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# The time course of photoinactivation of Photosystem II in leaves revisited

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| **Abstract:** | Since Photosystem II (PS II) performs the demanding function of water oxidation using light energy, it is susceptible to photoinactivation during photosynthesis. The time course of photoinactivation of PS II yields useful information about the process. Depending on how PS II function is assayed, however, the time course seems to differ. Here we revisit this problem by using two additional assays: (1) the quantum yield of oxygen evolution in limiting, continuous light and (2) the flash-induced cumulative delivery of PS II electrons to the oxidized primary donor (P700+) in PS I measured as a "P700 kinetics area". The P700 kinetics area is based on the fact that the two photosystems function in series: when P700 is completely photo-oxidized by a flash added to continuous far-red light, electrons delivered from PS II to PS I by the flash tend to re-reduce P700+ transiently to an extent depending on the PS II functionality, while the far-red light photo-oxidizes P700 back to the steady-state concentration. The quantum yield of oxygen evolution in limiting, continuous light indeed decreased in a way that deviated from a single negative exponential. However, measurement of the quantum yield of oxygen in limiting light may be complicated by changes in mitochondrial respiration between darkness and limiting light. Similarly, an assay based on chlorophyll fluorescence may be complicated by the varying depth in leaf tissue from which the signal is detected after progressive photoinactivation of PS II. On the other hand, the P700 kinetics area appears to be a reasonable assay, which is a measure of functional PS II in the whole leaf tissue and independent of changes in mitochondrial respiration. The P700 kinetics area decreased in a single negative exponential fashion during progressive photoinactivation of PS II in a number of plant species, at least at functional PS II contents 6% of the initial value, in agreement with the conclusion of Sarvikas et al. (Photosynth Res 103: 7-17). That is, the single-negative-exponential time course does not provide evidence for photoprotection of functional PS II complexes by photoinactivated, connected neighbours. |

REGULAR PAPER

# The time course of photoinactivation of Photosystem II in leaves revisited

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**Abstract**

Since Photosystem II (PS II) performs the demanding function of water oxidation using light energy, it is susceptible to photoinactivation during photosynthesis. The time course of photoinactivation of PS II yields useful information about the process. Depending on how PS II function is assayed, however, the time course seems to differ. Here we revisit this problem by using two additional assays: (1) the quantum yield of oxygen evolution in limiting, continuous light and (2) the flash-induced cumulative delivery of PS II electrons to the oxidized primary donor ($P700^+$) in PS I measured as a "P700 kinetics area". The P700 kinetics area is based on the fact that the two photosystems function in series: when P700 is completely photo-oxidized by a flash added to continuous far-red light, electrons delivered from PS II to PS I by the flash tend to re-reduce $P700^+$ transiently to an extent depending on the PS II functionality, while the far-red light photo-oxidizes P700 back to the steady-state concentration. The quantum yield of oxygen evolution in limiting, continuous light indeed decreased in a way that deviated from a single negative exponential. However, measurement of the quantum yield of oxygen in limiting light may be complicated by changes in mitochondrial respiration between darkness and limiting light. Similarly, an assay based on chlorophyll fluorescence may be complicated by the varying depth in leaf tissue from which the signal is detected after progressive photoinactivation of PS II. On the other hand, the P700 kinetics area appears to be a reasonable assay, which is a measure of functional PS II in the whole leaf tissue and independent of changes in mitochondrial respiration. The P700 kinetics area decreased in a single negative exponential fashion during progressive photoinactivation of PS II in a number of plant species, at least at functional PS II contents ≥ 6% of the initial value, in agreement with the conclusion of Sarvikas et al. (Photosynth Res 103: 7-17). That is, the single-negative-exponential time course does not provide evidence for photoprotection of functional PS II complexes by photoinactivated, connected neighbours.

**Key words**: Chlorophyll fluorescence • oxygen evolution • P700 • Photosystem II • photoinactivation of Photosystem II

**Abbreviations**

| | |
|---|---|
| Chl | Chlorophyll |
| $F_o$, $F_m$ | Chl fluorescence yield when PS II reaction centre traps are open and closed, respectively |
| $F_v$ | Variable Chl fluorescence yield (= $F_m - F_o$) |

| | | |
|---|---|---|
| 51 | $k_{pi}$ | Rate coefficient of photoinactivation of PS II |
| 52 | P700 | Special Chl pair in the PS I reaction centre |
| 53 | PS II | Photosystem II |
| 54 | $QY_{O2}$ | Quantum yield of oxygen evolution in continuous, limiting light |
| 55 | $\Sigma$ | Integrated flash-induced transient flow of electrons from PS II to $P700^+$ |

56 **Introduction**

57 Photosystem II (PS II) plays the unique role of water-splitting using light energy, liberating electrons and
58 protons that ultimately help to drive biosynthetic processes (Wydrzynski and Satoh 2005). Unfortunately, PS
59 II is "intrinsically suicidal" (van Gorkom and Schelvis 1993) during its normal function (Ewart 1896; Powles
60 1984; Krause 1988; Barber 1995; Adir et al. 2003; Vass and Cser 2011), and once photoinactivated, has to be
61 repaired if function is to be maintained (Kyle et al. 1984; Prásil et al. 1992; Aro et al. 1993; Chow 1994;
62 Melis 1999; Andersson and Aro 2001; Chow and Aro 2005).

