# A universal mathematical model of non-photochemical quenching to study short-term light memory in plants

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**The concept of plant memory triggers a quite controversial dispute on the definition, extent or even existence of such. Because plants are permanently exposed to rapidly changing environments it is evident that they had to evolve mechanisms enabling them to dynamically adapt to such fluctuations. Recognizing memory as a timed response to changes of external inputs through amplification and integration of multiple signals, here we study the short-term illumination memory in *Arabidopsis thaliana* by monitoring fluorescence emission dynamics. For this, we designed an experiment to systematically determine the extent of non-photochemical quenching (NPQ) after previous light exposure. We propose a simplified, mathematical model of photosynthesis that includes the key components required for NPQ activation. Due to its reduced complexity, our model is universally applicable to other species, which we demonstrate by adapting it to the shadow-tolerant plant *Epipremnum aureum*. We demonstrate that a basic mechanism of short-term light memory, which is based on two interacting components, can explain our experimental observations. The slow component, accumulation of zeaxanthin, accounts for the amount of memory remaining after relaxation in darkness, while the fast one, antennae protonation, increases quenching efficiency. With this combined theoretical and experimental approach we provide a unifying framework that helps to uncover general principles of key photoprotective mechanisms across species.**

Plants require light for photosynthesis, but excessive light is dangerous, because it can inflict irreparable damage to the photosynthetic apparatus. As sessile organisms, plants therefore require adaptive mechanisms to dynamically react to changing light conditions. A common strategy that has evolved in eukaryotic phototrophs [1] is the dissipation of excess absorbed energy as heat, through processes collectively termed as non-photochemical quenching of chlorophyll a fluorescence (NPQ) [2, 3]. It is moreover plausible to assume that plants can somehow store information about the illumination history to optimize their photosynthetic performance simultaneously avoiding damaging effects of over-excitation. Such an effect can be termed memory, defined as a timed response to changes to previously experienced light conditions. Here, we systematically investigate the possible effects of previous light exposure on the NPQ dynamics to quantify short-term memory in plants, elaborating on previous results linking NPQ to memory [4, 5, 6, 7].

Our knowledge on the molecular mechanisms of NPQ increased significantly over the past 40 years, where a breakthrough was achieved by discovering the role of the xanthophyll cycle in photoprotection [8] in the late 80s. Subsequently, a number of models were proposed providing strong evidence for at least two processes contributing to quenching: a zeaxanthin (Zx) dependent process [9] and another one dependent on the protonation of the PsbS protein [10]. Nevertheless, open questions on the specific roles, sites [11] and interactions of the quenching components led to the proposition of various contrasting models supported by *in vitro* studies. After testing various possibilities in computer models *in silico*, we implemented here the 4-state 2-site model of NPQ after [12], in which the strongest quenching is obtained when both components are active simultaneously, but each has an effect on its own.

Recent studies suggest that the photoprotective behavior in plants can be trained and related information is stored as a cellular light memory [7, 4]. Moreover, the memory of the NPQ state was linked to the hydrophobicity parameter (H-parameter) of xanthophylls [5], showing that increased light exposure will cause an increase in zeaxanthin concentration and make NPQ more responsive to further illumination.

Motivated by this apparent connection between the xanthophyll cycle and NPQ induction [13], we systematically investigated whether a memory of light exposure can be detected on the time-scale of minutes to hours. Using pulse amplitude modulated (PAM) chlorophyll fluorescence analysis we quantitatively investigated the effect of short-term

light memory on NPQ by comparing the fluorescence patterns of the first and second light periods. In the present study (see Fig. 1A), we examined two factors affecting the light memory in plants: intensity of incident light (varying from 100 to 900 $\mu$Em$^{-2}$s$^{-1}$) and the relaxation time between first and second light exposure (15, 30 or 60 min).

