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Chlorophyll fluorescence of *Chlamydomonas reinhardtii*: insights into the complexities

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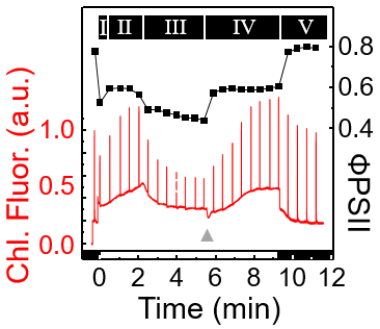
Summary

Tolerance of rapid changes in light intensity by photosynthetic organisms is facilitated by non-photochemical quenching (NPQ), a term with reference to quenching of chlorophyll fluorescence, the technique used in its discovery. Mechanisms of NPQ include dissipating excess light energy to heat (qE), the reversible attachment of light-harvesting complexes (LHC) to photosystems (state transitions, qT) and photoinhibition (qI). Chlorophyll is a ubiquitous pigment of photosynthetic organisms, found in LHC and the reaction centers of photosystem II and I (PSII; PSI). At room temperature, pulse-amplitude modulated (PAM) chlorophyll fluorescence protocols provide insights into PSII efficiency, thus a reasonable proxy for photosynthetic activity and CO_2 fixation. NPQ has a major impact on chlorophyll fluorescence intensity and is also quantified by PAM. Since NPQ mechanisms can occur simultaneously, they cause complexities in deciphering the signal. In algae, the ability for chlorophyll fluorescence in determining photosynthetic rates is not perfect, but it can still provide valuable information of processes affecting light harvesting. The aim of this report is to provide an overview of how various NPQ mechanisms in the model unicellular chlorophyte alga, *Chlamydomonas reinhardtii*, as well as environmental conditions, affect chlorophyll fluorescence. I also propose a PAM protocol enabling the kinetics associated with individual NPQ phases to be quantified in <20 min.



1. Why does photosynthesis need regulating?

Photosynthesis is remarkable. Not only has it led to all the oxygen that us aerobes need and breathe, it can maximize light use efficiency under highly changeable light intensities, and in accordance with the metabolic demands of the organism. Regulation is coordinated at multiple levels; at the (*1*) molecular level via energy-transfer processes involving carotenoids, chlorophylls and various proteins, (*2*) biochemical level with metabolite exchange, (*3*) sub-organelle level with supramolecular organization of protein complexes in the thylakoid membrane, (*4*) cellular level by chloroplast relocation, and (*5*) organism level, such as *via* phototaxis and heliotropism. These types of regulation are of critical importance, enabling photoautotrophy under highly contrasting environments encountered in nature (e.g. suboptimal environment, intermittent light and CO_2 supply). Moreover, regulation prevents damage from absorbance of excess light energy. Chlorophyll is a highly efficient photosensitizer that in an oxygen-rich environment, along with spillage of electrons from electron carriers, can generate a damaging level of reactive oxygen species (ROS), leading to photooxidative stress (Roach, Krieger-Liszkay 2019).

2. Modulating light-use efficiency

Light conditions are dynamically fluctuating, requiring light-harvesting systems to be dynamically regulated. Short-term acclimation (seconds to minutes) includes excess energy dissipation (energy-dependent quenching, qE) and energy redistribution of the light-harvesting apparatus (state transitions, qT), which can be activated in response to a low pH of the lumen and reduced redox state of the photosynthetic electron transfer system, respectively, thus forming direct feedback to energetic statuses. Collectively, regulatory mechanisms, such as qE and qT that control how efficiently and where exactly light energy is used (Figure 1; Table 1), are known as non-photochemical quenching (NPQ). The term 'quenching' refers to chlorophyll fluorescence, since this is partly how such mechanisms have been discovered and can also be monitored *in vivo* (Krause, Weis 1991). Sustained quenching associated with damaged PSII reaction centers (photoinhibition, qI) is also included in NPQ.

The qE component of NPQ reduces the quantum yield of chlorophyll fluorescence (i.e. induces quenching) in the light-harvesting antennae and is a pH-dependent process (Müller et al 2001). It activates due to a pH drop from accumulation of hydrogen ions in the thylakoid lumen, which leads to protonation of light-harvesting complexes (LHC) and absorbed light energy released non-radiatively as heat. In vascular plants, and some green algae, qE is associated with zeaxanthin (Demmig-Adams, Adams 1996; Quaas et al 2014; Goss, Lepetit 2015). A lowered pH activates violaxanthin de-epoxidase (VDE), which uses ascorbic acid as substrate to convert violaxanthin to zeaxanthin, which then may potentially directly quench or indirectly (e.g. affecting LHC conformational change) contribute to energy dissipation as heat (Figure 1, No. 2).

