

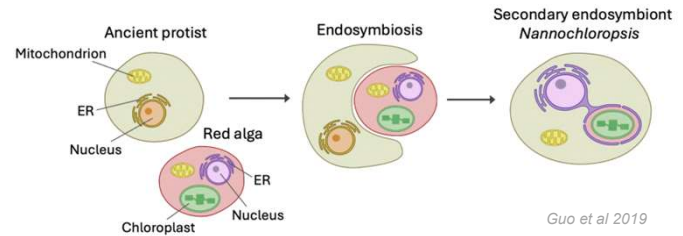
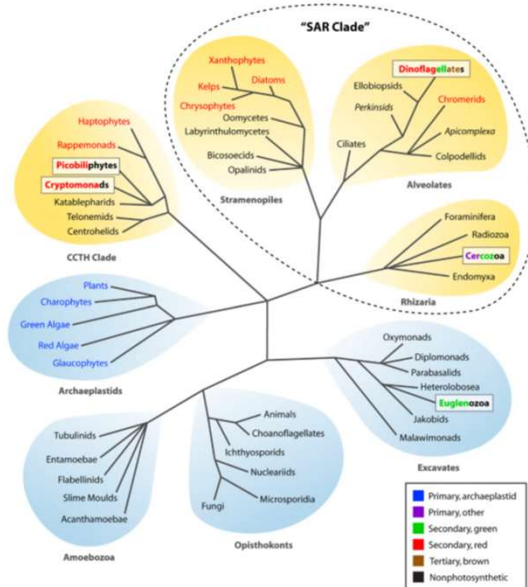
The Xanthophyll Cycle as the Main Driver of Non-Photochemical Quenching in *Nannochloropsis*

PhD candidate: Eleonora Mezzadrelli



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EVOLUTION



These species originated from a secondary endosymbiotic event in which a eukaryotic host cell engulfed a red alga and thus have a **different evolutionary history** with respect to plants and green algae

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Nannochloropsis is a microalga of the class Eustigmatophyceae within Heterokonta, a group which also includes diatoms and brown algae (Cavalier-Smith 2004; Riisberg et al. 2009). These species originated from a secondary endosymbiotic event in which a eukaryotic host cell engulfed a red alga and thus have a different evolutionary history with respect to plants and green algae (Archibald and Keeling 2002).

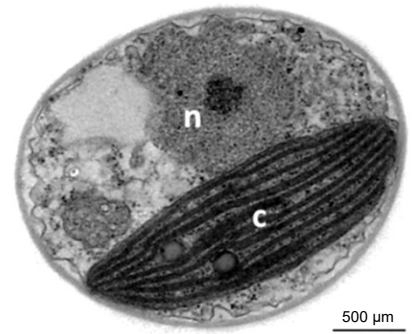
The process of secondary endosymbiosis describes the incorporation of a primary endosymbiotic eukaryote by a nonphotosynthetic protist. The microalga *Nannochloropsis* has evolved from an ancient protist that engulfed a red alga. In this process, the nucleus of the endosymbiont became dominant over the host nucleus. The chloroplast in the secondary endosymbiont is wrapped in four membranes of which the outer two membranes form a continuum with the nuclear endoplasmic reticulum (ER).

Nannochloropsis sp

- Industrially relevant for the high **TAG** content and its ability to accumulate large amounts of the valuable omega-3 fatty acid

PHOTOSYNTHETIC TRAITS:

- Harboring only **chlorophyll a**
- Exhibit a simple carotenoid composition with **violaxanthin** (up to 40% of tot car), **vaucheriaxanthin** and **b-carotene** as major pigments
- Violaxanthin is the major light harvesting pigment