Based on our results we constructed a mathematical model to test hypotheses regarding the mechanisms of the memory effect. We previously argued [14] that a major challenge of theoretical biology is to provide simple, yet robust mathematical models that are not specially tailored for one model organism but describe a variety of species. Only such cross-species models will allow discovery of common generalized principles while understanding the species-specific features. Existing mathematical models of high-energy state quenching (qE) are either too simplistic by aiming at reproducing the key biological features of quenching with minimal complexity [15] or too detailed and specialized focusing on quantification of the beneficial effect of qE under extreme light conditions in *Arabidopsis thaliana* [16]. Therefore, here we developed a simple model of the photosynthetic chain with maximally reduced parameter space, but including all important mechanistic aspects of NPQ to specifically study possible short-term light memory. Moreover, the model is designed for an easy adaptation to different organisms.

The model was initially calibrated for the model organism *Arabidopsis thaliana*, a sun-tolerant higher plant. Its universal application is demonstrated by adapting it to the non-model organism *Epipremnum aureum*, a shadow-tolerant, ornamental plant, for which measured kinetic parameters are sparse. Our model is able to realistically reproduce experimentally obtained fluorescence traces and simulate all main features of chlorophyll induction, including transient activation of NPQ [17], the dynamics of fluorescence relaxation in darkness and qualitative differences in the quenching response to different light intensities. Thus, the model serves as a theoretical framework in which the role of the main quenching components can be computationally assessed and existing hypotheses on short-term light memory can be tested.

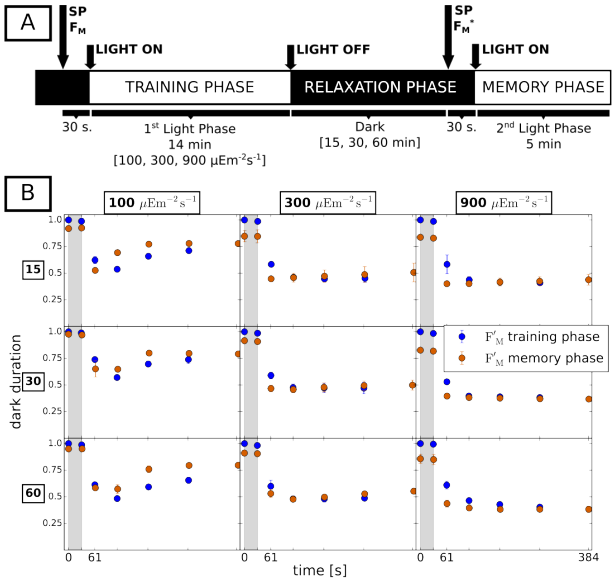


**Figure 1:** (A) Design of the experiment. Dark adapted plants were exposed to a first saturating pulse of light (SP) from which physiological parameters such as maximal fluorescence ($F_M$) and photosynthetic yield ($\Phi$PSII) were derived. After 30 s of darkness a second SP was applied and the light was switched on for a fixed (training) period of 14 minutes. SPs were applied in a defined sequence (see Fig. S1), yielding maximal fluorescence ($F'_M$). The training period was followed by a dark period, interrupted by six SP to follow the fluorescence relaxation dynamics. Subsequently, in the second (memory) period, the same illumination intensity as in the training period was applied. Each experiment was repeated three times for three light intensities (100, 300 and 900 $\mu$Em$^{-2}$s$^{-1}$) and different relaxation times (15, 30 and 60 min). (B) Comparison of $F'_M$ measured in the first (blue points) and second (red points) phase of light. The first measurement taken in the light (at 61 s) is lower in the memory phase than in the training phase indicating faster NPQ induction. Error bars indicate standard deviations for three replicates.

