSI-deficient mutant of *Synechocystis* sp. PCC 6803

Alonso M. Acuña, Pascal van Alphen, Filipe Branco dos Santos, Rienk van Grondelle, Klaas J. Hellingwerf, Ivo H.M. van Stokkum

ABSTRACT

Cyanobacterial thylakoid membranes are known to host photosynthetic and respiratory complexes. This hampers a straightforward interpretation of the highly dynamic fluorescence originating from photosynthetic units. The present study focuses on dark-to-light transitions in whole cells of a PSI-deficient mutant of the cyanobacterium *Synechocystis* sp. PCC 6803. The time-dependent cellular fluorescence spectrum has been measured, while having previously exposed the cells to different conditions that affect respiratory activity. The analysis method used allows the detected signal to be decomposed in a few components that are then assigned to functional emitting species. Additionally, we have worked out a minimal mathematical model consisting of sensible postulated species to interpret the recorded data. We conclude that the following two functional complexes play a major role: a phycobilisome antenna complex coupled to a PSI dimer with either two or no closed reaction centers. Crucially, we present evidence for an additional species capable of strongly quenching fluorescence, whose formation requires the presence of oxygen.

1. Introduction

The cyanobacterial thylakoid membrane harbors both photosynthetic and respiratory units, making electron transport a highly dynamic and complex process [1–3]. In the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*), three interwoven electron transport routes are typically regarded as dominant: i) linear photosynthetic electron transport involving the photosystems (PS) I and II, the cytochrome *b*/*f* complex (hereafter cyt *b*/*f*), the plastoquinone (PQ) and plastocyanin (PC) pools, ii) respiratory transport mainly involving the electron donors succinate dehydrogenase (SDH) and the type-I NAD(P)H dehydrogenase (NDH-1), cytochrome *c*, the cytochrome *bd*-type quinol oxidase (Cyd) and the aa*3*-type cytochrome *c* oxidase (COX) and iii) cyclic electron transport around PSI [4–7]. Additional possibilities include alternate respiratory terminal oxidases (ARTO) and electron transfer from PSI to flavodiiron proteins Flv1 and Flv3 as well as NDH-1 [8–13]. A balanced interplay of all these units is necessary in order to, for example, generate a favorable ATP:NADPH flux-ratio, typically of 3:2 [14], or alter this ratio to accommodate photoprotective and/or repair responses under stress conditions. Photoprotective mechanisms in *Synechocystis* reportedly include non-photochemical fluorescence quenching (NPQ) via the orange carotenoid protein (OCP) and state transitions (ST) [3,15]. While the former is a response to (high) blue light intensity that leads to photoactivation of OCP, which in turn, binds to the phycobilisome (PB) thereby quenching it [16–18], the latter refers to a change in the rate of excitation energy transfer (EET) to PSI vis-à-vis PSI [19–21]. In other words, while OCP activity enables an additional EET channel to avoid excitation energy reaching the reaction centers (RCs) in the first place, STs are a subtly regulated response to re-balance the EET ratio between the two photosystems during, for instance, a sudden change in the intensity of the incident radiation. Thus, PSI both plays a pivotal role in EET regulation and is a crucial vehicle for electron transport across the thylakoid membrane.

Cells of a PSI-deficient mutant of *Synechocystis* were subject to investigation in a recent study focusing on changes in chlorophyll (Chl) a fluorescence as a result of dark-to-light transitions [22]. Therein, the authors reported a quenched species, appearing under illumination conditions that ensured that OCP could not have been activated. This result unavoidably revives the fundamental question asked many years ago already by Vermaas et al. [23]: what happens to the excitations in the absence of PSI when the PSIIRC is closed? Since both OCP-related fluorescence quenching and ST must be ruled out, Acuña et al. [22]...
postulated a third quenching mechanism in order to explain the overall fluorescence decrease: a high-light protein (HiIp) would be at its origin. HiIps have indeed been suggested to act as non-photochemical quenchers in *Synechocystis* [24] and there is conclusive evidence that HiID, one of the members of the HiIp-family, binds Chlorophyll a (Chl a) and β-carotene (β-car), thus enabling a dissipative channel with energy being directly transferred from a Chl a Q_{B} state to a β-car S_{1} state [25,26]. Besides, in the specific case of the PSI-deficient mutant of *Synechocystis* the only photosynthetic species will be manifested and how: i) HiIp-containing cells have been shown to evolve much less singlet oxygen than the corresponding control strain [27], confirming the photoprotective role of HiID, and ii) the carotenoid content of HiID-containing fractions has been found to be relatively high [28], which makes the assumption of an HiID-related quenching channel all the more plausible.