In *C. reinhardtii*, zeaxanthin accumulation is not essential for qE induction and a different reductant than ascorbate is a substrate for VDE (Vidal-Meireles et al 2020). Instead, NPQ in *C. reinhardtii* is intricately linked to LHC-type Stress-Related (LHCSR) thylakoid membrane proteins, LHCSR1 and LHCSR3 (Figure 1, No. 1; Figure 2), which vascular plants do not have (Peers et al 2009).

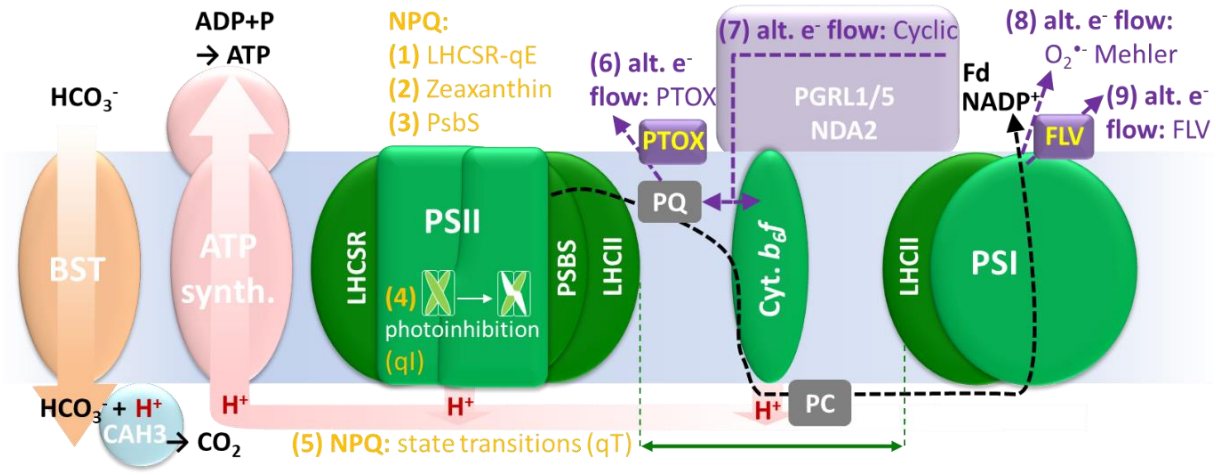


Figure 1. Schematic of protein complexes in the thylakoid membrane highlighting energy regulation, electron transfer pathways and transport of H^+ and HCO_3^- involved in photosynthesis of *Chlamydomonas reinhardtii*. Black and purple dashed lines denote "linear electron flow" and "alternative electron flow", respectively. See Table 1 for a description of the numbered mechanisms and abbreviations.

Table 1. Light energy regulation, alternative electron pathways and hydrogen ion transport in *Chlamydomonas reinhardtii*. Numbers correspond to mechanisms depicted in Figure 1.

Process		Short name	Description	*C. reinhardtii* mutants	Reference
Light energy regulation (NPQ)	1	LHCSR-qE	Main qE mechanism in *C. reinhardtii*, LHCSR3 protects from photo-oxidative stress and fluctuating light	*npq4, lhcsr1*	Ballotari et al 2016; Peers et al 2009; Roach et al 2020
	2	Zeaxanthin	Zeaxanthin accumulation is a ubiquitous high-light response. May function in qE	*npq1, npq2*	Niyogi et a, 1997
	3	PsbS	Facilitates recruitment of LHCSR, unclear involvement in NPQ	*psbs1*	Correa-Galvis et al 2016
	4	Photoinhibition (qI)	Light-associated damage to PSII, repairable within hours	*ftsh1-1*	Malnoë et al 2014
	5	State transitions (qT)	Movement of LHCII between PSII and PSI to balance energy distribution, active during a change in light intensity	*stt7-9, stt7-7, npq4stt7-9*	Depege et al 2003
Alternative electron pathways	6	PTOX	Plastid terminal oxidase, full reduction of O_2 to H_2O, safety valve	*ptox2*	Houille-Vernes et al 2011
	7	Cyclic	Reducing power re-invested back into ETC when greater ΔpH for qE or ATP is needed	*pgr5, pgrl1*	Buchert et al 2020 Jokel et al 2018
	8	ROS	Partial reduction of O_2 to ROS, safety valve	-	-
	9	FLV	Flavodiiron, full reduction of O_2 to H_2O before light-induced activation of CO_2 assimilation, safety valve	*flvB*	Chaux et al, 2017

