



Special issue in honor of Prof. Suleyman I. Allakhverdiev

REVIEW

Role of pheophytin in chlorophyll *a* fluorescence transients

G. GARAB 

*Department of Physics, Faculty of Science, University of Ostrava, Ostrava, Czech Republic
Institute of Plant Biology, HUN-REN Biological Research Centre, Szeged, Hungary*

Abstract

In this review, I revisit the groundbreaking discovery of pheophytin *a* (Pheo) in the primary charge separation of photosystem II (PSII) reaction centres (RCs) and draw attention to key observations that concern the origin of the variable chlorophyll *a* fluorescence (F_v) of PSII as well as the partitioning of the absorbed quanta between different de-excitation pathways. In particular, I recall data from the 1970s and 1980s, which have revealed photoreduction of Pheo in closed PSII (PSII_C) containing the primary quinone acceptor in reduced form, and in photoinhibited PSII with largely suppressed F_v . Taken together with recent experiments on the turnover of the $P_{680}^+Pheo^-$ radical pair and thoroughly documented large F_v values in PSII_C, these data invalidate the fundamental assumption of the so-called Q_A model of chlorophyll *a* fluorescence (ChlF): that PSII_C displays no photochemical activity, and thus the F_v/F_m ratio can be equated with the photochemical quantum efficiency of PSII (F_m , maximum fluorescence yield elicited by a saturating flash). Consequently, the co-dependent quantities – namely, the quantum efficiencies of the alternative pathways – cannot be derived from conventional ChlF measurements. It is proposed that monitoring the redox transients of Pheo and the $P_{680}^+Pheo^-$ radical pair may provide deeper insight into the photochemical activity and structural dynamics of PSII reaction centres, as well as the utilization or dissipation of absorbed excitation energy – thereby improving our understanding of the mechanisms underlying various biotic and abiotic stresses affecting PSII.

Keywords: chlorophyll *a* fluorescence induction; nonphotochemical quenching; pheophytin; photochemical quantum efficiency; photoinhibition; Q_A model.

Highlights

- Redox transients of pheophytin *a* and P_{680} play roles in chlorophyll *a* fluorescence
- The photochemical quantum yield of photosystem II containing reduced Q_A is not zero
- Quantum yield determinations of de-excitation pathways require tools beyond ChlF

Received 13 January 2026

Accepted 21 April 2026

Published online 14 May 2026

e-mail: garab.gyozo@gmail.com

Abbreviations: Chl – chlorophyll; ChlF – chlorophyll *a* fluorescence transients; DCMU – N¹-(3,4-dichlorophenyl)-N,N-dimethylurea; $F_{m/o}$ – maximum/minimum Chl *a* fluorescence yield in the dark-adapted state; F_v – variable Chl *a* fluorescence in the dark-adapted state; $F_v = F_m - F_o$; MTSF – multiple turnover saturating flash; PSII_{O/C/L} – open/closed/light-adapted state of PSII; NPQ – nonphotochemical quenching of Chl *a* fluorescence; RC – photochemical reaction centre; STSF – single-turnover saturating flash; Φ_D – quantum efficiency of the nonradiative de-excitation of the first singlet excited state of Chl *a*; Φ_F – quantum efficiency of Chl *a* fluorescence; $\Phi_{NPQ/NO}$ – quantum efficiency of the regulated/nonregulated nonphotochemical quenching of Chl *a* fluorescence; Φ_P – photochemical quantum efficiency of PSII.

Acknowledgements: I would like to express my special gratitude to two of my coworkers, Drs. Melinda Magyar and Gábor Sipka, for their key contributions in ChlF experiments and their enthusiastic and professional work; and also for providing Figures 2B (M.M.), and 1 and 3 (G.S.). I am indebted to Prof. Suleyman I. Allakhverdiev for his insightful discussions on this topic. I also extend my gratitude to Prof. Barry D. Bruce, guest editor of this Special Issue, and to the three anonymous reviewers of *Photosynthetica* for their critical evaluation of the manuscript and their valuable advice. This work was supported by the Grantová Agentura České Republiky (23-07744S to G.G.), and the European Union under the LERCO project (CZ.10.03.01/00/22_003/0000003) via the Operational Programme Just Transition.

Conflict of interest: The authors declare that they have no conflict of interest.

Introduction

Photosystem II (PSII) is a large multi-subunit pigment–protein complex embedded in the thylakoid membranes of oxygenic photosynthetic organisms. This enzyme, the water-plastoquinone oxidoreductase, is the ultimate source of virtually all reducing equivalents in the biosphere and is therefore often referred to as the engine of life (Barber 2004). Today, the basic photophysical and photochemical functions of its reaction centre complexes (RCs) are reasonably well understood (Shen 2015, Romero *et al.* 2017, Shevela *et al.* 2023, Hussein *et al.* 2024), thanks to decades of extensive research and key discoveries in numerous laboratories worldwide. One of the fundamental discoveries concerning the molecular architecture of the RC of PSII and its primary photochemistry has been the identification of pheophytin *a* (Pheo) as the primary electron acceptor of PSII (Klimov *et al.* 1977).

Fig. 1A shows that the electron transfer of PSII proceeds on the active branch of the RC, involving Pheo_{D1}. It also depicts the participation of the redox-active Tyr_Z, and the Mn₄CaO₅ cluster of the oxygen-evolving complex (OEC) on the donor side, and the non-heme iron (Fe), and the second, exchangeable plastoquinone molecule (Q_B) on the acceptor side. Fig. 1B displays the initial steps and approximate half-time values of the forward and backward electron transfer reactions.

As pointed out by Bruce *et al.* (2026), the discovery of Pheo as an active component in PSII RC, and its basic characterization (Klimov *et al.* 1977, 1979, 1980) were not accepted immediately by the community of photosynthesis researchers. Indeed, the 1980 *Annual Review of Plant Physiology* still used the conventionally accepted view on PSII photochemistry: “In the primary reaction of PSII, the reaction centre chlorophyll “P₆₈₀” donates an electron to the primary acceptor “Q”” (Velthuys 1980). However, a year later, Vermaas and Govindjee (1981) already pointed out that “There is an overwhelming evidence that Pheo is an intermediate in electron transport from the reaction

centre P₆₈₀ to the quinone Q”. (Here, Q stands for Q_A, the first, stable plastoquinone acceptor of PSII.)

Surprisingly, similar corrections in the field of chlorophyll *a* (Chl *a*) fluorescence induction and Chl *a* fluorescence transitions (ChlF), in general, despite some attempts (to be discussed in later paragraphs), had not been implemented for decades. This is especially remarkable given that, since the 1960s, ChlF has arguably been the most widely used technique for monitoring the functional activity of PSII and the fate of excitation energy. In fact, the “mainstream”, so-called Q_A model of ChlF has remained in use and was essentially unchanged since its foundational principles were established in the 1960s and 1970s – evidently, because of the immense successes of ChlF in numerous important discoveries. However, it must also be noted that the Q_A model has never been free of controversy, which, at least in part, can be attributed to the fact that the fundamentals of this model were established before the discovery of Pheo in PSII RCs. The controversial features of the Q_A model are often overlooked in primary photosynthesis texts, which implicitly or explicitly use the simplified photochemical model of Butler (1978) – as *e.g.*, in recent reviews of Calzadilla *et al.* (2025), van Amerongen and Croce (2025), Zuo (2025), and Konyukhov *et al.* (2026).

