Supplementary Material to

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

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

[a] Institute for Lasers, Life and Biophotonics, Faculty of Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1081, 1081 HV, Amsterdam, The Netherlands.
[b] Swammerdam Institute for Life Sciences, University of Amsterdam, 1098 XH, Amsterdam, The Netherlands.
[*] Authors have contributed equally to this work

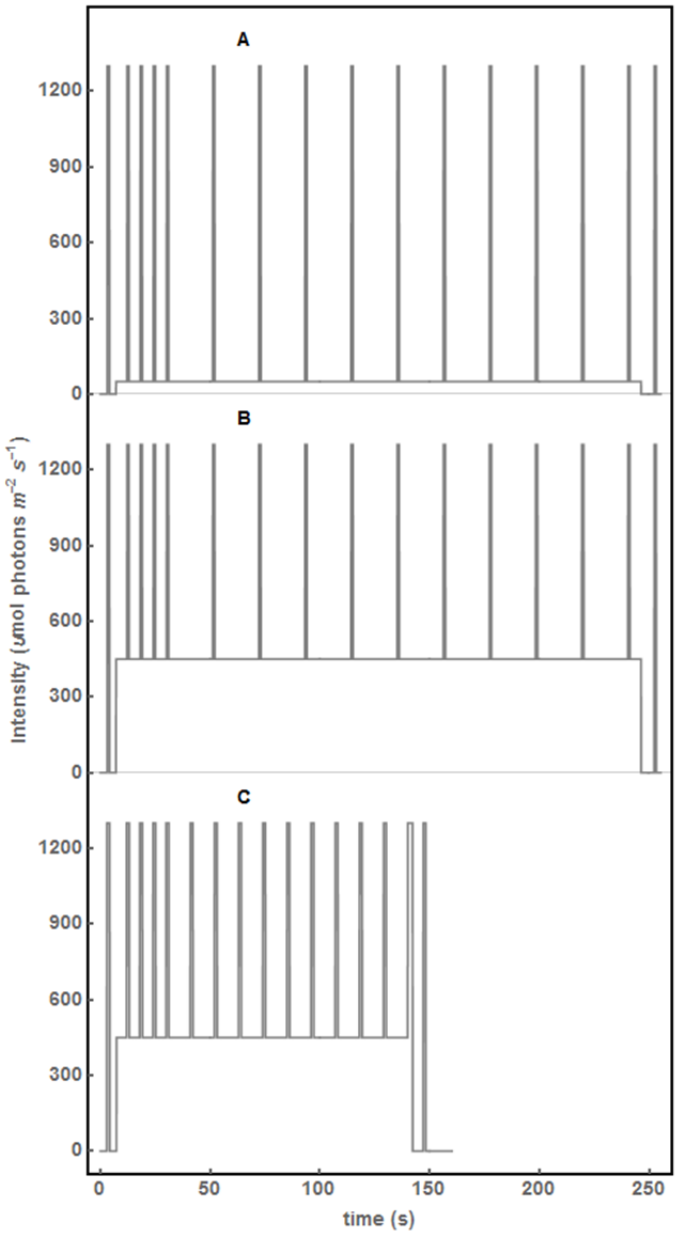

Figure S 1. Applied light (590 nm) protocol during experiments. The first and last saturation pulses (1300 µmol photons m^{-2} s^{-1}) are applied in darkness. The background light is either 50 µmol photons m^{-2} s^{-1} (A) or 450 µmol photons m^{-2} s^{-1} (B and C).

