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## **Short communication**

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**In diatoms, the transthylakoid proton gradient regulates the photoprotective non-photochemical fluorescence quenching beyond its control on the xanthophyll cycle**

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**Abbreviations:** Chl *a*, chlorophyll *a*; DCCD, N,N'-dicyclohexyl-carbodiimide; DEP, de-epoxidation; DD, diadinoxanthin; DT, diatoxanthin; LHC, light-harvesting complex; NPQ, non-photochemical fluorescence quenching; PSII; photosystem II; XC, xanthophyll cycle;  $\Delta$ pH, transthylakoid proton gradient.

## **Abstract**

In diatoms, the non-photochemical fluorescence quenching (NPQ) regulates photosynthesis during light fluctuations. NPQ is associated with an enzymatic xanthophyll cycle (XC) which is controlled by the light-driven transthylakoid proton gradient ( $\Delta\text{pH}$ ). In this report, special illumination conditions and chemicals were used to perturb the kinetics of the  $\Delta\text{pH}$  build-up, of the XC, and of NPQ. We found that the  $\Delta\text{pH}$ -related acidification of the lumen is also needed for NPQ to develop by switching the xanthophylls to an 'activated' state, probably via the protonation of light-harvesting antenna proteins. It confirms the NPQ model proposed for diatoms (Goss et al. 2006).

**Key words:** diatom; diatoxanthin; *Phaeodactylum tricorutum*; NPQ; xanthophyll cycle; transthylakoid delta pH

In order to maintain an optimal photosynthetic activity in a permanently changing light environment, plants and algae have evolved a number of mechanisms aimed at the feed-back regulation of the light-dependent reaction of photosynthesis (Niyogi 2000). The thermal dissipation of light energy absorbed in excess (so-called NPQ for non-photochemical chlorophyll (Chl) fluorescence quenching) by the light-harvesting antenna complex (LHC) of photosystem II (PSII) is one of the most important of the rapidly activated regulatory mechanisms (Külheim et al. 2002, Holt et al. 2004, Horton et al. 2005). It avoids energy imbalance within the photosynthetic apparatus and protects the PSII activity from high light stress. In higher plants, NPQ is triggered by the light-driven build-up of a transthylakoid proton gradient ( $\Delta pH$ ). The acidification of the lumen results in a protonation of specific sites of the PSII LHC antenna proteins (PsbS, CP26-29). Protonation induces conformational changes within the antenna leading to the dissipation of excess energy (Horton et al. 2005). The switch of the antenna to the dissipative quenching state is additionally regulated by the binding of de-epoxidised forms of xanthophylls (Holt et al. 2004, Horton et al. 2005) produced via a so-called xanthophyll cycle (XC). The XC results in the  $\Delta pH$ -dependent conversion of epoxidised to de-epoxidised forms of xanthophylls. Hence, in higher plants, both the protonation of the LHC antenna and the production of de-epoxidised xanthophylls are involved in NPQ.

Diatoms are a key taxon of the phytoplankton microalgae which dominate in deeply mixed waters (Falkowski et al. 2003) where they may experience large changes in irradiance. NPQ has been shown to allow the diatoms to keep photosynthesis effective in the fluctuating underwater light climate of the Northern marine waters (Kashino et al. 2002). The diatom NPQ process shows specific properties when compared to the NPQ of

plants/green algae (Ruban et al. 2004, Goss et al. 2006). The polypeptide organisation and composition of the LHC antenna is different with two major points regarding NPQ: 1) the plant minor components PsbS, CP26-29 are absent in diatoms (Armbrust et al. 2004), 2) the spatial arrangement of the polypeptides (Büchel 2003) as well as the nature and location of the xanthophylls are specific for diatoms (Lavaud et al. 2003, Guglielmi et al. 2005). The XC is also different from the one in plants. It is simpler and involves different pigments: the diadinoxanthin (DD) is converted into diatoxanthin (DT) by a de-epoxidase and vice versa (thanks to an epoxidase) depending on the  $\Delta\text{pH}$ . The XC enzymes show specific features: the de-epoxidase is activated at a higher luminal pH (Jakob et al. 2001, Grouneva et al. 2005) than in higher plants and its activity is strongly depending on the acidic form of the co-factor ascorbate (Grouneva et al. 2005). As a consequence, the DD de-epoxidase is characterised by a very rapid activation even when only small changes in the  $\Delta\text{pH}$  occur. The reactivity of the enzyme can be reduced by DCCD (N, N'-dicyclohexyl-carbodiimide) which binds to the domain which is responsible for the sensitivity of the enzyme to the pH (Jahns and Heyde 1999). The DT epoxidase is also very sensitive to a change in the  $\Delta\text{pH}$ , being inactivated by high  $\Delta\text{pH}$  conditions (Goss et al. 2006).