In this paper, I will provide a brief overview of the main characteristics of ChlF, the variable Chl *a* fluorescence (*F_v*), in particular. This will be followed by a section describing the fundamentals of the Q_A model – along with a list of substantial controversies. Separate sections will be dedicated to the sizeable photochemical activity of Pheo (and the turnover of the P₆₈₀⁺Pheo^{•−} radical pair) in closed RCs (PSII_C), containing Q_A[−] – findings that cannot be reconciled with the Q_A model. It will be demonstrated in the Appendix that the commonly employed equations, using the rate constants of competing processes for the quanta absorbed by PSII, must be complemented by taking into account the non-zero photochemical rates in PSII_C as well as in PSII_{NPQ} (in the presence of NPQ,

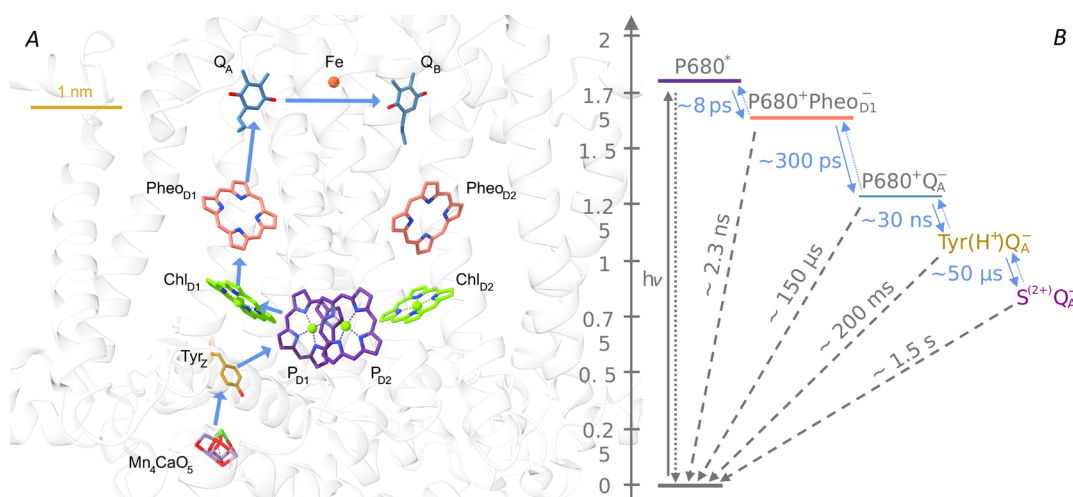


Fig. 1. Cofactor structure (A) and the route of charge separation and approximate electron transfer and back reaction time constants (B) in PSII RC of *Thermosynechococcus* (today, *Thermotichus*) *vulcanus* (pdb:5GTH). The figure is reproduced from Garab *et al.* (2023).

the regulated nonphotochemical quenching of Chl *a* fluorescence). The review will be closed with a short Conclusion – emphasizing that recording the redox transients of Pheo and/or of the $P_{680}^+Pheo^-$ radical pair, in combination with ChlF measurements, will most certainly aid in better understanding the structural and functional complexity of PSII RCs.

Basic observations of Chl *a* fluorescence induction kinetics

For brevity and to focus on the basic transients, I restrict the illustration of ChlF in dark-adapted samples to the fast rise kinetics between the minimum level F_0 (or O) and the maximum level, F_m (or P), and to the variable Chl *a* fluorescence $F_v = F_m - F_0$. (For slower transients and different experimental conditions, as well as for the case of NPQ, see e.g., Papageorgiou and Govindjee 2004, Tyystjärvi *et al.* 1999, Bernát *et al.* 2018, van Amerongen and Croce 2025.)

As shown in Fig. 2A, upon applying a long rectangular-profile flash excitation on a dark-adapted leaf of a vascular plant in the presence of DCMU, the fluorescence rapidly rises to its maximum level. This inhibitor, by blocking the electron transport between Q_A and Q_B , allows only one stable charge separation [DCMU, N' -(3,4-dichlorophenyl)- N,N -dimethylurea]. In thylakoid membranes with active whole-chain electron transport, the induction starts with a fast (~ 2 ms) O -to- J rise, which is traditionally termed the photochemical phase. This transition is followed by much slower J - I and I - P steps, which constitute the so-called thermal phase (Morin 1964, Delosme 1967, Stirbet and Govindjee 2012). Fig. 2B demonstrates that reaching F_m in the presence of DCMU requires several

single-turnover saturating flashes (STSFs) (cf. Joliot and Joliot 1979); further, it demonstrates that the STSF-induced fluorescence increments are essentially insensitive to doubling the flash intensity by using two synchronously fired STSFs (Garab 2024).

Fundamentals and controversial features of the Q_A model

Briefly, the Q_A model of ChlF of PSII rests on two pillars: (i) the RCs exist either in open ($PSII_O$) or in closed ($PSII_C$) states, displaying F_0 and F_m levels, respectively; and (ii) the quantum yield of PSII photochemistry of a dark-adapted sample is $\Phi_P = F_v/F_m$.

It was posited by Duysens and Sweers (1963) that the “RCs of PSII exist in two states, quenched and unquenched, containing oxidized and reduced quenchers, Q and QH ”. (In 1963, the nature of Q , its identity as Q_A , as the first, stable quinone acceptor of PSII, was not known.) These authors explained F_v as follows: “The excitation energy [...] is trapped if the centre is in oxidized state” but the “reduced reaction centre, QH , does not trap the excitation energy and thus does not quench the fluorescence”. Accordingly, “in order to reach F_m , it is necessary, and sufficient, to have Q_A completely reduced in all the active PSII centres” (Stirbet and Govindjee 2012).

The concept of Duysens and Sweers was further developed by Butler (1978), who performed experiments on isolated thylakoid membranes (TMs) at 77 K in the presence of an externally added quencher molecule (dibromothymoquinone) (Kitajima and Butler 1975) and employed a “simple photochemical model (a monomeric Chl *a* molecule) in which photochemistry (P), fluorescence (F), and nonradiative decay (D) all compete for the excitation energy in the antenna chlorophyll of PSII

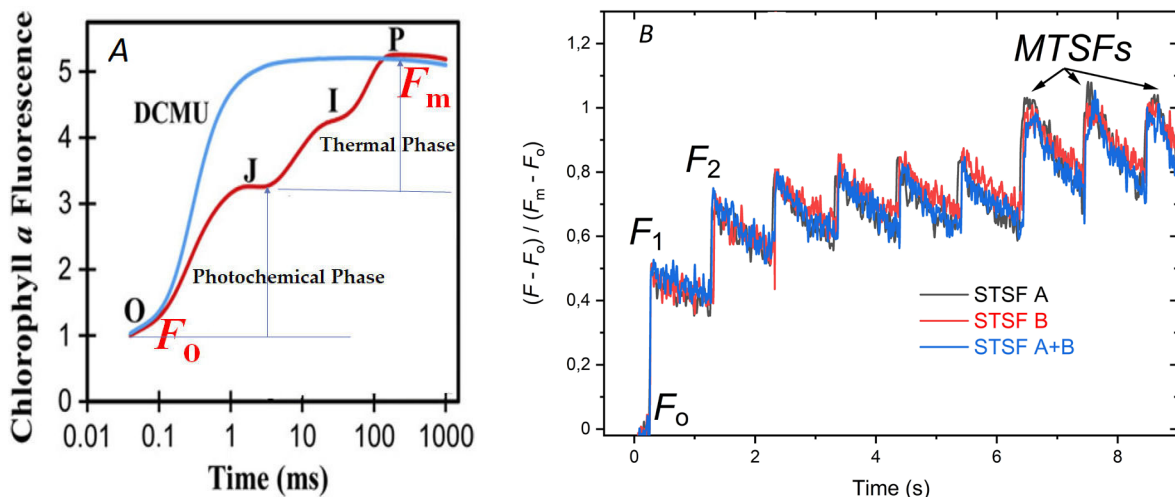


Fig. 2. Chl *a* fluorescence induction kinetics of dark-adapted samples, induced by intense rectangular-profile excitations (A) or by trains of single-turnover saturating flashes (STSFs), followed by multiple-turnover (MT) SFs (B). Note that the F_v/F_m ratios in these experiments were very similar, ≥ 0.8 . Panel A is modified from Stirbet and Govindjee (2011) with permission from Elsevier; it displays typical Chl *a* fluorescence transients at room temperature of an untreated and a DCMU-treated pea leaf. Panel B is reproduced from Garab (2024); it shows ChlF transients at 5°C of isolated DCMU-treated PSII core complexes (CCs) of *Thermosynechococcus vulcanus*. STSF A and STSF B indicate that the corresponding traces were elicited with the Xe flashes A and B, respectively. These flashes possess identical technical parameters. The label STSF A+B indicates that the given trace was recorded by synchronously firing the two flashes, A and B.

by first order rate processes”, characterized by the rate constants k_p , k_F , and k_D , respectively. Further, “photons absorbed by a photosynthetic organism have three fates” and “the sum of the quantum yields of each pathway has to be 1.00”. Hence, with open RC, the Φ_p yield of photochemistry is maximal: $\Phi_p = k_p/(k_F + k_p + k_D)$ and the fluorescence yield is $F_o = k_F/(k_F + k_p + k_D)$. “However, when the reaction centres are closed because the primary, electron acceptors are reduced, Φ_p goes to zero and the yield of fluorescence increases to a maximum”: hence, with $k_p = 0$, $F_m = k_F/(k_F + k_D)$; here D is used to include all other de-excitation routes. Using these equations, the quantum yield of photochemistry can readily be obtained as $\Phi_p = F_v/F_m$. For details of deriving this equation, see Blankenship (2021) and van Amerongen and Croce (2025). The Appendix reproduces this derivation but emphasizes that the turnover of Pheo and that of the primary radical pair, $P_{680}^+Pheo^-$, must be taken into account, because the assumption of $\Phi_p = 0$ ($k_p = 0$) does not hold.