The central aim of this study is to elucidate how cells of a PSI-deficient mutant of *Synechocystis* are able to cope with light stress during a dark-to-light transition. We do this by tracking the emitted cellular fluorescence spectrum over time [29,30]. The obtained results are analyzed (Section 3.1) using a method based on the Singular Value Decomposition (SVD) previously demonstrated in [22]. Then, we discuss the SVD-based results in terms of the parameters of a mathematical model (Section 3.2). The only photosynthetic fluorescent units present in the sample of this strain are the phycocyanin-containing PB antenna and the Chl a-containing PSII dimers. Any fluorescence dynamics should therefore be explained in terms of these species only. In the following Gedankenexperiment we make an attempt to anticipate which species will be manifested and how: i) a PB unable to transfer excitation energy to PSII should be visible as a pure PB-spectrum with a very high fluorescence quantum yield; ii) if the PB is coupled to a PSII-dimer while both RCs are closed, we also expect a high quantum yield, though with an additional chlorophyll-derived 680 nm signature; iii) in case of a functional PB-PSII complex with two photochemically (open) quenched RCs, we would expect a less strong 680 nm signature and a low fluorescence quantum yield; iv) should only one of the RCs in the PSII dimer be closed while the other would remain open, we would expect a fluorescence quantum yield that lies somewhere between ii) and iii); v) a PB-PSII complex being non-photochemically quenched would result in a low fluorescence quantum yield. Fig. 1 schematically depicts these species. We set out to measure time-resolved fluorescence spectra of whole cells of the PSI-deficient mutant of *Synechocystis* after preparing the cells with different dark-adaptation (DA) times, so as to systematically affect its respiratory activity. This resulted in the identification of a novel quencher that is insensitive to the respiratory inhibitor potassium cyanide (KCN), yet requires oxygen to function.

2. Materials and methods

2.1. Cell cultures

The PSI-deficient mutant of *Synechocystis* [31] was a gift from prof. C. Funk (Umeå University, Sweden) and was stored at ~ 80 °C in 15% glycerol. Prior to preparing a liquid culture, cells were streaked on agar plates containing BG-11 (Sigma) supplemented with 0.3% sodium thiosulfate, 35 μg/mL chloramphenicol and 10 mM glucose. The strain appeared incompatible with our usual agar, requiring the plates to be solidified using Difco Granulated agar (BD). The plates were incubated in an incubator (Versatile Environmental Test Chamber MLR-350H, Sanyo) with a humidified atmosphere of elevated CO_{2} (2%) kept at 30 °C. Incident light intensity was reduced to below 5 μmol photons m^{-2} s^{-1} by covering the plates with layers of paper.

Liquid cultures were prepared by inoculating 25 mL modified BG-11 (BG-11-PC, van Alphen et al. manuscript in preparation) supplemented with 10 mM glucose, 25 mM 1,4-Piperazinedipropansulfonic acid (PIPPS)-KOH buffered at pH 8.0, 5 μg/mL chloramphenicol in a 100-mL flask (FB33131, Fisherbrand). The nitrogen source was NaN_{3} except for the batch of cells discussed in Section 3.2.2 where NH_{4}Cl was used instead. The flasks were covered in multiple layers of paper to reduce the incident light intensity to below 5 μmol photons m^{-2} s^{-1} and were placed in a shaking incubator (Innova 43, New Brunswick Scientific), equipped with a custom LED panel containing LEDs of 632 nm (orange-red) and 451 nm (blue, both 8 nm full width at half maximum) at 120 rpm and 30 °C.

2.2. Spectrally resolved fluorescence induction

The multiple LED set-up described in [22,32] was placed right near the facilities where the *Synechocystis* cells were grown, ensuring thereby that the cells were in proper physiological conditions during the experiments. The illumination protocols (Fig. S 1) have been applied to ΔPSI cells of *Synechocystis*, taken from the same batch, diluted to an optical density at 730 nm (OD_{max}) of 0.4 in fresh medium (the only exception being Fig. 12). Two background (590 nm) light intensities

![Fig. 1. Visualization of possible fluorescent species in the *Synechocystis* ΔPSI mutant and their expected fluorescence quantum yield.](image-url)
μ1300

S1A) and 450 fi

we have measured under conditions preventing respiratory electron transfer to oxygen, realized in two different ways: chemically, using KCN (1 mM final concentration) which is known to inhibit respiratory activity [33,34]; and physically, by bubbling an N2/CO2 mixture into the cuvette containing the sample to generate an O2-deprived environment. Under these micro-oxic conditions, the oxygen concentration is assumed to be well below the Km of the terminal oxidases, such that the terminal oxidases are effectively shut down.