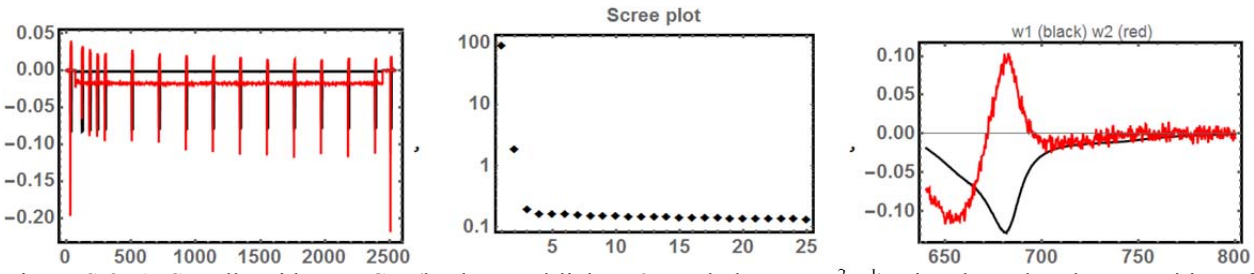

Figure S 2. ΔPSI cells without KCN (background light: 50 µmol photons m^{-2} s^{-1}): singular value decomposition of the dataset resulting in two singular values distinguishable from the noise (see the scree plot). From left to right: left singular vectors, logarithmic plot of the singular values and right singular vectors.

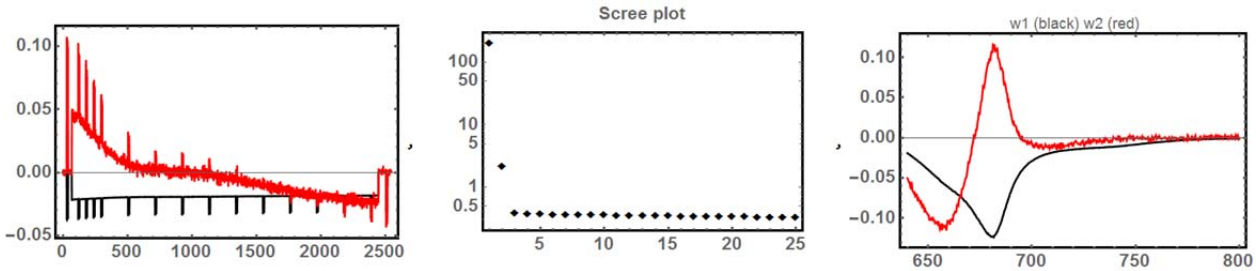

Figure S 3. ΔPSI cells previously treated with KCN: singular value decomposition of the dataset resulting in two singular values distinguishable from the noise (see the scree plot). From left to right: left singular vectors, logarithmic plot of the singular values and right singular vectors.