The light-dependent accumulation of DT is mandatory for NPQ, regulating its kinetics and extent in a quantity dependent way (Lavaud et al. 2002a, Ruban et al. 2004, Goss et al. 2006). As compared to land plants, the ratio xanthophylls over chlorophyll is much more larger (Wilhelm 1990, Lavaud et al. 2003). Consequently, NPQ in diatoms can reach higher values (Lavaud et al. 2002a). It has been recently shown that, once the antenna dissipative state is formed, the extent and kinetics of NPQ solely depend on

amount of DT and are independent from the  $\Delta\text{pH}$  (Goss et al. 2006), which explains the prolonged stability of NPQ after the illumination (Ruban et al. 2004). Still, it cannot be excluded that both the  $\Delta\text{pH}$  and DT are required to generate the antenna dissipative state (Lavaud et al. 2002b, Goss et al. 2006). This has been difficult to show because the kinetics of the build-up of  $\Delta\text{pH}$  and the accumulation of the DT are tightly related (Lavaud et al. 2002b, Ruban et al. 2004). Even though, in some cases DT can be present with no development of NPQ depending on the extent of the  $\Delta\text{pH}$  (Lavaud et al. 2002b, Goss et al. 2006). This observation and the specific properties of the XC regulation in diatoms were used to design a special experimental procedure aimed at uncoupling the relationship between  $\Delta\text{pH}$  and the XC and studying the effect on NPQ. For our experimental approach, we used five protocols (Prot.) and measured NPQ and DD de-epoxidation in *Phaeodactylum tricornutum* (Table 1). [Table 1 here.](#)

It has been previously shown in higher plants that pre-illumination of leaves changes the relationship between the  $\Delta\text{pH}$ , the XC and NPQ (Noctor et al. 1993) and accelerates the NPQ kinetics (Ruban et al. 2004), during the following high light exposure. We thus decided to apply this technique to the diatoms. In the two first protocols (Prot. n°1 and 2), the cells were pre-illuminated before inducing NPQ and DD de-epoxidation by high light (HL) exposure ( $2000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). The measurements during HL were compared with cells dark-adapted for the same time than the pre-illumination. In Prot. n°1, the cells were incubated at the growth light (GL) intensity ( $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) for a time long enough (45 min) to re-acclimate the cells to the growing conditions after the sampling and the preparation (see Lavaud et al. 2002a). We designed this experiment as a control to check the feasibility in diatoms of

accelerating the NPQ kinetics by pre-illuminating the cells. It is a preparatory procedure to Prot. n°2. After incubation of the cells to 45 min darkness and exposure to HL,  $NPQ_{1min}$  was 3.7 and its development paralleled the accumulation of DT formed by the de-epoxidation of DD (Table 2) according the previous observations (Lavaud et al. 2002a, Lavaud et al. 2004). When adapted to GL before the same HL exposure, NPQ was multiplied by a factor 1.6 (6 vs 3.7) similar to the increase of DT (Table 2). The irradiance used during the GL pre-incubation was not high enough to accumulate any DT (see Table 2). The cause of the acceleration is then likely to be a small difference in the  $\Delta pH$ , compared to the dark-adapted cells, due to the basal linear electron transport driven by the pre-illumination (Cruz et al. 2005). This small  $\Delta pH$  change might be sufficient to stimulate the DD de-epoxidase (Jakob et al. 2001, Grouneva et al. 2005). As the backward DT epoxidation reaction has the same (Lohr and Wilhelm 1999) or even higher (Goss et al. 2006) velocity in low light, no DT accumulated and consequently no NPQ developed (Lavaud et al. 2002b) during the GL pre-illumination. Nevertheless, in the conditions of Prot. n°1, the initial  $\Delta pH$  and the stimulated de-epoxidase lead to the concomitant acceleration of both DD de-epoxidation and NPQ kinetics during the following HL illumination as reported before in higher plants (Ruban et al. 2004). **Table 2 here.**