2.3. Additional elements of data analysis

Here we describe an extension of the spectral decomposition methods that have been described in [22]. Acquired fluorescence spectra (p wavelengths) at m time points can be represented by an (m × p) data matrix Ψ. The Singular Value Decomposition (SVD) procedure [35] decomposes Ψ according to:

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into an (m × m) matrix U, where the m columns are called the left singular vectors (lsv); the (m × p) diagonal matrix S whose diagonal elements (s1, s2, s3, ...) are called the singular values and the transpose of the (p × m) matrix V, V^T, whose rows are called the right singular vectors (rsv). Time-dependent characteristics are contained in the lsv, while the rsv are a linear combination of the species associated spectra (SAS). The original matrix Ψ can be satisfactorily reconstructed by means of the n most significant singular vectors yielding a (m × nλ) matrix Ψ_n. In Acuña, Kaha, Gwizdala, Snellenburg, Van Alphen, Van Oort, Kirilovsky, Van Gronelle and Van Stokkum [22] data matrices of rank n = 2 were analyzed. While the core of the method remains the same, we also present data matrices that are of rank n = 3, meaning that the transformation matrix applied to the singular vectors had to be correspondingly expanded.

We seek a mathematical transformation Ψ_n = U · S · (A^−1 · A) · V^T to resolve the SAS and their time-dependent concentrations. For a rank

were used: 50 μmol photons m\(^{-2}\) s\(^{-1}\) (referred to as low light; see Fig. S 1A) and 450 μmol photons m\(^{-2}\) s\(^{-1}\) (referred to as high light; see Fig. S 1B). The light intensity during the saturating pulses was 1300 μmol photons m\(^{-2}\) s\(^{-1}\). Each pulse (1 s) consists of ten data points since the time resolution is 100 ms. Cells have undergone varying DA times prior to application of the illumination protocol. Also, we have measured under conditions preventing respiratory electron

Table 1
Average (of seven points) levels of c1 and c2 at three different spots of the light protocol: first and last saturation pulses as well as average Fc level just before the background is turned off.

<table>
<thead>
<tr>
<th>DA (min) (Figure)</th>
<th>First pulse</th>
<th>Final Fc</th>
<th>Last pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c1</td>
<td>c2</td>
<td>c1</td>
</tr>
<tr>
<td>50 μmol photons m(^{-2}) s(^{-1})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34 S1</td>
<td>0.02</td>
<td>0.86</td>
<td>0.84</td>
</tr>
<tr>
<td>5 S4</td>
<td>0.03</td>
<td>0.97</td>
<td>0.84</td>
</tr>
<tr>
<td>1 S4</td>
<td>0.07</td>
<td>0.93</td>
<td>0.84</td>
</tr>
<tr>
<td>450 μmol photons m(^{-2}) s(^{-1})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34 + KCN [3]</td>
<td>0.02</td>
<td>0.98</td>
<td>0.28</td>
</tr>
<tr>
<td>34 + N2/CO2 [5]</td>
<td>0.03</td>
<td>0.97</td>
<td>0.29</td>
</tr>
<tr>
<td>1 (+N2/CO2) [5]</td>
<td>0.01</td>
<td>0.99</td>
<td>0.34</td>
</tr>
<tr>
<td>34 [6D]</td>
<td>0.02</td>
<td>0.98</td>
<td>0.60</td>
</tr>
<tr>
<td>5 [6D]</td>
<td>0.02</td>
<td>0.98</td>
<td>0.56</td>
</tr>
<tr>
<td>1 [6D]</td>
<td>0.02</td>
<td>0.98</td>
<td>0.40</td>
</tr>
</tbody>
</table>

we have measured under conditions preventing respiratory electron transfer to oxygen, realized in two different ways: chemically, using KCN (1 mM final concentration) which is known to inhibit respiratory activity [33,34]; and physically, by bubbling an N2/CO2 mixture into the cuvette containing the sample to generate an O2-deprived environment. Under these micro-oxic conditions, the oxygen concentration is assumed to be well below the Km of the terminal oxidases, such that the terminal oxidases are effectively shut down.

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We seek a mathematical transformation Ψ_n = U · S · (A^−1 · A) · V^T to resolve the SAS and their time-dependent concentrations. For a rank
\[ n = 3 \]

\[ A \text{ has the form:} \]

\[
\begin{pmatrix}
  a_{11} & a_{12} & a_{13} \\
  a_{21} & a_{22} & a_{23} \\
  a_{31} & a_{32} & a_{33}
\end{pmatrix}
\]

and the final decomposition of \( \Psi_{(n-2)} \) is expressed as a linear combination of three \( n_t \times 1 \) vectors corresponding to the concentrations and the \( 1 \times n_\lambda \) vectors corresponding to the SASs:\n
\[
\Psi_{(n-3)} = c_{11}\text{SAS}_{11} + c_{21}\text{SAS}_{21} + c_{31}\text{SAS}_{31}
\]  

(3)