63 Photoinactivation of PS II is easily revealed when repair is inhibited by an inhibitor of chloroplast-
64 encoded protein synthesis, e.g. lincomycin. In the absence of repair, a PS II complex, once photoinactivated,
65 remains inactive, enabling the gross loss of functional PS II to be studied during exposure to light. The time
66 course of photoinactivation of PS II in the absence of repair contains useful information about the
67 photoinactivation process. For example, when described as a single negative exponential, the time course is
68 characterized by the rate coefficient of photoinactivation ($k_{pi}$). This important parameter has been observed to
69 be directly proportional to the irradiance used (Tyystjärvi and Aro. 1996; Lee et al. 2001; Kato et al. 2003).
70 This linear relationship is indeed a consequence (Lee et al. 1999) of the reciprocity rule according to which,
71 other things being equal, equal dose of light gives equal effect regardless of the irradiance or the duration of
72 illumination; the reciprocity rule has been observed at moderate and high irradiance (Jones and Kok 1966;
73 Park et al. 1995a; Nagy et al. 1995; Lee et al. 1999), though not at low irradiance (Oguchi et al. 2009) or
74 during the light induction period (Shen et al. 1996). The value of $k_{pi}$ is a measure of the susceptibility of PS II
75 to photoinactivation, since a higher $k_{pi}$ corresponds to faster decrease in functional PS II. It is linearly
76 correlated with excess absorbed irradiance (Kato et al. 2003), that fraction of the absorbed light that is neither
77 utilized in photochemical conversion, nor dissipated in light-dependent or light-independent non-
78 photochemical quenching. Notwithstanding this linear relationship, an equally good linear correlation is
79 obtained between $k_{pi}$ and the entire fraction of absorbed irradiance not utilized in photochemical conversion

(Hendrickson et al. 2005). Either linear correlation suggests, in any case, that absorbed light energy not utilized in photochemical conversion has a likelihood of inducing photoinactivation of PS II.

Another useful piece of information can be obtained from the time course of photoinactivation of PS II after the time axis is converted to a photon exposure axis by multiplying time by the irradiance (in units of mol photons $m^{-2}$). The initial slope of the curve has units of μmol PS II photoinactivated per mol of incident photons, i.e., the maximum quantum yield of photoinactivation of PS II. The quantum yield of photoinactivation of PS II is maximum at near-zero photon exposure because all the PS II complexes are available to be photoinactivated. At higher photon exposure, PS II complexes that have already been photoinactivated absorb the light without contributing to the loss of functional PS II, so the quantum yield of PS II photoinactivation is lower (Park et al 1995b). In the extreme case where all PS II complexes are inactive, the quantum yield of photoinactivation is zero. The maximum quantum yield of photoinactivation of PS II is of the order of 1 μmol PS II photoinactivated after leaf tissue has absorbed $10^7$ photons (Park et al. 1995b), varying somewhat according to environmental conditions and plant species (Chow et al. 2005). While this quantum yield appears small, a square metre of leaf area (containing about 1 μmol PS II) may receive $>2 \times 10^7$ μmol photons during a sunny day. Therefore, the entire population of PS II may undergo photoinactivation during a sunny day, necessitating on-going repair in the light.

Therefore, for estimating the susceptibility of PS II to, or the quantum yield of, photoinactivation, an accurate description of the time course of PS II photoinactivation is important. Previous measurements by our group indicated that the decline of functional PS II may not follow a single negative exponential. i.e., photoinactivation of PS II may not be a first-order process. Instead, there appeared to be a population of PS II that was photoinactivated only slowly. To explain an apparently slowly-declining population of functional PS II, it was suggested that photoinactivated PS II complexes may help to photoprotect neighbouring, connected functional PS II complexes (Lee et al. 2001; Chow et al 2002).

On the other hand, work by the Tyystjärvi group has indicated that photoinactivation of PS II is a first-order process (Tyystjärvi et al. 1994; Tyystjärvi and Aro 1996). Further, in a recent paper from the same group, Sarvikas et al. (2010) concluded that photoinactivated PS II complexes do not protect the functional complexes. Their conclusion seems to be convincing, except for one potential complication which has not been ruled out. Their photoinactivation treatment of leaf tissue in the presence of lincomycin was followed by isolation of thylakoids for the assay of functional PS II activity by the light-saturated, uncoupled electron

transport to an exogenous PS II electron acceptor. As the photoinactivation treatment using high light progressed, increasing amounts of starch granules could be produced; if so, starch granules could disrupt the thylakoid-membrane system to increasing extents during progressive high-light treatment, such that more and more PS II (including any functional complexes) could be retained in the supernatant while thylakoid membranes were sedimented by centrifugation. This potential complication prompted us to revisit the time course of photoinactivation of PS II in leaf segments in the present study, using two assays of functional PS II other than chlorophyll florescence and oxygen yield per repetitive single-turnover flash. Furthermore, the *in vivo* measurement of the time course of photoinactivation of PS II in leaves is more important for understanding the photoinactivation mechanism(s) than in vitro, because sub-cellular fractions may have impaired regulation of thermal dissipation and electron flow from PSII (Kornyeyev, 2010). Our results appear to be consistent with the conclusion of Sarvikas et al. (2010), and do not constitute evidence in support of the hypothesis that photoinactivated PS II complexes photoprotect their functional, connected PS II neighbours. Why the decline of functional PS II did not follow a single negative exponential in our previous studies is discussed.

