



**HAL**  
open science

## The cytochrome *b\_6f* complex is not involved in cyanobacterial state transitions

Pablo I Calzadilla, Jiao Zhan, Pierre Sétif, Claire Lemaire, Daniel Solymosi,  
Natalia Battchikova, Qiang Wang, Diana Kirilovsky

### ► To cite this version:

Pablo I Calzadilla, Jiao Zhan, Pierre Sétif, Claire Lemaire, Daniel Solymosi, et al.. The cytochrome *b\_6f* complex is not involved in cyanobacterial state transitions. *The Plant cell*, 2019, 31 (4), pp.911-931. 10.1105/tpc.18.00916 . hal-02108309

**HAL Id: hal-02108309**

**<https://hal.science/hal-02108309>**

Submitted on 24 Apr 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

RESEARCH ARTICLE

## The Cytochrome *b<sub>6</sub>f* Complex is Not Involved in Cyanobacterial State Transitions

Pablo I. Calzadilla<sup>1\*</sup>, Jiao Zhan<sup>1,2\*</sup>, Pierre Sétif<sup>1</sup>, Claire Lemaire<sup>1</sup>, Daniel Solymosi<sup>3</sup>, Natalia Battchikova<sup>3</sup>, Qiang Wang<sup>2,4</sup> and Diana Kirilovsky<sup>1,a</sup>

<sup>1</sup> Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, 91198 Gif sur Yvette, France

<sup>2</sup> Key Laboratory of Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, Hubei, China

<sup>3</sup> Molecular Plant Biology Lab, Biochemistry Dept, Faculty of Science and Engineering, University of Turku, Turku, Finland

<sup>4</sup> Key Laboratory of Plant Stress Biology, State Key Laboratory of Cotton Biology, School of Life Sciences, Henan University, Kaifeng 475004, China

\*These authors contributed equally to this work

<sup>a</sup>Corresponding author: [diana.kirilovsky@cea.fr](mailto:diana.kirilovsky@cea.fr)

### Short title: Cyanobacterial state transitions revisited

**One-sentence summary:** Cyanobacterial state transitions, which balance photosystem activities during photosynthesis, depend on the plastoquinone pool redox state but not on cytochrome *b<sub>6</sub>f* or phosphorylation reactions.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors ([www.plantcell.org](http://www.plantcell.org)) is: Diana Kirilovsky ([diana.kirilovsky@cea.fr](mailto:diana.kirilovsky@cea.fr)).

### ABSTRACT

Photosynthetic organisms must sense and respond to fluctuating environmental conditions in order to perform efficient photosynthesis and to avoid the formation of dangerous reactive oxygen species. The excitation energy arriving at each photosystem permanently changes due to variations in the intensity and spectral properties of the absorbed light. Cyanobacteria, like plants and algae, have developed a mechanism, named state transitions, that balances photosystem activities. Here, we characterize the role of the cytochrome *b<sub>6</sub>f* complex and phosphorylation reactions in cyanobacterial state transitions using *Synechococcus elongatus* PCC 7942 and *Synechocystis* PCC 6803 as model organisms. First, large Photosystem II fluorescence quenching was observed in State II, which does not appear to be related to energy transfer from Photosystem II to Photosystem I (spillover). This membrane-associated process was inhibited by betaine, sucrose and high concentrations of phosphate. Then, using different chemicals affecting the plastoquinone pool redox state and cytochrome *b<sub>6</sub>f* activity, we demonstrate that this complex is not involved in state transitions in *S. elongatus* or

*Synechocystis* PCC6803. Finally, by constructing and characterizing 21 protein kinase and phosphatase mutants and using chemical inhibitors, we demonstrate that phosphorylation reactions are not essential for cyanobacterial state transitions. Thus, signal transduction is completely different in cyanobacterial and plant (green alga) state transitions.

## 1 INTRODUCTION

2           Photosynthetic organisms must cope with changes in the quality and quantity of  
3 incoming light. In order to survive and to optimize the use of light, they must adapt to  
4 changing environmental conditions by regulating the energy arriving at the reaction centers.  
5 Specific illumination of Photosystem II (PSII) or Photosystem I (PSI) creates an energy  
6 imbalance that leads to the over-reduction or over-oxidation of the intersystem electron  
7 transport chain. Murata (Murata, 1969) and Bonaventura and Myers (Bonaventura and Myers,  
8 1969) were the first to propose a mechanism, called “State transitions”, which rebalances the  
9 activity of reaction centers I and II. Two states were defined: State I, induced by light  
10 preferentially absorbed by PSI and characterized by a high PSII to PSI fluorescence ratio;  
11 State II, induced by light preferentially absorbed by PSII and characterized by a low PSII to  
12 PSI fluorescence ratio. The transition from one state to the other is triggered by changes in the  
13 redox state of the plastoquinone (PQ) pool (Allen et al., 1981; Mullineaux and Allen, 1990):  
14 oxidation of the PQ pool induces the transition to State I and its reduction induces the  
15 transition to State II.

16           In plants and green algae, reduction of the PQ pool induces the activation of a specific  
17 kinase that phosphorylates the membrane-bound light harvesting complex II (LHCII). The  
18 phosphorylated LHCII detaches from PSII and attaches to PSI during the transition from State  
19 I to State II. Oxidation of the PQ pool deactivates the kinase and a phosphatase  
20 dephosphorylates LHCII, which again migrates to PSII. The migration of LHCII from one  
21 photosystem to the other allows for a readjustment in the distribution of excitation energy  
22 arriving at PSI and PSII (see review (Minagawa, 2011)).

23           In red algae and cyanobacteria, the principal PSII antenna is the phycobilisome (PBS),  
24 a large extramembrane complex constituted by phycobiliproteins organized in a core from  
25 which rods radiate (reviews (Glazer, 1984; MacColl, 1998; Adir, 2008)). As a consequence,  
26 the processes involved in state transitions in these organisms differ. In red algae, the large  
27 fluorescence quenching induced by the illumination of dark-adapted cells is related to two  
28 different mechanisms: a PSII non-photochemical-quenching mechanism (qE) induced by a  
29 low luminal pH (Delphin et al., 1995; Delphin et al., 1996; Kowalczyk et al., 2013; Krupnik  
30 et al., 2013), in which the fluorescence quenching occurs at the level of the reaction centers

31 (Krupnik et al., 2013), and state transitions induced by changes in the redox state of the PQ  
32 pool, which involve changes in energy transfer from PSII to PSI (spillover) (Ley and Butler,  
33 1980; Kowalczyk et al., 2013). The relative importance of each mechanism varies among  
34 strains (Delphin et al., 1996; Kowalczyk et al., 2013). In cyanobacteria, the molecular  
35 mechanism of the PQ-pool dependent state transitions remains largely obscure. This process,  
36 which involves fluorescence changes occurring upon illumination of dark-adapted cells or  
37 under illumination with light absorbed more specifically by PSII or PSI, indeed remains an  
38 open question, despite the many studies resulting in the proposal of several hypotheses and  
39 models.

40 In the mobile-phycoobilisome model, the movement of phycobilisomes (PBSs) induces  
41 changes in direct energy transfer from PBS to PSII and PSI (Allen et al., 1985; Mullineaux  
42 and Allen, 1990; Mullineaux et al., 1997). This model attributes the low PSII fluorescence  
43 yield in State II to a lower amount of energy transfer from PBSs to PSII, together with larger  
44 energy transfer to PSI. The observations that PBSs are able to rapidly move on the thylakoid  
45 surface (Mullineaux et al., 1997) and that chemicals inhibiting PBS diffusion also inhibit state  
46 transitions support this model (Joshua and Mullineaux, 2004; Li et al., 2004; Li et al., 2006).  
47 In the spillover model, the energy transfer from PBS to PSII remains equal in both states, but  
48 the excess energy absorbed by PSII is transferred to PSI (spillover) in State II via a process  
49 involving the movement of photosystems (Ley and Butler, 1980; Bruce and Biggins, 1985;  
50 Olive et al., 1986; Biggins and Bruce, 1989; Biggins et al., 1989; Vernotte et al., 1992; El  
51 Bissati et al., 2000; Federman et al., 2000). The hypothesis that changes at the level of  
52 photosystems are responsible for state transitions is supported by various observations: state  
53 transitions occur in mutants lacking PBSs (Bruce et al., 1989; Olive et al., 1997; El Bissati et  
54 al., 2000); state transitions are accompanied by structural changes in membranes (Vernotte et  
55 al., 1992; Folea et al., 2008) and PSI monomerization/trimerization (Kruip et al., 1994;  
56 Schluchter et al., 1996; Aspinwall et al., 2004); and membrane fluidity influences state  
57 transitions (El Bissati et al., 2000). Nevertheless, there has been no clear demonstration that  
58 the spillover is larger in State II than in State I, although some studies have suggested this (see  
59 (Mullineaux et al., 1991; Bruce and Salehian, 1992)). It was also proposed that these two  
60 mechanisms coexist and are responsible for the fluorescence changes observed in state  
61 transitions: movement of PBS (changes in direct energy transfer from PBSs to photosystems)  
62 and movement of photosystems (changes in spillover) (Scott et al., 2006). However, more  
63 recent studies have questioned the definition of cyanobacterial state transitions as a rebalance  
64 of excitation energy arriving to one or another photosystem. The increase in fluorescence in

65 State I has principally been associated with the functional detachment of PBS from the  
66 photosystems (Kana et al., 2009; Kana, 2013; Chukhutsina et al., 2015), whereas the decrease  
67 in fluorescence in State II was mainly attributed to a specific fluorescence quenching of  
68 Photosystem II not involving spillover (Ranjbar Choubeh et al., 2018).

69 Furthermore, the states of plants and cyanobacteria in darkness differ: while plants are  
70 generally in State I, cyanobacteria are in State II (Aoki and Katoh, 1982; Mullineaux and  
71 Allen, 1986). In cyanobacteria, respiration and photosynthesis occur in the thylakoid  
72 membranes, and PQ, cytochrome (cyt) *b<sub>6</sub>f* and plastocyanin (or cyt *c<sub>6</sub>*) are electron carriers  
73 common to both electron transport chains (review (Mullineaux, 2014)). During respiration,  
74 the homologs of mitochondrial Complex I (NDH-1) and Complex II (Succinate  
75 dehydrogenase, SDH) reduce the PQ pool, and different oxidases oxidize it (for review see  
76 (Mullineaux, 2014)). Different cyanobacterial strains present different PQ pool reduction  
77 states in darkness, giving different levels of dark PSII fluorescence (see for ex (Misumi et al.,  
78 2016)). Upon illumination, PSI is activated and the PQ pool becomes more oxidized, leading  
79 to State I (Mullineaux and Allen, 1990; Campbell et al., 1998).

80 In plants and green algae, the redox sensor of the PQ pool is the cyt *b<sub>6</sub>f* complex,  
81 which interacts with a specific kinase of the major membrane chlorophyll antenna, LHCII  
82 (Wollman and Lemaire, 1988). Phosphorylation of LHCII trimers induces their detachment  
83 from PSII and partial (or total) attachment to PSI, inducing the transition to State II (Kyle et  
84 al., 1984). Two reports suggest that cyt *b<sub>6</sub>f* also plays a role in cyanobacterial state transitions  
85 (Mao et al., 2002; Huang et al., 2003). However, further evidence is still needed to confirm its  
86 direct involvement. In this sense, the relationship between phosphorylation and cyanobacterial  
87 state transitions is also an open question. Allen and coworkers suggested that specific types of  
88 phosphorylation could occur during state transitions (Allen et al., 1985), but this was not  
89 confirmed in more recent works. Nevertheless, analysis of phospho-proteomes showed that  
90 phosphorylation takes place in PBSs and photosystems (Yang et al., 2013; Chen et al., 2015;  
91 Spat et al., 2015). In addition, when residues Ser22, 49 and 154 and Thr94 of phycocyanin  
92 (PBS protein) were mutated to non-phosphorylatable amino acids in *Synechocystis* cells, the  
93 kinetic and amplitude of transition to State I induced by light illumination of dark adapted  
94 cells appeared to be affected (Chen et al., 2015). Some functions of Ser/Thr kinases (Spk)  
95 have already been described. For example, SpkA is involved in the control of cell motility  
96 (Kamei et al., 2001; Panichkin et al., 2006); SpkB participates in the oxidative stress response  
97 by phosphorylating glycyl-tRNA-synthetase  $\beta$ -subunit (Mata-Cabana et al., 2012); SpkE  
98 might be involved in the regulation of nitrogen metabolism (Galkin, 2003); SpkD might be

99 involved in adjusting the pool of TCA (tricarboxylic acid) cycle metabolites (Laurent et al.,  
100 2008); SpkG plays an essential role in high-salt resistance (Liang et al., 2011); SpkC, SpkF  
101 and SpkK are involved in the phosphorylation of the GroES chaperone protein (Zorina et al.,  
102 2014); and SpkG is involved in the phosphorylation of Fd5 (ferredoxin 5) protein (Angeleri et  
103 al., 2018).

104 As a whole, the molecular mechanism behind cyanobacterial state transitions is still a  
105 matter of discussion, although many hypotheses have been proposed. Therefore, we decided  
106 to further study this mechanism by specifically addressing the role of the cyt *b<sub>6</sub>f* complex in  
107 this process.

108 In the past decades, state transitions have mainly been studied in the cyanobacteria  
109 *Synechocystis* PCC 6803 (hereafter *Synechocystis*) (for ex: (Vernotte et al., 1992; Emlyn-  
110 Jones et al., 1999; McConnell et al., 2002; Kondo et al., 2009; Chukhutsina et al., 2015)),  
111 *Synechococcus* PCC7002 (McConnell et al., 2002; Dong and Zhao, 2008; Dong et al., 2009)  
112 and *Spirulina platensis* (Li et al., 2004; Li et al., 2006). In these strains, the changes in  
113 fluorescence related to state transitions (dark versus blue [or far-red] illumination) are rather  
114 small, which makes mechanistic studies difficult. The differences in PSII fluorescence in  
115 darkness (State II) and under blue-light illumination (State I) are significantly larger in  
116 *Synechococcus elongatus* strain than in *Synechocystis*. Therefore, in the current study, we  
117 characterized state transitions in *S. elongatus* and compared them to those in *Synechocystis*  
118 *PCC 6803*, which allowed us to obtain clearer conclusions. Our results confirm recently  
119 published data demonstrating that a large amplitude of PSII fluorescence quenching is  
120 induced in State II in *S. elongatus* (Ranjbar Choubeh et al., 2018). This PSII quenching  
121 appears to be unrelated to spillover. In addition, not only do we show that the results and  
122 arguments used to link the cyt *b<sub>6</sub>f* complex with state transitions were not conclusive, but we  
123 also demonstrate that cyt *b<sub>6</sub>f* and protein phosphorylation reactions do not participate in this  
124 process in cyanobacteria. Thus, different signaling pathways are involved in state transitions  
125 in cyanobacteria compared to plants and green algae.

126

## 127 **RESULTS**

### 128 **State transitions in *S. elongatus* and *Synechocystis***

#### 129 ***77 K fluorescence spectra in State II and State I***

130 The absorbance spectra indicate that, under our growth conditions, *S. elongatus*  
131 presents a higher phycocyanin (PC, absorbance at 620 nm) to chlorophyll (Chl, absorbance at  
132 680 nm) ratio than *Synechocystis* (Figure 1). In addition, the PSI to PSII ratio (measured by  
133  $(F_A, F_B)^-$  and  $\text{TyrD}^+$  EPR signals) was around 2–3 in *S. elongatus* and 4–5 in *Synechocystis*  
134 cells (for details, see Methods).

135 Figure 2 compares the low temperature (-196.15°C) 77 K fluorescence emission  
136 spectra of dark and blue-light adapted *S. elongatus* (A, C) and *Synechocystis* (B, D) cells. At  
137 this temperature, the fluorescence of both photosystems is visible, while at room temperature  
138 only PSII-related fluorescence is observed.

139 Supplemental Figures 1 and 2 show the Gaussian decomposition of these spectra,  
140 providing a visualization of different components of the spectra. When the PBSs were  
141 preferentially excited (excitation at 590 nm), we observed a large peak at 650-660 nm related  
142 to PC and allophycocyanin (APC) fluorescence, a peak at 683 nm related to the chlorophyll  
143 binding protein CP43 and the last emitters of PBS, a peak (or shoulder in *S. elongatus*) at 695  
144 nm corresponding to Reaction Center II and CP47, and finally a peak at 718 nm (*S. elongatus*)  
145 or 722 nm (*Synechocystis*) related to PSI fluorescence (Vandorssen et al., 1987; Siefermann-  
146 harms, 1988). The PSII-related peaks at 683 and 695 nm were higher in blue-light adapted  
147 cells (State I) than in dark-adapted cells (State II). The differences between the peaks in State  
148 I and II were larger in *S. elongatus* than in *Synechocystis*. Regarding PSI, the fluorescence  
149 was similar in dark- and light-adapted cells of both strains.

150 When the chlorophyll was preferentially excited (excitation at 430 nm), the PSI  
151 emission peak (at 718 nm in *S. elongatus* and 722 nm in *Synechocystis*) was the highest in  
152 both strains. In addition, the PSII-related peaks at 685 and 695 nm were much higher in *S.*  
153 *elongatus* than in *Synechocystis*, corresponding to a lower PSI to PSII ratio (2–3 in *S.*  
154 *elongatus* versus 4–5 in *Synechocystis*). The PSI related peaks were similar in darkness and  
155 blue-light illumination. The absence of changes in PSI-related fluorescence during state  
156 transitions was confirmed by normalizing the *S. elongatus* spectra with an external dye  
157 (Rhodamine B) (Figure 3). The PSII-related peaks increased upon blue-light illumination in  
158 both strains. Nevertheless, the emission at 695 nm increased more than the one at 683 nm  
159 (Supplemental Figures 1 and 2).

160

161 ***Effect of hyper-osmotic buffers on state transitions***

162 We investigated the effects of the hyper-osmotic buffers betaine (1 M, pH 7.0),  
163 sucrose (1 M) and phosphate (0.5 M, pH 7.5) on state transitions in *S. elongatus* cells (Figure  
164 4 and Supplemental Figure 3). Control cells were incubated in the absence of chemicals in the  
165 dark (cells-II) or under blue-light illumination (cells-I). Aliquots of these cells were rapidly  
166 frozen. Cells-II and cells-I were then incubated with chemicals for 5 min under identical  
167 conditions (dark for cells-II, blue-light illumination for cells-I). Cells-II were then transferred  
168 to blue-light for 5 minutes before freezing, whereas Cells-I were transferred to dark for 5  
169 minutes before freezing. 77 K fluorescence spectra were then measured with excitation at 430  
170 nm and at 590 nm. The first effect observed upon addition of betaine and sucrose was a large  
171 general quenching of fluorescence, suggesting that these chemicals have an effect not only on  
172 PBSs but also on membranes (Figure 4 and Supplemental Figure 3). The quenching effect of  
173 hyper-osmotic media was previously reported in *Synechocystis* by (Papageorgiou et al., 1999),  
174 who attributed it to alterations in membrane fluidity. Nevertheless, the PBS quenching  
175 seemed to be larger than that of Chl, since the 683/695 and 660/695 ratios decreased after the  
176 addition of betaine and sucrose with excitation at 590 nm. By contrast, phosphate addition had  
177 a more specific quenching effect on PBS fluorescence, as seen by the relatively large decrease  
178 in fluorescence at 660 nm (Supplemental Figure 3).