Hereafter, we will refer to these vectors using the simplified notation: \( c_{1}, c_{2}, c_{3}, \text{SAS}_{1}, \text{SAS}_{2} \) and \( \text{SAS}_{3} \). Furthermore, a number of criteria are followed to judge whether these transformed vectors are biophysically meaningful. With the expansion to a rank \( n = 3 \) matrix, the number of parameters to be estimated increases; this is why, additionally to the criteria presented in our previous work [22], we have added, for each SAS, SAS smoothness–penalties:

\[
\text{penalty}_{\text{smoothness}} = \sum_j w_j \left| \int \frac{\partial^2}{\partial t^2} \text{SAS}_j(\lambda) \right|
\]  

(4)

This is one among other contributions to the objective function that we seek to minimize. See the SI for the explicit objective function used in this work. For a rank \( n = 2 \) matrix, which is applicable for most of the data sets presented here, the description is simpler, and \( A \) has the form:

\[
A = \begin{pmatrix}
1 & a_{12} \\
0 & a_{22} \\
0 & a_{32}
\end{pmatrix}
\]

(5)

and the final decomposition of \( \Psi_{(n-2)} \) is expressed as:

\[
\Psi_{(n-2)} = c_{12}\text{SAS}_{12} + c_{22}\text{SAS}_{22}
\]  

(6)

Finally, each concentration value \( c_{nt} \) output at the position \( t \) that corresponds to a saturation pulse is multiplied by a parameter \( p_{tf} \) that accounts for the difference in light intensity between the background illumination and the saturation pulse. Thus, relative concentrations that are independent of the light intensity are estimated.

3. Results and discussion

3.1. Results from SVD analysis

In this section, we discuss results obtained following different protocols. First, we show results of ΔPSI cells exposed to high light background illumination, ambient O$_2$ and treated with KCN. (A) The SAS (Black: SAS$_1$; green: SAS$_2$) obtained after transformation of the singular vectors (shown in Fig. S 3). Panels B and C show a zoom view of the first and last pulse. (D) Time profiles. Key: orange: 450 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \); black: darkness. The colored bar on top illustrates the light regime with arrows indicating the beginning of a saturation pulse. Inset: The average deviation in the sum of concentrations from unity.
oxidases cannot function. Finally, we discuss results obtained from samples illuminated with 590 nm light of 450 μmol photons m$^{-2}$ s$^{-1}$ exposed to ambient oxygen.

3.1.1. Exposure to low light background illumination

After transformation of the singular vectors (Fig. S 2), we obtain the SAS shown in Fig. 2A (SAS1 and SAS2 in black and green, respectively). Panel D shows the corresponding concentration profiles of c1 (black) and c2 (green) during the entire experiment, while panel B (panel C) zooms into the first (last) saturation pulse. Fig. 2B and C show similar dynamics suggesting that during the second period of darkness the state of the cell is restored and light-induced photodamage can be excluded. As a reminder: one saturation pulse stretches over 1 s with 100 ms time resolution (i.e. ten data points); as light is turned off the signal returns to zero within one data point, meaning that we are unable to resolve relaxation dynamics and the changes in light intensity behave almost like step functions. When the light pulse is turned on at \( t = 3 \text{s} \), however, \( c_2 \) gradually builds up. This is due to the closure of RCs (consistent with the strong 680 nm signature of SAS2) which, though very fast, takes one data point (100 ms) to reach a maximum level \( (F_{\text{m}}) \). In other words: though not entirely resolved, the so-called OJIP induction phase [36] is, at least, visible. Conversely, we interpret \( c_1 \) as the population of complexes with open RCs since saturating light results in its sharp decrease (Fig. 2B, C and D). Thus, the interpretation of the two SAS in Fig. 2 can be given in terms of two species depicted in Fig. 1: SAS2 with the higher amplitude (high fluorescence quantum yield) as PB-PSII complexes with closed RCs (Fig. 1C) and SAS1, with low quantum yield and little 680 nm signature as PB-PSII complexes with open RCs (Fig. 1A). During saturation pulses, the decrease of \( c_1 \) is concomitant with a sharp increase of \( c_2 \), whereas during background illumination \( (F_0) \), the signal is a combination of both, and consists of fairly constant contributions from complexes with open and closed RCs, with, interestingly, the population of \( c_1 \) being the predominant one. The interpretation then is that the background light is able to close only a fraction (ca. 15%, Table 1) of the complexes in the sample as opposed to saturating light. With this background light intensity, the fluorescence dynamics are mainly explained by closing and re-opening of RCs (Fig. 2D) and there is no clear indication of the action of a Hill-type quencher (vide infra). This pattern systematically reproduces across different experiments with low intensity background light, with cells first dark-adapted for 34, 5 and 1 min (Fig. S 4).