The Q_A model became canonical in photosynthesis research and has been widely adopted in primary literature, reviews, and textbooks, and served the research on the mechanisms of photosynthesis (Genty *et al.* 1989, Vavilin *et al.* 1999, Papageorgiou and Govindjee 2004, Strasser *et al.* 2004, Farage *et al.* 2006, Baker 2008, Murchie and Lawson 2013, Porcar-Castell *et al.* 2014, Santabarbara *et al.* 2019, Taylor *et al.* 2019, Blankenship 2021, Gorbunov and Falkowski 2022). This widespread acceptance can largely be attributed to numerous observations showing that a high F_v/F_m ratio serves as a hallmark of healthy, fully functional PSII, whereas reduced values, generally, are indicative of photoinhibited or damaged RC. However, these interpretations have been challenged by concerns regarding a “lack of an adequate theoretical basis” (Holzwarth 1993, see also Trissl *et al.* 1993, Trissl 1994), as well as by the development of alternative or complementary models (Vredenberg 2008, 2011; Schansker *et al.* 2011), and the identification of multiple unresolved questions (Kalaji *et al.* 2014). Also, notable exceptions indicated that the F_v/F_m ratio does not necessarily correlate with PSII activity (Vavilin *et al.* 1999, Treves *et al.* 2016). Recent data have led to the conclusion that the interpretation of ChlF must be placed on new conceptual foundations (Garab *et al.* 2023, Garab 2025). Despite its known limitations and unresolved controversies, the F_v/F_m ratio continues to be widely – and frequently uncritically – used in the literature as a measure of the photochemical quantum efficiency of PSII (e.g., Zuo 2025, Chang *et al.* 2025, Huang *et al.* 2025, van Amerongen and Croce 2025). Most recently, Konyukhov *et al.* (2026) analyzed the “weak points” of the determination of the F_v/F_m ratio – focusing mostly on technical aspects, such as fluorescence spectroscopy, short vs. long flash excitation, and time resolution, and pitfalls related to the complexity of the biological material, including PSI activity, state transitions, and chlororespiration. Regrettably, many long-standing fundamental controversies and recent experimental

findings escaped the attention of these authors. This justifies revisiting the main controversial aspects of the Q_A model and advancing additional arguments.

(i) During the so-called thermal phase (see Fig. 2A), all RCs experience multiple excitations (Morin 1964, Delosme 1967, see also Neubauer and Schreiber 1987, Lazár and Pospíšil 1999), which are nevertheless unable to generate P.

(ii) The first STSF, closing all PSII RCs, was shown to induce F_1 ($<F_m$) and additional excitations were required to reach F_m (Joliot and Joliot 1979, Magyar *et al.* 2018, cf. also Laisk and Oja 2020) (see Fig. 2B). It has also been shown by Kolber *et al.* (1998) and Vredenberg *et al.* (2006) that to reach F_m , multiple single-turnover flashes are needed.

(iii) In addition, it has been discovered that, for completing each of the F_1 -to- F_2 -to- F_3 ... F_m increments, sufficiently long $\Delta\tau$ waiting times are required between consecutive STSFs (Magyar *et al.* 2018). Note that the existence of $\Delta\tau$ waiting times and the required multiple excitations explains the sigmoidicity of the O-to-P rise, induced by a long flash in the presence of DCMU. This has been proposed to reflect the composite character of the rise, containing more than one exponential component (Vredenberg 2011). The sigmoidicity is retained in monomeric and dimeric PSII core complexes (Sipka *et al.* 2021), ruling out its origin in energetic connectivity between PSII units, as explained in the Q_A model (Stirbet and Govindjee 2012).

(iv) The F_v/F_m ratio was found to be largely independent of the intensity of the excitation – using single or double STSFs (Fig. 2B) or varying the photon flux density of the long flashes between 0.9 and 15 mmol(photon) $m^{-2} s^{-1}$ (Schansker *et al.* 2011). For similar earlier observations, see Kolber *et al.* (1998).

(v) PSII_C is capable of gradually converting into the light-adapted charge-separated state (PSII_L) with improved stabilization of the charges; in fact, repetitive excitations have been shown to generate multiple intermediate states between PSII_C and PSII_L (Sipka *et al.* 2021).

(vi) The induction kinetics of DCMU-treated PSII core complex (CC) have been shown to depend strongly on the temperature in the cryogenic range (Magyar *et al.* 2018, Sipka *et al.* 2021) – in sharp contrast to the photoreduction of Q_A , which is an activationless process that is accelerated below 100 K (Kirmaier *et al.* 1985).

(vii) It has also been shown that the magnitude of F_1 and the amplitude of the initial phase of the O-to-P rise depend sharply on the pH between 6.5 and 7.0 (Magyar *et al.* 2025), in contrast to the photoreduction of Q_A , which is pH independent between 6.0 and 8.0 (Demeter *et al.* 1985). In broad terms, these data are in harmony with earlier hypotheses on donor-side effects (Vredenberg 2008, Klughammer *et al.* 2024) and on the dependence of fluorescence yield on the S-states of the OEC (Delosme and Joliot 2002, Ananyev and Dismukes 2005). Period-of-four oscillation of the fluorescence level,

associated with the activity of OEC, was also reported by Vredenberg *et al.* (2007).

(viii) As testified by experimental data at cryogenic temperatures, the direct contribution of the photoreduction of Q_A to F_v is small ($\leq 20\%$) (Schansker *et al.* 2011, Sipka *et al.* 2021, Magyar *et al.* 2025).

(ix) The O-to-P rise kinetic traces of DCMU-poisoned pea leaves between 0 and -100°C contain an essentially activationless fast kinetic component, being responsible for 19.6% of the rise, and two slower components with 13 and 16 kJ mol^{-1} activation energies (Schansker *et al.* 2011). Similar activation energies, 13.8 ± 0.8 and $11.3 \pm 3.8 \text{ kJ mol}^{-1}$, were obtained from F_m decay kinetics in spinach TMs and PSII CC of *T. vulcanus*, respectively, in cryogenic temperature ranges preventing the reoxidation of Q_A^- (Magyar *et al.* 2018).

Three further fundamental points, linked to the photochemical activity of Pheo in PSII, will be discussed in the section below.

Role of redox transients of Pheo in F_v

The following data, obtained in the Institute of Photosynthesis (Pushchino, USSR), the Bakh Institute of Biochemistry (Moscow, USSR), the Department of Biophysics (Moscow State University, Moscow, USSR), and the Institute of Microbiology (Třeboň, Czechoslovakia), are of pivotal relevance to the origin of F_v of PSII:

(i) Reversible photoreduction of Pheo was observed in pea subchloroplast particles at redox potentials between -50 mV and -550 mV , when Q_A was in the reduced form (Klimov *et al.* 1977). The redox potential of Pheo was determined first to be -610 mV on PSII particles, which were not capable of splitting water (Klimov *et al.* 1979). It should be noted here that, subsequently, Allakhverdiev *et al.* (2010) determined the redox potential in two cyanobacteria with different special-pair Chls. In *Synechocystis* sp. PCC 6803 and *Acaryochloris marina* MBIC 11017, whose special pairs consist of Chl *a* and Chl *d*, respectively, and obtained midpoint redox potential (E_m) values of $-536 \pm 8 \text{ mV}$ and $-478 \pm 24 \text{ mV}$. Further, measurements of the redox potentials of Q_A in the two cyanobacteria and in PSII with and without active OEC showed some variations in the energetics of PSII, but, in general, it was conserved, even though the redox potentials of Q_A and Pheo were sensitive to the molecular architecture and functional activity of PSII (Allakhverdiev *et al.* 2011).