Vascular plants and algae possess a PSII-associated protein, PsbS (Figure 1, No. 3), which in plants contributes to qE, but in algae is involved in qE via influencing accumulation of LHCSR (Correa-Galvis et al 2016; Redekop et al 2020). Since qE lowers the lifetime of chlorophyll fluorescence by dissipating the energy as heat, it leads to a detectable quenching of chlorophyll fluorescence (Krause, Weis 1991; Müller et al 2001). The governance of qE by pH means that processes affecting proton accumulation

in the lumen also affect qE. Protons accumulate in the lumen from water splitting by PSII and electron transfer through the cytochrome b_6f complex (cyt b_6f). Thus, the cyclic electron transfer pathway (Figure 1, No. 7) that recycles electrons into the electron transfer system also pumps protons into the lumen. Subsequently, ATP synthase uses the protonmotive force to convert ADP + P_i to ATP. Furthermore, conversion of HCO_3^- pumped into the lumen via Bestrophin-Like Proteins (BST) to CO_2 via carbonic anhydrase 3 (CAH3) (Mukherjee et al, 2019) also requires a proton, thereby influencing luminal pH and qE (Figure 1).

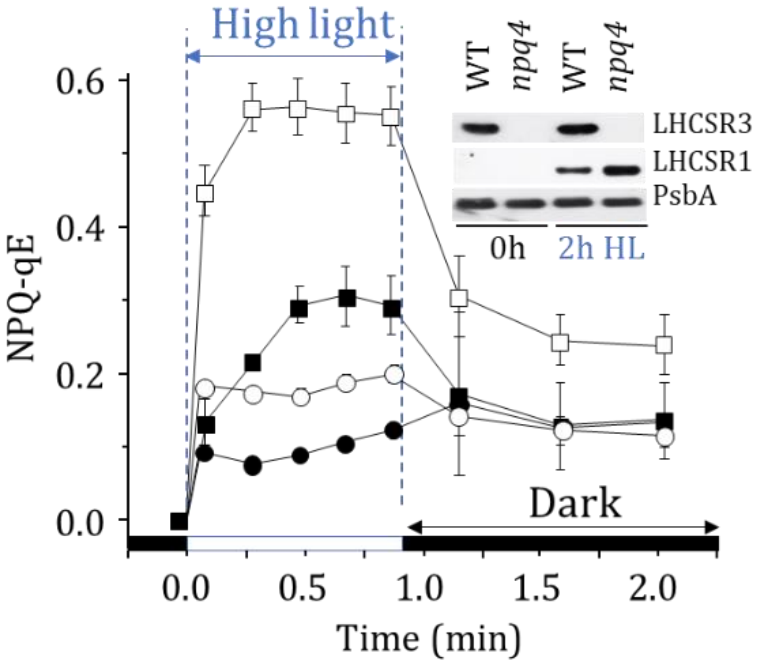


Figure 2. Thermal dissipation (qE) in response to light and dark. LHCSR1/3-dependent qE rapidly activates in response to high light (HL), while qE capacity increases after prolonged (2 h) HL treatment (open symbols), relative to 0 h HL (closed symbols). Using a *C. reinhardtii* mutant deficient in LHCSR3 (*npq4*; circles) the HL-induced increase in NPQ capacity is shown partly due to accumulation of LHCSR1 proteins (inset, Western Blot), that also occurs in photoautotrophic wild type (WT) cells (squares). The PSII reaction centre (PsbA) is loading control.

Light energy is also distributed between the two photosystems (PS), PSII and PSI, by movement of LHCII, in a process called state transitions, abbreviated as qT (Figure 1, No. 5). By changing the relative excitation of photosystems, state transitions influence redox poising of the photosynthetic electron transfer system (Rochaix 2011) and contribute to high-light acclimation (Allorent et al 2013; Bergner et al 2015). The translocation of LHCII between PSII and PSI is coordinated by its level of phosphorylation (Figure 3). In so-called state 1, LHCII is not phosphorylated and energetically coupled to PSII. In response to a reduced plastoquinone (PQ) pool, a Ser/Thr-phosphorylating (protein) kinase (STT7) phosphorylates LHC proteins, including LHCSR3, in a process involving the cyt b_6f (Vener et al 1997; Bergner et al 2015; Dumas et al 2017; Sarewicz et al 2021). Part of the phosphorylated pool of LHCII migrates to PSI where, in so-called state 2, it can act as antenna for PSI (Rochaix 2011). Dephosphorylation occurs via a PP2C-type phosphatase when the PQ pool is in an oxidised state. In *C. reinhardtii*, a major fraction of LHCII disassociates from PSII (Ünlü et al 2014; Nawrocki et al 2016), which decreases the level of chlorophyll fluorescence (Allorent et al 2013; Roach, Na 2017). Of note, *C. reinhardtii* has a large NADPH-dependent capacity for reducing the PQ pool in darkness (Bennoun 1982; Johnson, Alric 2012). This leads to *C. reinhardtii* phosphorylating LHC and inducing state 2 immediately after high-light treated cells are dark adapted (Figure 3; Roach, Na 2017).