## Materials and Methods

### Plant materials and growth conditions

*Spinacea oleracea* L. (cv. Yates hybrid 102) and *Gossypium hirsutum* L. (cv. Deltapine 90) plants were grown in a glasshouse at 28/18ºC (day/night) under natural light. *Alocasia macrorrhiza* (L.) G. Don was grown in the same glasshouse, but under green shade cloth that transmitted only 10% of the sunlight. *Capsicum annuum* L. (cv New Town no. 3) plants were grown at 24/21ºC (day/night) with a 12-h photoperiod (300 µmol photons $m^{-2}$ $s^{-1}$). The plants were provided weekly with a nutrient solution of 'Aquasol' (Hortico Ltd, Australia), supplemented by a slow release fertilizer 'Osmocote', Scott Australia Pty Ltd, Castle Hill). The *Monstera deliciosa* plant was grown indoors in room light.

### Photoinhibition treatment of leaf segments

Prior to photoinhibition treatment, leaf discs were allowed to take up lincomycin by floating them (for durations specified in the figure legends) on a solution of 1 mM solution in a clear plastic petri dish with their abaxial side facing air in darkness. Leaf discs continued to float on 1 mM lincomycin during illumination

with high or moderately high light, as specified. Illumination with white light was provided by an array of nine light-emitting diodes (Luxeon LEDs; Electus Distribution, NSW, Australia), with the light directed vertically upwards through a clear petri dish to the adaxial side of leaf discs.

Assay of PS II activity by chlorophyll fluorescence

Leaf segments were sampled after an illumination treatment, and dark-treated for 30 min before measurement of the ratio of variable to maximum chlorophyll (Chl) fluorescence yield, $F_v/F_m$, using a Plant Efficiency Analyser (Hansatech, King's Lynn, Norfolk, UK). See Stirbet and Govindjee (2011) for a review of the Chl fluorescence transient.

Assay of PS II activity by the oxygen yield

The content of functional PS II complexes was determined in leaf segments according to Chow et al. (1989, 1991). Repetitive single-turnover xenon flashes were applied at 10 Hz. The gross rate of flash-induced oxygen evolution was determined in 1% $CO_2$ in air using an oxygen electrode (Hansatech, King's Lynn, Norfolk, UK). A slight heating artifact was taken into account. The corrected gross rate of flash-induced oxygen evolution was compared with the flash frequency to obtain the $O_2$ per flash, from which the functional PS II content was obtained by assuming that each functional PS II evolved 1 $O_2$ after four flashes. This technique is discussed by W.S. Chow et al. (this issue).

Assay of PS II activity by the quantum yield of oxygen evolution in limiting light ($QY_{O2}$)

Oxygen evolution at steady limiting light (25 μmol photons $m^{-2}$ $s^{-1}$) was determined in 1% $CO_2$ in air using an oxygen electrode (Hansatech, King's Lynn, Norfolk, UK). The gross rate of oxygen evolution was obtained by algebraically subtracting the rate in post-illumination darkness from that in the preceding limiting light. The absorbed irradiance was calculated as the product of irradiance and the leaf absorptance (assumed to be 0.85). $QY_{O2}$ was obtained as the ratio of the gross rate of oxygen evolution to the absorbed irradiance. A large leaf disc (10 $cm^2$) was illuminated by limiting white light from a projector lamp. The large leaf area gave a good signal-to-noise ratio. To further minimize the variability, each disc was re-photoinhibited and then re-measured; in this way, the $QY_{O2}$ (normalized to the starting value) could be plotted as a function of cumulative illumination time for each leaf disc. The time course for four leaf discs was then averaged. This technique is discussed by W.S. Chow et al. (this issue).

Assay of PS II activity by $P700^+$ kinetics

P700 was discovered by Kok (1957) by an absorbance decrease at 703 nm associated with P700 oxidation. Photo-oxidized P700 after a flash is re-reduced by electrons arriving from PS II. Therefore, an empirical method was used whereby PS II functionality was assayed by a $P700^+$ signal (Losciale et al. 2008). Using this approach, we illuminated a leaf segment by continuous weak far-red light (wavelength 723 nm, ~10 μmol photons $m^{-2}$ $s^{-1}$) which oxidized about 85-90% of the total P700 at steady state. The remainder P700 could be oxidized momentarily by a single-turnover flash, after which electrons from PS II tended to reduce $P700^+$, while the continuous far-red light restored the steady-state [$P700^+$]. Thus, a dip in the $P700^+$ signal occurs transiently. The area between the dipping curve and the horizontal line representing the steady-state [$P700^+$] in continuous far-red light is a simple empirical measure of the summation of electron delivered by PS II. For example, if all PS II complexes were inactive, photo-oxidized P700 would remain oxidized after a flash, and there would be no 'dip'. This technique is discussed by W.S. Chow et al. (this issue).