3.1.2. KCN-treated cells exposed to high light background illumination

Fig. 3 shows the results obtained with a sample that was exposed to 450 μmol photons m$^{-2}$ s$^{-1}$ but previously treated with KCN, in order to inhibit terminal oxidase activity. Clearly, the first light pulse (Fig. 3B) induces a rise in \( c_2 \), which is by far the predominant species. Correspondingly, SAS2 (Fig. 3A) has also the higher fluorescence quantum yield and peaks at 681 nm. Therefore, we interpret it as a PB-PSII complex with both RCs closed (Fig. 1C). The interpretation of SAS1 is less straight forward than in the previous section, because: i) the population of \( c_2 \) instantly reaches a constant level with no sign of an induction phase nor are there correlated increasing/decreasing populations within the first 100 ms; and ii) only very small signs of open/closing RCs during saturation pulses are present throughout the experiment (Fig. 3D). Thus, SAS1 most probably consists of contributions from complexes with quenched species and little complexes with open RCs. As the sample goes through the illumination protocol, \( c_1 \) (\( c_2 \) gradually increases (decreases) while saturation pulses barely make any difference. Thus, this data matrix is interpretable in terms of the following two species: SAS2 would correspond to PB-PSII complexes with closed RCs (Fig. 1C) while SAS1 would correspond to a dynamic mixture of PB-PSII complexes non-photochemically quenched PB-PSII complexes (Fig. 1D) as well as some complexes with open RCs (Fig. 1A). Over time the quenched species become predominant, evidenced by the diminishing signs of open/closing RCs during the saturation pulses.
Fig. 4 shows an overlay of the four normalized SASs obtained up to this point: Indeed, the two SAS2, interpreted in the two experiments as being the same species, are virtually identical. The two SAS1 are very similar; in the case of the cells not treated with KCN, however, a less pronounced 680 nm signature indicates that energy transfer from the PB terminal emitters to PSII followed by photochemical quenching affects the SAS differently than the quenching of closed complexes by the unknown quencher, QX.

3.1.3. Exposure to high-light background illumination in a microoxic environment

In a further attempt to affect respiratory activity without risking an effect on photosynthesis, an experiment was conducted where cells were exposed to 450 μmol photons m⁻² s⁻¹ while a mixture of N₂/CO₂ was bubbled into the cuvette in order to deprive the cells of ambient oxygen. The results are shown in Fig. 5. The obtained SAS and the behavior during the saturation pulses are very similar to the results shown in Fig. 2. As in Fig. 2B and C, the first and last pulses in Fig. 5 clearly show opening/closing behavior and this seems to be a suitable interpretation for the SAS in this case as well. Due to the high background light intensity, however, the F₁ levels are now inverted: c₂ is as high as 65% (Table 1) indicating a higher relative concentration of complexes with closed PSII RCs. Otherwise, though there is a modest decrease in c₂ during the first 20 s of background illumination, once the concentration profiles even out, there is no sign of additional fluorescence quenching and most of the signal can be read as constant levels of fluorescence that toggle between distinct levels as a function of the light intensity. This is a remarkable observation, since, under the same background illumination, the KCN experiment reveals the system’s ability to quench fluorescence (Fig. 3) and it indicates that oxygen is required for QX to function.

3.1.4. Exposure to high light background illumination in an O₂-rich environment

Three experiments with different DA times are shown in Fig. 6. In all cases, cells were exposed to ambient oxygen without addition of KCN. The matrices were analyzed independently, yielding three pairs of concentration profiles shown in Fig. 6D and three pairs of SAS shown as insets therein. Fig. 6A–C and E–G show a zoom in into, respectively, the first and last saturation pulses. These SASs match well those of Fig. 4. Judging from the opening/closing dynamics and the gradual increase of the c₁ base level (concomitant with a decrease in the c₂ base level), the contribution of quenched PB-PSII complexes (Fig. 1D) again gradually increases over time. As previously observed under O₂-deprived conditions (Fig. 5), during the first 20 s of background illumination, c₂ decreases by 15–20% before F₁ levels off. However, contrary to those latter conditions, a new phase of fluorescence quenching sets in that lasts until the end of the light protocol. Remarkably, the second quenching phase seems to kick-off at earlier times for samples that underwent a longer DA period. While for the sample with the longest dark adaptation this phase sets in as early as ca. t = 50 s, for DA = 5 min it starts around 75 s and for DA = 1 min it only comes at times t > 140 s. Also, the relative concentration of c₁ during saturation...
pulses tends to reach higher and higher values throughout the experiment. Even after the system has gone through a short relaxation period of a few seconds in darkness, the last saturation pulse does not push the c₁ level down to zero (see Fig. 6E, F and G). Instead, values of around 30% (Table 1), similar to the KCN experiment, are observed in Fig. 6F and G. This again points at a quenched species being formed over time.