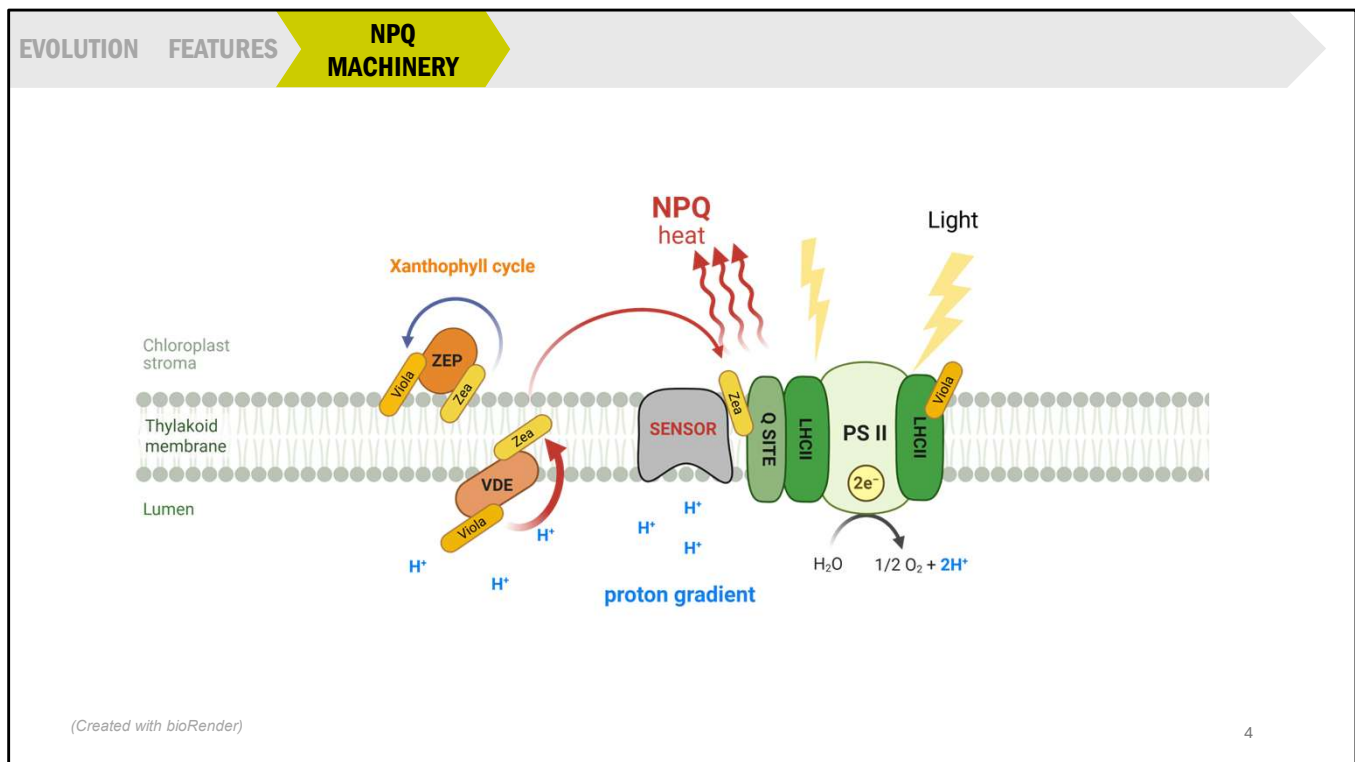


Meneghesso et al 2016

(Bina et al., 2017)