179 As previously observed (Joshua and Mullineaux, 2004; Li et al., 2004; Li et al., 2006),  
180 no fluorescence changes were detected in 77 K emission spectra obtained by excitation at 590  
181 nm when state transitions were tentatively induced in the presence of betaine, sucrose or  
182 phosphate (Figure 4 and Supplemental Figure 3). In addition, the chemicals also inhibited the  
183 fluorescence changes observed in the 77 K emission spectra obtained with 430 nm excitation  
184 (Figure 4 and Supplemental Figure 3). These results demonstrate that betaine, sucrose and  
185 phosphate also block the changes produced in the membranes. In conclusion, the effect of  
186 these hyper-osmotic buffers on state transitions could be due to the inhibition of PBS  
187 movement and/or processes occurring in the membrane.

188

### 189 ***State transitions kinetics and the redox state of the PQ pool in darkness***

190

191 Figure 5 shows typical traces of room temperature fluorescence kinetics measured  
192 with a PAM fluorometer in dark-adapted *S. elongatus* (A) and *Synechocystis* (B) cells  
193 successively illuminated by low intensities of blue and orange light. Dark-adapted cells  
194 presented a low dark maximal fluorescence ( $F_{md}$ ), indicating that the cells were in State II.  
195 Upon illumination with blue-light, which preferentially excites chlorophyll, a large and rapid



196 increase in  $F_m'$  was observed (arriving at a maximal  $F_{mb}'$  level), indicating the transition to  
197 State I. The ratio  $F_{vb}$  to  $F_{vd}$  ( $F_v$  = variable fluorescence =  $F_m - F_0$ ) was approximately 4.0 in *S.*  
198 *elongatus* but only 1.2 in *Synechocystis* cells (Figure 5). The dark  $F_{vd}/F_0$  was much smaller in  
199 *S. elongatus* than in *Synechocystis* (0.22 versus 1.09). By contrast, small differences were  
200 observed in the  $F_{vb}/F_0$  ratio (0.83 versus 1.26). These data suggest that dark-adapted *S.*  
201 *elongatus* cells were in a “stronger” State II than *Synechocystis* cells.

202 In order to elucidate whether this could be explained by a more reduced PQ pool in  
203 dark-adapted *S. elongatus*, we estimated the redox state of the PQ pool in each strain by  
204 measuring fluorescence induction curves in the absence and presence of DCMU, which  
205 inhibits electron transfer between the primary ( $Q_A$ ) and secondary ( $Q_B$ ) quinones in PSII  
206 (Figure 6). When dark-adapted cells are illuminated, the PQ pool becomes more reduced and  
207 the photochemical centers become partially closed. As a consequence, a concomitant  
208 fluorescence increase is observed until a steady state level is reached. The increase kinetics  
209 depends on both the initial redox state of the PQ pool and its rate of photochemical reduction  
210 under illumination. However, when DCMU is present, a maximum level of fluorescence is  
211 reached in which all the centers are closed and the rate of fluorescence increase depends only  
212 on the antenna size and is independent of the dark redox state of the PQ pool. Figure 7 (A and  
213 B) shows the fluorescence induction curves in the presence and absence of DCMU for *S.*  
214 *elongatus* and *Synechocystis* dark-adapted cells. The area between the curves is much larger  
215 for dark-adapted *Synechocystis* than for *S. elongatus* cells (59% vs. 33% of the DCMU area,  
216 respectively).

217 Because the area between the curves is at least partially proportional to the amount of  
218 dark-oxidized PQ (Bennoun, 1982; Srivastava et al., 1995), these results suggest that the PQ  
219 pool is more reduced in dark-adapted *S. elongatus* cells than in *Synechocystis*. However,  
220 under illumination, the rates of PQ reduction by PSII and of  $PQH_2$  (reduced PQ) reoxidation  
221 by PSI also affect the kinetics of fluorescence induction. These rates could be different  
222 between the strains, as the PSI to PSII and the phycobiliprotein to chlorophyll ratios are  
223 different in *Synechocystis* and *S. elongatus*. Therefore, we performed a control experiment in  
224 which the PQ dark reduction level in each strain was modified without changing the size of  
225 the antenna or PSI and PSII activities. The dark PQ redox state depends on the relative  
226 activities of NDH-1/SDH and of cyanide-sensitive terminal oxidases (Cyd and Cox) (see  
227 Figure 6); thus, the addition of sodium cyanide (NaCN) should lead to a large PQ-reduction  
228 level. A large effect of NaCN was observed in *Synechocystis* cells, in which the area between  
229 the curves (+/- DCMU) decreased from 59 in the absence of NaCN to 27% in its presence

230 (Figure 7). By contrast, the effect was much smaller in *S. elongatus* cells, with only a decrease  
231 from 33 to 24% (Figure 7). These results strongly suggest that the PQ pool was more strongly  
232 reduced in *S. elongatus* than in *Synechocystis*.

233 The illumination of blue-light adapted cells with orange light (which preferentially  
234 excites phycobiliproteins) induced a decrease in  $F_m'$  in both cyanobacterial strains (Figure 5).  
235 In *Synechocystis* cells, the steady state  $F_{m0}$  was similar to  $F_{md}$ , whereas in *S. elongatus*, the  
236  $F_{m0}$  was higher. As already mentioned, the “strength” of State II depends on the concentration  
237 of reduced PQ. Thus, our results indicate that the PQ pool was more strongly reduced in dark-  
238 adapted *S. elongatus* than in orange light-illuminated cells. This was not the case in  
239 *Synechocystis* cells, where the redox state of the PQ pool appeared to be similar under both  
240 conditions.

241

242 **Is cytochrome *b<sub>6</sub>f* involved in state transitions in *S. elongatus*?**

243 ***Effect of DMBQ and DBMIB on state transitions.***

244 Mao et al. (Mao et al., 2002) and Huang et al. (Huang et al., 2003) proposed that cyt  
245 *b<sub>6</sub>f* is involved in the signaling pathway of cyanobacterial state transitions, as observed in  
246 green algae and plants. They based their proposal on the results obtained by chemically  
247 inducing state transitions in *Synechocystis* and *Synechococcus* PCC 7002 using 2,6-  
248 dimethoxy-1,4-benzoquinone (DMBQ), p-benzoquinone (PBQ) and 2,5-dibromo-3-methyl-6-  
249 isopropyl-p-benzoquinone (DBMIB). DMBQ and PBQ accept electrons from the PQ pool  
250 (Preston and Critchley, 1988), while DBMIB inhibits cyt *b<sub>6</sub>f* activity by attaching to the Qo  
251 site (the PQH<sub>2</sub> binding site), preventing reoxidation of the PQ pool (Roberts and Kramer,  
252 2001) (Figure 6). These authors found that the addition of DMBQ (or PBQ) to dark-adapted  
253 cells induced an increase in  $F_{md}$ , which they attributed to a partial transition to State I  
254 triggered by oxidation of the PQ pool. The simultaneous addition of DMBQ and DBMIB  
255 inhibited this increase. Both authors hypothesized that under these conditions, the PQ pool  
256 remained oxidized, which led them to conclude that the binding of DBMIB to the cyt *b<sub>6</sub>f* Qo  
257 site was primarily involved in the transition to State II. However, none of these studies  
258 demonstrated that the PQ pool remained oxidized under these conditions.

259 Figure 8 shows the effect of DMBQ and DBMIB on  $F_{md}$  in dark-adapted *S. elongatus*  
260 cells. The addition of DMBQ (250  $\mu$ M) induced a slow increase in  $F_{md}$  (and  $F_0$ ) related to a  
261 partial transition to State I. Higher concentrations of DMBQ cannot be used because they  
262 induce fluorescence quenching. When DBMIB (20  $\mu$ M) was subsequently added, a rapid

263 decrease of  $F_{md}$  (and  $F_0$ ) was observed (Figure 8A). When both chemicals were  
264 simultaneously added, the DMBQ-induced increase in fluorescence was inhibited (Figure  
265 8B). These effects of chemicals were therefore similar to those previously observed in  
266 *Synechocystis* and *Synechococcus* PCC 7002 cells (Huang et al., 2003; Mao et al., 2003).

267 We then explored the dark redox state of the PQ pool in *S. elongatus* cells by  
268 analyzing the kinetics of fluorescence induction in the presence and absence of DCMU (as  
269 described in the previous section). The measurements were carried out in dark-adapted cells  
270 (15 min) in the presence of DMBQ alone (250  $\mu$ M) or DMBQ and DBMIB (5 and 10  $\mu$ M).  
271 The measurements were done after 3 min of incubation with DMBQ and at 1 and 3 min after  
272 the addition of DBMIB (Figure 9). As mentioned above, the PQ pool is reduced in dark-  
273 adapted *S. elongatus* cells: the area between the curves (+/- DCMU) is small and  $F_{md}$  is low  
274 (Figure 7A). The addition of DMBQ increased the level of  $F_{md}$  and the area between the two  
275 curves (+/- DCMU), suggesting that the PQ pool had become oxidized (Figure 9A). Since  
276 DMBQ also accepts electrons from the PQ pool during the light measurement, the larger area  
277 between the curves is also partially related to its activity during the measurement.

278 The addition of 5  $\mu$ M DBMIB induced a rapid decrease of  $F_{md}$  and of the area between  
279 the curves. The smaller area indicated that the PQ pool was more strongly reduced in darkness  
280 and during the light measurement in the presence of DBMIB (Figure 9B). Longer periods of  
281 incubation with DBMIB induced a larger decrease in area, indicating a larger reduction of the  
282 PQ pool even in the presence of DMBQ (Figure 9C). In line with these results, the addition of  
283 10  $\mu$ M DBMIB had a faster and larger effect (Figure 9D and E). Thus, the DMBQ-induced  
284 transition to State I is inhibited by DBMIB, likely because DBMIB treatment leads to the  
285 reduction of the PQ pool, even in the presence of DMBQ, at least in *S. elongatus*.

286 We further studied the effects of DBMIB by adding different concentrations of this  
287 chemical (2.5, 5, 10, 15 and 20  $\mu$ M) to *S. elongatus* cells adapted to State 1 by illumination  
288 with blue-green light or by adding DMBQ in darkness (Figure 8C). Under illumination, all  
289 concentrations of DBMIB efficiently induced a large transition to State 2 with rather similar  
290 kinetics (red curves). However, concentrations higher than 5  $\mu$ M were necessary to induce the  
291 transition to State 2 in darkness and in the presence of DMBQ. In this case, the rate and  
292 amplitude of the transition depended on DBMIB concentration. Thus, the same concentration  
293 of DBMIB had different effects under illumination and in darkness, in the presence of  
294 DMBQ. For example, 5  $\mu$ M DBMIB induced almost maximum fluorescence quenching under  
295 illumination but was unable to induce any quenching in darkness in the presence of DMBQ.  
296 Upon illumination of dark-adapted cells in the presence of DMBQ and different

297 concentrations of DBMIB (2.5, 5, 10, 15  $\mu$ M), larger fluorescence quenching was induced  
298 (Figure 8D). These results strongly suggest that the transition to State II is induced by the  
299 reduction of the PQ pool and not by the binding of DBMIB to the Qo site in *cyt b<sub>6</sub>f*.

300

### 301 *Effect of the TMPD on state transitions*

302

303 We then looked for another chemical compound that does not interact with *cyt b<sub>6</sub>f* and  
304 can oxidize the PQ pool in the presence of DBMIB to further confirm that this complex does  
305 not play a role in state transitions. *N,N,N'*-tetramethyl-*p*-phenylenediamine (TMPD)  
306 represents such a compound, since it restores oxygen evolution by reversing the inhibitory  
307 effect of DBMIB on the photosynthetic electron transport chain (Draber et al., 1970; Nanba  
308 and Katoh, 1985) (Figure 6). Nanba and Satoh (Nanba and Katoh, 1985) demonstrated that  
309 TMPD accepts electrons from the PQ pool and directly donates them to P700<sup>+</sup>, bypassing the  
310 DBMIB-poisoned *cyt b<sub>6</sub>f* complex. However, TMPD is also a good electron acceptor from  
311 PSI and can generate cyclic electron transfer around PSI (Hiyama and Ke, 1972) (Figure 6).  
312 This chemical has opposite effects on state transitions, depending on the preferential electron  
313 donor to TMPD (PQ pool versus PSI).

314 It is expected that, under blue-light in the absence of DBMIB (State I), the addition of  
315 TMPD should have no effect on fluorescence if TMPD is efficient at oxidizing reduced PQ,  
316 as the PQ pool should remain oxidized. However, exactly the opposite effect was observed: a  
317 large quenching of fluorescence was induced upon TMPD addition. The amplitude of  
318 quenching depended on the TMPD concentration (Supplemental Figure 4A-D). This effect  
319 can be explained by assuming that, under these conditions, TMPD is primarily involved in  
320 cyclic electron transport and functions poorly as an oxidizer of reduced PQ. Cyclic electron  
321 transport also limits linear electron flow through *cyt b<sub>6</sub>f* and consequently contributes to PQ  
322 reduction. In accordance with the poor efficiency of TMPD in oxidizing reduced PQ under  
323 these conditions, the addition of TMPD to DBMIB-poisoned cells led to no change in  
324 fluorescence, indicating that State II was maintained (Supplemental Figure 4E). In addition,  
325 TMPD did not hinder the effect of DBMIB on state transitions: large fluorescence quenching  
326 was observed even in the presence of TMPD (Supplemental Figure 4F).

327 In an attempt to modify the behavior of TMPD, we added methyl viologen (MV) to  
328 the reaction. MV is a good electron acceptor from PSI that could compete with TMPD at this  
329 level. This approach was found to be successful: the addition of DBMIB following that of  
330 TMPD led to only a small decrease in fluorescence, indicating that under these conditions,

331 TMPD was efficient at performing electron uptake from the PQ pool (Supplemental Figure  
332 4G). Notably, the presence of MV did not affect state transitions in the absence of TMPD  
333 (Supplemental Figure 4F and G).

334 More importantly, when TMPD was added in the presence of MV to largely quenched  
335 DBMIB-poisoned cells, it induced a large increase in fluorescence related to the transition to  
336 State 1 (Figure 10). The final Fv was 70% that of cells under blue-light illumination in the  
337 absence of chemicals. The transition to State 1 induced by TMPD under blue-light  
338 illumination was larger than that induced by DMBQ in darkness (55%). Thus, TMPD is able  
339 to reverse the effect of DBMIB by taking electrons from PQ and giving them to PSI,  
340 bypassing the inhibited cyt *b<sub>6</sub>f* complex. Similar results were obtained with *Synechocystis*  
341 cells using 3 mM MV and 7.5  $\mu$ M TMPD (Figure 10). In conclusion, the transition to State I  
342 can be induced even when cyt *b<sub>6</sub>f* is inhibited by DBMIB by partially oxidizing the PQ pool.

343

#### 344 **Are protein phosphorylation reactions required for cyanobacterial state transitions?**

345 In plants and green algae, conformational changes induced in the cyt *b<sub>6</sub>f* complex  
346 (especially in the Rieske protein) by the occupancy of the Qo site by a PQH<sub>2</sub> molecule (Zhang  
347 et al., 1998; Zito et al., 1999; Breyton, 2000; Finazzi et al., 2001) activates a specific kinase  
348 (STN7/Stt7) (Depege et al., 2003; Bellafiore et al., 2005)), which phosphorylates the mobile  
349 trimers of LHCII, inducing their detachment from PSII. As previously mentioned, at least two  
350 studies suggested that protein phosphorylation by one specific Ser/Thr kinase could also  
351 trigger cyanobacterial state transitions (Allen et al., 1985; Chen et al., 2015). To test this  
352 hypothesis, we created *Synechocystis* protein kinase and phosphatase mutants. *Synechocystis*  
353 has 12 genes encoding putative Ser/Thr kinases (SPTKs). Seven of these genes encode  
354 proteins belonging to the PKN2 subfamily (spkA to spkG) and five belonging to the ABC1  
355 subfamily (spkH to spkL) (Zorina, 2013). Each gene was individually deleted by replacing it  
356 with a kanamycin resistance cassette (see Methods for details). We also individually deleted  
357 genes encoding nine phosphatases: slr0328 (PTP family), sll1771, slr1860, sll1033, sll0602,  
358 slr0114, slr1983 (PPM family), sll1387 (PPP family) and slr0946. Thus, we created 12 single  
359 *Synechocystis* kinase mutants and 9 single *Synechocystis* phosphatase mutants. To determine  
360 if the mutants were affected in state transitions, we illuminated dark-adapted mutant cells with  
361 blue light to induce the transition to State I, followed by orange light to induce the transition  
362 to State II (Figure 11). All of the dark-adapted single mutants went to State I upon blue light  
363 illumination and then to State II during orange light illumination (Figure 11 and Supplemental  
364 Figure 5). The rates of decrease in fluorescence during the State I to State II transition were

365 similar in WT and mutant cells (Figure 11 and Supplemental Figure 5). These experiments  
366 indicate that no specific Ser/Thr kinase or phosphatase is involved in cyanobacterial state  
367 transitions, as it is the case in green algae and plants.

368 To confirm that phosphorylation reactions are not essential for cyanobacterial state  
369 transitions, we tested the effects of kinase and phosphatase inhibitors on *Synechocystis* and *S.*  
370 *elongatus*. Staurosporine and K252a (a derivative of staurosporine) are potent inhibitors of  
371 Ser/Thr and Tyr kinases that interact with their ATP binding sites ((Fernandez et al., 2006;  
372 Nakano and Omura, 2009)). NaF inhibits Ser/Thr and acid phosphatases and  $\text{Na}_3\text{VO}_4$  inhibits  
373 Tyr and alkaline phosphatases ((Delphin et al., 1995; McCartney et al., 1997) and references  
374 inside). Staurosporine and NaF were shown to inhibit state transitions in the green alga  
375 *Chlamydomonas reinhardtii* ((Delphin et al., 1995) and references inside). Figure 12 shows  
376 that the presence of staurosporine or K252a, which were added in excess (21  $\mu\text{M}$  and 1  $\mu\text{M}$ ,  
377 with the  $\text{IC}_{50}$  of these compounds 0.6  $\mu\text{M}$  and 96 nM, respectively (Nakano and Omura,  
378 2009)) did not inhibit state transitions in *Synechocystis* or *S. elongatus*.