### 3.2. Modeling dark-to-light transitions

#### 3.2.1. Rank 2 systems

To test whether the postulated species described in the sections above could indeed explain the observed dynamics, we constructed a minimal mathematical model (inspired by the model of Ebenhöh et al. [37]) that takes into account only species A, C and D from Fig. 1. We consider PB-PSII-dimer complexes that are fully closed by the action of light or open via an electron acceptor of the PQ pool. Additionally, fluorescence quenching can occur if QX attaches to the closed complex. Fig. 7 displays the photosynthetic and respiratory components we consider essential: we postulate three fluorescent components with two distinguishable spectral contributions. Following the interpretation of

![Fig. 7 Scheme summarizing the different species of the mathematical model postulated to describe the time-resolved fluorescence spectra of Synechocystis ΔPSI cells. PB-PSII-dimers complexes are closed by the action of light or opened via an electron acceptor of the PQ pool (horizontal transition). Additionally, fluorescence quenching can occur if quencher Qx attaches to a closed RC (vertical transition, detailed in the inset). A PB-PSII complex with two closed RCs (a) emits fluorescence as SAS₂; PB-PSII complexes with either two open RCs (b) or two closed and quenched RCs (c) emit as SAS₁. Key: kL, light-induced transition; kPQ, electron transfer rate to PQ pool; Keq, PQ equilibrium constant, kA, quencher attachment rate, kD, quencher detachment rate; kX, quencher specific deactivation rate, kbsf, cyt b₆f electron transfer rate; Keqbsf, cyt b₆f equilibrium constant; kCOX, terminal oxidase electron transfer rate. Further explanation in the main text and in the SI.](image)

#### Table 2

Key parameters of the model used to simulate the concentrations of the species depicted in Fig. 7 under different conditions. Common parameters for all conditions are the rates (in s⁻¹) $k_L = 10^{-5}$, $k_{QX} = 9 \cdot 10^{-3}$, $k_{at} = 0.6$, $k_{x,dec} = 0.02$, $k_A = 3.5 \cdot 10^{-4}$, $k_D = 5 \cdot 10^{-6}$, $k_{bsf} = 0.104$ and the equilibration constants $K_{eq} = 5 \cdot 10^3$ and $K_{eq,bsf} = 5 \cdot 10^{-4}$.

<table>
<thead>
<tr>
<th>Conditions [Figure]</th>
<th>$k_{COX}$ (s⁻¹)</th>
<th>$O_2$ conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$O_2 + DA = 34$ m [8B]</td>
<td>0.01</td>
<td>$4 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>$O_2 + KCN$ [9B]</td>
<td>0.001</td>
<td>$4 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>$\Delta O_2$ [10B]</td>
<td>0.01</td>
<td>$4 \cdot 10^{-4}$</td>
</tr>
<tr>
<td>$O_2 + DA = 34$ m [11B]</td>
<td>0.01</td>
<td>$4 \cdot 10^{-3}$</td>
</tr>
</tbody>
</table>

3.2. Modeling dark-to-light transitions

#### 3.2.1. Rank 2 systems

To test whether the postulated species described in the sections above could indeed explain the observed dynamics, we constructed a minimal mathematical model (inspired by the model of Ebenhöh et al. [37]) that takes into account only species A, C and D from Fig. 1. We consider PB-PSII-dimer complexes that are fully closed by the action of light or open via an electron acceptor of the PQ pool. Additionally, fluorescence quenching can occur if QX attaches to the closed complex. Fig. 7 displays the photosynthetic and respiratory components we consider essential: we postulate three fluorescent components with two distinguishable spectral contributions. Following the interpretation of

![Fig. 8 Spectral decomposition of fluorescence spectra of ΔPSI cells exposed to low background light after 34 min dark adaptation shown in Fig. 2D (A) and simulated concentrations (B) of PB-PSII-dimer complexes with open RCs (blue), with closed RCs and unquenched (green) or quenched (gray). Black represents the sum of blue and gray, and is observed as SAS₁.](image)
Fig. 2, there would be two species: a PB-PSII dimer complex with both RCs open as depicted in Fig. 7a, and another one with its RCs closed, as depicted in Fig. 7b. They would emit fluorescence as SAS1 and SAS2, respectively. The KCN experiment (Fig. 3D) supports the interpretation of SAS2 as originating from double-closed RCs, but the quenched component must be attributed to the action of a quencher QX. This would result in very similar SAS1 contributions but two fundamentally different molecular origins. This quenched species is depicted in Fig. 7c and the assumed mechanism for its formation is shown in the red box below: we postulate a light-induced excited state of the quencher Q\textsuperscript{X}\textsuperscript{*} that is populated during illumination (k\textsubscript{L}) and decays back to its non-activated counterpart, Q\textsubscript{X}\textsubscript{0}, with the specific deactivation rate k\textsubscript{X,dec}. Q\textsuperscript{X}\textsuperscript{*} is then able to bind to, presumably, PSII. The formation of this Q\textsubscript{X,bound} is described by an attachment rate k\textsubscript{A} and assumed to be proportional to the fraction of reduced PQ, PQH\textsubscript{2}, and to the concentration of O\textsubscript{2}. In a third step, the quencher detachment rate k\textsubscript{D} describes uncoupling of the quencher and a re-population of Q\textsubscript{X}\textsubscript{0}. As for the linear electron flow dynamics, the PQ pool is the main electron acceptor of Fig. 9. Spectral decomposition of fluorescence spectra of KCN-treated ΔPSII cells exposed to high background light after 34 min dark adaptation shown in Fig. 3D (A) and simulated concentrations (B) of PB-PSII-dimer complexes with open RCs (blue), with closed RCs and unquenched (green) or quenched (gray). Black represents the sum of blue and gray, and is observed as SAS1.