A special pre-illumination procedure (Prot. n°2) consisting in 3 successive 5 min steps of progressively increasing irradiance (PL, from 50 to 150 via 100  $\mu mol photons m^{-2} s^{-1}$ ) allowed breaking the proportionality between DT and NPQ. This pre-illumination procedure was designed regarding the results of Prot. n°1. Its first purpose has been to accelerate to a maximum the kinetics of both NPQ and DD de-epoxidation during HL by



driving the highest linear electron transport and consequently the highest  $\Delta\text{pH}$  without generating any NPQ and DT during the pre-illumination. The PL pre-illumination allowed reaching this situation (see Table 2). It has been tested that for a longer illumination at intermediate irradiance (like  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and that from  $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  on, whatever the illumination time, NPQ developed (not shown). Figure 1 shows a recording of the Chl *a* fluorescence emission changes induced by a 5 min HL illumination after pre-incubation of the cells with PL or darkness. The spikes resulted from short pulses of saturating intensity (down arrows). The maximum fluorescence yield,  $F_m$  was reached when such a pulse was applied to dark-adapted cells that have a low fluorescence yield ( $F_0$ ). During the illumination, NPQ ( $= (F_m - F_m')/F_m'$ ) rapidly developed, as shown by the lowering of the maximum yields reached during the pulses ( $F_m'$ ). **Figure 1 here**. When the cells were adapted to PL before HL exposure, the NPQ kinetics was accelerated during the first 30-45 s HL (see Inset Fig. 1A). It was already visible after few seconds of illumination (Fig. 1B, note the decrease after the G peak for pre-illuminated cells (Lazar 2006)). Nevertheless, in these conditions NPQ development and DD de-epoxidation were not accelerated to the same extent. The DT accumulated exceeded the amount which is effectively used for NPQ (Table 2, compare bold lines) (see also Lavaud et al. 2002b). Still, after 5 min HL (the time to reach steady-state conditions for Chl *a* fluorescence emission) the increase in NPQ and DT accumulation was similar (Table 2). In higher plants, de-epoxidised xanthophylls need to enter a so-called 'activated state' to drive NPQ (Holt et al. 2004, Horton et al. 2005) by binding to protonated LHC antenna proteins (such as PsbS). In diatoms, there is no PsbS (Armbrust et al. 2004), but a DT 'activated' state does exist (Ruban et al. 2004) arguing

for a LHC antenna protonation event as in higher plants. A possible explanation for the data of Prot. n°2 might be that the PL pre-illumination drove a linear electron transport (as seen by the increased steady-state  $F_S$  level (Fig. 1A) and the increased I shoulder (Fig. 1B) resulting from  $Q_A$  reduction) which generated a change in the  $\Delta pH$  which was high enough to ensure conditions for which the proportionality between the accumulation of DT and the protonation of LHC antenna polypeptides was lost: more DT molecules accumulated than LHC antenna sites have been protonated because of the high reactivity of the XC enzymes towards the  $\Delta pH$  (Jakob et al. 2001, Grouneva et al. 2005, Goss et al. 2006). Indeed, the DD de-epoxidase and the DT epoxidase are, respectively, rapidly activated and inactivated by high  $\Delta pH$  conditions driven by a sudden increase in light intensity.