379 To confirm that these kinase inhibitors are able to enter cyanobacterial cells and inhibit  
380 phosphorylation reactions, we tested their effects on the phosphorylation of the P<sub>II</sub> protein in  
381 *S. elongatus*. The P<sub>II</sub> protein (*glnB* gene product) is involved in the tight coordination of  
382 carbon and nitrogen assimilation. Its activity involves the phosphorylation and  
383 dephosphorylation of a Ser residue (Forchhammer and Tandeau de Marsac, 1995a, 1995b). In  
384 ammonium-grown cells, P<sub>II</sub> is completely dephosphorylated. The transfer of cells to medium  
385 lacking combined nitrogen induces phosphorylation of P<sub>II</sub> (Forchhammer and Tandeau de  
386 Marsac, 1995a, 1995b). The phosphorylation state of P<sub>II</sub> can be analyzed by gel  
387 electrophoresis in a phos-tag gel SDS page system and by immunoblot detection. In this  
388 system, phosphorylated proteins migrate more slowly than non-phosphorylated ones  
389 (Kinoshita and Kinoshita-Kikuta, 2011). Figure 12G shows that the presence of staurosporine  
390 and K252a completely inhibited the phosphorylation of the P<sub>II</sub> protein under nitrogen  
391 starvation conditions. This result indicates that both kinase inhibitors entered into *S. elongatus*  
392 cells and were able to inhibit protein phosphorylation.

393 Finally, phosphatase inhibitors (NaF and  $\text{Na}_3\text{VO}_4$ ) also did not affect state transitions  
394 (Figure 13). Nevertheless, NaF induced the partial inhibition of the oxygen evolving activity  
395 of PSII (Supplemental Figure 6) and a general decrease in fluorescence (Figure 13) in  
396 *Synechocystis*, indicating that this chemical entered the cells. In conclusion, our experiments  
397 show that phosphorylation reactions are not involved in cyanobacterial state transitions.

398

## 399 **DISCUSSION**

400 While the mechanism of state transitions in plants and green algae has been largely  
401 elucidated, it remains to be characterized in cyanobacteria. Contradictory hypotheses have  
402 been proposed about cyanobacterial state transitions based on studies addressing different  
403 aspects of the mechanism: the movement of PBS, spillover, reorganization of membrane  
404 complexes, involvement of Cyt *b<sub>6</sub>f* and/or phosphorylation reactions in signal transduction.  
405 However, none of these hypotheses has been definitively supported. Our results help to  
406 elucidate open questions about the mechanism behind the large fluorescence quenching  
407 observed in State II and the alleged role of the cyt *b<sub>6</sub>f* complex in the signaling pathway  
408 involved in cyanobacterial state transitions.

409

### 410 **The contributions of phycobilisome versus the membrane to state transitions**

411 It was previously shown that PBS can easily move and that high concentrations of  
412 betaine, sucrose and phosphate inhibit the diffusion of PBS and state transitions (Joshua and  
413 Mullineaux, 2004; Li et al., 2004; Li et al., 2006). Li and coworkers (Li et al., 2004) also  
414 observed that, in *Spirulina platensis*, betaine inhibits changes in 77 K emission spectra with  
415 430 nm excitation. However, the authors did not discuss this last result. Based on these works,  
416 it was concluded that the movement of PBS from one photosystem to the other was the main  
417 reason for the observed changes in fluorescence. By contrast, we demonstrated that these  
418 chemicals, in addition to hindering PBS movement, inhibit fluorescence changes that depend  
419 on membrane processes. No fluorescence change in 77 K emission spectra was detected in the  
420 presence of betaine, sucrose or phosphate upon cells illumination, not only when PBSs were  
421 excited but also when Chl was excited. Thus, based on these experiments, it cannot be  
422 concluded that the movement of PBS is the main contributor to state transitions in  
423 cyanobacteria.

424 Our experiments did not allow us to distinguish which changes, if any, occur at the  
425 level of PBS during state transitions: detachment of PBS from one or both photosystems or  
426 changes in energy transfer from PBS to one or the other photosystem. Nevertheless, we  
427 expect the contribution of these changes to be small in both *S. elongatus* and *Synechocystis*,  
428 since the main increase in fluorescence emission from State II to State I was related to PSII  
429 (G3, 695 nm emission peak) (Supplemental Figures 1 and 2).

430

### 431 **Photosystem II quenching is involved in State II**

432 Van Amerongen's group recently showed that state transitions involve a reversible  
433 quenching of PSII fluorescence independently of spillover changes in *S. elongatus* cells  
434 (Ranjbar Choubeh et al., 2018). They measured the fluorescence decay kinetics of cells in  
435 State II and State I with a streak camera using 430 nm or 577 nm excitation at 77 K. By  
436 performing global analysis of the data, the authors obtained decay-associated spectra. When  
437 430 nm excitation was used, PSII emission decreased, but the decay-associated spectra  
438 showed that PSI emission was similar in State I and State II. This argues against a change in  
439 spill-over during state transitions. This was also observed in *Synechocystis* cells (Ranjbar  
440 Choubeh et al., 2018).

441 Our results confirm these observations. We showed that both the 683 nm and 695 nm  
442 peaks decrease in State II (under Chl and PBS excitation), whereas the 718 nm peak did not  
443 change (see Supplemental Figures 1 and 2, including peak deconvolution to visualize different  
444 components of the spectra). The absence of changes in PSI-related fluorescence during state  
445 transitions was confirmed by normalizing the spectra with an external dye (Rhodamine B)  
446 (Figure 3). These results indicate that PSII emission is largely quenched in State II and that  
447 spillover from PSII to PSI does not contribute to this quenching. The PSII-related quenching  
448 mechanism remains to be elucidated.

449

#### 450 **The role of Cyt *b<sub>6f</sub>* in cyanobacterial state transitions**

451 One of the big questions that remain to be answered about the mechanism of  
452 cyanobacterial state transitions is how the signal is transmitted from the PQ pool to the PBS  
453 and/or photosystems to induce their movements or fluorescence quenching. In plants and  
454 green algae, the binding (and subsequent release) of PQH<sub>2</sub> in the Q<sub>o</sub> site of the cyt *b<sub>6f</sub>*  
455 complex plays a critical role in the activation of a specific Ser/Thr kinase that phosphorylates  
456 LHCII (Vener et al., 1995; Vener et al., 1997; Zito et al., 1999). The phosphorylated LHCII  
457 detaches from PSII and totally (or partially) associates with PSI (Vener et al., 1997; Wollman,  
458 2001). The role of the Q<sub>o</sub> site in state transitions was first suggested based on the effect of  
459 DBMIB in *Chlamydomonas*, where DBMIB inhibits State I to State II transition, although in  
460 its presence, the PQ pool is largely reduced (Finazzi et al., 2001). By contrast, in  
461 cyanobacteria, DBMIB induces the State I to State II transition. This occurs even in the  
462 presence of DCMU (which inhibits photoreduction of the PQ pool by PSII) or DMBQ (which  
463 oxidizes the PQ pool by taking electrons from PQH<sub>2</sub>) (present results and (Mao et al., 2002;  
464 Huang et al., 2003)). These authors proposed that the action of DBMIB is related to its



465 binding to the Qo site and not to the reduction of the PQ pool. The authors assumed that the  
466 PQ pool remains oxidized in the presence of both DBMIB and DMBQ (or PBQ).

467 Here, we demonstrated that this is not true, as the addition of DBMIB induced  
468 reduction of the PQ pool even in the presence of DMBQ. Moreover, we demonstrated that the  
469 same concentration of DBMIB has different effects on state transitions depending on the  
470 experimental conditions. For instance, DBMIB at 5  $\mu\text{M}$  was unable to induce the transition to  
471 State II in dark-adapted cells in the presence of DMBQ, whereas it induced large quenching in  
472 blue-green light-adapted cells in the absence or presence of DMBQ. The amplitude of  
473 fluorescence quenching induced by 10  $\mu\text{M}$  DBMIB was also larger under illumination than in  
474 darkness in the presence of DMBQ. Altogether, these experiments strongly suggested that the  
475 cyt *b<sub>6</sub>f* complex is not involved in cyanobacterial state transitions. The finding that the  
476 addition of TMPD to DBMIB-poisoned cells induced a large increase in fluorescence related  
477 to PQ oxidation and transition to State I indicates that DBMIB binding to the Qo site of cyt  
478 *b<sub>6</sub>f* is not involved in the transition to State II. Under these conditions, DBMIB remained  
479 attached to the Qo site, and the cyt *b<sub>6</sub>f* complex was inactive. As an alternative, it was recently  
480 proposed that the single chlorophyll *a* (Chl *a*) molecule present in cyt *b<sub>6</sub>f* could act as a redox  
481 sensor and signal transmitter during state transitions (Vladkova, 2016). This Chl *a* molecule is  
482 evolutionarily conserved and is present in all oxygen-evolving photosynthetic species  
483 (Vladkova, 2016). However, changes in this Chl *a* were induced by binding of DBMIB or  
484 PQH<sub>2</sub> to the Qo site. Thus, based on our results, the involvement of this Chl *a* molecule in  
485 cyanobacterial state transitions is not likely.

486 In addition, the characterization of 12 kinase and 9 phosphatase single mutants  
487 demonstrated that no specific protein kinase and/or phosphatase is necessary for  
488 cyanobacterial state transitions. More generally, the use of kinase and phosphatase inhibitors  
489 demonstrated that phosphorylation reactions are not essential for state transitions in  
490 *Synechocystis* and *S. elongatus*. Thus, signal transduction from the PQ pool to the antenna and  
491 the photosystems is completely different in cyanobacteria vs. green algae and plants.

492 While DCMU and DBMIB have opposite effects on cyanobacterial state transitions,  
493 they have the same effect on the transcription of photosynthetic genes (Alfonso et al., 1999;  
494 Alfonso et al., 2000; El Bissati and Kirilovsky, 2001). Both DCMU and DBMIB induced an  
495 increase in *psbA* transcription and a decrease in *psaE* transcription when added into  
496 *Synechocystis* cells under white and orange illumination. These findings strongly suggest that  
497 cyt *b<sub>6</sub>f* is involved in the redox transcriptional regulation of photosynthetic genes in  
498 *Synechocystis* (Alfonso et al., 1999; Alfonso et al., 2000; El Bissati and Kirilovsky, 2001). In

499 conclusion, *cyt b<sub>6</sub>f* is not involved in cyanobacterial state transitions, but it appears to be  
500 involved in redox transcriptional regulation.

501 Since it seems that in cyanobacteria, the principal effect of PQ reduction is an increase  
502 in PSII quenching and that *cyt b<sub>6</sub>f* is not involved in the signaling pathway, it is tempting to  
503 hypothesize that PSII itself senses the redox state of the PQ pool. Sensing cannot be linked to  
504  $Q_A^-$  accumulation because both the presence of DCMU and the reduction of the PQ pool  
505 increase  $Q_A^-$  concentration, but while DCMU induces the transition to State I, the reduction of  
506 the PQ pool induces the transition to State II. DCMU binding and over-reduction of the PQ  
507 pool were previously found to accelerate photoinhibition but through different mechanisms  
508 (Kirilovsky et al., 1994; Fufezan et al., 2005; Fischer et al., 2006). In line with this  
509 observation, the  $Q_B$  site could be modified differently in the presence of DCMU or PQH<sub>2</sub>,  
510 leading to different effects on  $Q_A$  redox potential, recombination reactions, and the generation  
511 of PSII quenching related to State II.

512 The PQ pool redox state could be sensed not only at the level of the  $Q_B$  site but also  
513 by the  $Q_C$  hydrophobic tunnel. The existence of this  $Q_C$  tunnel formed by *cyt b559* and *psbJ*  
514 was suggested by the X-ray crystallographic structural model of PSII of  
515 *Thermosynechococcus elongatus* at 2.9 Å resolution (Guskov et al., 2009). The function of  
516 this tunnel as a quinone binding site remains to be confirmed, since later structures at higher  
517 resolution did not contain a quinone in this hydrophobic pocket (Umena et al., 2011).  
518 However, *Synechocystis* mutants containing mutations around the proposed  $Q_C$  site show  
519 altered state transitions, making this site another interesting target of study (Huang et al.,  
520 2016).

521 In addition of these  $Q_{B/C}$  sites, there are other ways by which the redox state of the PQ  
522 pool could be sensed; the participation of other known proteins or novel factors in the  
523 signaling pathway cannot be ruled out. Overall, while the PSII-quenching mechanism and the  
524 redox sensor of the PQ pool remain to be elucidated, our results rule out the involvement of  
525 *cyt b<sub>6</sub>f* in this process.

526

## 527 **METHODS**

### 528 **Culture conditions and replicates**

529 The cyanobacteria *Synechocystis* PCC 6803 and *Synechococcus elongatus* (PCC 7942)  
530 strains were grown photo-autotrophically in BG11 medium (Herdman et al., 1973). The cells

531 were incubated in a rotary shaker (120 rpm) at 31 °C illuminated by fluorescence white lamps  
532 (50  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) under a CO<sub>2</sub> enriched atmosphere. The cells were maintained in  
533 their logarithmic phase of growth for all experiments. The kinase and phosphatase mutants  
534 were grown in the presence of kanamycin (40  $\mu\text{g/mL}$ ).

535 A biological replicate is a batch of cells on a particular day. The measurements were  
536 performed several times using the same batch of cells (technical replicates). The mean of each  
537 batch was calculated. The biologically independent experiments were performed on different  
538 days separated by at least a week. Thus, completely different cells were tested.

539

#### 540 **Construction of kinase and phosphatase mutants**

541 To obtain the kinase mutants ( $\Delta\text{spkB}$ ,  $\Delta\text{spkD}$ ,  $\Delta\text{spkE}$ ,  $\Delta\text{spkF}$ ,  $\Delta\text{spkG}$ ,  $\Delta\text{spkI}$ ,  $\Delta\text{spkJ}$ ,  
542  $\Delta\text{spkK}$ ,  $\Delta\text{spkL}$ ) a 500 bp fragment in the upstream region of each kinase gene was cloned into  
543 the pMD T-18 vector (Takara, Japan) and digested with XbaI. The PRL446 plasmid  
544 containing the kanamycin cassette was also digested with XbaI. Both linear fragments were  
545 ligated to generate the plasmid use to transform *Synechocystis* WT cells in order to obtain the  
546 knockout kinase mutants. The strategy to obtain the  $\Delta\text{spkA}$  and the  $\Delta\text{spkC}$  mutants was similar  
547 with only two minor modifications: 1) the plasmid containing the 500 bp upstream fragment  
548 was digested with SmaI instead of XbaI. 2) To obtain the final construction, the linearized  
549 plasmid was ligated to the kanamycin cassette with blunt ends, and the pPM-kinase-upper-  
550 kanamycin was obtained.

551 In parallel, a 500 bp fragment in the downstream region of each kinase gene was  
552 cloned into the pMD T-18 vector (Takara, Japan) and digested with SalI to generate the  
553 downstream fragment. Blunt ends were generated in the downstream fragment and in the  
554 pPM-kinase-upper-kanamycin linearized using the SacI/SphI enzyme. The resulting blunt-end  
555 DNAs were ligated together. After testing the direction of the inserted downstream fragment  
556 by PCR, the pPM-kinase-upper-kanamycin-down was used to transform WT *Synechocystis*  
557 cells.

558 To obtain the phosphatase mutants ( $\Delta\text{slr0328}$ ,  $\Delta\text{sll1771}$ ,  $\Delta\text{slr1860}$ ,  $\Delta\text{sll1033}$ ,  $\Delta\text{sll0602}$ ,  
559  $\Delta\text{slr0114}$ ,  $\Delta\text{slr1983}$ ,  $\Delta\text{sll1387}$  and  $\Delta\text{slr0946}$ ), a 500 bp fragment upstream of each  
560 phosphatase gene, the kanamycin cassette and a 500 bp fragment downstream of each  
561 phosphatase gene were spliced together using the PCR overlap extension method to obtain the  
562 upper-kanamycin-down DNA fragment for each phosphatase gene. The resulting DNA  
563 fragments were supplemented with a thymine at both termini and inserted into the T-cloning

564 vector pMD T-18 (Takara, Japan) to generate the final pPM-phosphatase-upper-kanamycin-  
565 down plasmids.

566 *Synechocystis* WT cells were transformed with these plasmids to obtain the knockout  
567 kinase and phosphatase mutants. The presence of the kanamycin cassette replacing the kinase  
568 and phosphatase genes and the complete segregation of each mutant were tested by PCR  
569 amplification and sequencing.

570 The oligonucleotides used in these constructions are described in Supplemental Table  
571 1.

572 The  $\Delta spkH$  *Synechocystis* mutant was kindly provided by Dr. Anna Zorina (Dimitry  
573 Los laboratory), and its construction is described in (Zorina et al., 2011).

## 574 **Fluorescence measurements**

### 575 *PAM fluorometer*

576 State transitions were monitored using a pulse amplitude modulated fluorometer  
577 (101/102/103-PAM; Walz, Effeltrich, Germany) in a 1x1 cm square stirred cuvette. All  
578 experiments were carried out at 31°C on dark-adapted (15 min) whole cells at a chlorophyll  
579 concentration of 2.5  $\mu\text{g/mL}$ . State I was induced by treatment with 85  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  of  
580 blue-green light (halogen white light filtered by a Corion cut-off 550-nm filter; 400 to 500  
581 nm). State II was induced by treatment with 25 (or 40)  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  of orange light  
582 (halogen white light filtered by a Melles Griot 03 FIV 046 filter; 600 to 640 nm) or by dark  
583 incubation. Saturating flashes (400 ms x 1200  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) were given to probe the  
584 maximum fluorescence level. The fluorescence parameters used in the analysis are the  
585 following:  $F_0$ , basal fluorescence;  $F_{\text{md}}$ , maximum fluorescence in darkness;  $F_{\text{m}}$ , maximum  
586 fluorescence under illumination;  $F_{\text{mb}}$ , maximum fluorescence under blue-light illumination;  
587  $F_{\text{mo}}$ , maximum fluorescence under orange light illumination;  $F_{\text{v}} = \text{variable fluorescence} = F_{\text{m}} -$   
588  $F_0$ ;  $F_{\text{vd}}$ , variable fluorescence in darkness;  $F_{\text{vb}}$ , variable fluorescence under blue-light  
589 illumination.

590 State transitions in the  $\Delta spkH$  mutant was measured in Turku (Finland) with a dual-  
591 PAM and compared to its own WT. State I was induced by treatment with 50  $\mu\text{mol photons}$   
592  $\text{m}^{-2} \text{ s}^{-1}$  of blue-light (460 nm) and then State II by treatment with 50  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  of  
593 orange light (635 nm). The measuring light was at 620 nm.