(ii) Analyses of the temperature dependences, in the range from -30 to -100°C , of key fluorescence parameters of isolated PSII particles and chloroplasts revealed the involvement of sizeable activation energies (Klimov *et al.* 1978).

(iii) It has been shown by Allakhverdiev *et al.* (1987) that different photoinhibitory treatments (under aerobic, anaerobic, and strongly reducing conditions), which all suppress F_v and the PSII electron transport from H_2O to *p*-benzoquinone, do not impair the primary charge

separation, *i.e.*, the formation of the $\text{P}_{680}^+\text{Pheo}^-$ primary radical pair.

Evidently, the photochemical activity of Pheo in the presence of Q_A^- cannot be reconciled with the Q_A model, according to which “reduced reaction centre, [containing] QH, does not trap the excitation energy”. Also, according to the Q_A model, the diminished F_v in photoinhibited PSII should indicate diminished photochemical rates. However, the persisting redox transients of Pheo indicated high rates of primary photochemistry in PSII. (Note that the main tenets of the Q_A model were laid down before the discovery of Pheo as the primary electron acceptor in PSII RC.)

To correlate the photochemical activity of PSII with F_v , Klimov *et al.* (1977) proposed that F_v arises from the recombination of the $\text{P}_{680}^+\text{Pheo}^-$ radical pair, which possesses a 2–4-ns lifetime. However, more detailed time-resolved spectroscopic investigations have shown that “the long-lived, nanosecond chlorophyll fluorescence is not charge-recombination luminescence but rather emission from equilibrated excited states of antenna chlorophylls” (Schatz *et al.* 1987). For further discussion, see also Dau (1994). An alternative model, taking into account the involvement of Pheo in F_v , was proposed by Vredenberg *et al.* (2009). These authors observed that the F_v/F_m value, induced by the first 35- μs long saturating flash, was 0.67, and the maximum, 0.8, could only be achieved by the second flash. These two F_v/F_m values were proposed to originate from single- and double-reduced Q_A , more precisely from $[\text{Pheo}Q_A]^-$ and $[\text{Pheo}Q_A]^{2-}$, respectively, in the Q_B -nonreducing RCs. To my knowledge, this hypothesis has not been substantiated. Also, it is difficult to reconcile it with more recent data, such as the multistep fluorescence increments, especially at low temperature, the rapid recombination of the primary radical pair in PSII_C, as well as the pH and temperature dependences of the main parameters of ChlF (see above).

At this point, it is also important to note that, in contrast to photoinhibition, heat-induced inactivation of PSII does lead to the loss of the primary charge separation, approximately parallel with the diminishment of the F_v/F_m ratio (Allakhverdiev *et al.* 1996). Hence, the same diminishing F_v/F_m ratios may be associated with very different photochemical rate constants. Thus, ChlF measurements *per se* may not provide correct information on the fate of absorbed quanta, and on the partitioning of the excitation energy between different pathways. Nevertheless, variations in the magnitude of the F_v/F_m ratio have important diagnostic value for the functional activity and structural dynamics of PSII. Note also that, as emphasized earlier (Garab 2024), because $\Phi_P \neq F_v/F_m$, the co-dependent quantity, the quantum efficiency of the so-called constitutive nonphotochemical quenching, Φ_{NO} , also loses its physical meaning (see Appendix). Correct interpretation of ChlF parameters requires careful assessment of all processes and complementary data. Also, multiple fluorescence levels between F_1 and F_m indicate multiple intermediate states between PSII_C and PSII_L, which must be taken into consideration. The accurate

determination of Φ_P , Φ_{NPQ} , and Φ_{NO} usually also requires knowledge of all the rate constants (see Appendix).

Regarding the physical mechanism(s) underlying the ChlF transients, it has been proposed that the gradual light-induced PSII_C-to-PSII_L transition, and the multistep F_I-to-F_m rise, originates from subtle conformational changes (most likely) on the donor side of the RC matrix, and dielectric relaxation processes driven by a combination of stationary and transient electric fields, due to the presence of Q_A⁻ and the rapidly recombining P₆₈₀⁺Pheo⁻ radical pair, respectively (Sipka *et al.* 2021, 2022; Magyar *et al.* 2025).

With regards to the more complex, O–J–I–P transients, additional mechanisms and processes must be taken into consideration (Garab 2025). It has earlier been established that the activity of photosystem I (Schansker *et al.* 2005), the occupancy of the Q_B binding site (Prášil *et al.* 2018), and donor-side effects (see above) play important roles. In addition, competition between the rise and decay of the Chl *a* fluorescence, governed, respectively, by the $\Delta\tau$ waiting times and the Q_A⁻ to Q_B electron transfer, must be taken into account.

Turnover rates of PSII photochemistry; rate constants associated with F_o and F_m

Since, as shown above, the photochemical quantum yield of closed PSII RCs does not drop to zero, *i.e.*, $k_P > 0$, it is of interest to estimate the “true” value of Φ_P

in different PSII states. This can, in principle, be done by measuring the turnover rate of the P₆₈₀⁺Pheo⁻ radical pair, which can be monitored by recording the decay kinetics of the absorbance transients at around 820 nm (see Fig. 1B). These transients on the nanoseconds-to-microseconds timescale reflect the decay of P₆₈₀⁺; the electron transfer between Pheo⁻ and Q_A occurs on the sub-ns scale.

Sipka *et al.* (2019) performed 819-nm absorbance transient (ΔA_{819}) measurements on dark-adapted DCMU-treated PSII CC upon single and multiple flash excitations (Fig. 3). In good agreement with the expectations, it was confirmed that only the first STSF is capable of generating stable charge separation. In contrast to the relatively slow decay of ΔA_{819} after the first flash (Fig. 3A), upon the second flash (Fig. 3B) or repetitive excitations, ΔA_{819} decayed with lifetimes of about 2 ns (~60%) and 10 ns (~30%), attributed to recombination of the primary radical pair.

These experiments have shown that the recovery of ΔA_{819} is relatively fast, ~75% and ~90% at 5 and 100 μ s, respectively (Fig. 3B inset). Conversely, the photochemically inactive time intervals of PSII_C are relatively short when compared to the average rate of PSII excitation and the re-opening of the RC, *via* the Q_A⁻ to Q_B electron transfer, which are in the range of several hundred microseconds. Hence, under realistic conditions, k_P and the photochemical quantum efficiency of PSII_C, albeit decreased, remain comparable to the dark-state values. According to Ostroumov *et al.* (2014), the quantum