Addition of exogenous ascorbate to the cells (Prot. n°3) confirmed this proposal. Ascorbate is one of the co-factors of the DD de-epoxidase. It shifts the activity of the enzyme at higher pH and consequently a lower  $\Delta pH$  is required for DD de-epoxidation (Grouneva et al. 2005). The presence of ascorbate lead to an increase of DD de-epoxidation without virtually any effect on NPQ at moderate light conditions (ML, 350  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , Table 3). For higher intensities (above the light for saturation of photosynthesis, 450  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), the effect of ascorbate on the relationship DT/NPQ was not visible anymore (data not shown), surely due to the fact that the change in the  $\Delta pH$  was too high and fast. [Table 3 here.](#)

Hence, as shown by Prot. n°2 and 3, increasing only the amount of DT molecules is not sufficient for enhancing NPQ, it needs a simultaneous acidification of the lumen

and subsequent capacity for switching to DT ‘activated’ molecules, probably via protonation of LHC antenna sites as recently proposed (Goss et al. 2006).

The relationship between  $\Delta\text{pH}$  and NPQ was further investigated by using DCCD to vary the extent of the light-dependent lumen acidification. DCCD inhibits the activity of the ATP synthase, by blocking the proton conductive channel, and thus the reflux of luminal protons (McCarty and Racker 1967). DCCD increases NPQ in diatoms in an incubation time-dependent way (Lavaud et al. 2002b). The amount of DCCD which penetrates the chloroplast is likely to be dependent on the incubation time. Prot. n°4 was designed to further test the influence of the DCCD incubation time on the kinetics of NPQ. It is a preparatory procedure to Prot. n°5. DCCD greatly enhanced the extent of NPQ at a low light irradiance (LL,  $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) which generated a small NPQ (0.09) in the absence of DCCD (Fig. 2A). At the low concentration of  $10 \mu\text{M}$ , the effect of DCCD on NPQ kinetics was dependent on the incubation time before the illumination even if after 5 min light the extent of both NPQ ( $1.7 \pm 0.1$ ) and DT accumulation ( $2 \pm 0.3 \text{ mol}/100 \text{ mol Chl } a$ ) were similar for 5 and 15 min incubation. In higher plants, at higher concentrations, DCCD binds to the proton binding sites of the CP26-29 and PsbS antenna polypeptides thus inhibiting NPQ (Bassi and Caffarri 2000, Horton et al. 2005). These polypeptides do not exist in diatoms (Armbrust et al. 2004) and the addition of DCCD at low concentrations enhances NPQ, as previously reported (Lavaud et al. 2002b), by enhancing the amount of protons which accumulate within the lumen. [Figure 2 here.](#)

Regarding the results of Prot. n°4, a dynamic study was then performed (Prot. n°5) where the effect of DCCD incubation time on NPQ and the DD de-epoxidation were

observed over 30 min to reach a steady-state of the penetration of DCCD in the chloroplast. As for Prot. n°3, ML irradiance was chosen to avoid a too high and fast change in the  $\Delta\text{pH}$  upon illumination, a better way to uncouple the NPQ and DD de-epoxidation kinetics. Without DCCD in the light conditions used, an NPQ of 1.3 formed concomitantly with DD de-epoxidation (15 %). For short DCCD incubation times, NPQ was enhanced with no parallel increase in DT; then the DD de-epoxidation suddenly increased (to above 25 %) (Fig. 2B). It is proposed that the uncoupling between NPQ and the DD de-epoxidation is due to the DCCD effect on the XC enzymes. DCCD reduces the de-epoxidase reactivity to changes in pH (Jahns and Heyde 1999), and simultaneously induces high  $\Delta\text{pH}$  which decreases the epoxidase activity (Goss et al. 2006). This situation generated a clear deviation from the linear NPQ-DT relationship usually observed (Lavaud et al. 2002a, Goss et al. 2006). Hence, it was possible to enhance the NPQ extent with a constant amount of already present DT molecules. In analogy to higher plants, we hypothesise that this was made possible through additional protonation of LHC antenna polypeptides and subsequent conformational changes (Horton et al. 2005, Goss et al. 2006).