# NPQ and plant light memory

## Higher sensitivity of NPQ in the memory phase

We first analyzed whether the quenching patterns differ between the two phases of light. We directly analyzed the originally measured maximal fluorescence ($F'_M(t)$) data instead of derived NPQ values (provided in Fig. S3), to avoid mathematical distortion of the kinetics and provide more reliable information on the mechanism [18]. Fluorescence measurements are a non-invasive method for monitoring photosynthetic dynamics, providing information on the photosynthetic efficiency, protection and energy dissipation. However, each measurement can only be relative [19] and therefore first each experimental data is normalized to the maximal fluorescence (measured after the first saturating pulse of light applied to a dark adapted plant: $F_M$) and then averages and standard deviations of the replicates are calculated. On Fig. 1B we visualize the maximal fluorescence kinetics in the first (training) and second (memory) light phase (shifted to the time 0). It can be observed that for all light intensities the first measurement in light (at 61 s) is consistently lower in the memory phase (see Tab. S1 for the statistical significance). This timed response to previously experienced illumination clearly demonstrates a short-term memory.

## Slow relaxation kinetics

To test whether this observation can be explained by an incomplete relaxation of NPQ during the dark period, we compared the last $F'_M$ in the relaxation phase (denoted $F_M{}^\star$) to $F_M$. The results confirmed that the observed lower $F'_M$ in the second light phase is, at least partially, caused by non-complete relaxation during shorter dark phases (Fig. S2). The analysis of the ratios of these two values highlights a combined effect of light intensity and the time spent in darkness on the ability of relaxing the fluorescence.

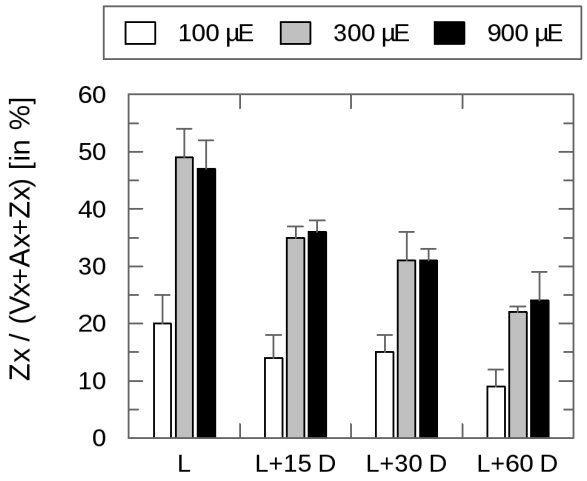


**Figure 2:** Pigment composition at the end of each phase (L=light, D=dark) presented as ratios of zeaxanthin (Zx) to total xanthophylls (Vx + Ax + Zx).

## Pigment concentration

To investigate if the memory can be associated with zeaxanthin-dependent quenching we measured the pigment composition at the end of each phase of the experiment (full analysis summarized on Fig. S5). Figure 2 shows that after exposing the samples for 15 minutes to high light intensities, Zx levels significantly increased up to 50% of all xanthophyll cycle pigments (sum of violaxanthin, antheraxanthin (Ax) and zeaxanthin). Simultaneously, one hour in dark was sufficient to reduce this by half, explaining lower quenching effects in samples kept for a longer periods in dark. This decrease was not as pronounced under illumination with the lowest light intensity.

# Mathematical Model

Based on our experimental results and our current understanding of NPQ, we developed a small kinetic model to verify our hypothesis on the induction of light memory. A general schematic of the model is shown in Fig. 3 and a mathematical description of the components as well as the source code to solve the system numerically can be found in the *SI Text*. Since the variable chlorophyll fluorescence originates from the antennae associated with PSII [20, 21], we limit our model to the photosynthetic reactions around photosystem II, reducing the system to only six differential equations and 41 parameters (Tab. S3). Because a number of applied simplifications (see *SI Text* for justification and details) may raise concern whether the system will exhibit a biologically meaningful steady state under extreme conditions, we investigated the stationary redox state of the plastoquinone pool (PQ) over different light intensities, demonstrating that it reaches plausible ratios of ∼50% for lower light intensities (see Fig. S4).