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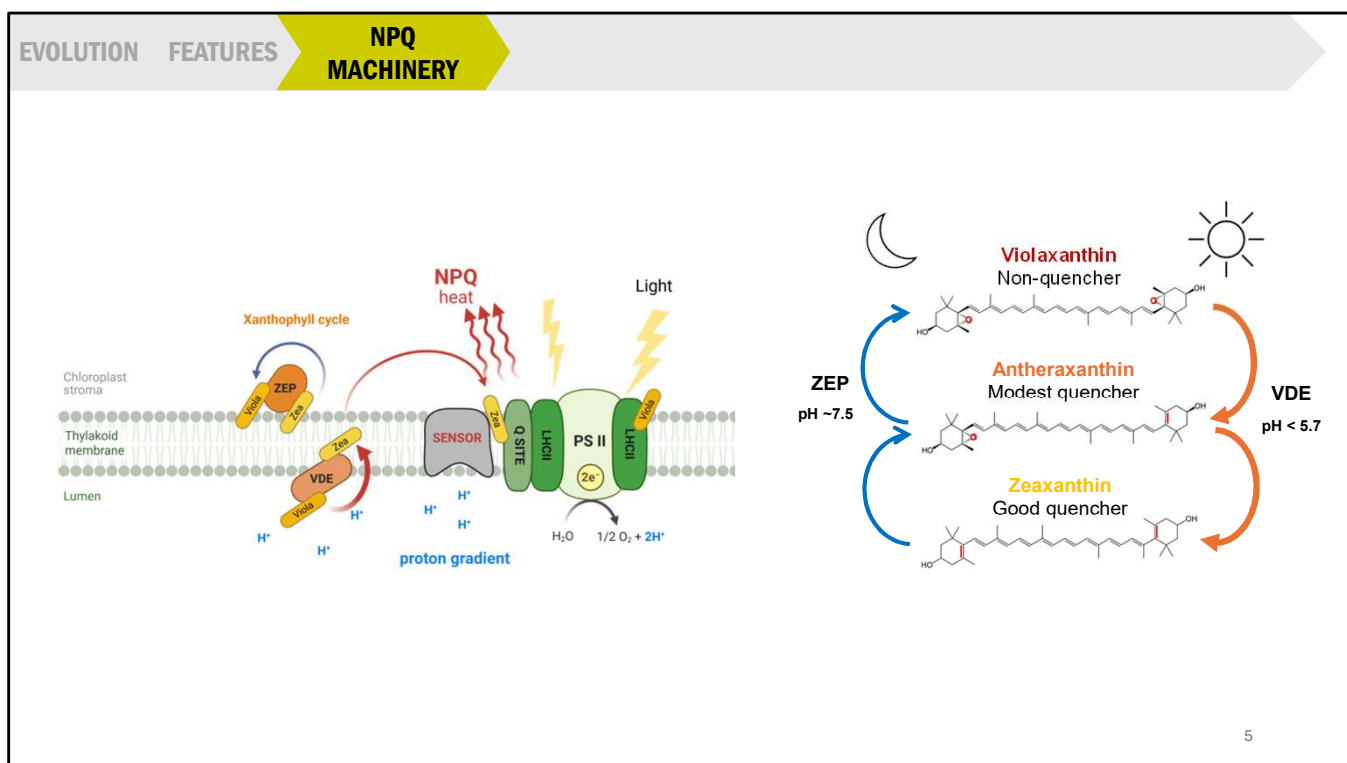
Species from the genus *Nannochloropsis* have emerged as promising organisms for biotechnological use due to their interesting cellular composition and easy manipulation.



In higher plants this activator is PsbS, which can sense the light-driven pH lowering in the lumen and induce antennal rearrangement (Kiss et al., 2008; Krishnan - Schmieden et al., 2021). Green algae, on the other hand, rely on LHCSR proteins, but also possess PsbS which might additionally support NPQ (Peers et al., 2009; Ballottari et al., 2016; Tibiletti et al., 2016; Girolomoni et al., 2020). In stramenopiles PsbS is absent, and NPQ activation is mediated by LHCX proteins (Bailleul et al., 2010; Ghazaryan et al., 2016; Park et al., 2019).

In the moss *Physcomitrium patens* thylakoid fractionation suggested that Zx binds to the NPQ activator LHCSR (Pinnola et al., 2013).

In the green alga *C. reinhardtii*, Zx is also incorporated in the NPQ activator LHCSR3, however, NPQ is only minorly regulated by Zx accumulation, but relies stronger on pH-sensing activity of LHCSR3 itself (Bonente et al., 2010; Ballottari et al., 2016; Troiano et al., 2021).