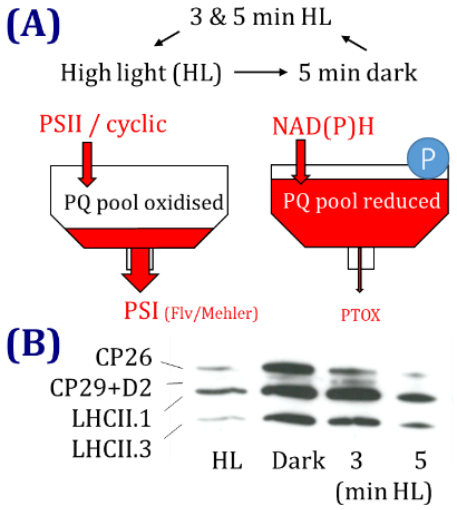


Figure 3. State transitions (qT) in response to light and dark. (A) The electron transfer system visualised as a 'sink' of electrons (e^-). In light, PSII activity pours e^- into the sink, which is emptied by PSI activity (redox state oxidised; state I). PTOX, FLV and Mehler may also contribute (Table 1). In the dark, PSII and PSI activity stop, but the sink can fill via Nda2 activity, leading to protein phosphorylation (blue P) of LHC proteins (redox state reduced; state II). **(B)** Western blot of phosphorylated proteins in response to sequential light treatments, from Roach and Na

3. Photosynthetic electron transfer pathways

The light-driven reaction of water splitting in PSII serves two main purposes: the release of protons and electrons. The build-up of a proton difference (ΔpH) across the thylakoid membrane is used, much like in mitochondria, to drive ATP synthase conversion of ADP + P_i to ATP, while electrons are used to reduce $NADP^+$ to NADPH (Figure 1). In the first part of the photosynthetic electron transfer system, electrons are passed from PSII to the PQ pool, subsequently to cyt b_6f, and finally via plastocyanin (PC) to PSI, where a second light-driven reaction is used to reduce stromal electron carriers, such as ferredoxin and eventually $NADP^+$ (Figure 1). Ultimately, ATP and NADPH are mainly used in the Calvin-Benson cycle to assimilate CO_2 into sugars. The additional release of O_2 from water-splitting in PSII also impacts photosynthetic electron transfer, but not directly, rather from photosynthesis operating in an oxidative environment. Oxygen can be used in at least three pathways as an electron-acceptor from the photosynthetic electron transfer system: (*1*) The plastoquinone oxidase (PTOX) oxidises plastoquinol to plastoquinone while reducing O_2 to H_2O (Figure 1, No. 6) and thus can poise the redox state of the PQ pool and influence qT (Houille-Vernes et al 2011); (*2*) Flavodiirons accept electrons from PSI while reducing O_2 to H_2O (Figure 1, No. 9), and are important when the Calvin-Benson cycle is inactive due to darkness and there is a sudden increase in light intensity, leading to a surge of electrons that cannot be used (Jokel et al 2015; Chaux et al 2017); (*3*) O_2 can also accept electrons directly in a non-enzymatic process called the Mehler reaction (Figure 1, No. 8), during which the ROS superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) are produced.

Any of the fore-mentioned processes that use O_2 as an electron acceptor avoid the formation of reducing equivalents (i.e. NADPH). Under stress or sub-optimal conditions when CO_2 assimilation is restricted, this can be important to prevent an imbalance of NADPH:ATP and an over-reduced electron transfer system and associated ROS production. When starch synthesis is compromised, a greater proportion of the electrons is directed toward O_2 reduction through both the FLVs and PTOX, suggesting an important role for starch synthesis in priming/regulating the Calvin-Benson cycle and electron transfer (Saroussi et al 2019). Another critical electron transfer process is the so-called cyclic electron transfer pathway (Figure 1, No. 7), either involving proton-gradient regulation (PGR) proteins [although controversially (Nawrocki et al 2019; Buchert 2020)], or NAD(P)H:ubiquinone reductase 2 (NDA2), proteins (Jans et al 2008).

Importantly, photoreduction of O_2, and especially the cyclic electron transfer pathway, promote ATP formation by using the light-driven reactions to create a ΔpH. Specifically relevant to algae, low CO_2 availability in the water-column has resulted in the evolution of carbon-concentrating mechanism (CCM) for RUBISCO, the first enzyme in carbon fixation, to efficiently access its substrate and prevent it reacting with O_2 (photorespiration). In H_2O, CO_2 dissolves into carbonic acid that disassociates to bicarbonate (HCO_3^-) and carbonate (CO_2^{2-}), which can be converted back to CO_2 in an acidic environment via CAH3 (Figure 1). Thus, the combined action of the cyclic electron transfer pathway and O_2 photoreduction generates a low luminal pH that is essential for CCM function (Burlacot et al 2022).