## Quencher requirements

The high-energy-state quenching represents the main component of NPQ and is induced on a time-scale of seconds to minutes [22]. Several factors are known to contribute to NPQ induction. A fast one requires the generation of a proton gradient (ΔpH) in the thylakoid membrane and a slow one is activated by low lumen pH. The quencher mechanism implemented in the model is based on a four state model [12], where the fully relaxed dark state (state I) is characterized by maximal Violaxanthin (Vx) concentration and de-protonated PsbS protein. In high light, a proton gradient is rapidly established, and PsbS acts as a proton sensor thus activates quenching, constituting the fast component of quencher (state II). Further reduction of the lumen pH (to 5.8) triggers de-epoxidation of Vx to Zx, leading to state III of a fully activated NPQ. From this, a transfer to darkness results in relaxation of ΔpH, and concomitant de-protonation of PsbS, leading to state IV, which still contributes to the overall quencher activity because of the slow relaxation of Zx (see Fig. 3).

These considerations lead to the overall equation for the quencher activity:

$$\begin{aligned} Q = \gamma_0 \cdot (1 - Z_s) \cdot [\mathrm{PsbS}] + \gamma_1 \cdot (1 - Z_s) \cdot [\mathrm{PsbS^P}] \\ + \gamma_2 \cdot Z_s \cdot [\mathrm{PsbS^P}] + \gamma_3 \cdot Z_s \cdot [\mathrm{PsbS}], \end{aligned} \quad (1)$$

where $Z_s = \frac{[\mathrm{Zx}]}{[\mathrm{Zx}]+k_{\mathrm{ZSat}}}$ reflects the contribution of Zx to the quencher and $k_{\mathrm{ZSat}}$ is a half-saturation constant. The overall concentration of xanthophylls ([Vx] + [Zx]) is assumed to be constant and the temporal changes of [Vx] and [PsbS] are determined by appropriate differential equations (described in the *SI Text*, Eq. 9 and 10). The $\gamma$ parameters were fitted to the fluorescence traces and their effect on the steady state and quencher activity was extensively studied using metabolic control analysis [23] (Fig. S6). The residual quenching from state I ($\gamma_0$) originates from other not included or not even identified players and was calibrated to yield the base quenching as observed for quenching mutants [9].

## Transiently generated NPQ

It was observed that illuminating dark-adapted plants with nonsaturating light transiently induces a strong non-photochemical quenching [20, 13], which is explained by the transient generation of a transthylakoid pH gradient [24]. This transient NPQ relaxes within a few minutes when the ΔpH is reduced due to delayed activation of ATP and NADPH consuming reactions, including the $H^+$-ATPase [25]. In our simplified model focusing on PSII, we therefore included a delayed activation of ATPase, by which we could realistically reproduce the overall dynamics of transient NPQ activation upon dark-light transition. Thus, ATP synthesis is mediated by an active form of ATPase (denoted ATPase*), which dynamically changes over time and directly depends on the light availability:

$$\begin{aligned} \frac{d\mathrm{ATPase^*}}{dt} = & k_{\mathrm{actATPase}} \cdot \mathrm{H(PFD)} \cdot \mathrm{ATPase} \\ & - k_{\mathrm{deactATPase}} \cdot (1 - \mathrm{H(PFD)}) \cdot \mathrm{ATPase^*}, \end{aligned} \quad (2)$$

where H(x) is the Heaviside function and PFD is the light intensity.