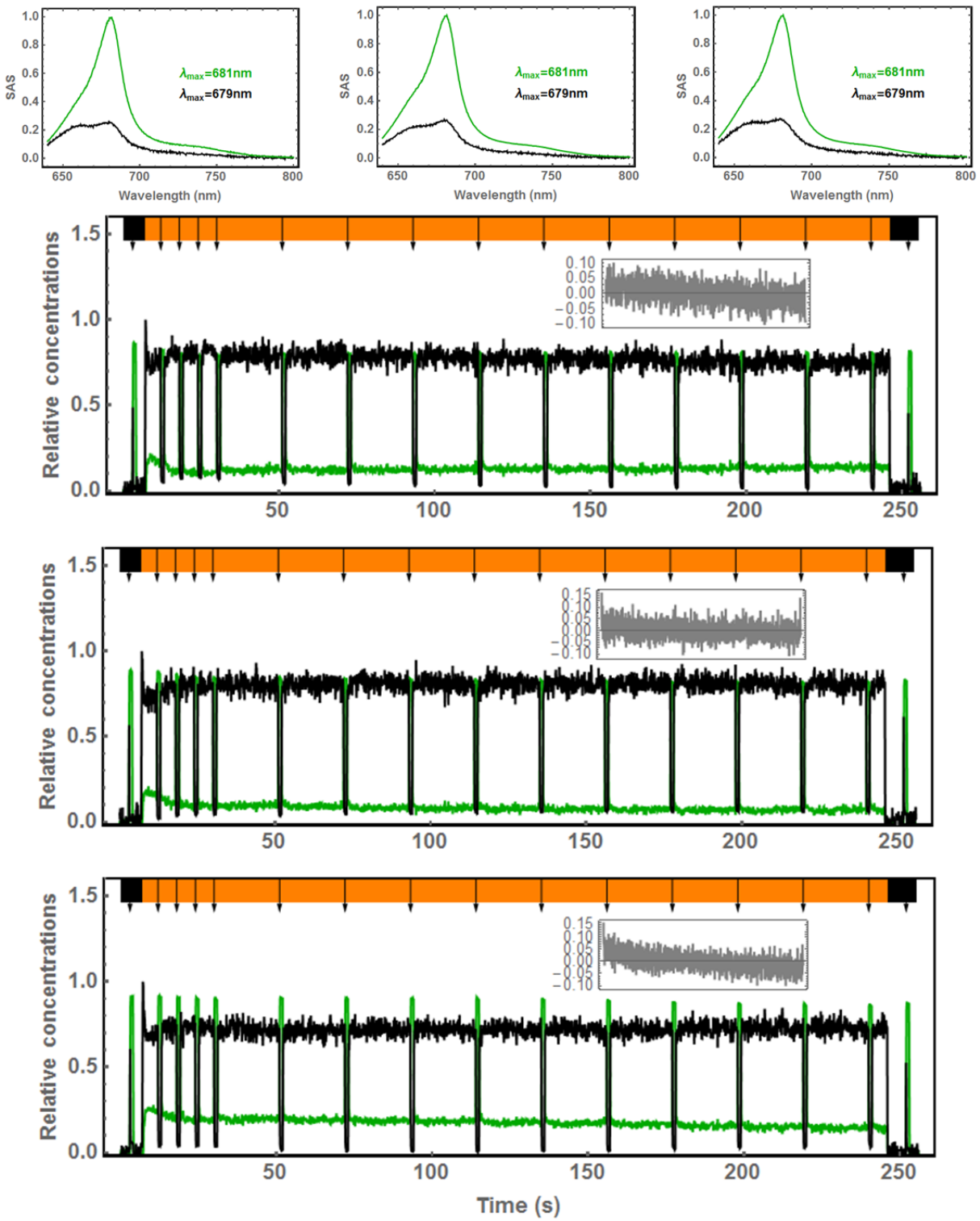

Figure S 4. Concentration profiles of the two components c_1 (black) and c_2 (green) corresponding to the SAS shown above (*left*: DA=34; *center*: DA=5; *right*: DA=1min) of ΔPSI cells exposed to low light background illumination and O_2. *Key to concentration panels*: *top*: DA time= 34 min, *middle*: DA time=5 min; *bottom*: DA time=1 min. The colored bar on top illustrates the light regime: 50 µmol photons m^{-2} s^{-1} of 590 nm light (orange) or darkness (black) with arrows indicating the beginning of a saturation pulse. *Insets*: the average deviation in the sum of concentrations from unity.

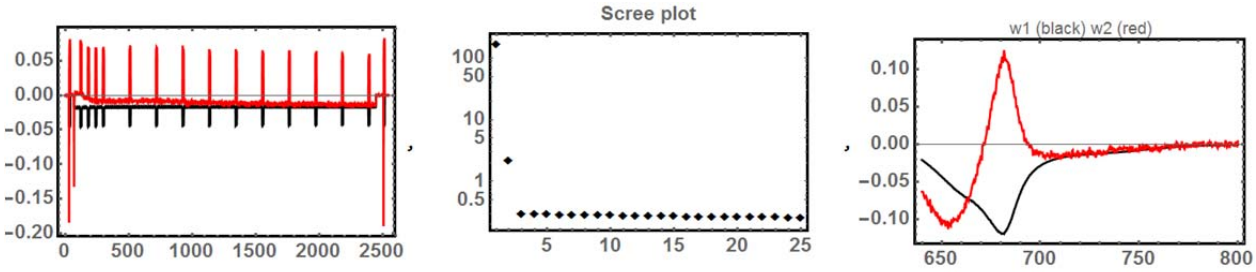

Figure S 5. Dark-adapted (34 min) ΔPSI cells without KCN exposed to a mixture of N_2/CO_2: singular value decomposition of the dataset resulting in two singular values distinguishable from the noise (see the scree plot). From left to right: left singular vectors, logarithmic plot of the singular values and right singular vectors.