**Results and Discussion**

Assaying the functional PS II content by the gross $O_2$ yield per repetitive, single-turnover flash, Lee et al. (2001) reported an apparently slowly-photoinactivated PS II population during prolonged illumination in the presence of lincomycin. This was matched by a slowly-declining value of the Chl fluorescence parameter, $1/F_o - 1/F_m$. On the other hand, Sarvikas et al. (2010) reported that thylakoids isolated from photoinhibited leaves exhibited PS II activity that decreased in a strictly single-exponential fashion. Therefore, we sought other measures of PS II in leaf tissue to re-examine the time course of photoinactivation.

One such measure is $QY_{O2}$, the quantum yield of oxygen evolution under continuous limiting light in $CO_2$-enriched air (Björkman and Demmig 1987; Evans 1987). The rationale is that photoinactivated PS II complexes would still absorb light but would not perform photosynthetic $O_2$ evolution, thus lowering the quantum yield of oxygen evolution in limiting light. Indeed, Chow et al. (2002) reported that $QY_{O2}$ measured under continuous limiting light was directly proportional to the $O_2$ yield per single-turnover flash in wild-type *Arabidopsis*. An advantage of measuring $QY_{O2}$ is that a large leaf disc could be used, thereby improving the signal-to-noise ratio. Fig. 1 shows semi-log plots of the decrease of $QY_{O2}$ as a function of cumulative time of illumination (at 2000 μmol photons $m^{-2}$ $s^{-1}$) in the presence of lincomycin, in spinach, capsicum and *Alocasia* leaf discs. In all three cases, the decrease of $QY_{O2}$ deviated from a single-exponential decay, which is represented by the dashed line in each case if extrapolated from the first two time points. In the case of

*Alocasia*, leaf discs were illuminated in two ways: either the adaxial side was illuminated at 2000 μmol photons $m^{-2}$ $s^{-1}$ or both sides were illuminated simultaneously at 1000 μmol photons $m^{-2}$ $s^{-1}$ each. Illumination of both sides accelerated photoinactivation somewhat compared with illumination of one side only, but deviation from a single negative exponential occurred in both treatments.

However, measurement of gross oxygen evolution, either as $O_2$ yield per repetitive, single-turnover flash or as $QY_{O2}$ using continuous limiting light, could suffer one drawback. Both measures rely on an algebraic subtraction of the signal in darkness from that in repetitive flash illumination or continuous limiting light. If mitochondrial respiration were slower even in limiting light (as has been reported for *Eucalyptus* by Atkin et al. 2000) or during repetitive flash illumination, compared with darkness, both measures could over-estimate the functional PS II content. Indeed, the quantum yield of $O_2$ evolution in continuous limiting light, 0.106 mol $O_2$ per mol absorbed photons among diverse C3 plant species (Björkman and Demmig 1987), corresponding to 0.106 × 4 = 0.42 mol electrons per mol absorbed photons, may be too high (A. Laisk et al. this issue); the highest value measured by A. Laisk et al. was 0.33 mol electrons per mol absorbed photons, using very brief flashes. In continuous limiting light, if all PS II complexes were completely non-functional, a residual light-minus-dark signal, due to lower mitochondrial respiration in the light, could in principle persist after prolonged photoinhibitory illumination and could account for the apparent slowly-declining population of PS II.

Likewise, Chl fluorescence parameters (such as $F_v/F_m$ or $1/F_o - 1/F_m$) have their own inherent complications. The Chl fluorescence signal is detected from an initially shallow depth of leaf tissue. The depth may well increase during the time course of photoinactivation of PS II, as a greater contribution to the signal comes from deeper tissue when PS II complexes in shallower tissue become photoinactivated; in deeper tissue, photoinactivation of PS II tends to be less severe because of shading by shallower tissue (Oguchi et al. 2011). To avoid the above potential complications, we sought yet another measure of PS II activity in leaf tissue that is independent of oxygen measurements and of Chl fluorescence detection.

The $P700^+$ signal may provide such a measure. The rationale for using a PS I signal to assay PS II functionality is based on the fact that the two photosystems work in series in electron transfer. When P700 is photo-oxidized by a flash superimposed on continuous background far-red light, PS II is also excited to transfer electrons which subsequently arrive at PS I to reduce $P700^+$. The cumulative delivery of electrons from PS II to $P700^+$ depends on the content of functional PS II. Previously, we used a simple model of flash-