594 When mentioned, 2,6-dimethoxy-1,4-benzoquinone (DMBQ, 250  $\mu\text{M}$ ) and/or 2,5-  
595 dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB, 2.5 to 20  $\mu\text{M}$ ), methyl viologen (2

596 mM or 3 mM) or *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD, 7.5 or 10  $\mu$ M) were  
597 added to the stirred cuvette.

598 When mentioned, staurosporine (21  $\mu$ M), K252a (1.07  $\mu$ M), NaF (50 or 100 mM) or  
599  $\text{Na}_3\text{VO}_4$  (1 mM) were added to dark-adapted cells. The kinase inhibitors were incubated for  
600 90 min and the phosphatase inhibitors for 1 hour. Longer incubations gave the same results.  
601 State transitions were then measured.

602

### 603 *PQ pool redox state estimations*

604 The PQ pool redox state was estimated by measuring fluorescence induction curves in  
605 the presence and absence of DCMU in a PSI fluorometer (PSI Instruments, Brno, Czech  
606 Republic). Whole cells (Chl concentration 2.5  $\mu\text{g}/\text{mL}$ ) were dark-adapted for 15 min at 31°C,  
607 and illuminated (orange light 180  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ,  $\lambda = 630 \text{ nm}$ ) in the 1-ms to 1-s time  
608 range with or without 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, 10  $\mu$ M). The  
609 measuring light for these experiments was blue ( $\lambda = 460 \text{ nm}$ ), and detection was in the far-red  
610 region ( $\geq 695 \text{ nm}$ ). The fluorescence induction curves were followed in each case, and the  
611 area between them was considered to be proportional to the oxidation state of the PQ pool. In  
612 addition, measurements in the presence of DMBQ (250  $\mu$ M) and/or DBMIB (5 or 10  $\mu$ M),  
613 with or without DCMU (10  $\mu$ M) were performed.

614

### 615 *Fluorescence emission spectra*

616 77 K Fluorescence emission spectra were monitored in a CARY Eclipse  
617 spectrophotometer (Varian, Santa Clara, USA). In all cases, whole cells (Chl concentration  
618 5.0  $\mu\text{g}/\text{mL}$ ) were dark-adapted for 15 min before the measurements. Then, spectra were  
619 recorded corresponding to State II. For State I spectra, cells were illuminated for 5 min with  
620 85  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  of blue-green light (halogen white light filtered by a Corion cut-off  
621 550-nm filter; 400 to 500 nm). Samples were collected in RMN tubes and frozen by  
622 immersion in liquid nitrogen. Excitation was made at 430 nm or 590 nm, and emission was  
623 scanned from 620 nm to 800 nm. All the spectra were normalized by the signal intensity at  
624 800 nm. When Rhodamine B (0.4  $\mu$ M) was added as an internal standard, excitation was  
625 made at 430 nm and emission was scanned from 550 nm to 800 nm. In these cases, the spectra  
626 were normalized to the Rhodamine B peak at 568 nm.

627 To address the effects of high osmotic buffers in state transitions, betaine (1 M),  
628 sucrose (1 M) or  $K_2HPO_4/KH_2PO_4$  buffer (0.5 M, pH 7.5) was added to cells pre-adapted to  
629 State I or State II. The cells were incubated with the different buffers for 5 min, before State II  
630 or I was induced by darkness or blue-light illumination (5 min), respectively. Samples were  
631 taken before and after 5 min incubation with the chemicals and at the end of the light/dark  
632 treatment. Excitation was at 430 nm or 590 nm, and emission was scanned from 620 nm to  
633 800 nm. All of the spectra were normalized by the signal intensity at 800 nm.

634

### 635 **EPR measurements**

636 To assess the PSI/PSII ratio in the cyanobacterial strains, reduced  $F_A/F_B$  Fe-S centers  
637 and  $TyrD^+$  were measured as an estimation of PSI and PSII levels, respectively. First, 300 mL  
638 of cells ( $OD_{800} = 1.0$ ) were harvested and washed with 50 mL of washing buffer (50 mM  
639 HEPES pH 8.0, 5 mM  $MgCl_2$ ). The cells were then centrifuged at 6000 rpm for 10 min at 20  
640 °C and washed again with 25 mL of washing buffer. This step was repeated and the cells were  
641 washed with 2 mL of buffer. Finally, the cells were centrifuged at 3500 rpm for 10 min at 20  
642 °C and re-suspended in 500  $\mu$ L of buffer.

643 Calibrated EPR tubes were prepared with 150  $\mu$ L of concentrated cells and 20  $\mu$ L of  
644 200 mM potassium ferricyanide. The tubes were illuminated for 30 sec and incubated in  
645 darkness for 5 sec before freezing. This treatment elicited full oxidation of  $TyrD^+$  with no or  
646 very little  $P700^+$  (a few percent). In case some  $P700^+$  was present, its contribution was  
647 subtracted in order to obtain a pure  $TyrD^+$  line shape before spin quantitation. The spectra  
648 were recorded at 20 K with an ESR300D X-band spectrometer (Bruker, Rheinstetten,  
649 Germany), using a  $TE_{102}$  resonator equipped with a front grid for sample illumination within  
650 the cavity. Illumination was performed using a halogen lamp (250 W). The temperature was  
651 controlled with a helium cryostat (Oxford Instruments, UK). Samples were first measured in  
652 darkness for  $TyrD^+$  spectra and  $F_A/F_B$  baseline, and then, after 2 min illumination at 20 K, for  
653 the singly reduced  $F_A/F_B$  spectra. The  $F_A/F_B$  difference light-induced spectrum was used for  
654 quantitation after suppressing the  $P700^+$  signal. Isolated PSI was used to check that charge  
655 separation was 100% efficient at 20 K by comparing the dithionite-reduced ( $F_A^-/F_B^-$ ) spectrum  
656 (2 spins per  $P700$ ) to the light-induced singly reduced  $F_A/F_B$  difference spectrum (1 spin per  
657 PSI). The following EPR parameters were used: for  $TyrD^+$ , modulation amplitude: 2 G,  
658 microwave power: 2  $\mu$ W, number of scans: 8. For  $F_A/F_B$  spectra, modulation amplitude: 10 G,  
659 microwave power: 0.8 mW, number of scans: 2. These microwave powers were found to be  
660 non-saturating at 20 K. The singly reduced ( $F_A/F_B$ ) and  $TyrD^+$  relative spin amounts were

661 calculated by double integration of the EPR signals, with correction for differences in  
662 microwave power and modulation amplitude.

663

#### 664 **Determining the effects of kinase inhibitors by Phos-tag<sup>TM</sup> gel SDS-PAGE**

665 *S. elongatus* cells were pre-grown in fresh BG11 medium. When the cells were at 0.8  
666 OD<sub>800</sub>, they were harvested and washed with nitrogen-free BG11 medium (BG11<sup>-N</sup>). The  
667 pellet was re-suspended in BG11 medium containing 5 mM NH<sub>4</sub>Cl (BG11<sup>NH4</sup>) as the nitrogen  
668 source and incubated for 2 hours. The culture was then separated into four samples. One  
669 sample was kept in the same BG11<sup>NH4</sup> medium to obtain a completely dephosphorylated P<sub>II</sub>  
670 protein.

671 A second sample was washed and transferred to BG11<sup>-N</sup> medium for two hours to  
672 obtain a fully phosphorylated P<sub>II</sub>. The other two samples (after the two-hour incubation in the  
673 presence of NH<sub>4</sub>Cl) were supplemented with a final concentration of 21 μM staurosporine and  
674 1.07 μM K252a, respectively, and incubated for an additional hour. Finally, both samples  
675 were washed and transferred into new BG11<sup>-N</sup> medium in the presence of inhibitors and  
676 incubated for two more hours. The cells of all the samples were washed quickly with pre-  
677 chilled 50 mM Tris-HCl (pH 6.8) and harvested by centrifugation at 4 °C.

678 The cell-free extracts were prepared as follows: the cells were resuspended in 500 μL  
679 of 50 mM Tris-HCl (pH 6.8) with protease (1 mM caproic acid, 1 mM phenylmethylsulfonyl  
680 fluoride, 1 mM benzamidine) and phosphatase inhibitors and broken via 5 cycles of vortexing  
681 (1 min) in the presence of glass beads. Unbroken cells were removed by centrifugation and  
682 the supernatant was recovered. The phosphorylation state of P<sub>II</sub> was tested by loading the  
683 samples onto a normal 15% SDS-PAGE gel with or without 50 μM Zn-Phos-tag (Kinoshita  
684 and Kinoshita-Kikuta, 2011). The gels were washed with 10 mM EDTA for 3×15 min,  
685 followed by a 10 min washing with Phos-tag gel running buffer. The gels were then blotted  
686 onto polyvinylidene difluoride membranes using a Trans-blot Turbo system (Bio-Rad  
687 Laboratories, Hercules, CA). The P<sub>II</sub> band was revealed by immunoblotting with an anti-P<sub>II</sub>  
688 antibody (dilution 1:2500, kindly provided by Prof. Karl Forchhammer) using a chemo-  
689 luminescent detection system (Pierce).

690

#### 691 **Accession numbers**

692 Sequence data from this article can be found in the GenBank/EMBL libraries under the  
693 following accession numbers: *spkA* (*sll1575*), AB046597; *spkB* (*slr1697*), AB046598; *spkC*

694 (*slr0599*), AB046599; *spkD* (*sll0776*), AB046600; *spkE* (*slr1443*), AB046602; *spkF*  
695 (*slr1225*), AB046601; *spkG* (*slr0152*), BAA18552; *spkH* (*sll0005*), BAA10206; *spkI*  
696 (*sll1770*), BAA17672; *spkJ* (*slr0889*), BAA17617; *spkK* (*slr1919*), BAA17147; *spkL*  
697 (*sll0095*), BAA10646; *slr0328*, BAA10030; *sll1771*, BAA17671; *slr1860*, X75568; *sll1033*,  
698 BAA16771; *sll0602*, BAA10367; *slr0114*, BAA10651; *slr1983*, BAA18225; *sll1387*,  
699 BAA18237; *slr0946*, ALJ68053.

700

701

## 702 **Supplemental Data**

703 **Supplemental Figure 1.** Gaussian decomposition of the 77 K fluorescence emission spectra  
704 of *S. elongatus* WT strains.

705 **Supplemental Figure 2.** Gaussian decomposition of the 77 K fluorescence emission spectra  
706 of *Synechocystis* strains.

707 **Supplemental Figure 3.** 77 K fluorescence emission spectra of WT *S. elongatus* cells treated  
708 with sucrose or phosphate buffer.

709 **Supplemental Figure 4.** Fluorescence changes induced by TMPD in the presence or absence  
710 of MV.

711 **Supplemental Figure 5.** State transitions in *Synechocystis* kinase and phosphatase mutants.

712 **Supplemental Figure 6.** Oxygen evolving activity in the presence of NaF.

713 **Supplemental Table 1.** Oligonucleotides used for the construction of the kinase and  
714 phosphatase mutants.

715

## 716 **ACKNOWLEDGMENTS**

717 The authors thank Dr A. Zorina for the gift of the  $\Delta$ *spkH* mutant to the Turku's laboratory and  
718 Prof Karl Forchhammer for the gift of the anti-PII antibody. This work was supported by  
719 grants from the Agence Nationale de la Recherche (RECYFUEL project (ANR-16-CE05-  
720 0026)), and from the European Union's Horizon 2020 research and innovation program under  
721 grant agreement no. 675006 (SE2B). P.C. salary is financed by RECYFUEL (ANR project).

722 The research was also supported by the Centre National de la Recherche Scientifique  
723 (CNRS), the Commissariat à l'Energie Atomique (CEA) and the National Natural Science  
724 Foundation of China (31700107/31770128). The French Infrastructure for Integrated  
725 Structural Biology (FRISBI) ANR-10-INBS-05 also partially supported this research.

726



727 **AUTHOR CONTRIBUTIONS**

728 P.C. performed all the experiments related to the characterization of state transitions and the  
729 role of cyt *b<sub>6</sub>f* and analyzed data; J.Z. constructed all the kinase and phosphatase mutants and  
730 realized almost all the experiments related with the role of phosphorylations and analyzed the  
731 data; P.S. designed, performed, analyzed and supervised experiments; C.L, N. B. and Q. W.  
732 designed and supervised some experiments, D.K. conceived the project, designed and  
733 supervised almost all the experiments, analyzed data; the article was written by D.K., P.S. and  
734 P.C.

735

736 **REFERENCES**

- 737 **Adir N** (2008) Structure of the phycobilisome antennae in cyanobacteria and red algae. *In* P Fromme,  
738 ed, Photosynthetic Protein Complexes: A Structural Approach. WILEY-VCH Verlag GmbH &  
739 Co. KGaA, Weinheim, pp 243-274
- 740 **Alfonso M, Perewoska I, Constant S, Kirilovsky D** (1999) Redox control of psbA expression in  
741 cyanobacteria *Synechocystis* strains. *Journal of Photochemistry and Photobiology B: Biology*  
742 **48**: 104-113
- 743 **Alfonso M, Perewoska I, Kirilovsky D** (2000) Redox control of psbA gene expression in the  
744 cyanobacterium *Synechocystis* PCC 6803. Involvement of the cytochrome *b<sub>6</sub>f* complex. *Plant*  
745 *Physiol* **122**: 505-516
- 746 **Allen JF, Bennett J, Steinback KE, Arntzen CJ** (1981) Chloroplast protein phosphorylation couples  
747 plastoquinone redox state to distribution of excitation energy between photosystems.  
748 *Nature* **291**: 25-29
- 749 **Allen JF, Sanders CE, Holmes NG** (1985) Correlation of membrane-protein phosphorylation with  
750 excitation-energy distribution in the cyanobacterium *Synechococcus* 6301. *FEBS Lett.* **193**:  
751 271-275
- 752 **Angeleri M, Zorina A, Aro EM, Battchikova N** (2018) Interplay of SpkG kinase and the Slr0151 protein  
753 in the phosphorylation of ferredoxin 5 in *Synechocystis* sp. strain PCC 6803. *FEBS letters* **592**:  
754 411-421
- 755 **Aoki M, Katoh S** (1982) Oxidation and reduction of plastoquinone by photosynthetic and respiratory  
756 electron transport in a cyanobacterium *Synechococcus* sp. *Biochim. Biophys. Acta* **682**: 307-  
757 314
- 758 **Aspinwall CL, Sarcina M, Mullineaux CW** (2004) Phycobilisome Mobility in the Cyanobacterium  
759 *Synechococcus* sp. PCC7942 is Influenced by the Trimerisation of Photosystem I. *Photosynth*  
760 *Res* **79**: 179-187
- 761 **Bellafiore S, Barneche F, Peltier G, Rochaix JD** (2005) State transitions and light adaptation require  
762 chloroplast thylakoid protein kinase STN7. *Nature* **433**: 892-895
- 763 **Bennoun P** (1982) Evidence for a respiratory chain in the chloroplast. *Proc Natl Acad Sci U S A* **79**:  
764 4352-4356
- 765 **Biggins J, Bruce D** (1989) Regulation of excitation-energy transfer in organisms containing  
766 phycobilins. *Photosynth Res* **20**: 1-34
- 767 **Biggins J, Tanguay NA, Frank HA** (1989) Electron-transfer reactions in Photosystem-I following  
768 vitamin-K1 depletion by ultraviolet-Irradiation. *FEBS Lett.* **250**: 271-274
- 769 **Bonaventura C, Myers J** (1969) Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*.  
770 *Biochim Biophys Acta* **189**: 366-383

771 **Breyton C** (2000) Conformational changes in the cytochrome b6f complex induced by inhibitor  
772 binding. *J Biol Chem* **275**: 13195-13201

773 **Bruce D, Biggins J** (1985) Mechanism of the light-state transition in photosynthesis : V. 77 K linear  
774 dichroism of *Anacystis nidulans* in state 1 and state 2. *Biochim Biophys Acta* **810**: 295-301

775 **Bruce D, Brimble S, Bryant DA** (1989) State transitions in a phycobilisome-less mutant of the  
776 cyanobacterium *Synechococcus* sp. PCC 7002. *Biochim Biophys Acta* **974**: 66-73

777 **Bruce D, Salehian O** (1992) Laser-induced optoacoustic calorimetry of cyanobacteria. The efficiency of  
778 primary photosynthetic processes in state 1 and state 2. *Biochim Biophys Acta* **1100**: 242-  
779 250

780 **Campbell D, Hurry V, Clarke AK, Gustafsson P, Öquist G** (1998) Chlorophyll fluorescence analysis of  
781 cyanobacterial photosynthesis and acclimation. *Microbiol Mol Biol Rev* **62**: 667-683

782 **Chen Z, Zhan J, Chen Y, Yang M, He C, Ge F, Wang Q** (2015) Effects of Phosphorylation of beta  
783 Subunits of Phycocyanins on State Transition in the Model Cyanobacterium *Synechocystis* sp.  
784 PCC 6803. *Plant Cell Physiol* **56**: 1997-2013

785 **Chukhutsina V, Bersanini L, Aro EM, van Amerongen H** (2015) Cyanobacterial Light-Harvesting  
786 Phycobilisomes Uncouple From Photosystem I During Dark-To-Light Transitions. *Sci Rep* **5**:  
787 14193

788 **Delphin E, Duval JC, Etienne AL, Kirilovsky D** (1996) State transitions or Delta pH-dependent  
789 quenching of photosystem II fluorescence in red algae. *Biochemistry* **35**: 9435-9445

790 **Delphin E, Duval JC, Kirilovsky D** (1995) Comparison of state 1 state 2 transitions in the green alga  
791 *Chlamydomonas reinhardtii* and in the red alga *Rhodella violacea*: Effect of kinase and  
792 phosphatase inhibitors. *Biochimica Et Biophysica Acta-Bioenergetics* **1232**: 91-95

793 **Depege N, Bellafiore S, Rochaix JD** (2003) Role of chloroplast protein kinase Stt7 in LHClI  
794 phosphorylation and state transition in *Chlamydomonas*. *Science* **299**: 1572-1575

795 **Dong C, Tang A, Zhao J, Mullineaux CW, Shen G, Bryant DA** (2009) ApcD is necessary for efficient  
796 energy transfer from phycobilisomes to photosystem I and helps to prevent photoinhibition  
797 in the cyanobacterium *Synechococcus* sp. PCC 7002. *Biochim Biophys Acta* **1787**: 1122-1128

798 **Dong C, Zhao J** (2008) ApcD is required for state transition but not involved in blue-light induced  
799 quenching in the cyanobacterium *Anabaena* sp. PCC7120. *Chi Sci Bull* **53**: 3422-3424

800 **Draber W, Trebst A, Harth E** (1970) On a new inhibitor of photosynthetic electron-transport in  
801 isolated chloroplasts. *Z Naturforsch B* **25**: 1157-1159

802 **El Bissati K, Delphin E, Murata N, Etienne A, Kirilovsky D** (2000) Photosystem II fluorescence  
803 quenching in the cyanobacterium *Synechocystis* PCC 6803: involvement of two different  
804 mechanisms. *Biochim Biophys Acta* **1457**: 229-242