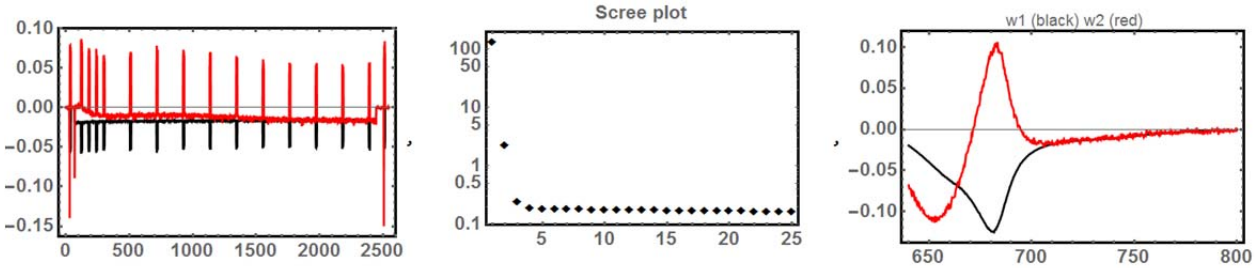

Figure S 6. Dark-adapted (1 min) ΔPSI cells without KCN exposed to a mixture of N_2/CO_2: singular value decomposition of the dataset resulting in two singular values distinguishable from the noise (see the scree plot). From left to right: left singular vectors, logarithmic plot of the singular values and right singular vectors.

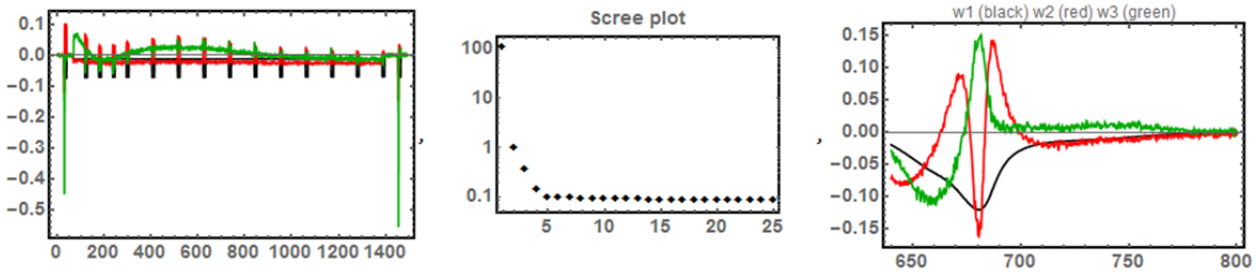

Figure S 7. ΔPSI cells without KCN (background light: 450 µmol photons m^{-2} s^{-1}, see exact light protocol in Figure S 1C): singular value decomposition of the dataset resulting in at least three singular values distinguishable from the noise (see the scree plot). From left to right: left singular vectors, logarithmic plot of the singular values and right singular vectors.