Altogether, these observations strongly support the hypothesis that in diatoms, the  $\Delta\text{pH}$  plays a central role in the NPQ process not only in controlling the activity of the XC enzymes. As protons accumulate in the lumen during illumination, protonation of LHC antenna polypeptides is likely to occur, as in higher plants, enabling the switch of DT molecules to an 'activated' quenching state previously reported (Ruban et al. 2004); which is an essential step in the development of NPQ. In diatoms, the mechanism of NPQ might be similar to that in higher plants (Holt et al. 2004, Horton et al. 2005), confirming

the model recently proposed (Goss et al. 2006). Nevertheless, one has to keep in mind that the organisation and xanthophyll/protein composition of the diatom LHC (Büchel 2003, Lavaud et al. 2003, Guglielmi et al. 2005) as well as the regulation of the XC (Jakob et al. 2001, Grouneva et al. 2005) are very different from higher plants. These specific characteristics probably explain why the extent and kinetics of NPQ in diatoms show striking features when compared to higher plants (Ruban et al. 2004). An important question which still remains to be solved is the capacity for protonation of LHC polypeptides to drive alone (i. e. without the presence of DT) a basal NPQ via LHC conformational changes such as in plants (Holt et al. 2004, Horton et al. 2005). So far there is no clear evidence for such a mechanism in diatoms (Lavaud et al. 2002b, Goss et al. 2006). Because of the characteristics of the XC enzymes, only an *in vitro* approach would allow to clarify this point. The recent improvement in the isolation of native LHC complexes from diatoms (Büchel 2003, Lavaud et al. 2003, Guglielmi et al. 2005) now provides the possibility to follow the same successful approach as in higher plants (Ruban et al. 1994).

Hence, in diatoms, there is a very tight and concerted activation of the XC and of the NPQ process via the  $\Delta\text{pH}$  build-up. This is surely due to the fact that the de-epoxidase is very rapidly activated by the acidification of the lumen (Jakob et al. 2001, Grouneva et al. 2005) while the epoxidase is simultaneously inhibited by the increase of  $\Delta\text{pH}$  (Goss et al. 2006) ensuring a fast supply of NPQ with DT molecules (Lavaud et al. 2002b). This feature is important for a fast switch of the LHC function to a photoprotective state of energy dissipation in a highly variable underwater light climate. In this context, it is noteworthy that the first-order reaction time constant of the XC in diatoms is faster than

the time needed for the irradiance to double in the coastal/estuarine turbulent waters where the diatoms flourish (MacIntyre et al. 2000).

## Methods

We used five experimental protocols (Prot.) and measured NPQ and DD de-epoxidation. All the details for the experimental approach are given in Table 1 and the corresponding parts of the text. The photosynthetic properties as well as the growing and preparation for experiments of the low and high NPQ cells of *Phaeodactylum tricornutum* Böhlin were described previously (Lavaud et al. 2002a, Lavaud et al. 2003). Because high NPQ cells exhibit faster NPQ kinetics (Lavaud et al. 2002a), they were preferred for Prot. n°1 and 2. Xanthophyll pigment analyses were performed by HPLC as described (Lavaud et al. 2003). The DD de-epoxidation (DEP) extent was calculated as  $(DT/DD+DT) \times 100$  where diadinoxanthin (DD) is the epoxidised form and diatoxanthin (DT) is the de-epoxidised form. NPQ ( $= (F_m - F_m')/F_m'$ ) was calculated from standard modulated Chl *a* fluorescence measurements (see the text for details) performed with a PAM-101 fluorometer (Walz, Effeltrich, Germany) as described (Lavaud et al. 2004). Fluorescence induction kinetics (FIK) of *P. tricornutum* cells were obtained with a home-made fluorometer as described (Parésys et al. 2005). Ascorbate (Sigma) was obtained from a 2 M fresh stock solution in distilled water and DCCD (Sigma) from a fresh 1 M stock solution in absolute ethanol. For all the measurements, sodium bicarbonate was added to the algal suspension (4 mM final, from a freshly prepared 0.2 M stock solution) to prevent any limitation of the photosynthetic rate by carbon supply.