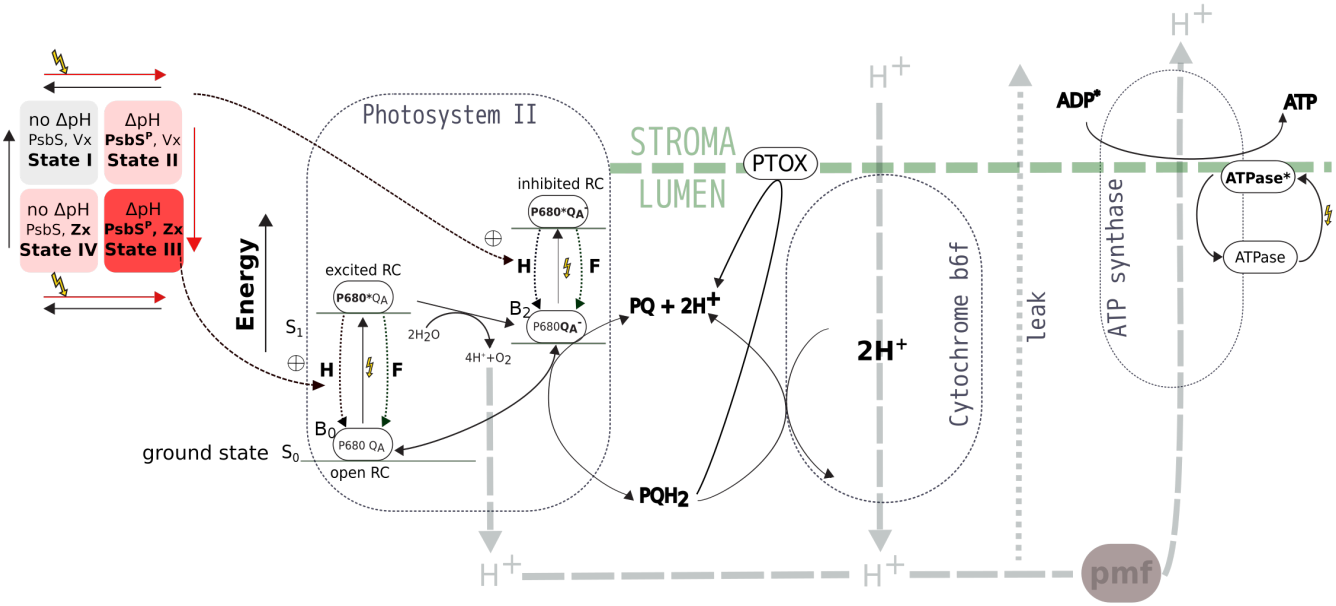


**Figure 3:** Scheme of the components included in the simplified mathematical model including a detailed description of internal processes occurring inside photosystem II (for more details see the *SI Text*) that are directly affected by the four state quencher. The plastoquinone pool (PQ) is reduced by PSII, which also releases protons into the lumen by the oxygen evolving complex. PQ can be oxidized by cytochrome b6f and PTOX. Oxidation by cyt b6f is coupled to proton translocation from stroma to lumen. Protonation of the lumen drives the production of ATP through the ATPase. The ATPase is active in the light and inactive in the dark. Protons can also passively leak out of the thylakoid lumen.

## Dynamic simulations

Our model is capable of reproducing the fluorescence traces simulating various PAM protocols using low, moderate and high light intensities. In the model description, fluorescence is not a system variable. We therefore calculate it from the rate at which excited chlorophyll will revert to its ground state through fluorescence ($k_F$) and not quenching ($k_H$) or photochemistry ($k_P$), and use the fact that the signal is proportional to the occupation of the two ground states of PSII RCs ($B_0$ and $B_2$ in Fig. 3) [15, 26]:

$$\Phi = \frac{k_F}{k_H \cdot Q + k_F + k_P}[B_0] + \frac{k_F}{k_H \cdot Q + k_F}[B_2], \qquad (3)$$

where $k_H \cdot Q$ is the rate of NPQ, modulated by the quencher activity $Q$ (Eq. 1). By reading out the fluorescence values we can reproduce *in silico* all experimentally obtained fluorescence measurements (Fig. 5, S3).

A clear benefit of computer simulations is the possibility of following the dynamics of otherwise hard to measure molecules. In Fig. 4 we demonstrate how lumenal pH changes in the response to different light intensities and how quenching components saturate under high light and relax in the darkness. The trajectories in Fig. 4A visualize the different time scales on which the system operates and illustrate the memory. The quencher remains active, which can be seen because the trajectories do not revert back to the initial dark state after the dark relaxation phase.

