## Shimakawa et al. - Supplementary Material

Fig. S1. Experimental design of photoinhibition treatments.



Yellow wedges represent photoinhibitory treatment with continuous light, blue flashes represent photoinhibitory treatment with repetitive saturating pulses.

**Fig. S2.** Absorption spectra of PSI isolated from leaves treated with continuous light at room temperature (C/RT), continuous light at low temperature (C/LT), and repetitive flashes at room temperature (F/RT).



Absorption spectra were normalized to an OD of 1.70 at the red absorption maximum of 678 nm.

**Fig. S3.** Flash-absorption measurements at 800 nm at varied laser intensities. The samples are those studied in Fig. 2.



Same experimental conditions as those of Fig. 2 except the laser intensity varied from 1.6% (0.4 mJ) to 100% (25 mJ) by using different numbers of 50% neutral density filters. Parts A, B and C correspond to C/RT, C/LT and F/RT PSI, respectively. All data were normalized to the same OD of 1.70 at red absorption maximum. For each PSI, all kinetics were normalized to the same signal size at 10 ms, using the 100% signal as the reference. This allows the saturation properties of the fast  $\mu$ s signals to be compared.

**Fig. S4**: Identification of the P700 triplet state (<sup>3</sup>P700) spectrum in PSI treated with continuous light at low temperature (C/LT). C/LT treated PSI (same preparation as the one studied in Fig. 3) was studied by flash-absorption spectroscopy between 730 and 970 nm.



Laser excitation was ca. 0.75 mJ at 700 nm, giving about 60% of the maximal extent of charge separation. A global fit with a single exponential component and a constant was performed on all kinetic traces, giving a  $t_{1/2}$  = 7.3 µs for the decay. Part A shows a few kinetic traces, at 740, 820 and 870 nm, with fits in continuous lines extrapolated to time zero.

Part B: the preexponential factors of the decay phase and the constant values are shown as open red squares and closed black circles, respectively. The red and black spectra exhibit quite different shapes, which are in full agreement with formerly determined ( ${}^{3}P700 - P700$ ) and ( $P700^{+} - P700$ ) difference spectra, respectively (see Figure 2 in Setif (1981)).





Kinetics in the presence of 4 mM sodium ascorbate and 40  $\mu$ M DCPIP were measured at 820 nm with C/RT, C/LT and F/RT PSI on time scales of 80 ms and 300 ns for parts A/C and B/D, respectively. The samples are those studied in Figure 4 and data were normalized to an OD<sub>1 cm</sub> at 12 at red maximum. The 600 nm laser intensity was 2.6 and 0.13 mJ in parts A/B and C/D, respectively. The intensity of 2.6 mJ was quasi-saturating PSI photochemistry, as judged by a decrease of 5% amplitude in the slow decaying component with a 47% intensity (1.25 mJ). The C/RT kinetics at 0.13 mJ laser energy were fitted with 3 exponential components and a constant, with the best fit curve shown in cyan in parts C/D. The fitted curve has the following equation:

 $\Delta A \times 10^3 = 1.60 \, e^{-t/0.01042} + 0.13 \, e^{-t/0.4003} + 0.22 \, e^{-t/5.513} + 4.06,$ 

with time in  $\mu$ s (decay kinetics of 10.4 ns, 400 ns and 5.5  $\mu$ s).

The kinetics shown in Fig. 3 were obtained after subtracting the decaying part of the fitted curve (*i.e.*, without the constant) from all 3 kinetics. This subtraction procedure assumes that the decaying signal is due to antenna signals and that these antenna signals are identical in the 3 types of PSI.