induced redox kinetics of P700 in continuous background far-red light to estimate the cumulative delivery of flash-induced electrons (Σ) from PS II to $P700^+$. Σ exhibited a single linear correlation with the gross $O_2$ yield per repetitive, single-turnover flash for all the plant species examined (Losciale et al. 2008), as discussed by W.S. Chow et al. (this issue). However, Σ (normalized to the total P700 signal) differed greatly from the independently-determined stoichiometry of the two photosystems. Here we used, instead of Σ, a simple area bounded by the P700 redox kinetics curve and the horizontal line corresponding to the steady-state $[P700^+]$ in continuous far-red light (shaded area in Fig. 2A) as an empirical assay of PS II content. We term this area the "P700 kinetics area". Fig. 2B shows that the P700 kinetics area was directly proportional to the gross $O_2$ yield per repetitive, single-turnover flash during progressive photoinactivation of PS II, in both capsicum and spinach. An advantage of using the P700 kinetics area is that its measurement is not time-consuming, so that many replicate leaf segments can be conveniently measured, thereby minimizing the standard error of the mean. Another advantage is that it is a whole-tissue measurement, as demonstrated by the observation that the area (after normalizing the signal to the maximum photo-oxidizable P700 signal) measured in the reflectance mode is similar when measured from the adaxial or abaxial sides (Oguchi et al. 2011).

Fig. 3 show that the P700 kinetics area declined in a single-exponential fashion with photon exposure (= irradiance × time) at two irradiances, namely, 720 and 1500 μmol photons $m^{-2}$ $s^{-1}$. The finding that the points follow one single-exponential decay in the presence of lincomycin confirms the reciprocity rule (Jones and Kok 1966; Park et al. 1995a; Nagy et al. 1995; Lee et al. 1999). If the P700 kinetics area were plotted against time for each irradiance, each treatment would exhibit a single-exponential decay with photoinhibition time. That is, there did not appear to be any population of PS II that underwent slower photoinactivation. Below ~6%, the P700 kinetics area appeared to deviate from the single-exponential decay. Perhaps a small percentage of PS II electrons could not reach $P700^+$; if so, the P700 kinetics area would tend to under-estimate the functional PS II slightly. Mostly, however, the P700 kinetics area showed a single-exponential decay of functional PS II content down to ~ 6% of the original value, akin to the conclusions of the Sarvikas et al. (2010).

Fig. 4 also depicts the decrease in the P700 kinetics area in capsium (A), cotton (B) and *Monstera deliciosa* (C) with illumination time. In all cases, the decrease followed a single negative exponential for areas ≥ 6% of the initial value. In capsicum, as in spinach, the area, when less than ~6%, was below the dashed line (Fig. 4A), indicating deviation from a single negative potential at very low functional PS II

content. Possibly, some electrons released in water oxidation in PS II did not reach $P700^+$. This could occur, for example, by charge recombination of an electron on the acceptor side of PS II with electron-deficient components on the donor side of PS II. In addition, $O_2$ may intercept the electrons on the acceptor side of PSII. In any case, a small percentage of PS II electrons could be shunted away from $P700^+$. If so, this could be a drawback of using the P700 kinetics area as an assay for a *very low* content of functional PS II.

In cotton, there was a slight lag phase, presumably because floating cotton leaf discs for only 2 h in darkness on lincomycin solution was not sufficient to completely inhibit repair of PS II during the initial period of illumination. By contrast, in *Monstera* leaf discs that had been floated on a lincomycin solution overnight in darkness, no lag phase was apparent (Fig. 4C). In *Monstera* leaf discs, interestingly, $F_v/F_m$ declined ahead of the P700 kinetics area; probably $F_v/F_m$ mainly measured PS II functionality at shallower depths of leaf tissue where photoinactivation was more severe. $F_v/F_m$ did not follow a single-exponential decay, but exhibited a slowly-decreasing component; presumably the Chl fluorescence signal was increasingly contributed from deeper tissue where photoinactivation of PS II was less severe because of shading by chloroplasts in shallower tissue (Oguchi et al. 2011).

## Conclusions

The gross $O_2$ yield per repetitive, single-turnover flash and $QY_{O2}$ in steady limiting light both tend to over-estimate functional PS II in leaf segments if mitochondrial respiration is less in the light than in darkness. Similarly, an assay based on Chl fluorescence also tends to over-estimate functional PS II, since the depth in leaf tissue from which Chl fluorescence is detected may increase during the time course of photoinactivation; in deeper tissue, PS II is photoinactivated to a lesser extent (Oguchi 2011). In these three assays of PS II functionality, therefore, deviation from a single-exponential decay of functional PS II content does not constitute evidence for photoprotection of functional PS II by photoinactivated, connected neighbours, contrary to what has been suggested earlier (Lee et al, 2001; Chow et al. 2002, 2005). Notwithstanding this correction, however, it is still a strong possibility that photoinactivated PS II complexes are strong quenchers of excitation energy within themselves, judging from their short Chl fluorescence lifetime (Matsubara and Chow 2004).

The use of the whole-tissue measurement of the P700 kinetics area appears to be a reasonable assay of functional PS II content, free from the complications outlined above for the other approaches. The P700 kinetics area decreased in a single negative exponential fashion down to ~ 6% of the initial value, despite the

possibility that a small percentage of PS II electrons may not reach $P700^+$. Therefore, our results are consistent with those of Sarvikas et al. (2010), who used thylakoids isolated from photoinhibited leaves to assay PS II functionality.