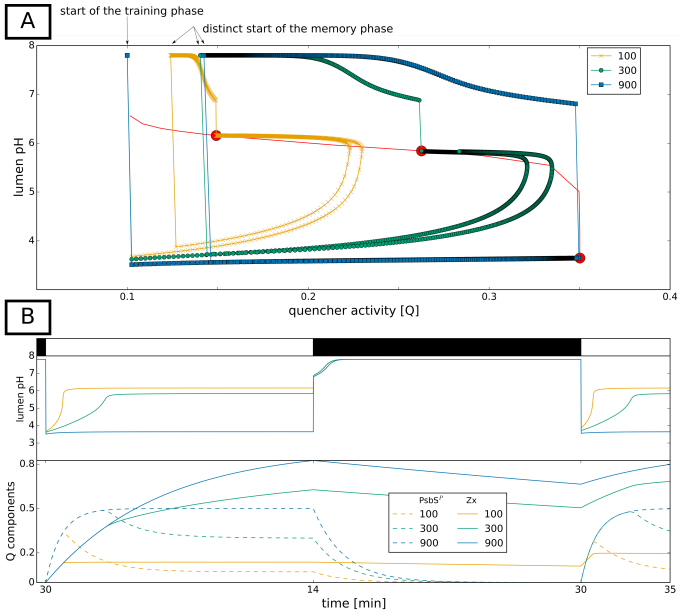


**Figure 4:** (A) The phase plane trajectories of the quenching variable (Q) and the lumenal pH during the light memory protocol. The red line depicts stationary states calculated for different light intensities. The markers on the trajectories are set in regular intervals of 1 s to visualise the different time scales on which the system is operating. (B) Internal variables for three different light intensities. The upper panel shows how the ability of relaxing the lumenal pH is lost with increasing light intensity. The bottom panel shows the dynamics of the quenching components (solid line for the relative zeaxanthin concentration, dashed line for the ratio of protonated PsbS).

## Application to a non-model organism

It is a well recognized issue in the field of systems biology that modeling biological processes often requires acquiring a number of parameters [27] of which some might have a physical meaning, some may be a rough approximation

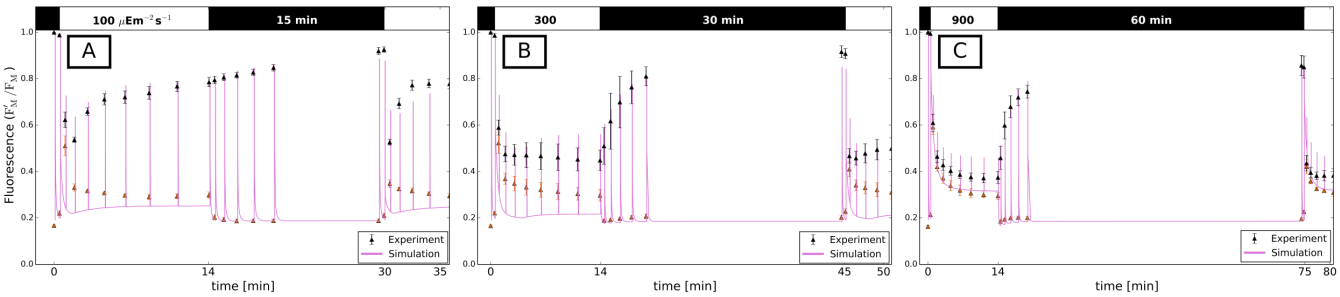


**Figure 5:** Pulse amplitude modulation traces of wild type *Arabidopsis* obtained experimentally (with error bars) and simulated by the model for three different setups. The model is capable of reproducing the transient drop in fluorescence in the first minute of light exposure under low light intensity (A). This property is lost under higher light intensities (B). The model also correctly reproduces the relaxation in darkness after one hour (C).

of measured values, others may be fitted and some may be simply impossible to measure with current techniques. Much focus was put on developing optimization algorithms that will ease parameter estimation, but the only safe way to reduce the risk of over-fitting is to minimize the dimensions of the parameter space. One of the difficulties of using available and published kinetic models is their often huge parameter space, which makes them hard to adapt to study analogous mechanisms in other species. To demonstrate that our model is of sufficient simplicity to allow for an easy adaptation to a new and not extensively studied organism, we have adapted our model to the ornamental, shadow-tolerant plant *Epipremnum aureum*, also referred to as *Pothos*. This choice was motivated by the finding that shade-tolerant plants are characterized by longer lasting memory for leaf illumination, as compared to plants found in semi-arid climates [6].