4. A very brief guide to the origin of chlorophyll fluorescence

Pulse-amplitude modulated (PAM) chlorophyll fluorescence is a non-invasive measurement of PSII activity. It can be used to calculate linear photosynthetic electron transfer rates towards an estimation of carbon assimilation, and serve as an invaluable indicator of organism 'health'. Within the thylakoid membrane, LHC that are also called 'antenna', facilitate in capturing photons to 'excite' electrons in pigments to higher energetic levels and subsequently trap this energy (Figure 1). Absorbed excitation energy has one of three fates, it can be (*1*) passed to the reaction centre chlorophylls (PSII and PSI) to drive and increase photochemistry, (*2*) released as heat (i.e. thermal dissipation, including an increase in qE), or (*3*) emitted as fluorescence which decreases energy available for fluorescence. Moreover, chlorophyll fluorescence intensity is also intricately linked to the redox state of the electron transfer system, because when electrons can no longer be passed from PSII into the PQ pool (i.e. it is already fully reduced) more energy is available for fluorescence.

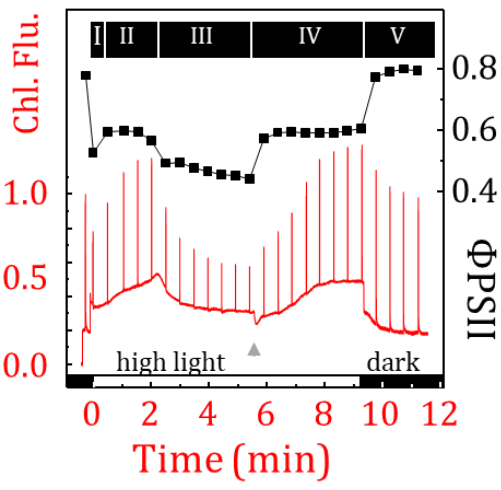


Figure 4. Effect of light-induced CO_2 depletion on NPQ. Placing high-light acclimated cells into darkness induces qT state 2 (Figure 3) and LHCII to decouple from PSII, decreasing F_m. Re-treating cells with high-light (white bar, X axis), causes transition to state 1 (Figure 3) and LHCII to couple with PSII, increasing F_m (red spikes; 0-2 min), and activation of qE (F_m quenching, 2-5 min) as CO_2 depletes. 1 mM HCO_3^- was added just before 6 min (grey arrow) relaxing qE. Effects on ΦPSII are shown (squares, right Y-axis) and corresponding NPQ phases I to V (top black boxes) are described in the text.

In practice, saturating pulses of light that fully reduce the PQ pool lead to maximum fluorescence (F_m), and are repeatedly applied (e.g. 20 s intervals), as evident by the fluorescence spikes in Figure 4. The ratio between F_m and minimum fluorescence just before the pulse (F_o in dark; F_t in light) is used to calculate the quantum efficiency of PSII (i.e. potential photochemistry), which in the dark is at a maximum (so-called F_v/F_m), and lower in the light (so-called ΦPSII) because NPQ is active. NPQ is calculated by changes in F_m (see below). For a review on chlorophyll fluorescence measurements read

Maxwell and Johnson (2000). The ΦPSII changes depend on CO_2 availability and NPQ, as shown in a PAM quenching kinetics of photoautotrophic *C. reinhardtii* cells (Figure 4).

5. Overlapping NPQ mechanisms confound chlorophyll fluorescence measurements

NPQ is calculated from a chlorophyll fluorescence analysis by $(F_m°-F_m')/F_m'$, with $F_m°$ and F_m' measured before (usually dark-acclimated) and during (e.g. in light) treatment, respectively. Thus, quenching of F_m is indicative of NPQ. In Figure 4, the change in F_m reveals five distinct NPQ phases during a dark-light-dark treatment of pre-high-light acclimated *C. reinhardtii* cells. These phases have been resolved using mutants deficient qE (i.e. *npq4*) and qT (i.e. *stt7*) and with the H^+ ionophore nigericin (Allorent et al 2013; Roach, Na 2017), and are (*1*) immediate qE of state 2 cells, (*2*) qT transition from state 2 to 1, (*3*) continued induction of qE of state 1 cells as CO_2 depletes, (*4*) relaxation of qE, in this case after HCO_3^- was added, as also occurs with addition of nigericin (Figure S1), and (*5*) qT transition from state 1 to 2 of cells in the dark (Figure 4). When photosynthesis is hindered due to low CO_2 availability, activation of qE lowers ΦPSII since an increase in light energy is partitioned to thermal dissipation rather than driving photochemistry. At room temperature, the level of fluorescence from PSI and associated antenna is lower, and often disregarded, although for accurate calculation of electron transfer rates it needs consideration (Pfündel et al 2013). At much lower temperatures (e.g. at 77 K; –196 °C), biochemical and physiological processes that modulate chlorophyll fluorescence are mostly abolished, and the fluorescence emissions of PSI and PSII are distinguishable. An aliquot of cells measured in Figure 4 was removed for analysis at 77 K (Figure 5). Here, it becomes obvious that the ratio of fluorescence originating from PSII and PSI changes throughout the treatment as qE activates and relaxes, and qT transitions occur between states 1 and 2.