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**Figure Legends**

**Fig. 1** Semi-log plots of the decrease in the quantum yield of $O_2$ evolution measured in continuous limiting light (25 μmol $m^{-2}$ $s^{-1}$) and 1% $CO_2$ in air, as a function of cumulative time of illumination of leaf discs (10 $cm^2$) of (A) spinach, (B) capsicum and (C) *Alocasia macrorrhiza*. Leaf discs were floated on 1.0 mM lincomycin overnight before photoinhibition treatment at 2000 μmol photons $m^{-2}$ $s^{-1}$. The adaxial side of leaf discs were illuminated, except for some of the *Alocasia* leaf discs which were illuminated on both sides, each side at 1000 μmol photons $m^{-2}$ $s^{-1}$. Values are means ± S.E (n = 4). The dashed lines represent single-exponential decay if extrapolated from the first two time points. Measurements were made after the samples had been dark-treated for about 20 min following removal from the high light.

**Fig. 2** The use of a P700 kinetics area to assay functional PS II. (A). An example of the $P700^+$ kinetics signal. Continuous background far-red light oxidized about 90% of the total photo-oxidizable P700. A saturating, single-turnover flash momentarily oxidized the remainding P700, before electrons arrive from PS II after the flash, tending to reduce $P700^+$. The continuous far-red light brought the [$P700^+$] back to the steady-state level (horizontal line). The area bounded by the horizontal line and the dipping curve is termed the "P700 kinetics area", and is used as an empirical measure of the functional PS II content. (B). the P700 kinetics area is linearly correlated with the $O_2$ yield per repetitive, single turn-over flash. Functional PS II content was varied by progressive photoinactivation of PS II in leaf discs in the presence of lincomycin. Measurements were made after the samples had been dark-treated for about 30 min following removal from the high light.

**Fig. 3** Semi-log plots of the decrease in the P700 kinetics area with photon exposure (= irradiance × time), given to spinach leaf discs in the presence of lincomycin at two irradiances: 720 and 1500 μmol photons $m^{-2}$ $s^{-1}$. Spinach leaf discs were floated on 1 mM lincomycin solution overnight before the photoinhibition treatment. Values are means ± S.E. (n = 4). Measurements were made after the samples had been dark-treated for about 30 min following removal from the high light.

**Fig. 4** Semi-log plots of the decrease in the P700 kinetics area with illumination time in leaf discs of (A) capsicum, (B) cotton (n = 2), and (C) *Monstera deliciosa* (± S.E, n = 4). The irradiance was 1500 μmol photons $m^{-2}$ $s^{-1}$ in each case. Leaf discs were floated overnight on 1 mM lincomycin solution in darkness in (A), and (C), but only for 2 h in (B). Measurements were made after the samples had been dark-treated for about 30 min following removal from the high light.

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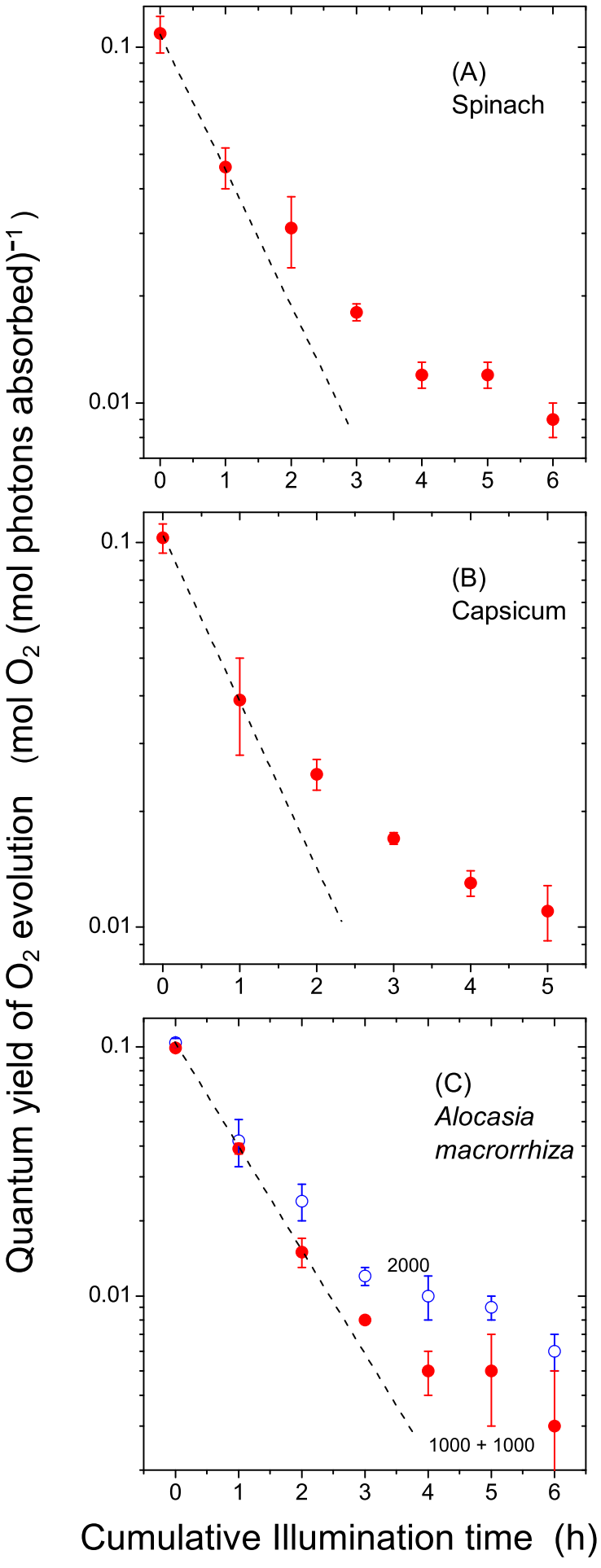