We therefore collected the fluorescence data for *Pothos* using the same experimental setup as described above for *Arabidopsis* (see Figs. S1 and S2). The analysis of the NPQ dynamics demonstrated that under identical conditions this plant exceeds the quenching capacity of *Arabidopsis* (Fig. S3) confirming similar observations on other shadow-tolerant plants [6]. In fact, under moderate light *Pothos* already behaves like *Arabidopsis* exposed to high light. With no additional information available, we assumed that the basic principle of the protecting mechanism is the same in the two plant species, but since *Pothos* exhibits higher quenching capacity, we can reflect it by increasing the parameter $\gamma_2$. We measured the chlorophyll content in both species (Tab. S2) and found a 70% higher content in *Pothos* than in *Arabidopsis*. With limited information on the electron transport chain protein abundance, we kept the same values of all internal parameters as for *Arabidopsis* and explained the more sensitive quenching response to light by increasing the factor converting photon flux density to light activation rate. With only those two changes in the parameter space we reproduced the experimentally observed fluorescence and photosynthetic yield kinetics for *Pothos* (Fig. 6). Our simulation results allow us to hypothesize that the enhanced quenching capacity can be explained by a more efficient energy transfer from the chlorophylls to the quencher. Possible molecular explanations might be an increased number of quenching sites or a closer spatial arrangement.

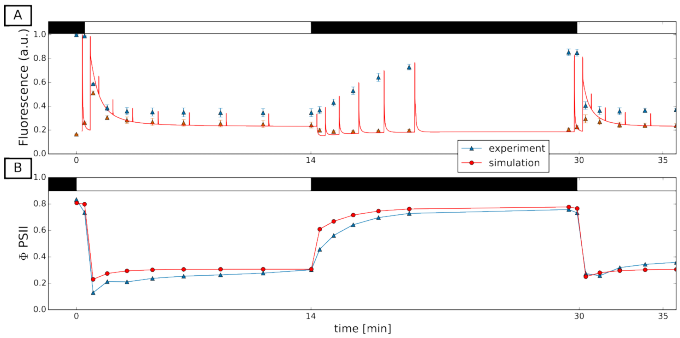


**Figure 6:** (A) Measured and simulated fluorescence traces for *Pothos* exposed to light of 100 $\mu$Em$^{-2}$s$^{-1}$ intensity. Minimal change into the parameter space of the model enabled reproducing all features of the fluorescence dynamics. (B) Inhibitory effect of light on the photosynthetic activity (expressed in terms of maximal yield) is reproduced in our simulation.

## Results and Discussion

We have presented a new mathematical model of NPQ, and employed the model not only to accurately describe the rapidly reversible components of non-photochemical quenching, such as the previously published models [15, 16], but further used it to explain the phenomenon of short-term light memory. Our model accurately simulates the changes in the fluorescence yield at low, moderate and high light intensities. It further provides an explanation for the higher extent of quenching observed for plants which have previously been illuminated. Moreover, it supports the notion that the same organizational principles of photoprotective mechanisms are present in plants as different as *Pothos* and *Arabidopsis*.

The concept of plant memory is still quite controversial, especially in the context of plant intelligence [28, 29]. In this work, we do not intend to discuss or even provide arguments for the latter. Rather, we demonstrated with a simple experimental setup with two light exposure periods separated by a varying relaxation time that the extent of quenching does indeed depend on how long ago and how much light a plant has previously experienced – a behavior which can safely be termed memory. We could demonstrate experimentally and theoretically that this light memory can be attributed to the slow quenching component associated with the de-epoxidation of violaxanthin to zeaxanthin,

which is triggered by low lumenal pH. In the dark, epoxidation of zeaxanthin to violaxanthin occurs slow, so that even after 30-60 minutes the conversion is not complete. In a second exposure to light, the rapidly protonated antennae PsbS-H further contribute to quenching with an increased efficiency when zeaxanthin is still present.