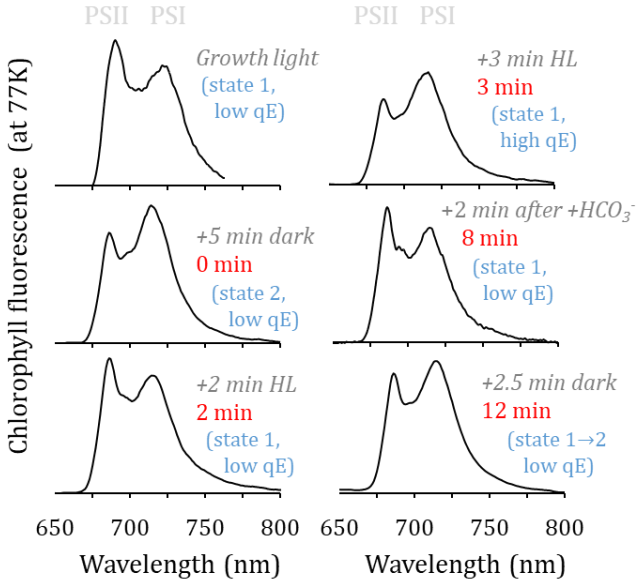


Figure 5. Effect of light treatments and CO_2 depletion on chlorophyll fluorescence measured at 77 K. Fluorescence peaks at 685 and 715 nm correspond to PSII and PSI emission, respectively (excitation at 440 nm). Samples were from cells treated in Figure 4 at 0, 2, 3, 8 and 12 min (red), with major influence of most recent treatment (*italic* grey) and interpreted NPQ state (blue) given for each measurement.

Although different aspects of NPQ are regulated by distinct mechanisms (Table 1), it is known that individual processes affect others, such as transition to state 1, increasing antenna size inducing a more rapid activation of qE (Allorent et al 2013), and LHCSR3 also influencing qT (Roach, Na 2017). Further complexity arises from temporal overlap between, e.g. NPQ phases I-III activated in response to light, which each have opposing effects on fluorescence. Similarly, if HCO_3^- had not been added to cells in Figure 4, qE would have relaxed later in the dark (increasing F_m as shown in Figure 6A) while cells transition to state 2 (decreasing F_m). What also becomes obvious from such analyses is that the light acclimation state of cells has a significant impact on NPQ phases. For example, not only does the qE capacity increase in high-light acclimated cells (Figure 2), but qT is also amplified (Figure 6A). This may involve a change in activity level of processes described in Table 1. In various NPQ mutants the increase in F_m between 0.5 and 3 min (NPQ phase II; Figure 4), attributed to transition to state 1, consistently shows a positive relationship with the level qE after 10 min high-light (NPQ phase III; Figure 4), suggesting that the qE protein in *C. reinhardtii*, LHCSR3, has a role in qT (Figure 6B). In agreement with this, some increase in F_m occurred in *stt7-9* (Figure 6B), indicating processes in addition to LHC de-phosphorylation are involved in NPQ phase II.