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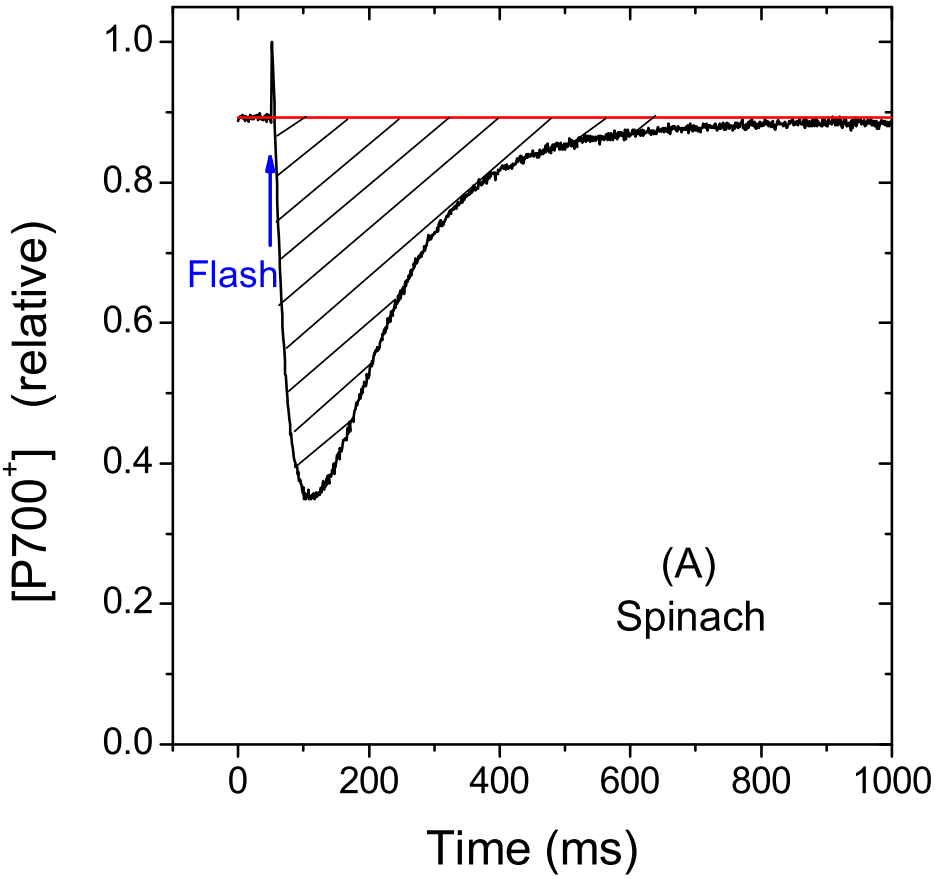