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## Figure legends

Figure 1. (A) Chlorophyll (Chl) *a* fluorescence emission changes in *Phaeodactylum tricornutum* as measured with a PAM-fluorometer during a 5 min high light (HL, 2000  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) exposure (arrow up: light on). Cells were pre-illuminated in three steps of 5 min with progressively increasing irradiance (PL, 50/100/150  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) (continuous line) or not (15 min darkness, dotted line). Inset: enlargement of the first 1 min of HL illumination. (B) Fluorescence induction kinetics (FIK) of cells pre-illuminated (continuous line) or not (dotted line). The letters refer to the FIK phases in diatoms (Lazar 2006). See the text for details. Fig. 1A and 1B stand for Prot. n°2.

Figure 2. (A) Effect of DCCD (N,N'-dicyclohexyl-carbodiimide ) (10  $\mu\text{M}$  final) on the development of the non-photochemical quenching (NPQ) (5 min light exposure, 100  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) in *P. tricornutum*. The times refer to the DCCD incubation time in the dark prior to the illumination. (B) DCCD (10  $\mu\text{M}$  final) incubation time effect on NPQ (closed circles) and the xanthophyll de-epoxidation activity (closed triangles for the diatoxanthin (DT) amount; open triangles for the de-epoxidation (DEP) extent) in *P. tricornutum*. Incubation was performed in the dark prior to the illumination (5 min, 350  $\mu\text{E m}^{-2} \text{s}^{-1}$ ). Values are mean of three measurements  $\pm$  SD. Fig. 2A and 2B stand for Prot. n°4 and 5, respectively.

**Table 1** Descriptions of the five experimental protocols (Prot.) used.

Prot.	Cells	Pre-incubation	Light treatment
1	High NPQ	45 min Darkness or GL	HL, 5 min
2	High NPQ	15 min Darkness or PL	HL, 5 min
3	Low NPQ	15 min darkness, +/- 100 mM ascorbate	ML, 5 min
4	Low NPQ	15 min darkness, +/- 10 $\mu$ M DCCD DCCD incubation times: 5 and 15 min	LL, 5 min
5	Low NPQ	30 min darkness, +/- 10 $\mu$ M DCCD DCCD incubation times: 5 to 30 min	ML, 5 min

NPQ, non-photochemical fluorescence quenching; DCCD, N,N'-dicyclohexylcarbodiimide. Because high NPQ cells exhibit faster NPQ kinetics (Lavaud et al. 2002a), they were preferred for Prot. n°1 and 2. Ascorbate is a co-factor for the diadinoxanthin de-epoxidase. DCCD is an inhibitor of the ATP synthase and it delays the reactivity of the de-epoxidase towards the transthylakoid  $\Delta$ pH (see the text for details). Light intensities: GL, growing light (50  $\mu$ mol photons  $m^{-2} s^{-1}$ ); PL, progressively increasing light (3x 5 min 50, 100, 150  $\mu$ mol photons  $m^{-2} s^{-1}$ ); LL, low light (100  $\mu$ mol photons  $m^{-2} s^{-1}$ ); GL/PL/LL were chosen to not induce any diatoxanthin and no NPQ (see the text for details); ML, moderate light (350  $\mu$ mol photons  $m^{-2} s^{-1}$ ) just below the irradiance for saturation of photosynthesis (450  $\mu$ mol photons  $m^{-2} s^{-1}$ , Lavaud et al. 2002a); HL, high light (2000  $\mu$ mol photons  $m^{-2} s^{-1}$ ) allowing to rapidly (in few seconds) obtain a large NPQ (Lavaud et al. 2002a).

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**Table 2** Effect of different pre-illumination treatments (Prot n°1 and 2) on the non-photochemical quenching (NPQ), the diatoxanthin (DT) accumulation (in mol/100 mol Chl *a*) and the diadinoxanthin (DD) de-epoxidation (DEP in %) in *Phaeodactylum tricornutum* cells during a high light (HL) exposure (for Prot. n°2, data are from the experiments shown in Fig. 1A).