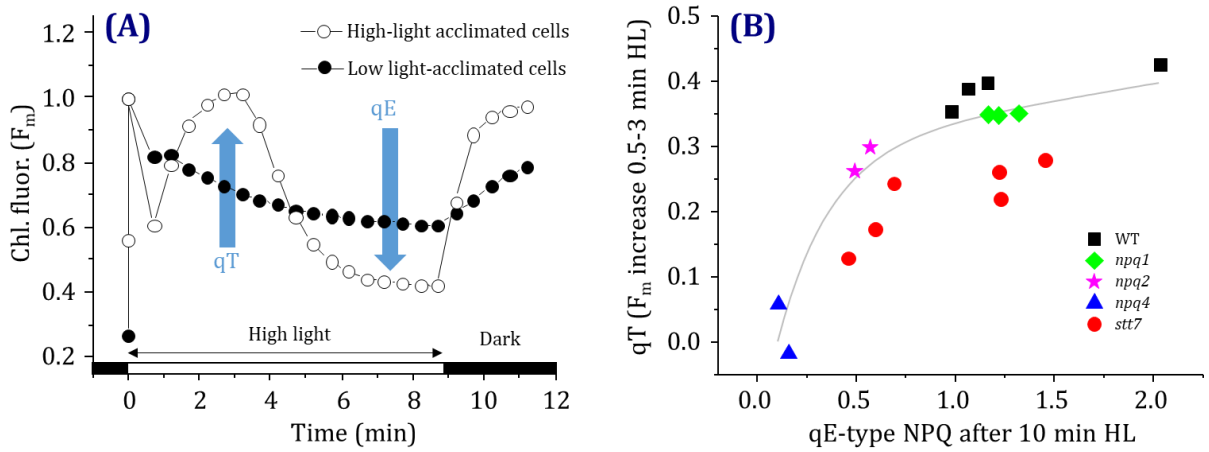


Figure 6. Effect of light-acclimated state on activation of NPQ processes. (A) *C. reinhardtii* photoautotrophic cells were either low- or high-light acclimated and dark adapted for 10 min before measuring chlorophyll fluorescence at 450 µmol photons·m^{-2}·s^{-1} (high-light) and darkness. Symbols are F_o at 0 min, and F_m thereafter (both normalised to F_m°), with influence of qT transition to state 1 and qE activation depicted with blue arrows. **(B)** Correlation of F_m increase between 0.5 and 3 min after dark to high-light treatment, with qE after 10 min high-light, in *C. reinhardtii* wild type (WT) and various NPQ mutants (see Table 1) high-light acclimated for 0-4 h.

Accelerated metabolism in high-light acclimated cells and cross-organelle metabolite exchange, potentially leading to more rapid fluctuations in NADPH and ATP levels, no doubt contribute to energy regulation, although our understanding of such short-term metabolism at the sub-cellular level is poor. Coupling non-invasive measurements, such as chlorophyll & NAD(P)H fluorescence, and net O_2 flux, with profiling of primary metabolites will provide a more holistic understanding of how photosynthesis responds to changes in light intensity.

6. A protocol for quantifying NPQ phases with PAM chlorophyll fluorescence in liquid *C. reinhardtii* cultures

Considering the potential complexity of deciphering PAM chlorophyll fluorescence of *C. reinhardtii*, there is a need for protocols that allow distinguishing the individual NPQ components. The suggested protocol has been created to enable one mechanism at a time to dominate, enabling accurate quantification of associated chlorophyll fluorescence kinetics. As shown in Figure 7, specific sequences of light treatment of defined duration (Figure 7A) enable kinetics associated with each of the NPQ phases (in brackets), introduced in Figure 4, to be quantified in a photoautotrophic liquid culture in <20 min. These include: **0-0.5 min** (I: immediate qE), **0.5-2 min** (IIa: rapid qT transition to state 1), **3-5 min** (IIb: slower qT transition to state 1), **5-7 min** (III: CO_2 depletion-induced qE), **9-10 min** (IV: qE relaxation), **11-13 min** (V: qT transition to state 2), and an additional phase **14-18 min** (VI: qT transition to state 1) in response to far-red light under which PSI activity occurs greater than PSII activity, oxidising the PQ pool (Figure 4). For calculating qE, F_m measured at the end of far-red light treatment when cells are in state 1 should be used rather than F_m before actinic light treatment when cells are in state 2, otherwise qE will be underestimated. Importantly, consideration needs to be given to acclimation time to any change in trophic status or light intensity before performing experiments. At least 24 h is recommended if cells are to be considered acclimated to a specific state, but breaking or training circadian rhythms can take longer (Mittag et al 2005).