805 **El Bissati K, Kirilovsky D** (2001) Regulation of *psbA* and *psaE* expression by light quality in  
806 *Synechocystis* species PCC 6803. A redox control mechanism. *Plant Physiol.* **125**: 1988-2000

807 **Emlyn-Jones D, Ashby MK, Mullineaux CW** (1999) A gene required for the regulation of  
808 photosynthetic light harvesting in the cyanobacterium *Synechocystis* 6803. *Mol Microbiol* **33**:  
809 1050-1058

810 **Federman S, Malkin S, Scherz A** (2000) Excitation energy transfer in aggregates of Photosystem I and  
811 Photosystem II of the cyanobacterium *Synechocystis* sp. PCC 6803: Can assembly of the  
812 pigment-protein complexes control the extent of spillover? *Photosynth Res* **64**: 199-207

813 **Fernandez P, Saint-Joanis B, Barilone N, Jackson M, Gicquel B, Cole ST, Alzari PM** (2006) The Ser/Thr  
814 protein kinase PknB is essential for sustaining mycobacterial growth. *J Bacteriol* **188**: 7778-  
815 7784

816 **Finazzi G, Zito F, Barbagallo RP, Wollman FA** (2001) Contrasted effects of inhibitors of cytochrome  
817 b6f complex on state transitions in *Chlamydomonas reinhardtii*: the role of Qo site occupancy  
818 in LHClI kinase activation. *J Biol Chem* **276**: 9770-9774

819 **Fischer BB, Eggen RI, Trebst A, Krieger-Liszakay A** (2006) The glutathione peroxidase homologous  
820 gene *Gpxh* in *Chlamydomonas reinhardtii* is upregulated by singlet oxygen produced in  
821 photosystem II. *Planta* **223**: 583-590

822 **Folea IM, Zhang P, Aro EM, Boekema EJ** (2008) Domain organization of photosystem II in  
823 membranes of the cyanobacterium *Synechocystis* PCC6803 investigated by electron  
824 microscopy. *FEBS Lett* **582**: 1749-1754

825 **Forchhammer K, Tandeau de Marsac N** (1995a) Functional analysis of the phosphoprotein PII (glnB  
826 gene product) in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *J Bacteriol* **177**:  
827 2033-2040

828 **Forchhammer K, Tandeau de Marsac N** (1995b) Phosphorylation of the PII protein (glnB gene  
829 product) in the cyanobacterium *Synechococcus* sp. strain PCC 7942: analysis of in vitro kinase  
830 activity. *J Bacteriol* **177**: 5812-5817

831 **Fufezan C, Drepper F, Juhnke HD, Lancaster CRD, Un S, Rutherford AW, Krieger-Liszkay A** (2005)  
832 Herbicide-induced changes in charge recombination and redox potential of Q(A) in the T4  
833 mutant of *Blastochloris viridis*. *Biochemistry* **44**: 5931-5939

834 **Galkin AN, Mikheeva, L.E. and Shestakov, S.V.** (2003) The insertional inactivation of genes encoding  
835 eukaryotic-type serine/threonine protein kinases in the cyanobacterium *Synechocystis* sp.  
836 PCC 6803. *Microbiology* **72**

837 **Glazer AN** (1984) Phycobilisome - a macromolecular complex optimized for light energy-transfer.  
838 *Biochim Biophys Acta* **768**: 29-51

839 **Guskov A, Kern J, Gabdulkhakov A, Broser M, Zouni A, Saenger W** (2009) Cyanobacterial  
840 photosystem II at 2.9-Å resolution and the role of quinones, lipids, channels and chloride. *Nat*  
841 *Struct Mol Biol* **16**: 334-342

842 **Hiyama T, Ke B** (1972) Difference spectra and extinction coefficients of P 700. *Biochim Biophys Acta*  
843 **267**: 160-171

844 **Huang C, Yuan X, Zhao J, Bryant DA** (2003) Kinetic analyses of state transitions of the  
845 cyanobacterium *Synechococcus* sp. PCC 7002 and its mutant strains impaired in electron  
846 transport. *Biochim Biophys Acta* **1607**: 121-130

847 **Huang JY, Chiu YF, Ortega JM, Wang HT, Tseng TS, Ke SC, Roncel M, Chu HA** (2016) Mutations of  
848 Cytochrome b559 and PsbJ on and near the QC Site in Photosystem II Influence the  
849 Regulation of Short-Term Light Response and Photosynthetic Growth of the cyanobacterium  
850 *Synechocystis* sp. PCC 6803. *Biochemistry* **55**: 2214-2226

851 **Joshua S, Mullineaux CW** (2004) Phycobilisome diffusion is required for light-state transitions in  
852 cyanobacteria. *Plant Physiol* **135**: 2112-2119

853 **Kamei A, Yuasa T, Orikiawa K, Geng XX, Ikeuchi M** (2001) A eukaryotic-type protein kinase, SpkA, is  
854 required for normal motility of the unicellular cyanobacterium *Synechocystis* sp. strain PCC  
855 6803. *J Bacteriol* **183**: 1505-1510

856 **Kana R** (2013) Mobility of photosynthetic proteins. *Photosynth Res* **116**: 465-479

857 **Kana R, Prasil O, Komarek O, Papageorgiou GC, Govindjee** (2009) Spectral characteristic of  
858 fluorescence induction in a model cyanobacterium, *Synechococcus* sp. PCC 7942. *Biochim*  
859 *Biophys Acta* **1787**: 1170-1178

860 **Kinoshita E, Kinoshita-Kikuta E** (2011) Improved Phos-tag SDS-PAGE under neutral pH conditions for  
861 advanced protein phosphorylation profiling. *Proteomics* **11**: 319-323

862 **Kirilovsky D, Rutherford AW, Etienne AL** (1994) Influence of DCMU and Ferricyanide on  
863 Photodamage in Photosystem II. *Biochemistry* **33**: 3087-3095

864 **Kondo K, Mullineaux CW, Ikeuchi M** (2009) Distinct roles of CpcG1-phycobilisome and CpcG2-  
865 phycobilisome in state transitions in a cyanobacterium *Synechocystis* sp. PCC 6803.  
866 *Photosynth Res* **99**: 217-225

867 **Kowalczyk N, Rappaport F, Boyen C, Wollman FA, Collen J, Joliot P** (2013) Photosynthesis in  
868 *Chondrus crispus*: the contribution of energy spill-over in the regulation of excitonic flux.  
869 *Biochim Biophys Acta* **1827**: 834-842

870 **Kruip J, Bald D, Boekema E, Rogner M** (1994) Evidence for the existence of trimeric and monomeric  
871 photosystem-I complexes in thylakoid membranes from cyanobacteria. *Photosynth. res.* **40**:  
872 279-286

873 **Krupnik T, Kotabova E, van Bezouwen LS, Mazur R, Garstka M, Nixon PJ, Barber J, Kana R, Boekema**  
874 **EJ, Kargul J** (2013) A reaction center-dependent photoprotection mechanism in a highly  
875 robust photosystem II from an extremophilic red alga, *Cyanidioschyzon merolae*. *J Biol Chem*  
876 **288**: 23529-23542

877 **Kyle DJ, Ohad I, Arntzen CJ** (1984) Membrane protein damage and repair: Selective loss of a  
878 quinone-protein function in chloroplast membranes. *Proc Natl Acad Sci U S A* **81**: 4070-4074

879 **Laurent S, Jang J, Janicki A, Zhang CC, Bedu S** (2008) Inactivation of spkD, encoding a Ser/Thr kinase,  
880 affects the pool of the TCA cycle metabolites in *Synechocystis* sp. strain PCC 6803.  
881 *Microbiology* **154**: 2161-2167

882 **Ley AC, Butler WL** (1980) Energy distribution in the photochemical apparatus of *Porphyridium*  
883 *cruentum* in State-I and State-II. *Biochim. Biophys. Acta.* **592**: 349-363

884 **Li D, Xie J, Zhao J, Xia A, Li D, Gong Y** (2004) Light-induced excitation energy redistribution in  
885 *Spirulina platensis* cells: "spillover" or "mobile PBSs"? *Biochim Biophys Acta* **1608**: 114-121

886 **Li H, Li D, Yang S, Xie J, Zhao J** (2006) The state transition mechanism - simply depending on light-on  
887 and -off in *Spirulina platensis*. *Biochim Biophys Acta* **1757**: 1512-1519

888 **Liang C, Zhang X, Chi X, Guan X, Li Y, Qin S, Shao HB** (2011) Serine/threonine protein kinase SpkG is a  
889 candidate for high salt resistance in the unicellular cyanobacterium *Synechocystis* sp. PCC  
890 6803. *PLoS One* **6**: e18718

891 **MacColl R** (1998) Cyanobacterial phycobilisomes. *J Struct Biol* **124**: 311-334

892 **Mao HB, Li GF, Ruan X, Wu QY, Gong YD, Zhang XF, Zhao NM** (2002) The redox state of  
893 plastoquinone pool regulates state transitions via cytochrome b<sub>6</sub>f complex in *Synechocystis*  
894 sp. PCC 6803. *FEBS Lett* **519**: 82-86

895 **Mao L, Wang Y, Hu X** (2003) pi-pi stacking interactions in the peridinin-chlorophyll-protein of  
896 *Amphidinium carterae*. *J. Phys. Chem. B* **107**: 3963-3971

897 **Mata-Cabana A, Garcia-Dominguez M, Florencio FJ, Lindahl M** (2012) Thiol-based redox modulation  
898 of a cyanobacterial eukaryotic-type serine/threonine kinase required for oxidative stress  
899 tolerance. *Antioxid Redox Signal* **17**: 521-533

900 **McCartney B, Howell LD, Kennelly PJ, Potts M** (1997) Protein tyrosine phosphorylation in the  
901 cyanobacterium *Anabaena* sp. strain PCC 7120. *J Bacteriol* **179**: 2314-2318

902 **McConnell MD, Koop R, Vasil'ev S, Bruce D** (2002) Regulation of the distribution of chlorophyll and  
903 phycobilin-absorbed excitation energy in cyanobacteria. A structure-based model for the  
904 light state transition. *Plant Physiol* **130**: 1201-1212

905 **Minagawa J** (2011) State transitions--the molecular remodeling of photosynthetic supercomplexes  
906 that controls energy flow in the chloroplast. *Biochim Biophys Acta* **1807**: 897-905

907 **Misumi M, Katoh H, Tomo T, Sonoike K** (2016) Relationship Between Photochemical Quenching and  
908 Non-Photochemical Quenching in Six Species of Cyanobacteria Reveals Species Difference in  
909 Redox State and Species Commonality in Energy Dissipation. *Plant Cell Physiol* **57**: 1510-1517

910 **Mullineaux CW** (2014) Co-existence of photosynthetic and respiratory activities in cyanobacterial  
911 thylakoid membranes. *Biochim Biophys Acta* **1837**: 503-511

912 **Mullineaux CW, Allen JF** (1986) The state 2 transition in the cyanobacterium *Synechococcus* 6301 can  
913 be driven by respiratory electron flow into the plastoquinone pool. *FEBS Lett* **205**: 155-160

914 **Mullineaux CW, Allen JF** (1990) State 1 - State 2 transitions in the Cyanobacterium *Synechococcus*  
915 6301 are controlled by the redox state of electron carriers between Photosystem I and  
916 Photosystem II. *Photosynth. Res.* **23**: 297-311

917 **Mullineaux CW, Griebenow S, Braslavsky SE** (1991) Photosynthetic energy storage in cyanobacterial  
918 cells adapted to light-states 1 and 2. A laser-induced optoacoustic study. *Biochim Biophys*  
919 *Acta* **1060**: 315-318

920 **Mullineaux CW, Tobin MJ, Jones GR** (1997) Mobility of photosynthetic complexes in thylakoid  
921 membranes. *Nature* **390**: 421-424

922 **Murata N** (1969) Control of excitation transfer in photosynthesis. I. Light-induced change of  
923 chlorophyll a fluorescence in *Porphyridium cruentum*. *Biochim Biophys Acta* **172**: 242-251

924 **Nakano H, Omura S** (2009) Chemical biology of natural indolocarbazole products: 30 years since the  
925 discovery of staurosporine. *J Antibiot (Tokyo)* **62**: 17-26

926 **Nanba M, Katoh S** (1985) Restoration by tetramethyl-p-phenylenediamine of photosynthesis in  
927 dibromothymoquinone-inhibited cells of the cyanobacterium *Synechococcus* sp. *Biochim*  
928 *Biophys Acta* **809**: 74-80

929 **Olive J, Ajlani G, Astier C, Recouvreur M, Vernotte C** (1997) Ultrastructure and light adaptation of  
930 phycobilisome mutants of *Synechocystis* PCC 6803. *Biochim. Biophys. Acta* **1319**: 275-282

931 **Olive J, Mbina I, Vernotte C, Astier C, Wollman FA** (1986) Randomization of the Ef particles in  
932 thylakoid membranes of *Synechocystis* 6714 upon transition from State-I to State-II. *FEBS*  
933 *Lett.* **208**: 308-312

934 **Panichkin VB, Arakawa-Kobayashi S, Kanaseki T, Suzuki I, Los DA, Shestakov SV, Murata N** (2006)  
935 Serine/threonine protein kinase SpkA in *Synechocystis* sp. strain PCC 6803 is a regulator of  
936 expression of three putative pilA operons, formation of thick pili, and cell motility. *J Bacteriol*  
937 **188**: 7696-7699

938 **Papageorgiou GC, Govindjee, Nimuro M, Stamatakis K, Alygizaki-Zorba A, Murata N** (1999) Light-  
939 induced and osmotically-induced changes in chlorophyll a fluorescence in two *Synechocystis*  
940 sp. PCC 6803 strains that differ in membrane lipid unsaturation. *Photosynth Res* **59**: 125-136

941 **Preston C, Critchley C** (1988) Interaction of electron acceptors with thylakoids from halophytic and  
942 non-halophytic species. *Photosynth Res* **16**: 187-202

943 **Ranjbar Choubeh R, Wientjes E, Struik PC, Kirilovsky D, van Amerongen H** (2018) State transitions in  
944 the cyanobacterium *Synechococcus elongatus* 7942 involve reversible quenching of the  
945 photosystem II core. *Biochim Biophys Acta* **1859**: 1059-1066

946 **Roberts AG, Kramer DM** (2001) Inhibitor "double occupancy" in the Q(o) pocket of the chloroplast  
947 cytochrome b6f complex. *Biochemistry* **40**: 13407-13412

948 **Schluchter WM, Shen GH, Zhao JD, Bryant DA** (1996) Characterization of *psaI* and *psaL* mutants of  
949 *Synechococcus* sp strain PCC 7002: A new model for state transitions in cyanobacteria.  
950 *Photochem Photobiol* **64**: 53-66

951 **Scott M, McCollum C, Vasil'ev S, Crozier C, Espie GS, Krol M, Huner NP, Bruce D** (2006) Mechanism  
952 of the down regulation of photosynthesis by blue light in the Cyanobacterium *Synechocystis*  
953 sp. PCC 6803. *Biochemistry* **45**: 8952-8958

954 **Spat P, Macek B, Forchhammer K** (2015) Phosphoproteome of the cyanobacterium *Synechocystis* sp.  
955 PCC 6803 and its dynamics during nitrogen starvation. *Front Microbiol* **6**: 248

956 **Srivastava A, Strasser RJ, Govindjee** (1995) Polyphasic rise of chlorophyll a fluorescence in herbicide-  
957 resistant D1 mutants of *Chlamydomonas reinhardtii*. *Photosynth Res* **43**: 131-141

958 **Umena Y, Kawakami K, Shen JR, Kamiya N** (2011) Crystal structure of oxygen-evolving photosystem  
959 II at a resolution of 1.9 Å. *Nature* **473**: 55-60

960 **Vener AV, Van Kan PJ, Gal A, Andersson B, Ohad I** (1995) Activation/deactivation cycle of redox-  
961 controlled thylakoid protein phosphorylation. Role of plastoquinol bound to the reduced  
962 cytochrome bf complex. *J Biol Chem* **270**: 25225-25232

963 **Vener AV, van Kan PJ, Rich PR, Ohad I, Andersson B** (1997) Plastoquinol at the quinol oxidation site  
964 of reduced cytochrome bf mediates signal transduction between light and protein  
965 phosphorylation: thylakoid protein kinase deactivation by a single-turnover flash. *Proc Natl*  
966 *Acad Sci U S A* **94**: 1585-1590

967 **Vernotte C, Picaud M, Kirilovsky D, Olive J, Ajlani G, Astier C** (1992) Changes in the Photosynthetic  
968 Apparatus in the cyanobacterium *Synechocystis* sp PCC 6714 Following Light-to-Dark and  
969 Dark-to-Light Transitions. *Photosynthesis Research* **32**: 45-57

970 **Vladkova R** (2016) Chlorophyll a is the crucial redox sensor and transmembrane signal transmitter in  
971 the cytochrome b6f complex. Components and mechanisms of state transitions from the  
972 hydrophobic mismatch viewpoint. *J Biomol Struct Dyn* **34**: 824-854

973 **Wollman FA** (2001) State transitions reveal the dynamics and flexibility of the photosynthetic  
974 apparatus. *Embo J* **20**: 3623-3630

975 **Wollman FA, Lemaire C** (1988) Studies on kinase-controlled state transitions in Photosystem II and  
976 *b<sub>6</sub>f* mutants from *Chlamydomonas reinhardtii* which lack quinone-binding proteins.  
977 *Biochimica et Biophysica Acta* **933**: 85-94

978 **Yang MK, Qiao ZX, Zhang WY, Xiong Q, Zhang J, Li T, Ge F, Zhao JD** (2013) Global phosphoproteomic  
979 analysis reveals diverse functions of serine/threonine/tyrosine phosphorylation in the model  
980 cyanobacterium *Synechococcus* sp. strain PCC 7002. *J Proteome Res* **12**: 1909-1923