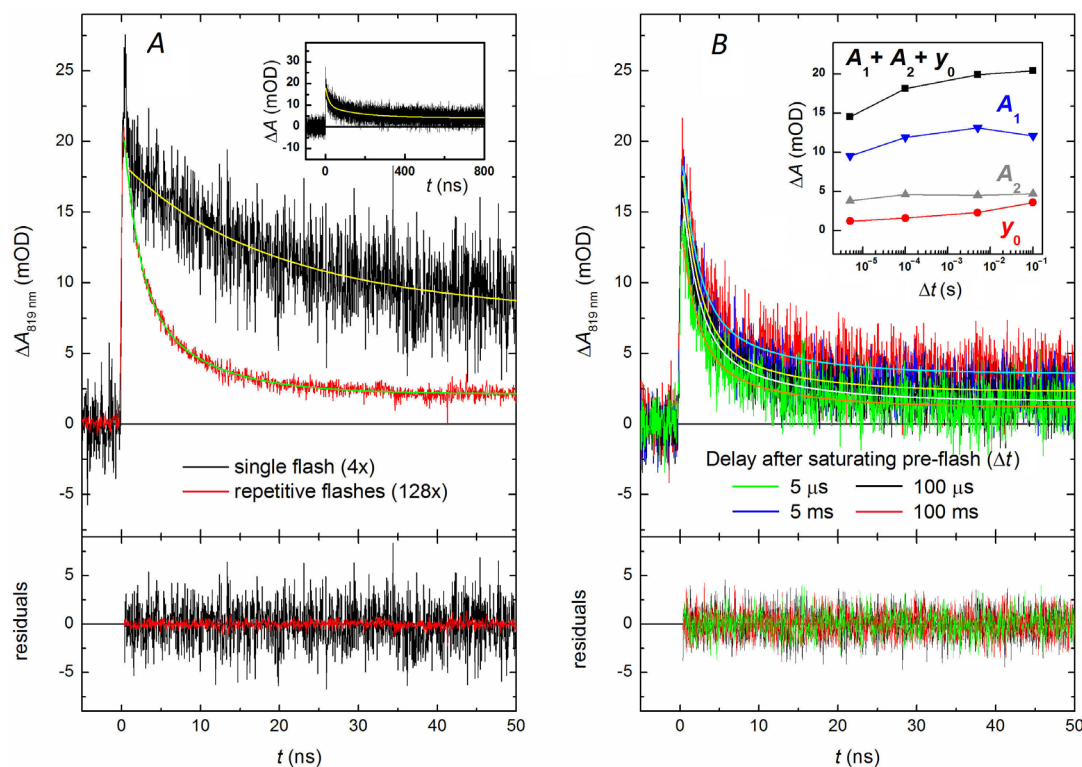


Fig. 3. Traces and parameters of ΔA_{819} in DCMU-treated, dark-adapted PSII CC from *Thermosynechococcus vulcanus*. (A) Single flash excitation and repetitive (2 s⁻¹) flash excitations. (B) Traces recorded upon a second flash excitation, which followed a saturating (pre-)flash after different time intervals, as indicated; the smooth lines represent global biexponential fits of the curves; inset: dependence of the fitted amplitudes on the delay between the first (pre-)flash and the actinic flash. The figure is reproduced from Sipka *et al.* (2019).

efficiency of PSII upon closing the RC decreases from 89 to 76%. Note that these authors, similar to Butler (1978), define k_p as the rate constant that is associated with the primary photochemical charge separation, as opposed to the dissipative pathways, k_{IC} (internal conversion) and k_{ISC} (intersystem crossing), and k_F (see also Appendix). When starting with active dark-adapted PSII, the charges can be stabilized by secondary electron transfer steps, and may eventually lead to oxygen evolution, the production of NADPH, and ATP synthesis; alternatively, in the presence of reduced Q_A , the charges will recombine (Fig. 3). Evidently, to monitor the fate of the photochemically produced charges, additional rate constants must be determined, which requires techniques independent of conventional ChLF measurements.

By taking into account the high turnover rate of the primary charge separation, the F_v/F_m ratio cannot be equated with the quantum efficiency of PSII. It must also be noted, however, that in our earlier work (Garab *et al.* 2023), this key argument, *i.e.*, that $\Phi_p > 0$ and $k_p > 0$, was not used. Earlier, it was pointed out that the implicit assumptions of Butler (1978) – that “none of the rate constants change as the traps go from open to closed, and that all the fluorescence that is observed in both the F_o and F_m states comes from a homogeneous system in which all chlorophyll excited states are equivalent” (quoted from Blankenship 2021) – do not hold true. Thus, it was argued that $F_o = k_{Fo}/(k_{Fo} + k_{p_o} + k_{D_o})$ should be compared with $F_m = k_{Fm}/(k_{Fm} + k_{D_m})$. However, Garab *et al.* (2023) overlooked the facts that PSII_C largely retains its primary photochemical activity (Klimov *et al.* 1977, 1979; Allakhverdiev *et al.* 1987, Ostroumov *et al.* 2014, Sipka *et al.* 2019, Garab 2025); *i.e.*, $k_{p_m} \neq 0$. Thus, the correct expressions for F_m and F_i are as follows: $F_m = k_{Fm}/(k_{Fm} + k_{p_m} + k_{D_m})$ and $F_i = k_{Fi}/(k_{Fi} + k_{p_i} + k_{D_i})$, where $i = 1, 2, 3, \dots$ stands for the i -th intermediate between F_o and F_m . (For the derivation of these equations and further comments, see Appendix.) Evidently, neither the F_v/F_m ratio nor any F_i/F_o -type ratio provides a valid measure of the photochemical quantum yield of PSII or of co-dependent quantities such as Φ_{NO} (*cf.* Garab 2024).

Conclusion

With this short review, I wish – belatedly and with sincere apologies – to give credit to Slava Klimov, Suleyman Allakhverdiev, and their colleagues for their demonstration of redox turnover of Pheo (and P_{680}) in the presence of Q_A^- and in photoinhibited PSII.

Data showing that PSII_C possesses nonzero photochemical activity invalidate the Q_A model and demonstrate that PSII quantum efficiency parameters conventionally derived from ChLF measurements are not physically valid.

Finally, I would like to stress that monitoring the redox transients of Pheo and the formation and decay of the $P_{680}^+Pheo^-$ radical pair, in combination with ChLF measurements and other complementary tools, may lead to a deeper understanding of the photophysics, photochemistry, and structural dynamics of PSII under different, physiologically important conditions.

References

- Allakhverdiev S.I., Feyziev Y.M., Ahmed A. *et al.*: Stabilization of oxygen evolution and primary electron transport reactions in photosystem II against heat stress with glycinebetaine and sucrose. – *J. Photoch. Photobio. B* **34**: 149-157, 1996.
- Allakhverdiev S.I., Šetlíková E., Klimov V.V., Šetlík I.: In photoinhibited photosystem II particles pheophytin photoreduction remains unimpaired. – *FEBS Lett.* **226**: 186-190, 1987.
- Allakhverdiev S.I., Tomo T., Shimada Y. *et al.*: Redox potential of pheophytin *a* in photosystem II of two cyanobacteria having the different special pair chlorophylls. – *PNAS* **107**: 3924-3929, 2010.
- Allakhverdiev S.I., Tsuchiya T., Watabe K. *et al.*: Redox potentials of primary electron acceptor quinone molecule (Q_A^-) and conserved energetics of photosystem II in cyanobacteria with chlorophyll *a* and chlorophyll *d*. – *PNAS* **108**: 8054-8058, 2011.
- Ananyev G., Dismukes G.C.: How fast can Photosystem II split water? Kinetic performance at high and low frequencies. – *Photosynth. Res.* **84**: 355-365, 2005.
- Baker N.R.: Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. – *Annu. Rev. Plant Biol.* **59**: 89-113, 2008.
- Barber J.: Engine of life and big bang of evolution: a personal perspective. – *Photosynth. Res.* **80**: 137-155, 2004.
- Bernát G, Steinbach G., Kaňa R. *et al.*: On the origin of the slow M–T chlorophyll *a* fluorescence decline in cyanobacteria: interplay of short-term light-responses. – *Photosynth. Res.* **136**: 183-198, 2018.
- Bhattacharjee S., Neese F., Pantazis D.A.: Triplet states in the reaction center of Photosystem II. – *Chem. Sci.* **14**: 9503-9516, 2023.
- Blankenship R.E.: *Molecular Mechanisms of Photosynthesis*. 3rd Edition. Pp. 352. Wiley, Chichester 2021.
- Bruce B.D., Tatsuya T., Govindjee G. *et al.*: A life in light: celebrating the 75th birthday of Professor Suleyman I. Allakhverdiev. – *Photosynthetica* **64**, 2026.
- Butler W.L.: Energy distribution in photochemical apparatus of photosynthesis. – *Annu. Rev. Plant Physiol.* **29**: 345-378, 1978.
- Calzadilla P.I., Krieger-Liszka A., Johnson G.N.: Chlorophyll fluorescence and photosynthesis. – In: Lagario M.G. (Ed.): *Fluorescence of Living Plants*. Comprehensive Series in Photochemical and Photobiological Sciences. Vol. 1. Pp. 19-37. Springer, Cham 2025.
- Chang K.W., Tang H., Fu L.J. *et al.*: Recent advances in plant stress analysis using chlorophyll *a* fluorescence. – *Photosynthetica* **63**: 359-373, 2025.
- Dau H.: Molecular mechanisms and quantitative models of variable photosystem II fluorescence. – *Photochem. Photobiol.* **60**: 1-23, 1994.
- Delosme R.: [Studies on the induction of fluorescence in green algae and chloroplasts under intense illumination.] – *BBA-Bioenergetics* **143**: 108-128, 1967. [In French]
- Delosme R., Joliot P.: Period four oscillations in chlorophyll *a* fluorescence. – *Photosynth. Res.* **73**: 165-168, 2002.
- Demeter S., Rózsa Z., Vass I., Sallai A.: Thermoluminescence study of charge recombination in Photosystem II at low temperatures. I. Characterization of the Z_v and A thermoluminescence bands. – *BBA-Bioenergetics* **809**: 369-378, 1985.
- Duysens L.N.M., Sweers H.E.: Mechanism of two photochemical reactions in algae as studied by means of fluorescence. – In: Japanese Society of Plant Physiologists (Ed.): *Microalgae and Photosynthetic Bacteria*. Pp. 353-372. University of Tokyo Press, Tokyo 1963.