Protocol	Pre-illumination	HL illumination time	NPQ	DT	DEP extent
n°1	No	0 min	0.005	0	0
	(45 min dark)	1 min	3.7	3.2	21
		45 min, GL	0 min	0.001	0
			1 min	6.0	5.7
n°2	No	0 s	0.002	0	0
	(15 min dark)	30 s	<b>1.3</b>	<b>1.8</b>	<b>12</b>
		5 min	6.3	7.5	50
	15 min PL	0 s	0.004	0	0
		30 s	<b>1.7</b>	<b>3.5</b>	<b>28</b>
		5 min	6.3	7.8	52

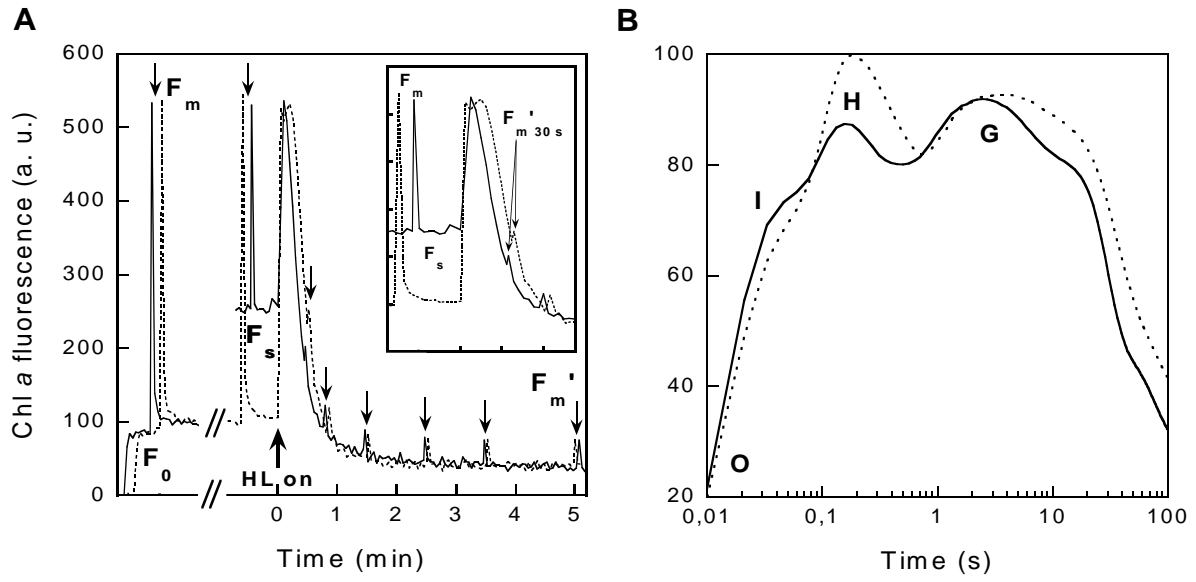
Light intensities: GL, growing light ( $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ); PL, progressively increasing light (3x 5 min 50, 100, 150  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ); HL, high light ( $2000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). DD+DT was  $14.7 \pm 0.3 \text{ mol/100 mol Chl } a$ .

**Table 3** Effect of exogenous ascorbate (Prot. n°3) on the non-photochemical quenching (NPQ) and the DD de-epoxidation (DEP) in *P. tricornutum*.

Ascorbate (mM)	NPQ	DT (mol/100 mol Chl <i>a</i> )	DD+DT (mol/100 mol Chl <i>a</i> )	DEP extent (%)
0	1.3 ± 0.1	1.3 ± 0.1	9.3 ± 0.1	15.0 ± 0.2
100	1.4 ± 0.2 (2.0 ± 0.3)	2.1 ± 0.3	9.4 ± 0.1	22.2 ± 3.1

DD, diadinoxanthin, DT, diatoxanthin. Between brackets: NPQ value usually reached for DT = 2.1 ± 0.3 mol/100 mol Chl *a* (Lavaud et al. 2002a). For low NPQ cells, the maximal NPQ value is about 3 (Lavaud et al. 2002a). Values are mean of three measurements ± SD.

**Figure 1 – Lavaud & Kroth**



**Figure 2 – Lavaud & Kroth**