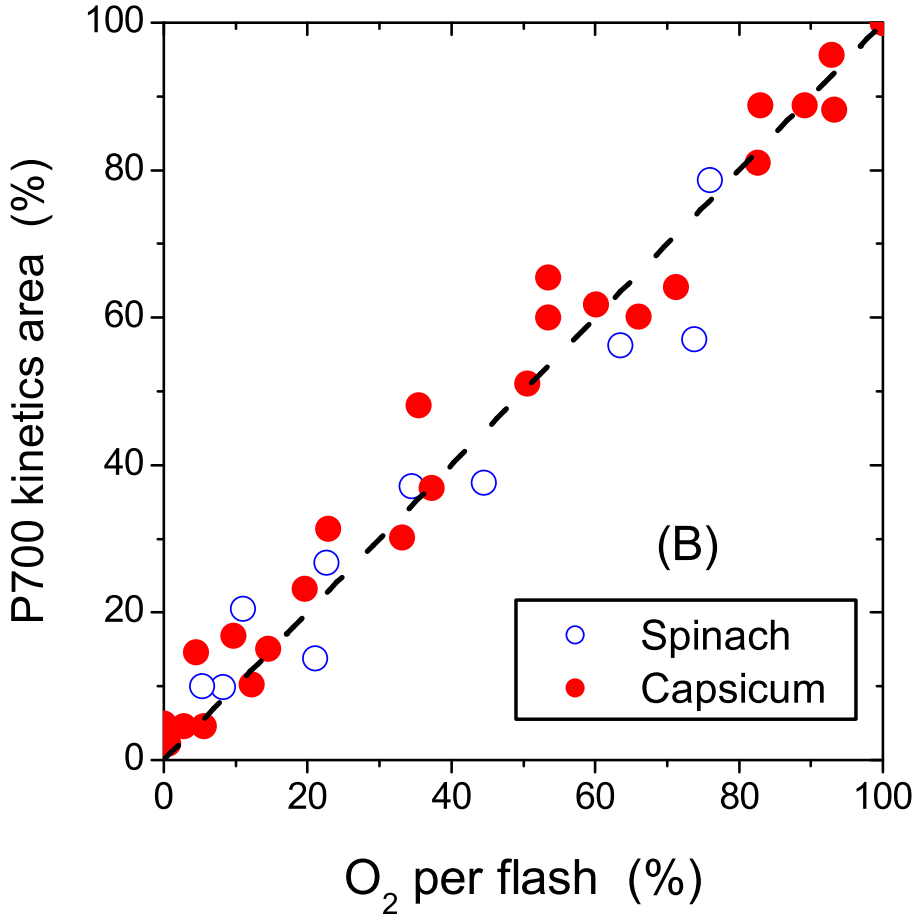

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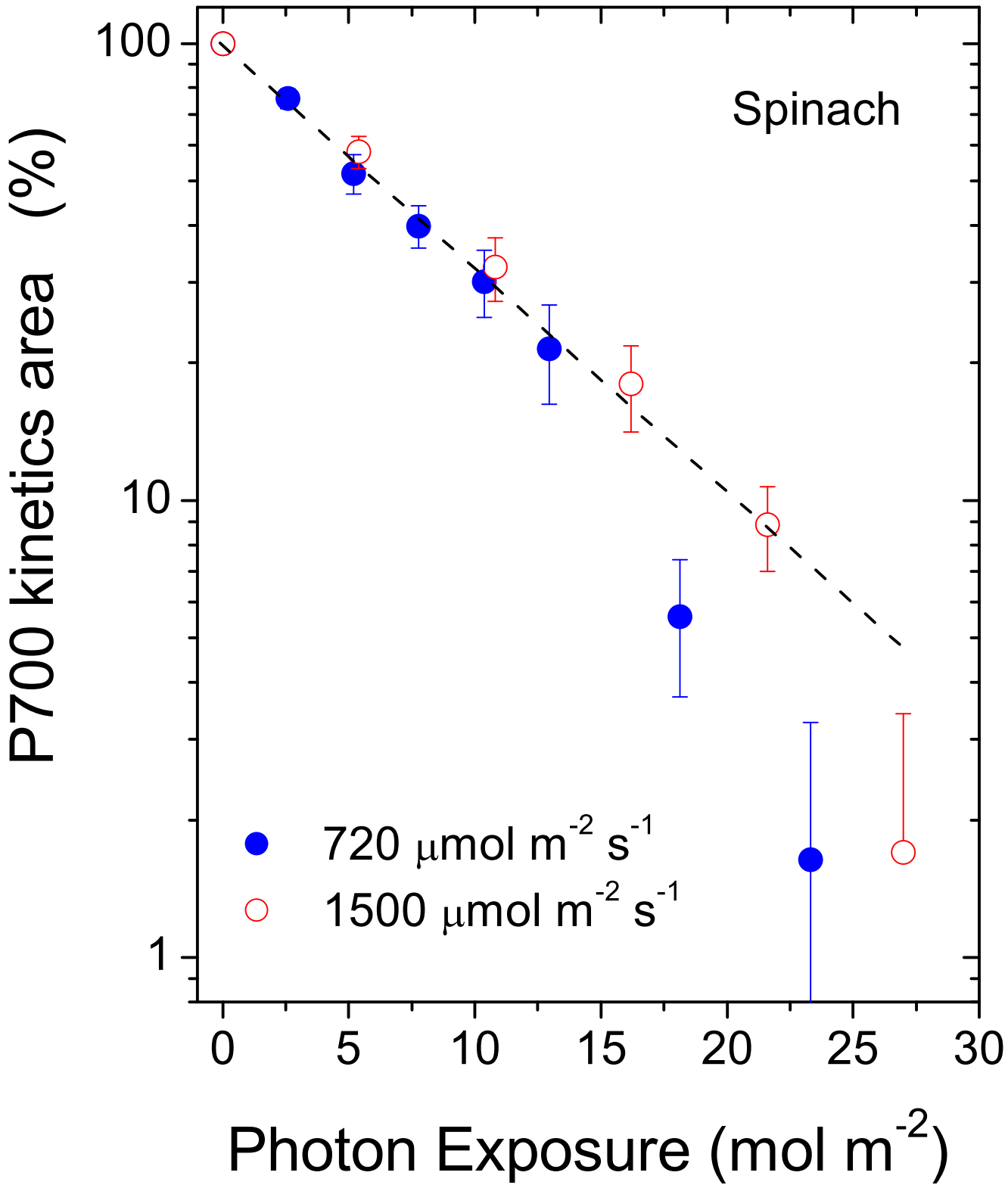

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