- Farage P.K., Blowers D., Long S.P., Baker N.R.: Low growth temperatures modify the efficiency of light use by photosystem II for CO₂ assimilation in leaves of two chilling-tolerant C₄ species, *Cyperus longus* L. and *Miscanthus × giganteus*. – Plant Cell Environ. **29**: 720-728, 2006.
- Farooq S., Chmeliov J., Wientjes E. *et al.*: Dynamic feedback of the photosystem II reaction centre on photoprotection in plants. – Nat. Plants **4**: 225-231, 2018.
- Garab G.: Revisiting the nonregulatory, constitutive nonphotochemical quenching of the absorbed light energy in oxygenic photosynthetic organisms. – Photosynthetica **62**: 204-208, 2024.
- Garab G.: Revisiting the Q_A model of chlorophyll-*a* fluorescence induction: new perspectives to monitor the photochemical activity and structural dynamics of photosystem II. – Photosynth. Res. **163**: 54, 2025.
- Garab G., Magyar M., Sipka G., Lambrev P.H.: New foundations for the physical mechanism of variable chlorophyll *a* fluorescence. Quantum efficiency versus the light-adapted state of photosystem II. – J. Exp. Bot. **74**: 5458-5471, 2023.
- Genty B., Briantais J.-M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – BBA-Gen. Subjects **990**: 87-92, 1989.
- Genty B., Harbinson J., Cailly A.L., Rizza F.: Fate of excitation at PS II in leaves: the nonphotochemical side. – In: The Third BBSRC Robert Hill Symposium on Photosynthesis. Abstract no. P28. University of Sheffield, Department of Molecular Biology and Biotechnology, Sheffield 1996.
- Gorbunov M.Y., Falkowski P.G.: Using chlorophyll fluorescence to determine the fate of photons absorbed by phytoplankton in the world's oceans. – Annu. Rev. Mar. Sci. **14**: 213-238, 2022.
- Holzwarth A.R.: Is it time to throw away your apparatus for chlorophyll fluorescence induction? – Biophys. J. **64**: 1280-1281, 1993.
- Horton P.: Developments in research on non-photochemical fluorescence quenching: emergence of key ideas, theories and experimental approaches. – In: Demmig-Adams B., Garab G., Adams III W., Govindjee (Ed.): Non-Photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria. Advances in Photosynthesis and Respiration. Vol. 40. Pp. 73-95. Springer, Dordrecht 2014.
- Huang D., Cheng C.-Q., Zhang H.-Y. *et al.*: Heat shock transcription factor-mediated thermal tolerance and cell size plasticity in marine diatoms. – Nat. Commun. **16**: 3404, 2025.
- Hussein R., Graça A., Forsman J. *et al.*: Cryo-electron microscopy reveals hydrogen positions and water networks in Photosystem II. – Science **384**: 1349-1355, 2024.
- Joliot P., Joliot A.: Comparative study of the fluorescence yield and of the C550 absorption change at room temperature. – BBA-Bioenergetics **546**: 93-105, 1979.
- Kalaji H.M., Schansker G., Ladle R.J. *et al.*: Frequently asked questions about *in vivo* chlorophyll fluorescence: practical issues. – Photosynth. Res. **122**: 121-158, 2014.
- Kirmaier C., Holten D., Parson W.W.: Temperature and detection-wavelength dependence of the picosecond electron-transfer kinetics measured in *Rhodospseudomonas sphaeroides* reaction centers. Resolution of new spectral and kinetic components in the primary charge-separation process. – BBA-Bioenergetics **810**: 33-48, 1985.
- Kitajima M., Butler W.L.: Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. – BBA-Bioenergetics **376**: 105-115, 1975.
- Klimov V.V., Allakhverdiev S.I., Demeter S., Krasnovskii A.A.: [Pheophytin reduction in Photosystem II of chloroplasts in relation to oxidation-reduction potential of the medium.] – Dokl. Akad. Nauk SSSR **249**: 227-230, 1979. [In Russian]
- Klimov V.V., Allakhverdiev S.I., Pashchenko V.Z.: [Measurement of the activation energy and life-time of the fluorescence of photosystem 2 chlorophyll.] – Dokl. Akad. Nauk SSSR **242**: 1204-1207, 1978. [In Russian]
- Klimov V.V., Allakhverdiev S.I., Shutilova N.I., Krasnovsky A.A.: [Investigation of pheophytin photoreduction and chlorophyll P-680 photooxidation with preparations of photosystems 2 from pea and *Chlamydomonas reinhardtii* chloroplasts.] – Sov. Plant Physiol. **27**: 315-326, 1980. [In Russian]
- Klimov V.V., Klevanik A.V., Shuvalov V.A., Krasnovsky A.A.: Reduction of pheophytin in the primary light reactions of photosystem II. – FEBS Lett. **82**: 183-186, 1977.
- Klughammer C., Schlosser F., Schreiber U.: Flash-kinetics as a complementary analytical tool in PAM fluorimetry. – Photosynth. Res. **161**: 151-176, 2024.
- Klughammer C., Schreiber U.: Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorimetry and the Saturation Pulse method. – PAM Appl. Notes **1**: 27-35, 2008.
- Kolber Z.S., Prášil O., Falkowski P.G.: Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. – BBA-Bioenergetics **1367**: 88-106, 1998.
- Konyukhov I.V., Stirbet A., Rubin A.B., Govindjee G.: A reexamination of the Kitajima and Butler (1975) model for relating chlorophyll *a* fluorescence to photochemistry. – Photosynthetica **64**: 12-25, 2026.
- Kramer D.M., Johnson G., Kiirats O., Edwards G.E.: New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. – Photosynth. Res. **79**: 209-218, 2004.
- Laisk A., Oja V.: Variable fluorescence of closed photochemical reaction centers. – Photosynth. Res. **143**: 335-346, 2020.
- Lazár D., Pospíšil P.: Mathematical simulation of chlorophyll *a* fluorescence rise measured with 3-(3',4'-dichlorophenyl)-1,1-dimethylurea-treated barley leaves at room and high temperatures. – Eur. Biophys. J. **28**: 468-477, 1999.
- Magyar M., Sipka G., Domonkos I. *et al.*: The role of protonation processes in the gradual formation of the light-adapted charge-separated state of photosystem II. – Front. Photobiol. **3**: 1623224, 2025.
- Magyar M., Sipka G., Kovács L. *et al.*: Rate-limiting steps in the dark-to-light transition of Photosystem II – revealed by chlorophyll-*a* fluorescence induction. – Sci. Rep.-UK **8**: 2755, 2018.
- Morin P.: [Studies of chlorophyll fluorescence kinetics *in vivo*, during the first moments following the onset of illumination.] – J. Chim. Phys. **61**: 674-680, 1964. [In French]
- Murchie E.H., Lawson T.: Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. – J. Exp. Bot. **64**: 3983-3998, 2013.
- Neubauer C., Schreiber U.: The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination. 1. Saturation characteristics and partial control by the Photosystem-II acceptor side. – Z. Naturforsch. C **42**: 1246-1254, 1987.
- Ostroumov E.E., Khan Y.R., Scholes G.D., Govindjee: Photophysics of photosynthetic pigment-protein complexes. – In: Demmig-Adams B., Garab G., Adams III W., Govindjee (Ed.): Non-Photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria. Advances in Photosynthesis and Respiration 40. Pp. 97-128. Springer, Dordrecht 2014.
- Papageorgiou G.C., Govindjee: Chlorophyll *a* Fluorescence:

- A Signature of Photosynthesis. *Advances in Photosynthesis and Respiration*. Vol. 19. Pp. 818. Springer, Dordrecht 2004.
- Park S., Steen C.J., Fischer A.L., Fleming G.R.: Snapshot transient absorption spectroscopy: toward *in vivo* investigations of nonphotochemical quenching mechanisms. – *Photosynth. Res.* **141**: 367-376, 2019.
- Porcar-Castell A., Tyystjärvi E., Atherton J. *et al.*: Linking chlorophyll *a* fluorescence to photosynthesis for remote sensing applications: mechanisms and challenges. – *J. Exp. Bot.* **65**: 4065-4095, 2014.
- Prášil O., Kolber Z.S., Falkowski P.G.: Control of the maximal chlorophyll fluorescence yield by the Q_B binding site. – *Photosynthetica* **56**: 150-162, 2018.
- Romero E., Novoderezhkin V.I., van Grondelle R.: Quantum design of photosynthesis for bio-inspired solar-energy conversion. – *Nature* **543**: 355-365, 2017.
- Ruban A.V.: Nonphotochemical chlorophyll fluorescence quenching: mechanism and effectiveness in protecting plants from photodamage. – *Plant Physiol.* **170**: 1903-1916, 2016.
- Santabarbara S., Monteleone F.V., Remelli W. *et al.*: Comparative excitation-emission dependence of the F_v/F_m ratio in model green algae and cyanobacterial strains. – *Physiol. Plantarum* **166**: 351-364, 2019.
- Schansker G., Tóth S.Z., Kovács L. *et al.*: Evidence for a fluorescence yield change driven by a light-induced conformational change within photosystem II during the fast chlorophyll *a* fluorescence rise. – *BBA-Bioenergetics* **1807**: 1032-1043, 2011.
- Schansker G., Tóth S.Z., Strasser R.J.: Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl *a* fluorescence rise OJIP. – *BBA-Bioenergetics* **1706**: 250-261, 2005.
- Schatz G.H., Brock H., Holzwarth A.R.: Picosecond kinetics of fluorescence and absorbance changes in photosystem II particles excited at low photon density. – *PNAS* **84**: 8414-8418, 1987.
- Shen J.-R.: The structure of photosystem II and the mechanism of water oxidation in photosynthesis. – *Annu. Rev. Plant Biol.* **66**: 23-48, 2015.
- Shevela D., Kern J.F., Govindjee G., Messinger J.: Solar energy conversion by photosystem II: principles and structures. – *Photosynth. Res.* **156**: 279-307, 2023.
- Sipka G., Magyar M., Mezzetti A. *et al.*: Light-adapted charge-separated state of photosystem II: structural and functional dynamics of the closed reaction center. – *Plant Cell* **33**: 1286-1302, 2021.
- Sipka G., Müller P., Brettel K. *et al.*: Redox transients of P680 associated with the incremental chlorophyll-*a* fluorescence yield rises elicited by a series of saturating flashes in diuron-treated photosystem II core complex of *Thermosynechococcus vulcanus*. – *Physiol. Plantarum* **166**: 22-32, 2019.
- Sipka G., Nagy L., Magyar M. *et al.*: Light-induced reversible reorganizations in closed Type II reaction centre complexes: physiological roles and physical mechanisms. – *Open Biol.* **12**: 220297, 2022.
- Stirbet A., Govindjee: On the relation between the Kautsky effect (chlorophyll *a* fluorescence induction) and photosystem II: basics and applications of the OJIP fluorescence transient. – *J. Photoch. Photobiol. B* **104**: 236-257, 2011.
- Stirbet A., Govindjee: Chlorophyll *a* fluorescence induction: a personal perspective of the thermal phase, the J-I-P rise. – *Photosynth. Res.* **113**: 15-61, 2012.
- Strasser R.J., Tsimilli-Michael M., Srivastava A.: Analysis of the chlorophyll *a* fluorescence transient. – In: Papageorgiou G.C., Govindjee (Ed.): *Chlorophyll *a* Fluorescence: A Signature of Photosynthesis*. *Advances in Photosynthesis and Respiration*. Pp. 321-362. Springer, Dordrecht 2004.
- Szczepaniak M., Sander J., Nowaczyk M. *et al.*: Charge separation, stabilization, and protein relaxation in photosystem II core particles with closed reaction center. – *Biophys. J.* **96**: 621-631, 2009.
- Taylor C.R., van Ieperen W., Harbinson J.: Demonstration of a relationship between state transitions and photosynthetic efficiency in a higher plant. – *Biochem. J.* **476**: 3295-3312, 2019.
- Treves H., Raanan H., Kedem I. *et al.*: The mechanisms whereby the green alga *Chlorella ohadii*, isolated from desert soil crust, exhibits unparalleled photodamage resistance. – *New Phytol.* **210**: 1229-1243, 2016.
- Trissl H.-W.: Response to Falkowski *et al.* – *Biophys. J.* **66**: 925-926, 1994.
- Trissl H.-W., Gao Y., Wulf K.: Theoretical fluorescence induction curves derived from coupled differential equations describing the primary photochemistry of photosystem II by an exciton-radical pair equilibrium. – *Biophys. J.* **64**: 974-988, 1993.
- Tyystjärvi E., Koski A., Keränen M., Nevalainen O.: The Kautsky curve is a built-in barcode. – *Biophys. J.* **77**: 1159-1167, 1999.
- van Amerongen H., Chmeliov J.: Instantaneous switching between different modes of non-photochemical quenching in plants: consequences for increasing biomass production. – *BBA-Bioenergetics* **1861**: 148119, 2020.
- van Amerongen H., Croce R.: Nonphotochemical quenching in plants: mechanisms and mysteries. – *Plant Cell* **37**: koaf240, 2025.
- Vavilin D.V., Ermakova-Gerdes S.Y., Keilty A.T., Vermaas W.F.J.: Tryptophan at position 181 of the D2 protein of photosystem II confers quenching of variable fluorescence of chlorophyll: implications for the mechanism of energy-dependent quenching. – *Biochemistry* **38**: 14690-14696, 1999.
- Velthuys B.R.: Mechanism of electron flow in photosystem II and toward photosystem I. – *Annu. Rev. Plant Physiol.* **31**: 545-567, 1980.
- Vermaas W.F.J., Govindjee: The acceptor side of photosystem II in photosynthesis. – *Photochem. Photobiol.* **34**: 775-793, 1981.
- Vredenberg W.J.: Analysis of initial chlorophyll fluorescence induction kinetics in chloroplasts in terms of rate constants of donor side quenching release and electron trapping in photosystem II. – *Photosynth. Res.* **96**: 83-97, 2008.
- Vredenberg W.: Kinetic analyses and mathematical modeling of primary photochemical and photoelectrochemical processes in plant photosystems. – *Biosystems* **103**: 138-151, 2011.
- Vredenberg W., Durchan M., Prášil O.: On the chlorophyll *a* fluorescence yield in chloroplasts upon excitation with twin turnover flashes (TTF) and high frequency flash trains. – *Photosynth. Res.* **93**: 183-192, 2007.
- Vredenberg W., Durchan M., Prášil O.: Photochemical and photoelectrochemical quenching of chlorophyll fluorescence in photosystem II. – *BBA-Bioenergetics* **1787**: 1468-1478, 2009.
- Vredenberg W., Kasalicky V., Durchan M., Prášil O.: The chlorophyll *a* fluorescence induction pattern in chloroplasts upon repetitive single turnover excitations: accumulation and function of Q_B -nonreducing centers. – *BBA-Bioenergetics* **1757**: 173-181, 2006.
- Wientjes E., van Amerongen H., Croce R.: Quantum yield of charge separation in Photosystem II: functional effect of changes in the antenna size upon light acclimation. – *J. Phys. Chem. B* **117**: 11200-11208, 2013.
- Zuo G.: Non-photochemical quenching (NPQ) in photoprotection: insights into NPQ levels required to avoid photoinactivation and photoinhibition. – *New Phytol.* **246**: 1967-1974, 2025.

Appendix

In this section, first, I present the simplified photochemical model of PSII (Butler 1978), along with its more recent formulations by Blankenship (2021) and van Amerongen and Croce (2025). The role of Pheo in the calculated photochemical and nonphotochemical quantum efficiencies, derived from ChlF measurements, will be discussed in later paragraphs.