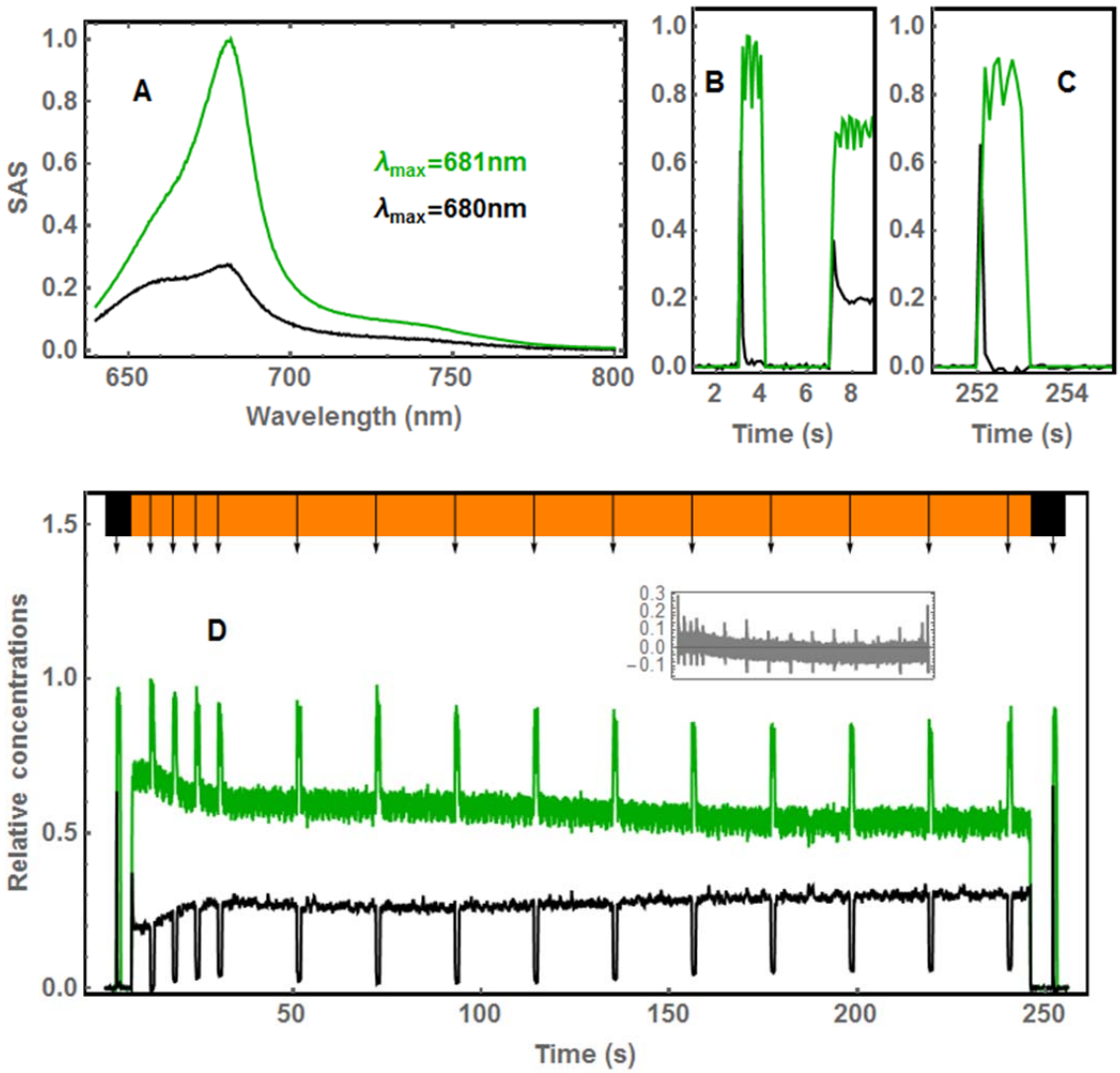

Figure S 8. Spectral decomposition of fluorescence spectra of ΔPSI cells under microoxic conditions after 1 min dark adaptation. (A) The SAS (Black: SAS_1; green: SAS_2) obtained after transformation of the singular vectors (shown in Figure S 6). Panels B and C show a zoom view of the first and last pulse. (D) Time profiles. *Key: orange*: 450 µmol photons m^{-2} s^{-1}; *black*: darkness. The colored bar on top illustrates the light regime with arrows indicating the beginning of a saturation pulse. *Inset*: the average percentual deviation in the sum of concentrations from unity is less than 2%.