Fig. 10. Spectral decomposition of fluorescence spectra of ΔPSII cells under microoxic conditions exposed to high background light after 34 min dark adaptation shown in Fig. 5D (A) and simulated concentrations (B) of PB-PSII-dimer complexes with open RCs (blue), with closed RCs and unquenched (green) or quenched (gray). Black represents the sum of blue and gray, and is observed as SAS1.
PSII and works at the rate $k_{\text{PQ}}$. A potential back transfer from the PQ pool to the photosystem is considered in the equilibrium constant $K_{\text{eq}}$. Furthermore, the electrons are carried to the cyt $b_6f$ whose turnover rate is $k_{\text{b}6\text{f}}$ and which is in equilibrium with the PQ pool with $K_{\text{eq,b}6\text{f}}$. Finally, the action of the terminal oxidases as acceptors of the PC pool is summed up in the parameter $k_{\text{COX}}$ that stands for the last step in the linear electron transport chain. The corresponding system of ordinary differential equations is described in the SI.

Using this model, we set out to simulate results from the analysis presented above. The simulation of the result of the experiment with low background illumination (Fig. 2) is shown in Fig. 8A. A first set of parameters inspired by the model of Matuszyńska et al. [38] was used to simulate the concentration profiles of Fig. 8B. The parameters are collated in Table 2. The general toggle behavior between complexes with open and closed RCs is reproduced fairly well using these values for $k_L$ and $k_{\text{PQ}}$. Moreover, the choice of the equilibrium constants $K_{\text{eq},b_6f}$ and $K_{\text{eq,b}6\text{f}}$ and the rates for cyt $b_6f$ and the terminal oxidases $k_{\text{b}6\text{f}}$ and $k_{\text{COX}}$ lead to similar $F_o$ and $F_i$ levels. Note that the simulated concentrations predict the behavior of the species during periods of darkness between the first (last) pulse and the onset (ending) of background illumination, whereas the original data matrix shows a gap because during such dark periods, effectively, no measurement was carried out.

The challenge for the model is to now reproduce the behavior found in the other datasets while tweaking only the parameters that reflect the conditions under which the experiment was carried out. Between the experiments depicted in Figs. 2 and 3, there are two main differences: the background light intensity increases by a factor of 9 and KCN-sensitive electron sinks are chemically blocked. The first parameter, $k_L$, is adjusted by inputting a different light profile (Fig. S 1), with the right background level. As for the action of KCN on the sample, we decrease the $k_{\text{COX}}$ parameter by an order of magnitude: this should reflect, on the one hand, the fact that there is an overall decrease in the turnover rate of terminal oxidases. On the other hand, $k_{\text{COX}}$ not being zero, accounts for the assumption that the thylakoid membrane may harbor additional, non-KCN-sensitive electron sinks [39] or that KCN may affect the reduction rate of e.g. cyt f [40], ultimately leading to a different, though non-zero, action of the terminal oxidases. Additionally, a mutant which lacks all terminal oxidases has a significantly affected PAM profile [41], compatible with that of KCN-treated cells presented in this work. Small changes in the parameters $k_{\text{COX}}$ and $k_L$ already lead to Fig. 9B (changed parameters are written in bold in Table 2). A higher background light intensity leads to an overall increase of the $c_2$ level. Additionally, a considerable drop in $k_{\text{COX}}$ translates in the inability to drain electrons from the PC pool which, then again, leads to complete reduction of the PQ pool and a further increase in the $F_s$ level, almost to the point that saturation pulses barely stand out (almost no variable fluorescence.

Fig. 11. Spectral decomposition of fluorescence spectra of ΔPSI cells exposed to high background light after 1 min of dark adaptation shown in Fig. 6D, top (A) and simulated concentrations (B) of PB-PSII-dimer complexes with open RCs (blue), with closed RCs and unquenched (green) or quenched (gray). Black represents the sum of blue and gray, and is observed as SAS1.