This conclusion is supported experimentally by direct comparison of fluorescence traces, demonstrating that light memory is affected by the length of darkness, which again determines the residual levels of zeaxanthin at the last point of darkness (Fig. 2). Further theoretical support is provided by our simulations of the mathematical model of NPQ, which has been constructed based on widely accepted quenching mechanisms [12] (Fig. 4).

Despite its simplicity, the model structure allows testing various hypothesis on the molecular mechanisms of quenching in mutants impaired in their quenching capabilities. Our simulations for the npq4 mutant, which is lacking the pigment-binding protein associated with photosystem II, but has a normal xanthophyll cycle, are in agreement with previously published work on that mutant [30] (Fig. S7). Although mutant analysis is not in the focus of this research, this again demonstrates the flexible use and adaptability of our model and indicates its value when interpreting experimental results.

The carefully chosen selective model reduction helps to identify common underlying principles of NPQ in different photosynthetic organisms, and we expect that the model should be easily adapted even to distantly related species such as diatoms, where despite a different molecular nature of the xanthophylls, the cycle still operates according to the same principles as in higher plants [1].

Finally, thanks to the modular structure of the model, it should be a straightforward exercise to utilize this work in the context of a more detailed model of photosynthesis, such as the model on state-transitions in *Chlamydomonas reinhardtii* previously published by us [26], which does not include any mechanistic details of energy-dependent quenching.

## Concluding remarks

We have demonstrated that there exists an observable difference in the quenching dynamics when plants have previously experienced light exposure. Two components of NPQ interact to generate this short-term light memory. The slower one, accumulation of zeaxanthin, accounts for the amount of memory lasting after relaxation in darkness, while the fast one increases the efficiency of quenching. However, our experiments do not provide evidence for an acceleration of quenching activity by previous light exposure. Rather, we propose to explain the consistently lower $F'_M$ in the first seconds of the second light period by accumulation of Zx only. Therefore, plants with active short-term memory of previously experienced light initiate their photoprotection with some head-start, but at the same speed. Moreover, our computational model supports hypotheses on why shadow-tolerant plants exhibit a higher quenching capacity. Together with this manuscript we provide all necessary files to repeat and perform further experiments *in silico*, therefore we encourage our readers to treat this adaptation as an example of how our model can be used to test hypotheses regarding NPQ in other, also less studied organisms.

**Plant materials and growing conditions** *Arabidopsis* (*Arabidopsis thaliana* ecotype Columbia 0) wild-type and *Pothos* (*Epipremnum aureum*) were grown in soil at the temperature of 23°C under light intensity of 90-100 $\mu Em^{-2}s^{-1}$ with a 16 hours light/8 hours night regime. Detached leaves from three-weeks old *Arabidopsis* and two-months old *Pothos* plants were used for the measurements. To ensure perfect relaxation, plants were dark adapted for at least 12 h before the measurements.

**Fluorescence measurements** Chlorophyll fluorescence measurements were taken using PAM-2000 fluorometer (Heinz Walz, Germany) with external Halogen lamp and a DT-Cyan special interference filter (eliminating $\lambda >$ 700 nm) mounted directly on the lamp. A leaf attached to the measuring window of the fluorometer was illuminated by a train of weak probing flashes (475 nm). The average intensity of measuring light was $0.1\mu Em^{-2}s^{-1}$ and saturating pulses of light was 2650 $\mu Em^{-2}s^{-1}$. The exact times of pulse application are to be found in the *SI Text*.

**Pigment analysis** For pigment extraction, frozen plant material was homogenized in presence of cold acetone. Unsolubilized material was removed by short centrifugation and the pigment content of the acetone extract was analyzed by reversed-phase HPLC according to Frber *et al.* [31].

**Simulations** The differential equations were numerically solved using the SciPy library, a Python-based ecosystem of open-source software for mathematics, science and engineering. We provide the open source code that can reproduce all figures presented in this paper, including instructions in the *SI Text*.

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