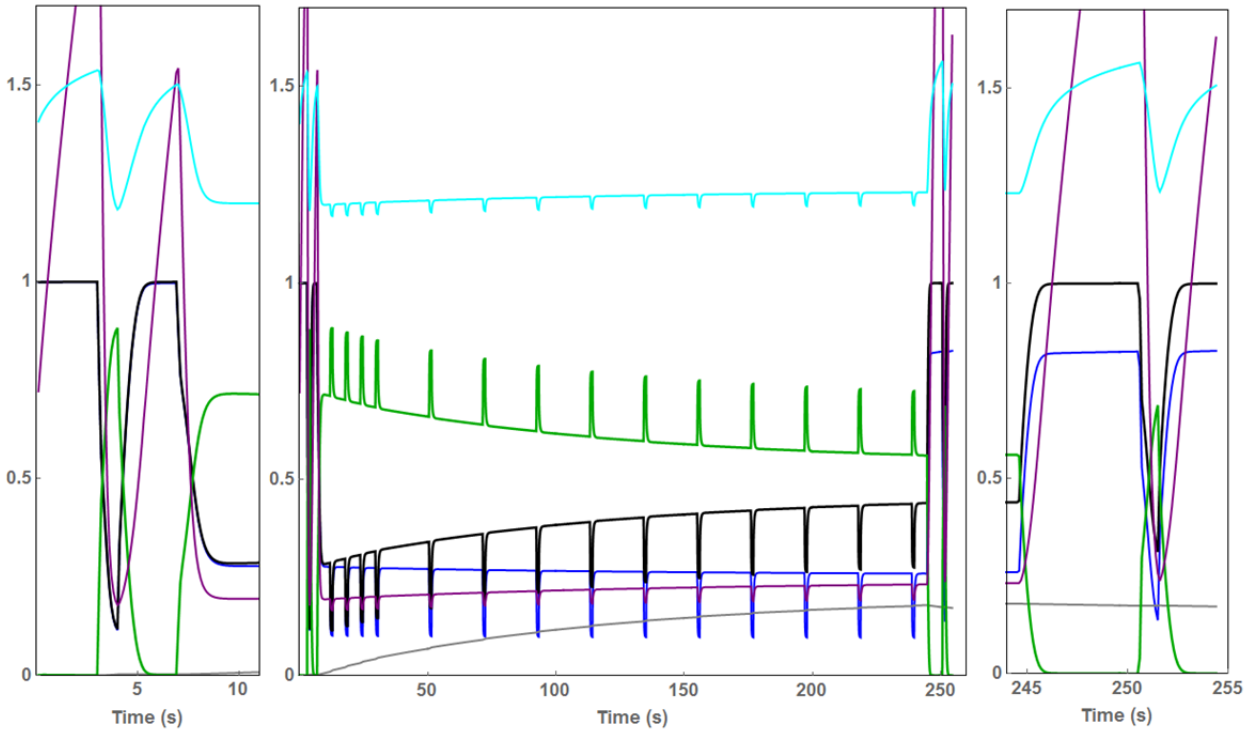

Figure S 9. Simulated concentrations of PB-PSII-dimer complexes (reproduced from Figure 11B) with open RCs (blue), closed RCs, unquenched (green) or quenched (gray). Black represents the sum of blue and grey, and is observed as SAS_1. In addition, the time profiles of the PQ (purple) and PC (cyan) pools throughout the experiment are shown. The redox state of the PQ pool is separately shown in Figure 12.

Objective Function

Definition of residuals to be minimized by the objective function:

$$residuals = R_m + R_s + R_c + R_{Smooth}$$

whereby R_m stands for the deviation from a constant sum of concentrations, R_s increases with negativity of the SAS, R_c increases with negativity of the concentration profiles and R_{Smooth} increases as the second derivative of the SAS increases.

$$R_{m,i} = \sum_i c_{ti} - \overline{\sum_i c_{ti}}$$

$$R_{s,j} = \begin{cases} 0 & if\ SAS_{j\lambda} > 0 \\ \sum_\lambda SAS_{j\lambda} & if\ SAS_{j\lambda} < 0 \end{cases}$$

$$R_{c,i} = \begin{cases} 0 & if\ c_{ti} > 0 \\ \sum_t c_{ti} & if\ c_{ti} < 0 \end{cases}$$

$$R_{Smooth} = \sum_i \left| \int \frac{\partial^2}{\partial \lambda^2} SAS_i(\lambda) \right|$$