Fig. 12. The redox state of the PQ pool predicted by the model for ΔPSI cells exposed to high background light after 1 min of dark adaptation. The full concentration profile is shown in Fig. S 9.
Concomitantly, the absence of O₂ directly impacts the quencher kinetics of zero. The last pulse of the KCN-treated sample (Fig. 3C) decreases to 30% instead of being completely quenched.

Fig. 11. The fluorescence spectrum over time (solid gray curve) and is observed as the black SAS1. The deactivation of QX happens slowly enough that after turning the background light off, the gray level lingers on. The remaining QX explains why the black level in the last pulse of the KCN-treated sample (Fig. 3C) decreases to 30% instead of zero.

As a next step, we now restore the k_COX parameter to its original value and decrease, instead, the concentration of O₂ by one order of magnitude. The result is shown in Fig. 10: restoring k_COX is a sensible choice given the magnitude of the observed variable fluorescence. Concomitantly, the absence of O₂ directly impacts the quencher kinetics (cf. the SI). Since nearly no quencher is formed, the overall levels of both c₁ and c₂ remain fairly constant throughout the experiment, as indeed observed. This would reinforce the hypothesis that QX is operative in the presence of oxygen in order to hamper the formation of singlet oxygen or any other ROS.

In principle, restoring the oxygen value to its original value should then describe the experiment series shown in Fig. 6. This is illustrated in Fig. 11. The first striking observation is that, although variable fluorescence and an overall decrease of the F_i level in c₂ are reproduced to some extent, the data shows particular moments in time where fluorescence quenching sets in. The first decrease in c₂ happens within the first 20–40 s of background illumination and it is visible in all samples. Depending on the length of the DA, a second phase sets in with c₂ steadily decreasing until the end of the light protocol. There are two clear exceptions: i) in the case of low background light and ii) in the microoxic environment, the second phase does not set in.

The current model, however, does not yet explain how dark-adaptation relates to the specific evolution of the fluorescence spectrum over time. Nevertheless, the model does offer a prediction concerning the behavior of the redox potential throughout the experiment: the redox state of the PQ pool (generally considered as a key regulator in cyanobacteria; see e.g. [9]) follows the trend of the SAS2 concentration and is predicted to be much higher (Fig. 12) than reported for the wild type under high light [42]. This is in line with the high degree of reduction of the PQ pool observed in this mutant by indirect measurements in similar light conditions [5]. It is not known whether the biochemical mechanism(s) of state transitions are still operative in the PSI-deletion strain. The ease of modulation of the redox state of the PQ pool might suggest they are; the actual regulatory parameter, however, may be the redox state of the cytb6f complex [42].

3.2.2. Beyond the rank 2 analysis

Further limitations to the model arise with data matrices of higher rank. Fig. 13 shows a dataset where the fluorescence signal was acquired with another light protocol where the sampling frequency of the saturation pulses was increased (Fig. S 1C) and with another batch of ΔPSI cells. The response is highly dynamic and visual inspection of the singular value scree plot (Fig. S 7) already points out that, at least, a third component is clearly distinguishable from the noise. Here too, the black and green are interpreted as a PB-PSII dimer complex with closed and open RCs, respectively (compare with SASs in Fig. 6A), and display during the first 50 s a mutual exchange in populations with the third component (red) nearly being constant (Fig. 6D). The third component could be an intermediate state such as the one depicted in Fig. 1B where one RC in the PSII dimer is closed but not the other. An alternative may include a PB-PSII complex with closed RCs but able to alleviate the excitation pressure by transferring energy to e.g. Flv2/4 [43], resulting in less back-transfer to PB which manifests as a less pronounced 660 nm emission than that of SAS2. Additional measurements of expression levels of the Flv2/4 proteins would have to support this hypothesis, but regardless of the mechanism, none of these two interpretations would contradict the behavior of the (red) time profile c₃, which decreases during the pulses, contributing thus to the population of c₂ (which systematically has maximal values during saturation periods). This alternating pattern in the concentration of certain complexes illustrates the dynamic character of the response to light exposure. It is unclear...
which and how many molecular origins such a re-arrangement of complexes might be resulting from. Recently, however, small-angle neutron scattering experiments in Synechocystis cells have revealed a substantial re-configuration of the interthylakoidal space in the event of a dark-to-light transition [44,45]. Also, in the case of the PAL mutant lacking phycobilisomes, a rapid (2–4 min) re-organization of the thylakoidal surface is observed which the authors suggest could originate from surface charges [41]. These interpretations, as well as the speculation that the contribution of the fourth singular value (Fig. 7) may be non-negligible present a scenario, however, that goes beyond the scope of the minimal model presented in this work.

4. Closing remarks

In the series of experiments that were all analyzed as independent rank 2 systems, the two SASs are interpreted as follows: SAS2: PB-PSII scope of the minimal model presented in this work.

References

[31] P.H. Lambrev, M. Nilkens, Y. Mileslavina, P. Jahn, A.R. Holzwarth, Kinetic and