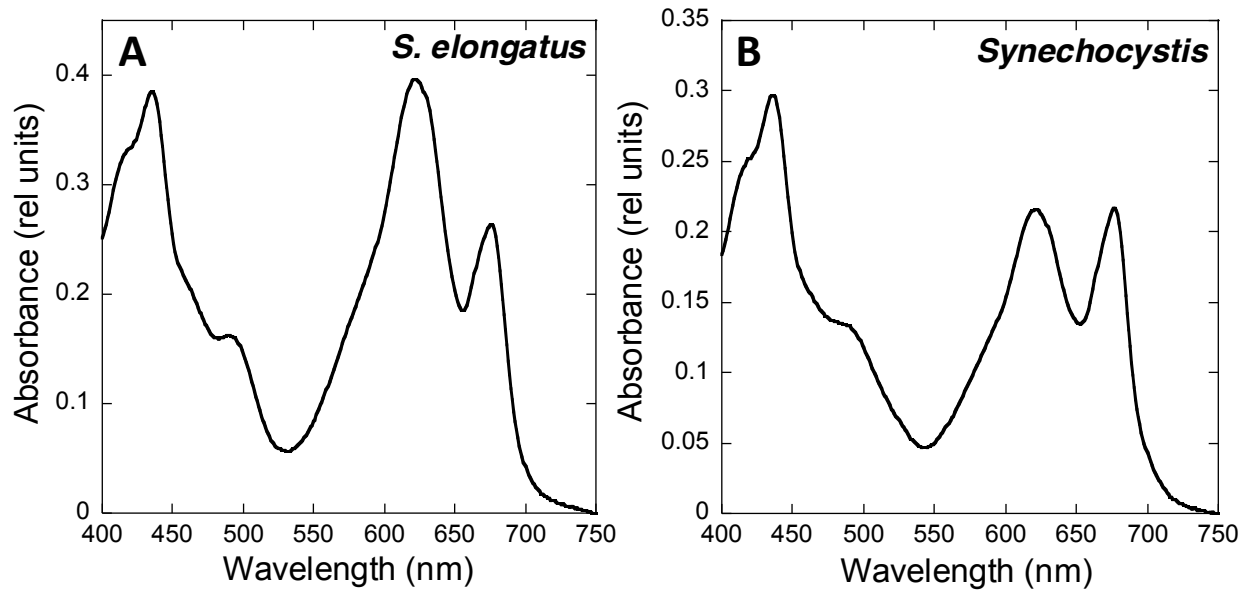
981 **Zhang Z, Huang L, Shulmeister VM, Chi YI, Kim KK, Hung LW, Crofts AR, Berry EA, Kim SH** (1998)  
982 Electron transfer by domain movement in cytochrome *bc<sub>1</sub>*. *Nature* **392**: 677-684

983 **Zito F, Finazzi G, Delosme R, Nitschke W, Picot D, Wollman FA** (1999) The Qo site of cytochrome *b<sub>6</sub>f*  
984 complexes controls the activation of the LHCII kinase. *Embo J* **18**: 2961-2969

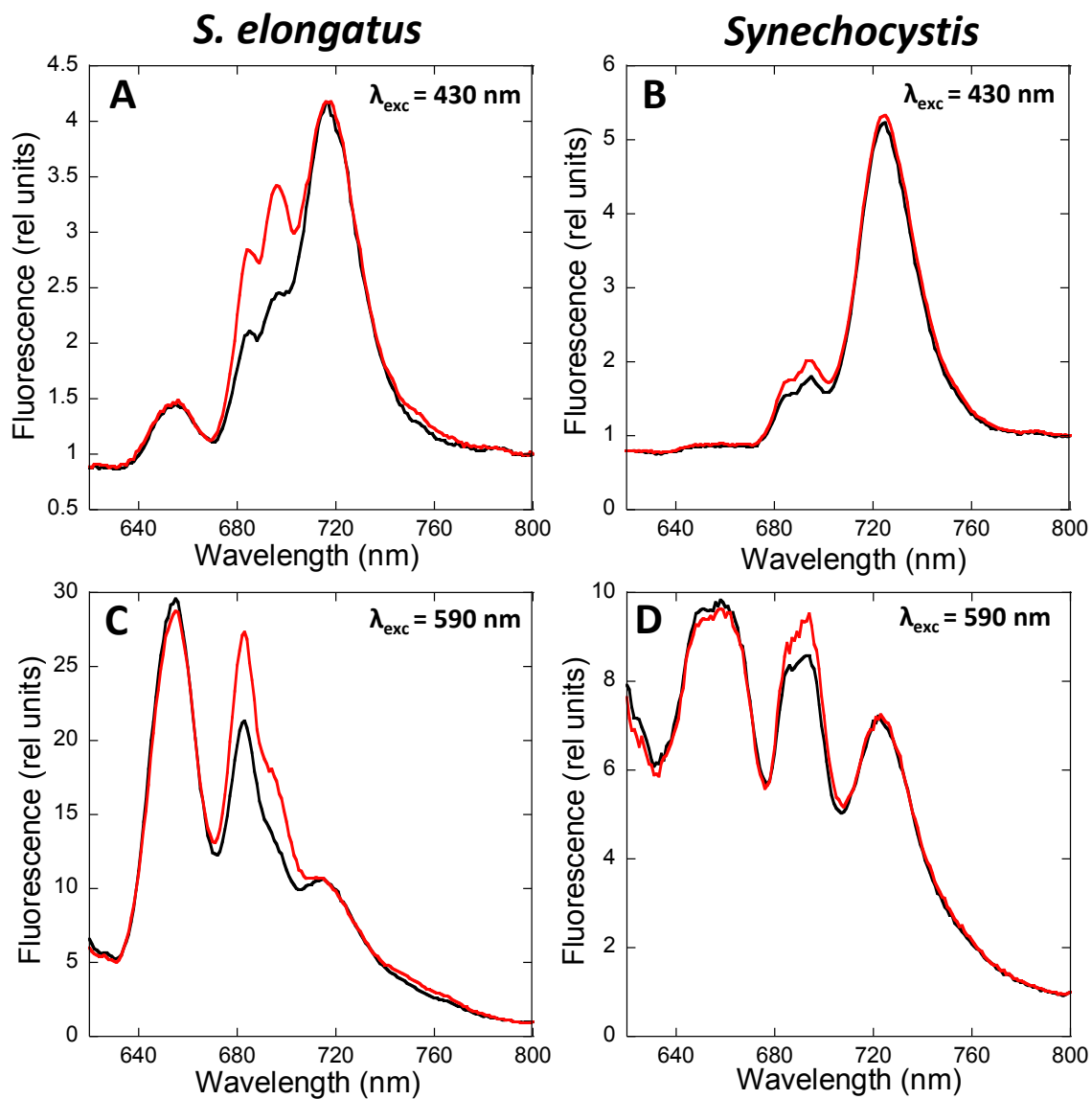
985 **Zorina A** (2013) Eukaryotic protein kinases in cyanobacteria. *Russian Journal of Plant Physiol* **60**: 589-  
986 596

987 **Zorina A, Stepanchenko N, Novikova GV, Sinetova M, Panichkin VB, Moshkov IE, Zinchenko VV,**  
988 **Shestakov SV, Suzuki I, Murata N, Los DA** (2011) Eukaryotic-like Ser/Thr protein kinases  
989 SpkC/F/K are involved in phosphorylation of GroES in the cyanobacterium *Synechocystis*.  
990 *DNA Res* **18**: 137-151

991 **Zorina AA, Bedbenov VS, Novikova GV, Panichkin VB, Los' DA** (2014) Involvement of  
992 serine/threonine protein kinases in the cold stress response in the cyanobacterium  
993 *Synechocystis* sp. PCC 6803: Functional characterization of SpkE protein kinase. *Mol Biol* **48**:  
994 390-398

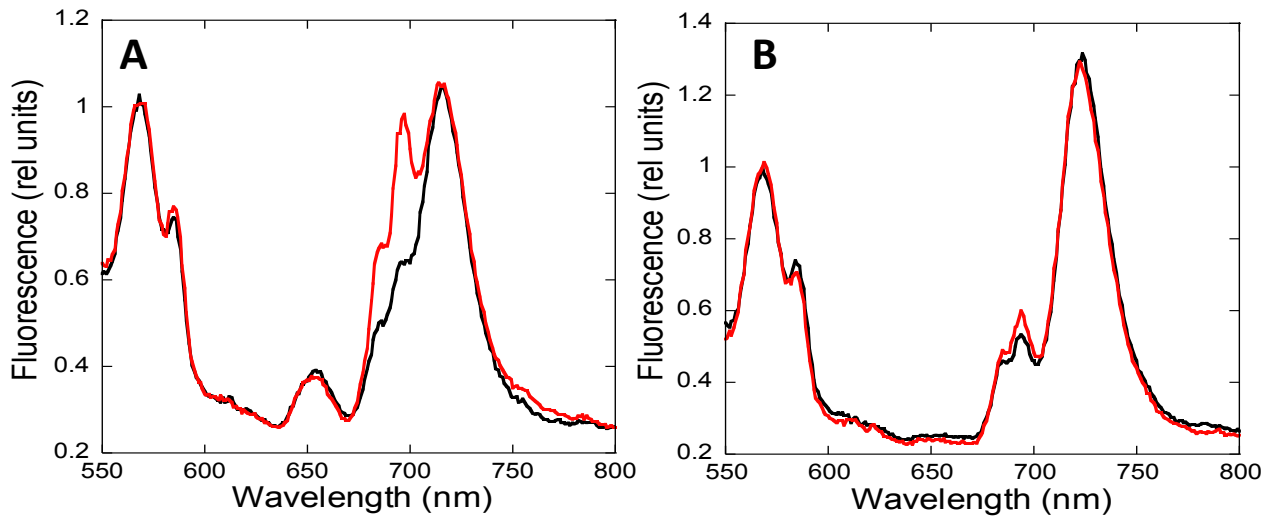


**Figure 1. Absorption spectra of *S. elongatus* (A) and *Synechocystis* (B) cells.** The spectra were recorded at a Chl concentration of 2.5  $\mu\text{g}/\text{mL}$ . The curves shown are the average of 3 independent biological replicates (as described in the Methods).

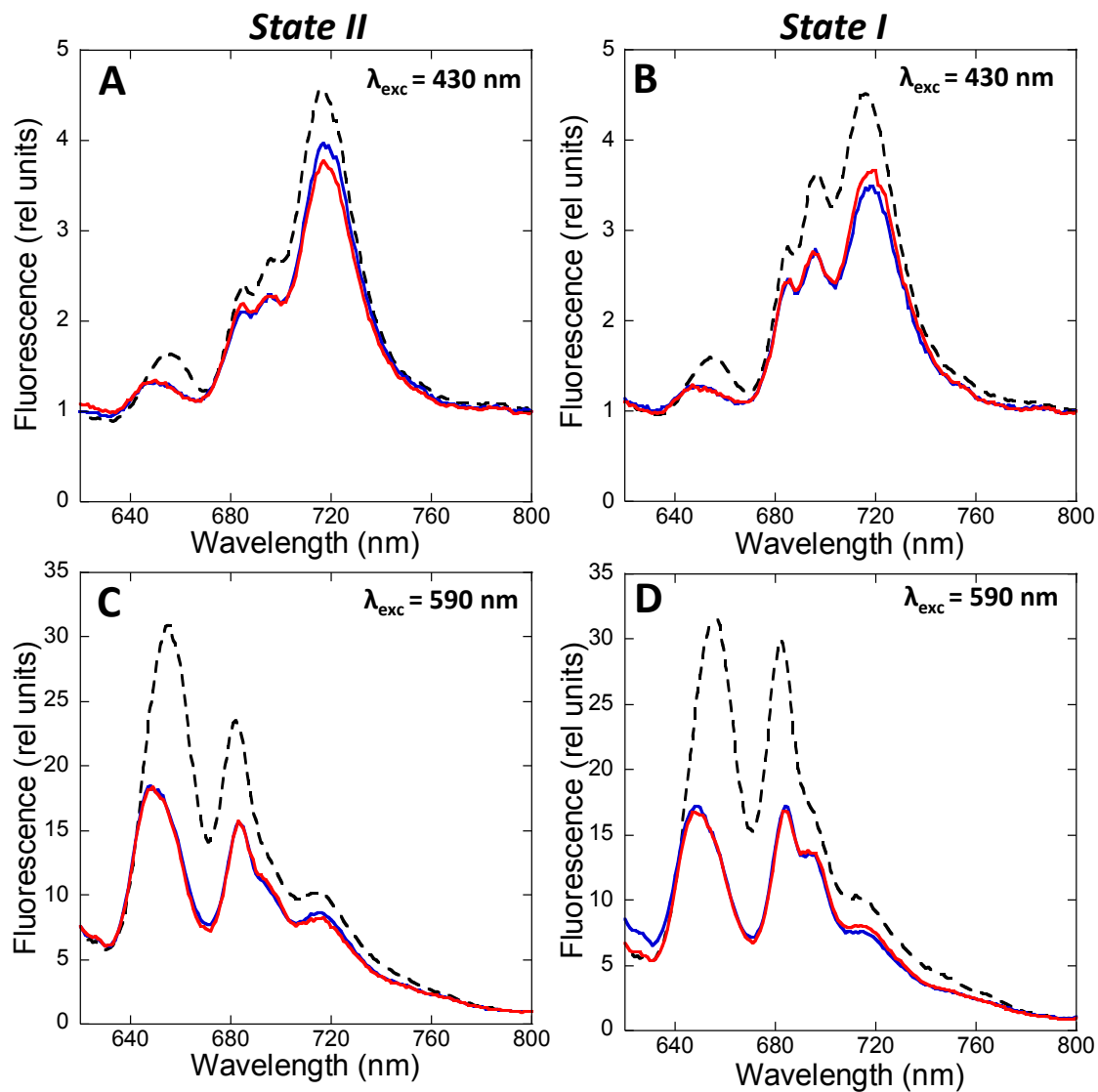


**Figure 2. State transitions in *S. elongatus* and *Synechocystis*.** 77 K fluorescence emission spectra of dark (black, State II) and blue-light (red, 85  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , State I) adapted *S. elongatus* (A and C) and *Synechocystis* (B and D) cells. The excitation was done at 430 nm (A and B) and 590 nm (C and D). Normalization was done at 800 nm, and the spectra are the average of at least 3 independent biological replicates.

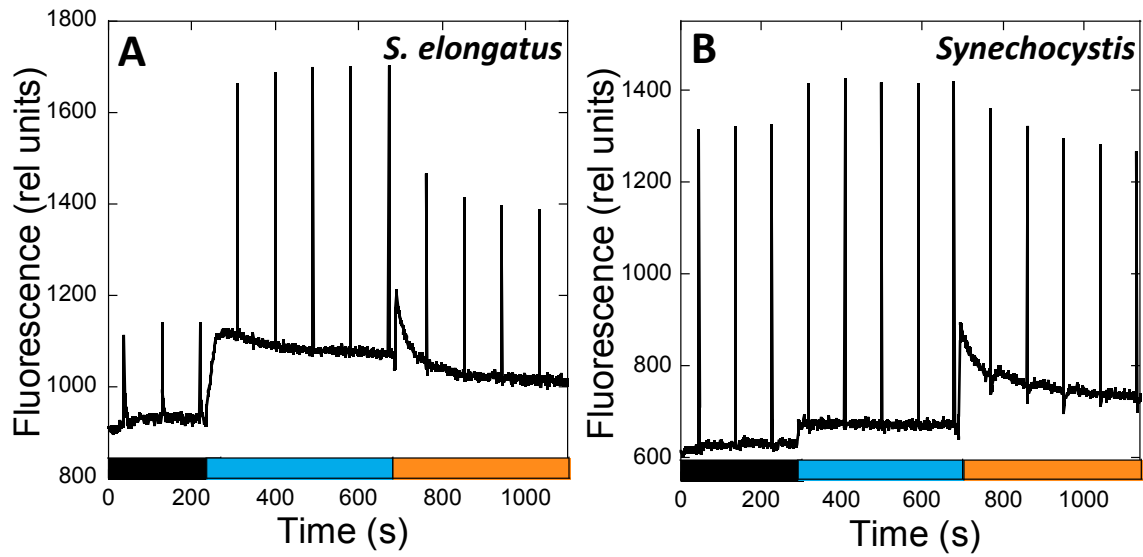




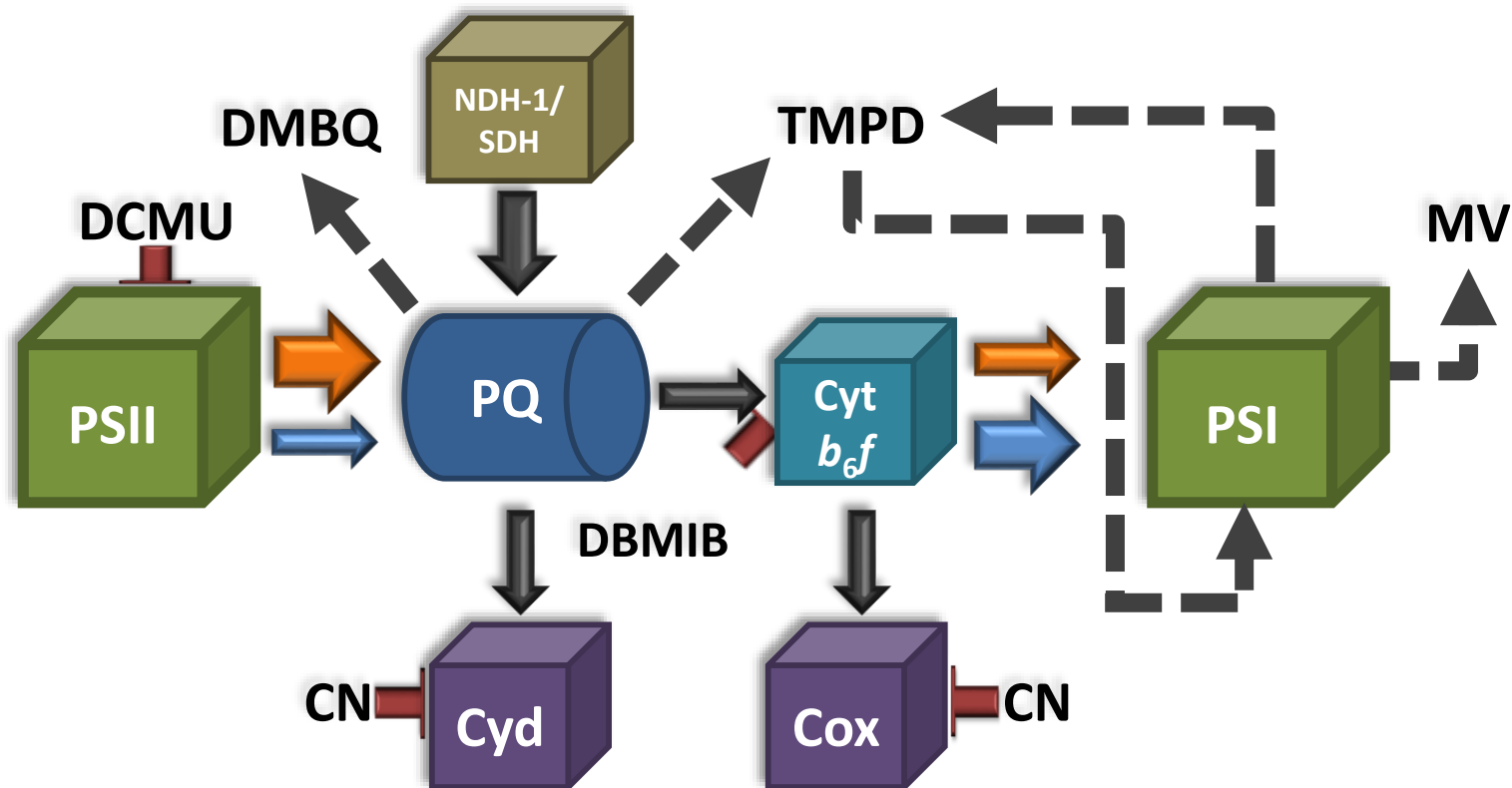
**Figure 3. 77 K fluorescence emission spectra of WT *S. elongatus* (A) and *Synechocystis* (B) cells using Rhodamine B as an internal standard.** Cells were dark adapted for 15 min before the measurements (dark lines) or incubated under blue light illumination for 5 min (red lines) before the addition of Rhodamine B (0.4  $\mu$ M). The excitation was done at 430 nm. Normalization was done at the peak of Rhodamine B at 568 nm, and the spectra are the average of at least 3 independent biological replicates.