The penalties are applied using a specific weight to each of the residuals' criteria:

$$PENALTY_{Total} = \sum_i w_{m,i} \cdot R_{m,i} + \sum_j w_{s,j} \cdot R_{s,j} + \sum_i w_{c,i} \cdot R_{c,i} + \sum_j w_j \cdot \left| \int \frac{\partial^2}{\partial \lambda^2} SAS_j(\lambda) \right|$$

whereby $i,j = 1,2,3$

Mathematical model

The mathematical model behind Figure 7 is a system of ordinary differential equations that considers the turnover rates of PS II, cyt b_6f and terminal oxidases as well as the action of light that is captured in the function $\ell(t)$ yielding different levels of illumination as a function of time, as illustrated in Figure S 1. Part of it is similar to the model of [37].
The rate of closing of open RCs (c_0) by light (k_L), which leads to a population increase in closed RCs (c_1), is:

$$v_{l,0} = k_L \cdot \ell(t) \cdot c_0$$

The PQ pool (k_{PQ}) is the main electron sink. An oxidized PQ pool, PQ(t), accepts PSII-generated electrons, thereby lowering the population of closed RCs (c_1), but at the same time, an equilibrium constant (K_{eq}) is included; it considers an eventual back transfer to the PSII due to a reduced PQ pool, PQH_2(t), resulting in a decrease in the population of open RCs (c_0):

$$v_{PSII} = -k_{PQ} \cdot PQ(t) \cdot c_1 + \frac{k_Q}{K_{eq}} \cdot PQH_2(t) \cdot c_0$$

Additionally, the population c_1 can decrease by the action of a quencher Q_x. The assumption is that Q_x first has to be activated by light to Q_x^*:

$$v_{Qx} = \frac{k_L}{k_{act}} \cdot \ell(t) \cdot Q_x$$

This excited state would also have an intrinsic decay rate $k_{x,dec}$:

$$v_{x,dec} = k_{x,dec} \cdot Q_x^*$$

and as long as it lives, and provided reactive oxygen species are being formed, it attaches (k_A) to the PB-PSII complex (forming the species $Q_{x,bound}$) thereby quenching its fluorescence:

$$v_{x,A} = k_A \cdot O_2 \cdot PQH_2 \cdot Q_x^* \cdot c_1$$

where the probability for quenching is considered proportional to the concentration of oxygen O_2, the degree of PQ reduction (PQH_2) and the population c_1. The population of quenched complexes c_2 decreases as the quencher detaches:

$$v_{x,D} = k_D \cdot c_2$$

As for the electron acceptors for the PQ pool, the turnover rate of the cyt b_6f complex is assumed to increase as the PQ pool is reduced while the PC pool is strongly oxidized; conversely, a strongly reduced PC pool, as well as low incoming electron flux from the PQ pool slow down the rate of cytochrome b_6f. This relationship is reflected in the following expression [37]:

$$v_{b6f} = k_{b6f} \cdot \left(PQH_2 \cdot PC^2 - \frac{PQ \cdot PC_{red}^2}{K_{eq,b6f}} \right)$$

where PC is the fraction of oxidized plastocyanin and PC_{red} the fraction of reduced plastocyanin and $K_{eq,b6f}$ is an equilibrium constant specific to the cyt b_6f. Finally, the linear electron transport chain leads to terminal oxidases, summed up in the term k_{COX}, that capture electrons from the reduced PC pool:

$$v_{COX} = k_{COX} \cdot PC_{red}$$

Hence, the system of differential equations that we set out to solve is the following:

$$\frac{d}{dt} c_1 = v_{l,0} + v_{PSII} - v_{x,A} + v_{x,D}$$

$$\frac{d}{dt} c_2 = v_{x,A} - v_{x,D}$$

$$\frac{d}{dt} PQ(t) = v_{b6f} - v_{PSII}$$

$$\frac{d}{dt} PC(t) = -2v_{b6f} + v_{COX}$$

$$\frac{d}{dt} Q_x^*(t) = v_{Qx} - v_{x,dec} - v_{x,A}$$

$$\frac{d}{dt} Q_{x,bound}(t) = v_{x,A} - v_{x,D}$$

with:

$$c_0 = c_{total} - c_1 - c_2$$

$$Q_x = Q_{total} - Q_x^* - Q_{x,bound}$$