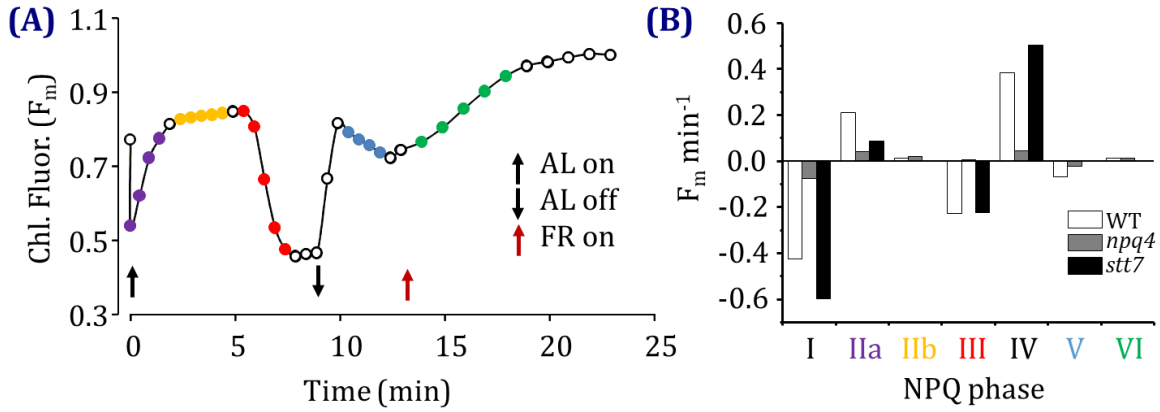


Figure 7. A PAM protocol to semi-quantify NPQ phases. (A) Changes in F_m in response to actinic light (AL on) of 450 µmol photons·m^{-2}·s^{-1}, darkness (AL off), and far-red light (FR on) of maximum intensity using a PAM 2500 (Walz, Effeltrich, Germany). 2 mL of wild type *C. reinhardtii* culture at 7.5 µg chlorophyll·mL^{-1} and acclimated to 250 µmol photons·m^{-2}·s^{-1} were incubated in a 3 mL cuvette and dark-treated for 15 min before starting and constantly stirred with a mini rotating magnetic disk during measurement. Saturating pulses to measure F_m were every 30 s, or 2 min in FR. Colour symbols and NPQ phases are explained in the text. **(B)** Kinetics of each NPQ phase calculated for photoautotrophic WT, *npq4* and *stt7-9* expressed as change in F_m min^{-1}.

For proof of concept of the PAM protocol and confirmation of NPQ phases, the *npq4* and *stt7-9* mutants were measured in the same way (Figure 7B), showing that NPQ phases I, IIa, III and IV are heavily influenced by LHCSR3, while IIb, V and VI are STT7-dependent, as reported by Roach and Na (2017). Of note, the *stt7-9* mutant is 'leaky' and

can still phosphorylate LHCSR3, but insufficiently phosphorylates other LHC to enable qT (Bergner et al 2015). It is worth considering that longer dark-treatments before measurement increase the duration of phase IIb (Roach, Na 2017). Furthermore, the intensity of actinic light during measurement exerts an influence, with a higher light intensity activating qE earlier and reducing the time before induction of phase III. The density of culture also has a major influence, not only on the intensity of chlorophyll fluorescence signal, but also on how quickly CO_2 depletes from the media, and how much self-shading occurs within the culture (Vera-Vives et al 2022). Finally, if cells have been high-light treated before measurement, then recovery from qI may lead to a gradual increase in F_m over time as PSII is repaired. This has implications for calculating NPQ, since reference is typically made to the first F_m before light treatment during fluorescence measurements.

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Abbreviations

BST	Bestrophin-like proteins
CAH3	carbonic anhydrase 3
cyt b_6f	cytochrome b_6f complex
Fd	ferredoxin
FLA	flavodiiron
F_m/F_o	maximum/minimum chlorophyll fluorescence values
LHC	light-harvesting complex
LHCSR	LHC-stress-related protein
NPQ	non-photochemical quenching
PAM	pulse-amplitude modulated
PC	plastocyanin
ΔpH	trans-membrane hydrogen ion difference
ΦPSII	relative quantum yield of PSII
PsbS	subunit S of PSII
PSII/PSI	photosystem II/I
PQ	plastoquinone
LHC	light-harvesting complex
LHCSR	LHC-stress-related protein
NPQ	non-photochemical quenching
qE	thermal dissipation-type NPQ
qI	photoinhibition-type NPQ
qT	state transition-type NPQ
ROS	reactive oxygen species
STT7	STT7 kinase

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Supplementary figure

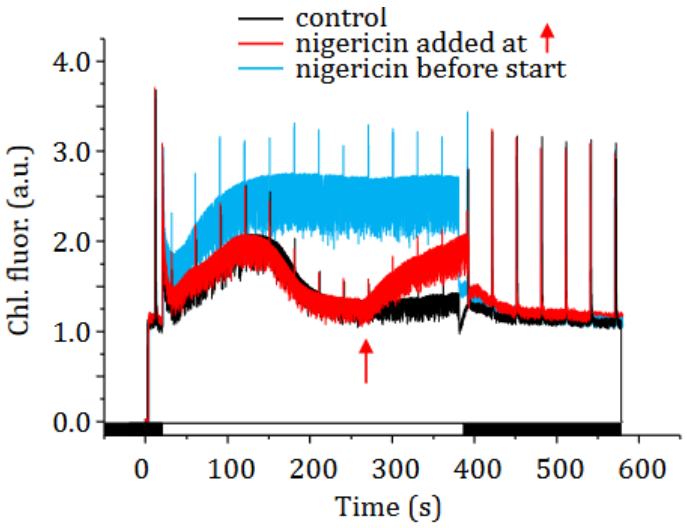

Figure S1. Effect of 10 µM nigericin, either added just **before** or **during** (at arrow), compared to **control (without)**, on chlorophyll fluorescence traces of high-light acclimated and 10 min dark-treated cell wall-less (cw15) photoautotrophic *Chlamydomonas reinhardtii*. Light intensity during the analysis was 476 µmol photons·m^{-2}·s^{-1} (white bar X-axis). Saturating pulses, leading to chlorophyll fluorescence spikes (F_m) were made every 30 s.