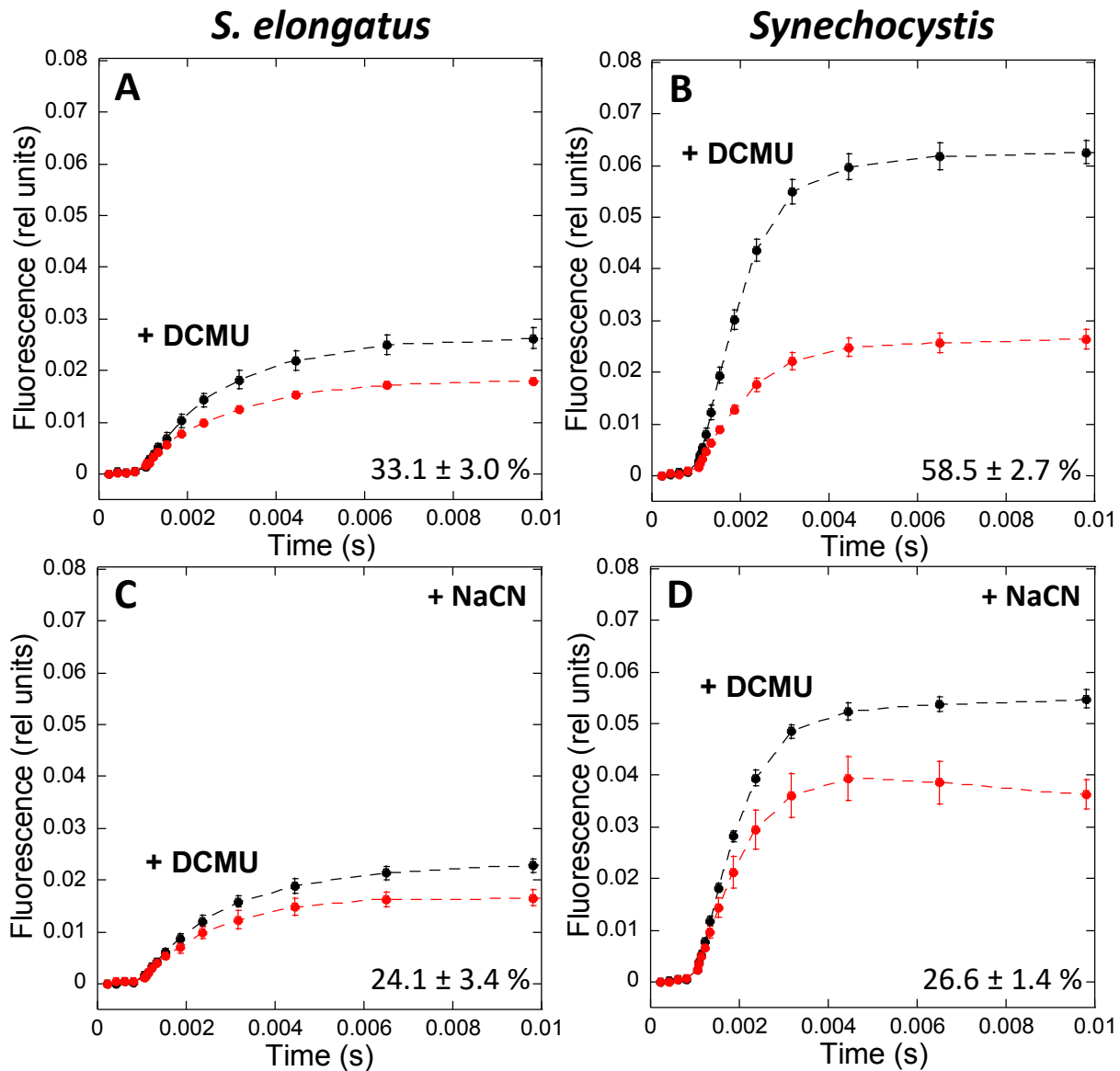
**Figure 4. 77 K fluorescence emission spectra of *S. elongatus* cells treated with betaine.** Cells were dark adapted for 15 min (A and C) or incubated under blue-light illumination ( $85 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) for 5 min (B and D). 77 K spectra were measured (dashed lines). Then, samples were taken after 5 min incubation with betaine (1 M) and measured (blue lines). Finally, State I (A and C) or State II (B and D) was tentatively induced by blue light illumination ( $85 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) or darkness, respectively, for 5 min more (red lines). The excitation was done at 430 nm (A and B) or 590 nm (C and D). Normalization was done at 800 nm, and the spectra are the average of at least 3 independent biological replicates.



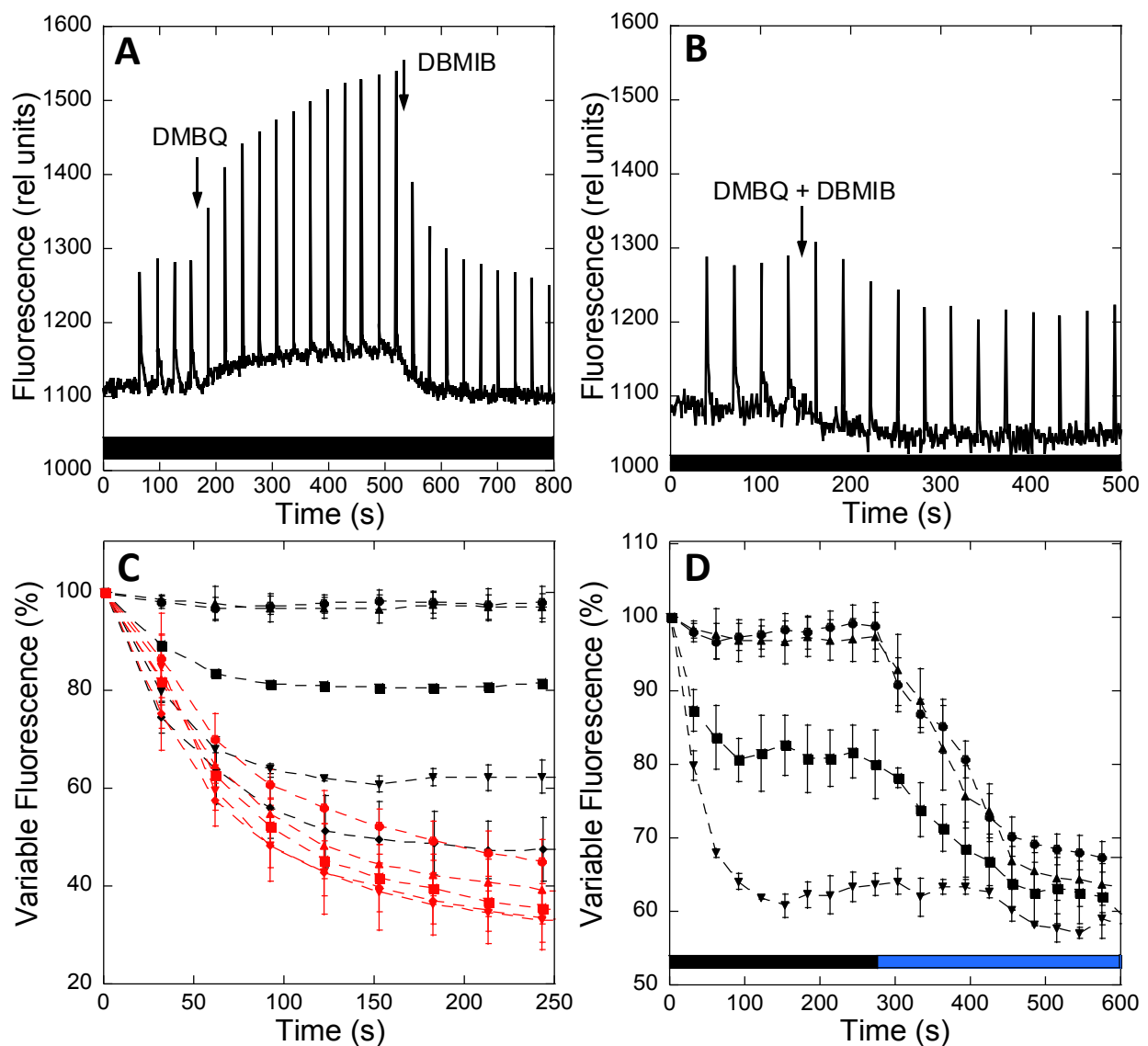
**Figure 5. State transitions in *S. elongatus* (A) and *Synechocystis* (B).** The fluorescence changes were followed with a PAM fluorometer. Dark-adapted cells (Chl concentration 2.5  $\mu\text{g}/\text{mL}$ ) were successively illuminated with blue light ( $85 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and orange light ( $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Saturating pulses ( $400 \text{ ms} \times 1200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) were applied every 90 sec.



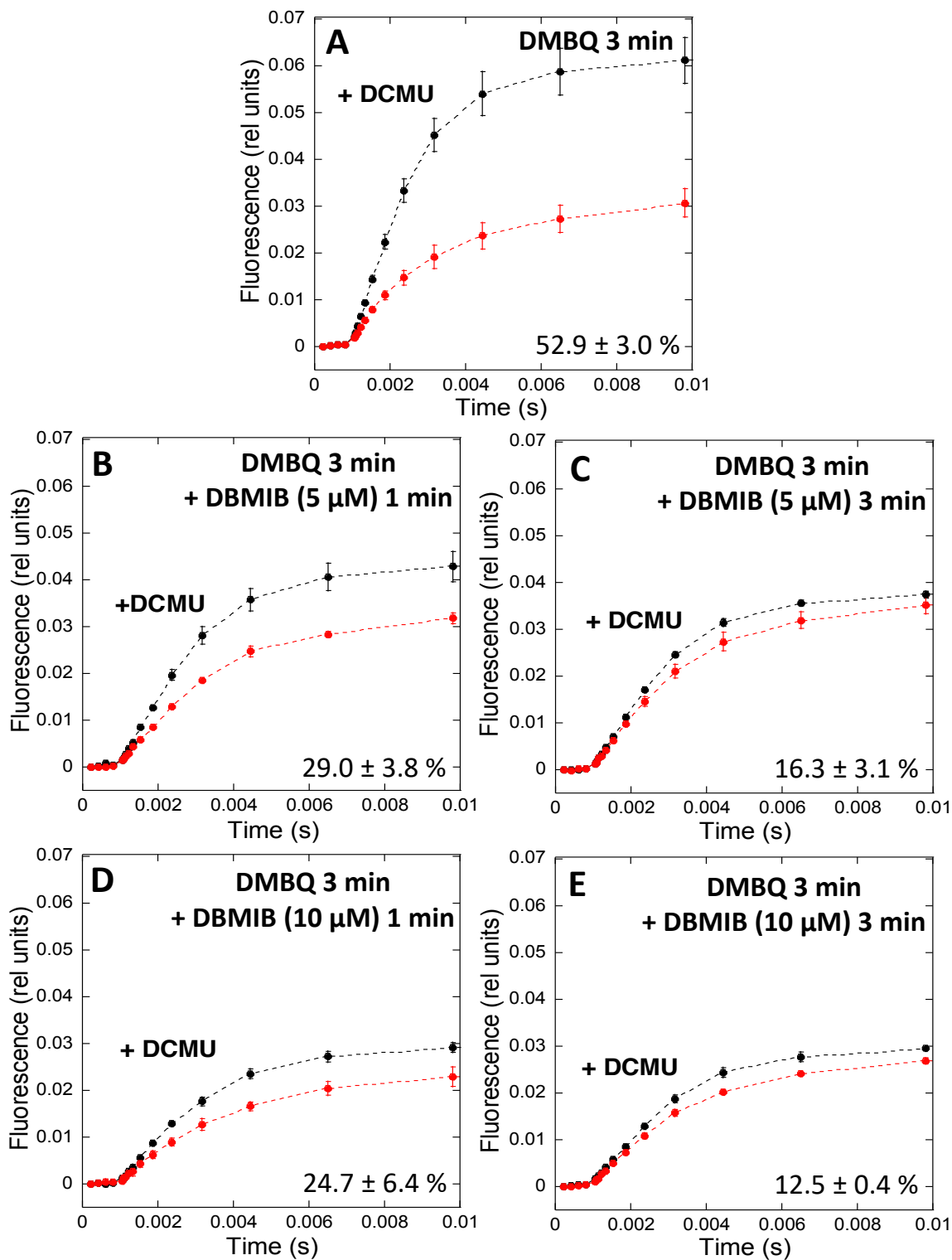
**Figure 6. Schematic model of the effects of different chemicals on the intersystem electron transport chain in cyanobacteria.** The arrows indicate the direction of electron transport between different protein complexes/chemical compounds. The size of each arrow is proportional to the quantity of electrons transfer under different conditions. Orange and blue arrows indicate electron transfer under orange or blue light illumination, respectively. Black solid arrows indicate electron transfer occurring under dark and light conditions. Dashed arrows indicate electrons taken/given by the added chemicals. Red lines indicate the inhibition of the different complexes by the given chemicals. Protein complexes: PSII, photosystem II; PSI, photosystem I; PQ, plastoquinone pool; Cyt  $b_6f$ , cytochrome  $b_6f$ ; NDH-1, NAD(P)H dehydrogenase type 1; SDH, succinate dehydrogenase; Cyd, cytochrome bd-type quinol oxidase; Cox, cytochrome c oxidase. Chemical compounds: DCMU; 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMBQ, 2,6-dimethoxy-1,4-benzoquinone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; MV, methyl viologen; CN, cyanide.



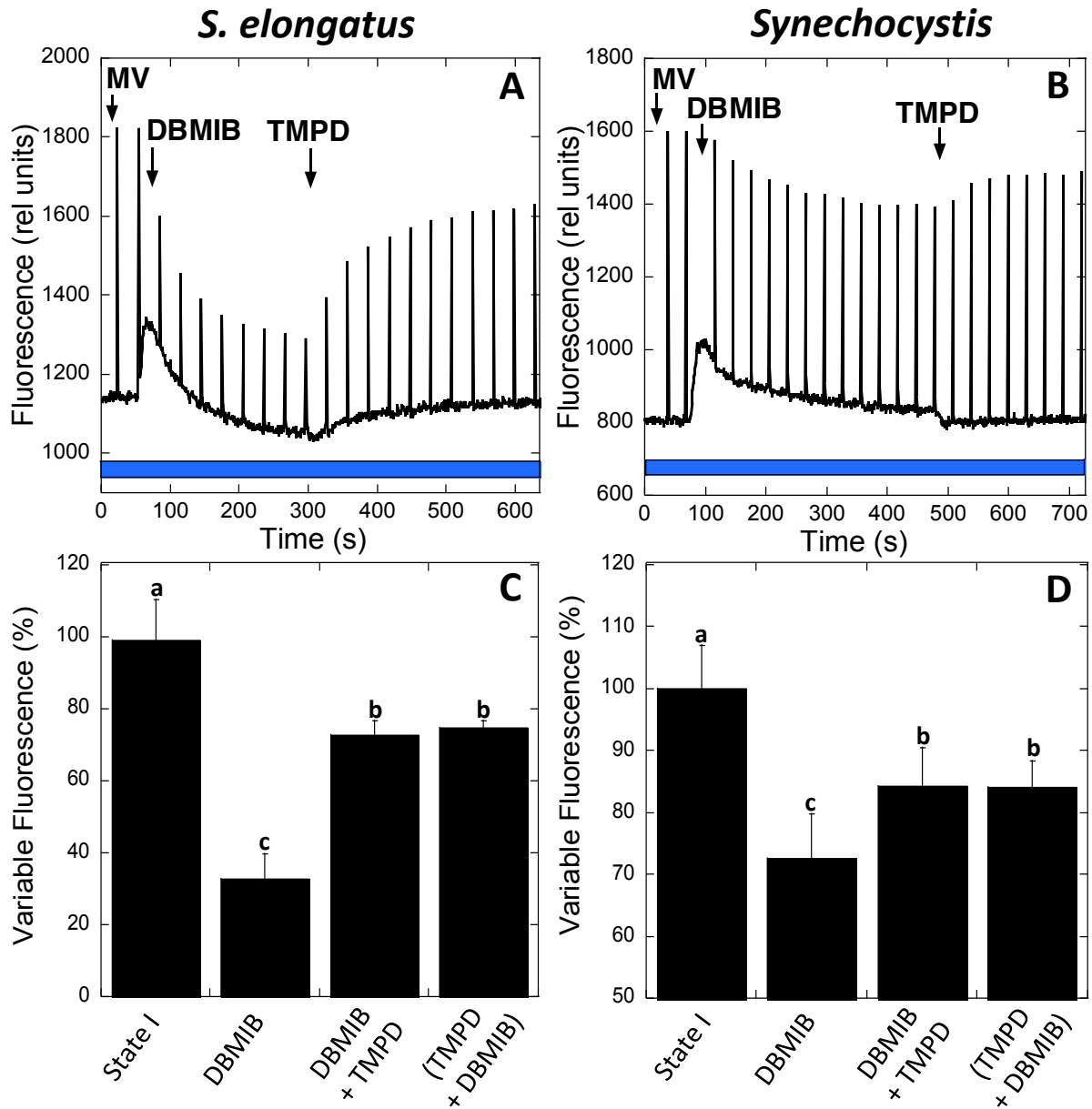
**Figure 7. Fluorescence induction in the absence and presence of NaCN in *S. elongatus* and *Synechocystis* cells.** Whole cells (Chl concentration 2.5  $\mu\text{g}/\text{mL}$ ) were dark adapted for 15 min at 31°C before illumination with orange light (180  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). (A and B) Fluorescence induction in the absence of NaCN. (C and D) Fluorescence induction after 3 min incubation in the presence of NaCN (80  $\mu\text{M}$ ). Black lines: fluorescence induction in the presence of DCMU (10  $\mu\text{M}$ ). Red lines: fluorescence induction in the absence of DCMU. The detection wavelength was  $\geq 695 \text{ nm}$  (far-red). The curves are the average of at least 3 independent biological replicates. The percentages shown are the area between the curves relative to the area under the curve with DCMU. The errors bars correspond to the standard deviation of the data shown.