In general, as pointed out by Butler (1978), variations in ChlF are ultimately determined by changes in the k_P , k_D , and k_F rate constants of photochemistry (P), heat dissipation (D), and fluorescence (F), respectively, processes which compete for the absorbed excitation energy of PSII. Assuming first order rates, the Φ quantum efficiencies can be given as a function of these rate constants, e.g., for Φ_P :

$$\Phi_P = k_P / (k_P + k_D + k_F).$$

Evidently, the sum of the quantum efficiencies of all competing processes must be 1.0:

$$\Phi_P + \Phi_D + \Phi_F = 1.0.$$

This equation provides the framework to determine the partitioning of the absorbed quanta among the different de-excitation pathways in PSII.

In the general case, allowing NPQ, the maximum level may decrease from F_m to F_m' . The magnitude of NPQ is conventionally determined using ChlF measurements:

$$NPQ = (F_m - F_m') / F_m'.$$

Note that, at the same time, F_o may also change to F_o' .

It was recognized by Genty *et al.* (1996) and later emphasized and further elaborated by other authors (Kramer *et al.* 2004, Klughammer and Schreiber 2008, Calzadilla *et al.* 2025) that it is important to introduce an additional nonphotochemical quenching component of Chl *a* fluorescence, NO, representing the so-called nonregulated (or constitutive) energy dissipation pathway. The introduction of NO warrants that the sum of quantum efficiencies equals 1.0. This is because $\Phi_P < 1.0$, and Φ_{NPQ} can be zero or very small. Hence:

$$\Phi_D = \Phi_{NPQ} + \Phi_{NO}.$$

ChlF is often used to determine the partitioning of the absorbed quanta between different de-excitation pathways in PSII by using the measured fluorescence levels and the appropriate rate constants.

Accordingly, the fluorescence intensity in PSII_o can be given as:

$$F_o = C \times k_F / (k_F + k_{IC} + k_{ISC} + k_P),$$

where C is a constant, specific to the measuring device.

As van Amerongen and Croce (2025) and Blankenship (2021) have shown, here, we discriminate between direct and indirect dissipation pathways, corresponding to internal conversion (IC) of the singlet excited state of Chl *a* and the formation of triplet states *via* intersystem crossing (ISC), respectively.

By accepting the assumption of Butler (1978), that upon the transition of PSII_o-to-PSII_c, Φ_P and k_P go to zero and F_o rises to F_m , and adopting the terminology of van Amerongen and Croce (2025), we obtain:

$$F_m = C \times k_F / (k_F + k_{IC} + k_{ISC}).$$

By combining ISC and IC again, and ignoring C , we can write:

$$F_o = k_F / (k_F + k_P + k_D)$$

and

$$F_m = k_F / (k_F + k_D).$$

From these equations, we obtain the widely used term for PSII photochemical quantum efficiency, found in photosynthesis texts (Blankenship 2021):

$$\Phi_P = F_v / F_m.$$

In the presence of excess light, and assuming again $\Phi_P = 0$ and $k_P = 0$, which, within the framework of the Q_A model, is a plausible assumption, the quantum efficiency of NPQ is:

$$\Phi_{NPQ} = k_{NPQ} / (k_F + k_D),$$

which may be written as

$$\Phi_{NPQ} = k_{NPQ} / (k_F + k_{NO} + k_{NPQ}).$$

It is to be noted, however, that these equations do not apply for NPQ with PSII_o, because, even in the Q_A model, k_P in PSII_o is >0 . It is interesting to note here that the k_{NPQ} rates have been shown to differ for open and closed RCs (Farooq *et al.* 2018, van Amerongen and Chmeliov 2020).

Van Amerongen and Croce (2025) have pointed out that NPQ can also be determined by measuring the average lifetimes, using the overall physical correlation between the de-excitation rate constants and the average fluorescence lifetimes. For a fluorophore, the fluorescence lifetime, τ , and the quantum yield, Φ_f , are related by the equation $\Phi_f = \tau / \tau_0$, where τ_0 is the natural radiative lifetime. Hence, an excited-state lifetime is defined by the same rate constants that are used for the yields. For example, in PSII_o (F_o condition), the lifetime can be given as

$$\tau_0 = 1 / (k_F + k_{IC} + k_{ISC} + k_P).$$

It is essential to note that in all PSII states, the decays comprise more than one, typically three, lifetime components (e.g., Szczepaniak *et al.* 2009, Wientjes *et al.* 2013, Sipka *et al.* 2021).

By measuring the lifetimes, artefacts, such as reabsorption of the fluorescence emission, and non-PSII contributions to F_v , e.g., from photosystem I, can be excluded. In harmony with the fluorescence yields, in excess light, the average lifetimes typically decrease from ~ 2 ns to several hundred picoseconds, clearly illustrating that NPQ, by reducing the excitation pressure on the RC, acts as a photoprotective mechanism (Wientjes *et al.* 2013). In plants, NPQ is arguably the most substantial photoprotective mechanism (Horton 2014, Ruban 2016).

It must be stressed here that all these ChLF yield- or lifetime-based determinations of the different quantum yields in PSII_{C/L/NPQ/NO} states relied on the assumption that in RCs containing Q_A in reduced form $\Phi_P = 0$ (and $k_P = 0$). As shown in previous sections, this assumption does not hold true.

As pointed out in the main text, the fact that $\Phi_P \neq 0$ could have been established by using the data on Pheo turnover (Klimov *et al.* 1977, 1979; Allakhverdiev *et al.* 1987), but it was Ostroumov *et al.* (2014) who clarified it first that, upon closing the RC, Φ_P decreases only marginally from about 89% to only about 76%. In excess light, generating NPQ, *i.e.*, in PSII_{NPQ}, the photochemical quantum yield of PSII decreases further, but its value may remain substantial; its magnitude evidently depends on the intensity of the actinic light and the magnitude of NPQ.

Concerning the partitioning of the excitation energy, it must be seen that—using conventional ChLF measurements—it is not possible to discriminate between “efficient” and “inefficient” photochemical yields, leading to stable and transient charge-separated states, respectively. This would require independent experiments, such as oxygen polarimetry, Hill reaction, electrochromic absorbance transients, *etc.* Also, since $\Phi_P \neq F_v/F_m$, the co-dependent quantity $\Phi_{NO} = 1 - F_v/F_m$ loses its physical meaning; and determination of Φ_{NPQ} may also be difficult, given that its most convenient determination would be to use the equation:

$$\Phi_{NPQ} = 1 - (\Phi_P + \Phi_{NO} + \Phi_F).$$

Evidently, Φ_F can be determined in different PSII states. Under excess light conditions, the “ChLF-silent” dissipated

energy might be large because NPQ often does not account for all photosynthetically unused quanta. Transient absorption techniques might aid the determination of the quantum yields and rate constants of the different de-excitation pathways (Park *et al.* 2019, Sipka *et al.* 2019). The values of Φ_P might also be determined by independent techniques. These may thus lead to good estimates of the Φ_P , Φ_{NO} , and Φ_{NPQ} quantum yields.

In general, all quantum yields can be obtained from the rate constants. ChLF measurements carry key information, but the above analysis shows that, except for the equations describing F_o , the formulations should be expanded to include all relevant state-specific rate constants. Changes in fluorescence levels and fluorescence lifetime measurements are highly informative for determining k_F . For internal conversion, some simplifications may be applied, *e.g.*, $k_{IC(O)} = k_{IC(C)}$ [here, (O) and (C) refer to PSII_O and PSII_C, respectively]. With sizeable NPQ components, invariance of k_{IC} , compared to PSII_C with zero NPQ, is highly unlikely. With regards to the rate constants of intersystem crossing, k_{ISC} , Chl *a* triplet states in PSII in excess light most probably originate from the recombination of the primary radical pair (Park *et al.* 2019, Bhattacharjee *et al.* 2023). The accumulation of carotenoid triplets, which might be part of the protective mechanism *via* quenching harmful Chl *a* triplets may be recognized from transient quenching of ChLF (Klughammer *et al.* 2024). The magnitude of k_P in different states can most readily be determined *via* measuring the turnover of Pheo (Klimov *et al.* 1979, Allakhverdiev *et al.* 1987) or the absorbance transients at around 820 nm (Sipka *et al.* 2019).