**Figure 8. Fluorescence changes induced by chemicals.** *S. elongatus* cells were dark adapted (15 min) before the successive addition of DMBQ (250 μM) and DBMIB (20 μM) (A) or the simultaneous addition of DMBQ (250 μM) and DBMIB (20 μM) (B). The measurements were done using a PAM fluorometer (measuring light 650 nm). Saturating pulses (400 ms x 1200 μmol photons m<sup>-2</sup> s<sup>-1</sup>) were applied every 30 sec. Typical experiments are shown. (C) Variable fluorescence (F<sub>v</sub>) traces of different concentrations of DBMIB, added at time 0, when State I was induced by blue light (red lines, 85 μmol photons m<sup>-2</sup> s<sup>-1</sup>) or by DMBQ (250 μM) in darkness (dark lines). The values were normalized to the variable fluorescence of state I (induced by light or DMBQ, respectively). (D) F<sub>v</sub> traces of different concentrations of DBMIB when State I was induced by DMBQ (250 μM) in darkness, and the cells were then illuminated with blue light (85 μmol photons m<sup>-2</sup> s<sup>-1</sup>). The values were normalized to the variable fluorescence of state I induced by DMBQ. The averages of at least 3 independent biological replicates are shown. The errors bars correspond to the standard deviation of the data shown. (●) 2.5 μM DBMIB; (▲) 5 μM DBMIB; (■) 10 μM DBMIB; (▼) 15 μM DBMIB; (◆) 20 μM DBMIB.

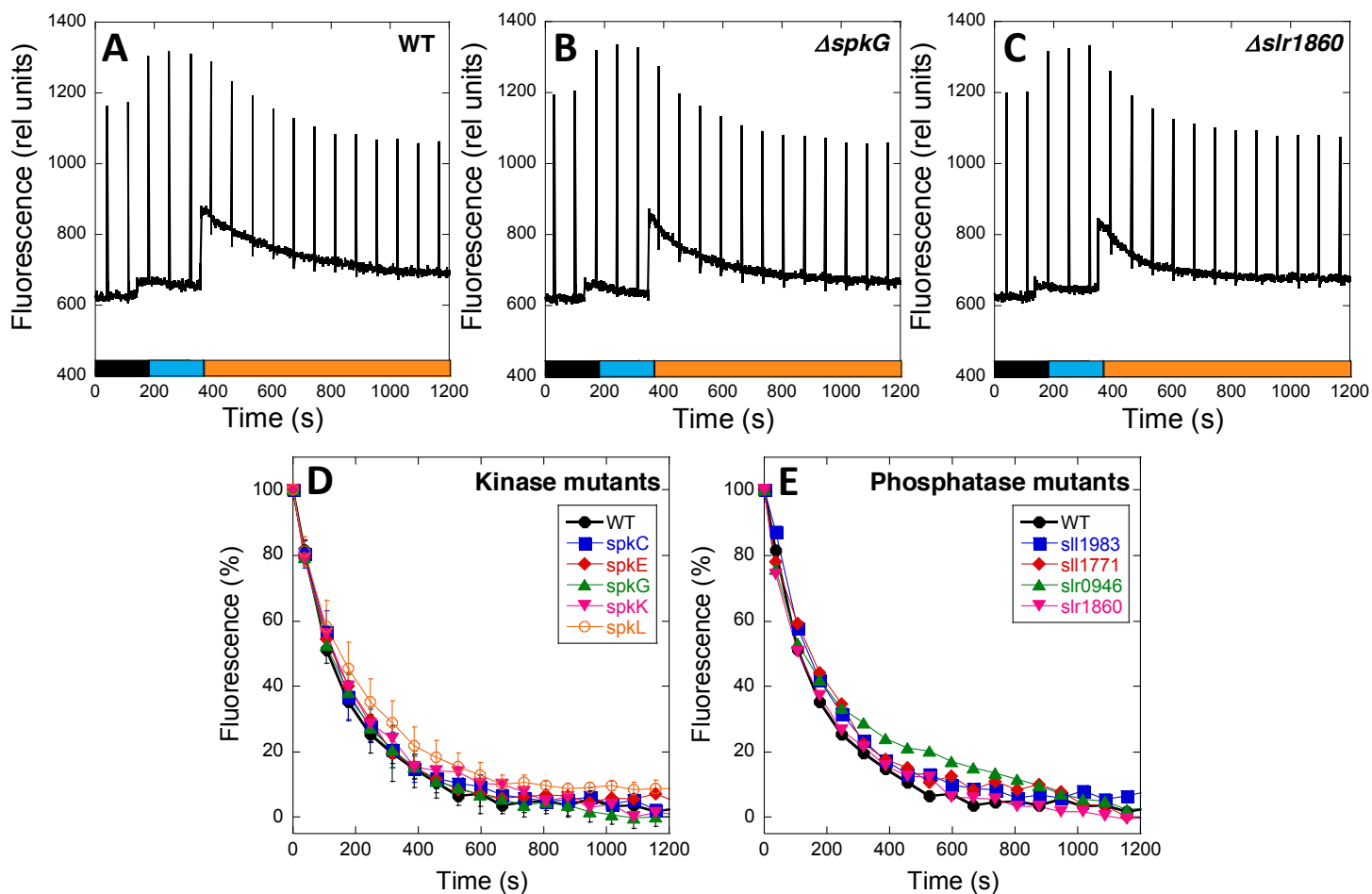


**Figure 9. Estimation of the PQ pool redox state.** Fluorescence induction in the presence (dark lines) or absence (red lines) of DCMU (10 μM) in dark-adapted *S. elongatus* cells. Whole cells (Chl concentration 2.5 μg/mL) were dark adapted for 15 min (at 31°C) before illumination with orange light (180 μmol photons m<sup>-2</sup> s<sup>-1</sup>). (A) cells were incubated for 3 min with DMBQ (250 μM); (B and C) cells were incubated first with DMBQ (250 μM) for 3 min and then with DBMIB (5 μM) for 1 min or 3 min, respectively; (D and E) cells were incubated first with DMBQ (250 μM) for 3 min and then with DBMIB (10 μM) for 1 min or 3 min, respectively. Black lines: fluorescence induction in the presence of DCMU. Red lines: fluorescence induction in the absence of DCMU. The detection wavelength was ≥ 695 nm (far-red). The curves are the average of at least 3 independent biological replicates. The percentages shown are the area between the curves relative to the area under the curve with DCMU. The errors bars correspond to the standard deviation of the data shown.

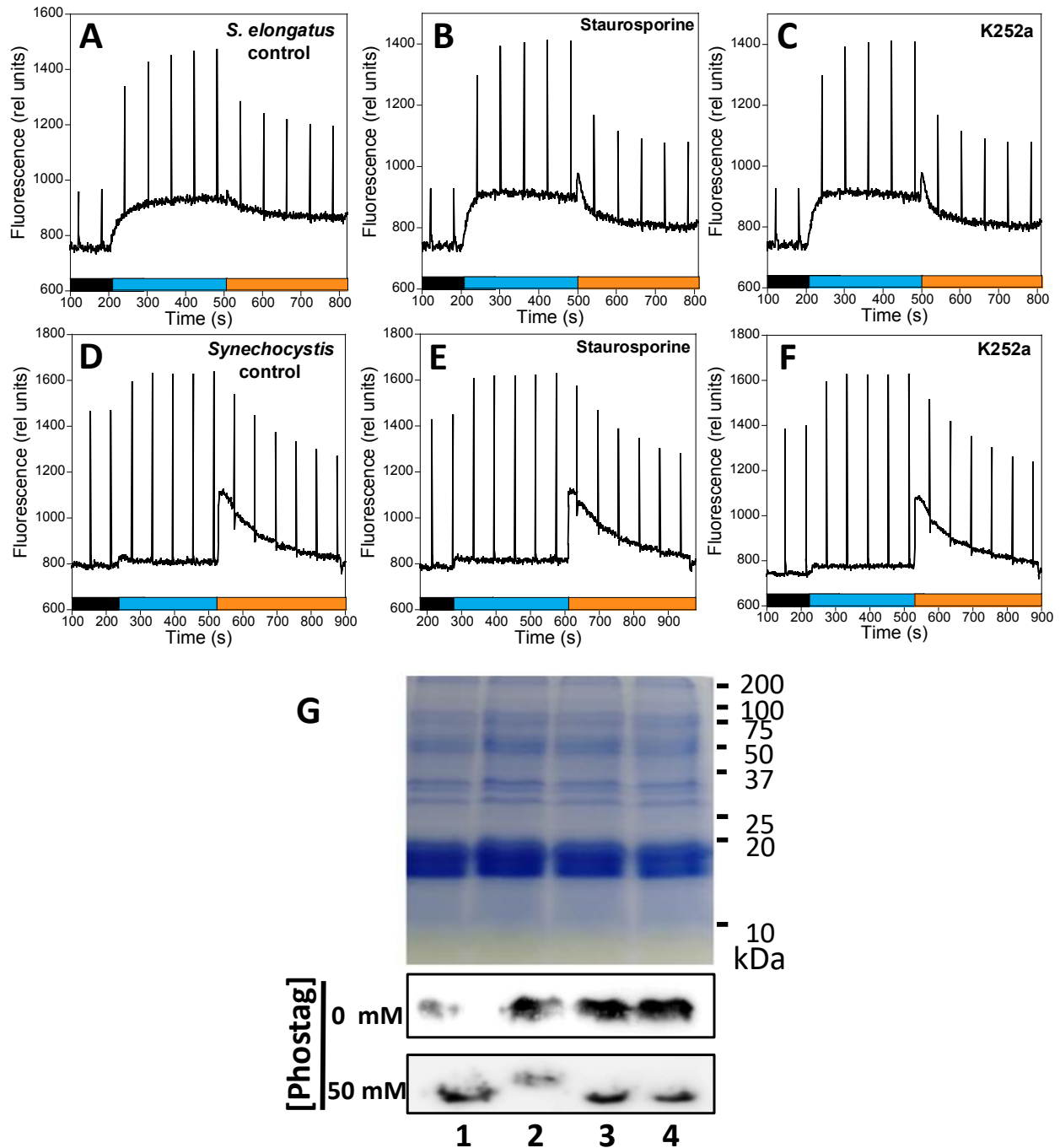


**Figure 10. Fluorescence changes induced by TMPD in the presence of MV and DBMIB.** *S. elongatus* (A) and *Synechocystis* (B) cells were dark adapted (15 min) before the transition to State I by blue light illumination ( $85 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) in the presence of MV (2 mM or 3 mM, respectively). DBMIB ( $10 \mu\text{M}$  for *S. elongatus* or  $7.5 \mu\text{M}$  for *Synechocystis*) was then added. After reaching a steady State II, TMPD ( $10 \mu\text{M}$ ) was added. Saturating pulses were applied every 30 sec. (C and D) Graphs show the percentage of variable fluorescence ( $F_v$  %) for *S. elongatus* and *Synechocystis*, respectively, after blue light illumination (State I); after the addition of DBMIB; DBMIB and then TMPD or when both chemicals were added at the same time. The mean and standard deviation of 4 independent biological replicates are shown. Columns without common letters differ significantly (Tukey test,  $p < 0.05$ ).

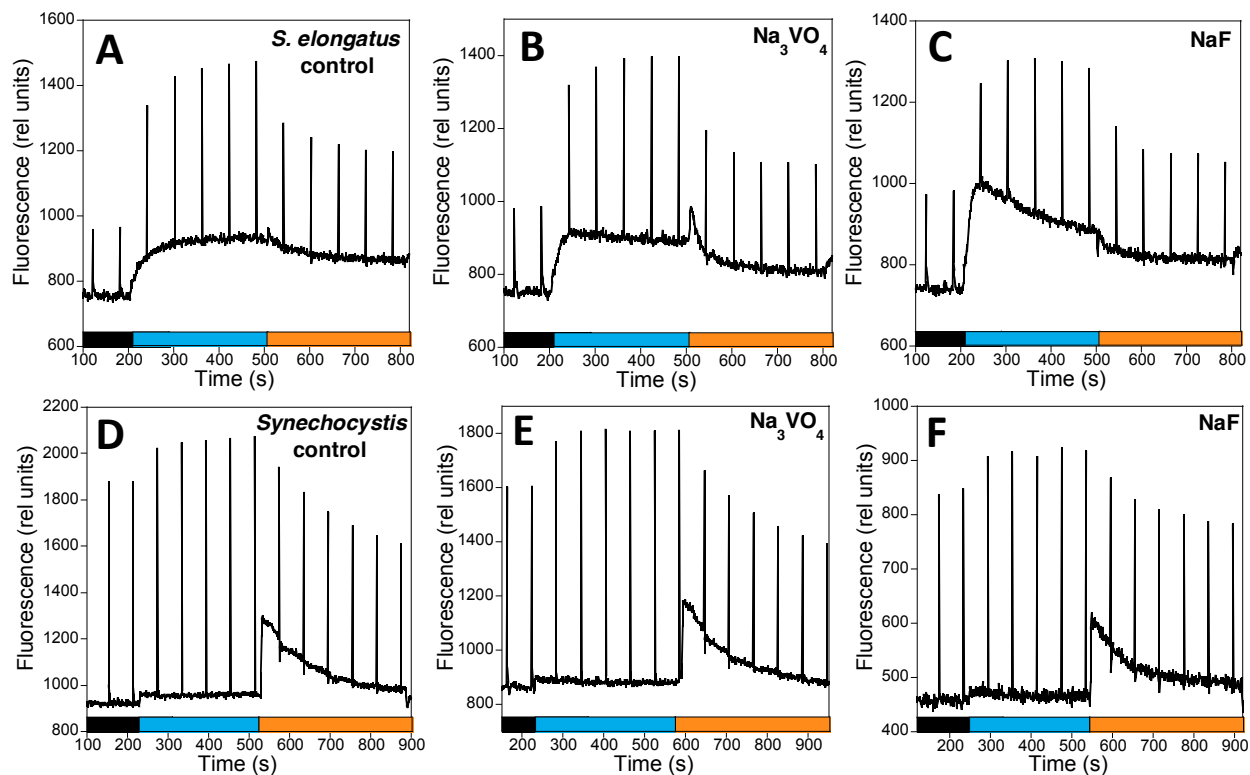




**Figure 11. State transitions in *Synechocystis* kinase and phosphatase mutants.** The fluorescence changes were followed with a PAM fluorometer. Dark-adapted cells (Chl concentration 2.5  $\mu\text{g}/\text{mL}$ ) were successively illuminated with blue light (85  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and orange light (40  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Typical experiments are shown for *Synechocystis* WT (A),  $\Delta spkG$  (B) and  $\Delta slr1860$  (C) cells. Saturating pulses (400 ms x 1200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) were applied every 70 sec. (D and E) Fv (%) decrease induced by orange illumination of blue-light adapted cells (State I to State II transition) of kinase and phosphatase mutants, respectively. The values were normalized, with the Fv of State I equal to 100% and the Fv of State II equal to 0%. (D) The values for WT and  $\Delta spkC$ ,  $\Delta spkE$ ,  $\Delta spkG$ ,  $\Delta spkK$  and  $\Delta spkL$  mutants are shown. (E) The values for WT and  $\Delta slr1983$ ,  $\Delta slr1771$ ,  $\Delta slr0946$  and  $\Delta slr1860$  mutants are shown. The averages of at least 3 independent biological replicates are shown. The errors bars correspond to the standard deviation of the data shown.



**Figure 12. Effects of kinase inhibitors on state transitions and PII phosphorylation.** *S. elongatus* (A-C) and *Synechocystis* (D-F) cells were incubated in the absence and presence of kinase inhibitors for 90 min in darkness. Then, the cells (Chl concentration 2.5  $\mu\text{g}/\text{mL}$ ) were successively illuminated with blue light ( $85 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and orange light ( $40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and the fluorescence changes were followed using a PAM fluorometer. (A and D) Control cells containing DMSO (0.1% v/v). (B and E) Cells treated with Staurosporine (21  $\mu\text{M}$ ). (C and F) Cells treated with K252a (1.07  $\mu\text{M}$ ). Saturating pulses (400 ms x  $1200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) were applied every 60 sec. (G) Gel electrophoresis containing phostag to detect phosphorylated proteins and immunoblot using an anti-PII protein. (1) BG11 medium; (2) nitrogen depleted BG11 medium; (3), nitrogen depleted BG11 medium supplemented with K252a (1.07  $\mu\text{M}$ ); (4), nitrogen depleted BG11 medium supplemented with staurosporine (21  $\mu\text{M}$ ).



**Figure 13. Effect of phosphatase inhibitors on state transitions.** *S. elongatus* (A-C) and *Synechocystis* (D-F) cells were incubated in the absence and presence of phosphatase inhibitors for 90 min in darkness. Then, the cells (Chl concentration 2.5  $\mu\text{g}/\text{mL}$ ) were successively illuminated with blue light (85  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and orange light (40  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and the fluorescence changes were followed using a PAM fluorometer. (A and D) Control cells; (B and E) cells treated  $\text{Na}_3\text{VO}_4$  (1 mM); (C and F) cells treated with NaF (50 mM). Saturating pulses (400 ms x 1200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) were applied every 60 sec.

**The cytochrome b6f complex is not involved in cyanobacterial state transitions**

Pablo I. Calzadilla, Jiao Zhan, Pierre Sétif, Claire Lemaire, Daniel Solymosi, Natalia Battchikova, Qiang Wang and Diana Kirilovsky  
*Plant Cell*; originally published online March 8, 2019;  
DOI 10.1105/tpc.18.00916

This information is current as of March 9, 2019

|                                 |   |
|---------------------------------|---|
| <b>Supplemental Data</b>        | <a href="/content/suppl/2019/03/08/tpc.18.00916.DC1.html">/content/suppl/2019/03/08/tpc.18.00916.DC1.html</a>   |
| <b>Permissions</b>              | <a href="https://www.copyright.com/ccc/openurl.do?sid=pd_hw1532298X&amp;issn=1532298X&amp;WT.mc_id=pd_hw1532298X">https://www.copyright.com/ccc/openurl.do?sid=pd_hw1532298X&amp;issn=1532298X&amp;WT.mc_id=pd_hw1532298X</a> |
| <b>eTOCs</b>                    | Sign up for eTOCs at:<br><a href="http://www.plantcell.org/cgi/alerts/ctmain">http://www.plantcell.org/cgi/alerts/ctmain</a>  |
| <b>CiteTrack Alerts</b>         | Sign up for CiteTrack Alerts at:<br><a href="http://www.plantcell.org/cgi/alerts/ctmain">http://www.plantcell.org/cgi/alerts/ctmain</a>   |
| <b>Subscription Information</b> | Subscription Information for <i>The Plant Cell</i> and <i>Plant Physiology</i> is available at:<br><a href="http://www.aspb.org/publications/subscriptions.cfm">http://www.aspb.org/publications/subscriptions.cfm</a>        |