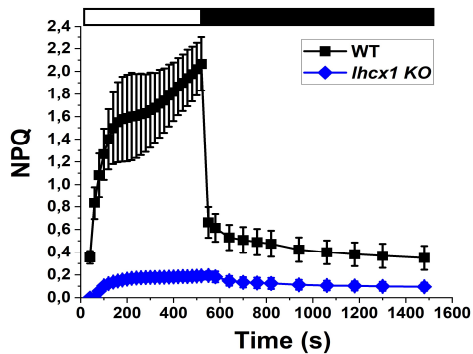


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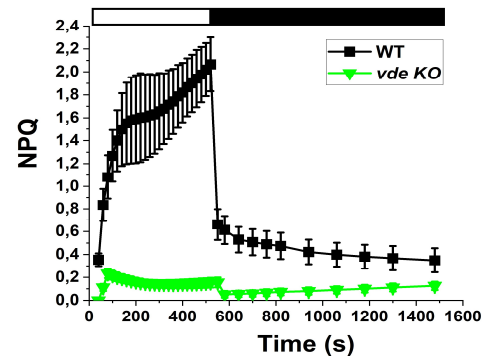
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NPQ activation is mediated by **LHCX1**
protein (homolog protein of LHCSR)



(Perin et al., 2023)

NPQ is **Zeaxanthin dependent** but
Zeaxanthin accumulation alone is not
sufficient to activate NPQ

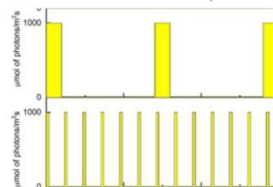
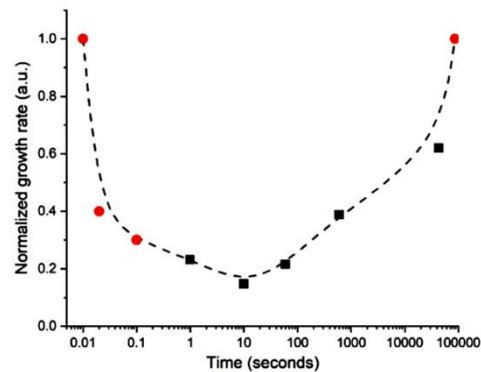


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In *Nannochloropsis* LHCX1 is a homolog of green alga LHCSR that is necessary for NPQ. (LHCX1 KO are not able to activate NPQ such as the VDE KO → no zeaxanthin conversion)

However, biochemical data for pigment binding of PsbS and LHCX1 was never shown (Crouchman et al., 2006; Giovagnetti et al., 2022) and there is structural data arguing against the permanent incorporation of Zx (Fan et al., 2015), raising the hypothesis that Zx might also act as an allosteric activator to initiate antennal rearrangement (Crouchman et al., 2006).

Light fluctuations were designed to expose cells to an optimal average photon flux corresponding to $150 \mu\text{mol photons m}^{-2}\text{s}^{-1}$.



(Bellan et al. 2020)

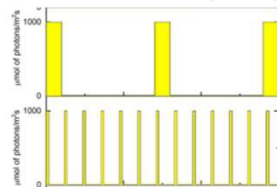
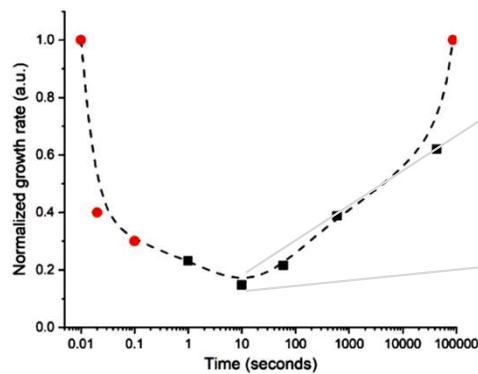
(Sforza et al. 2012)

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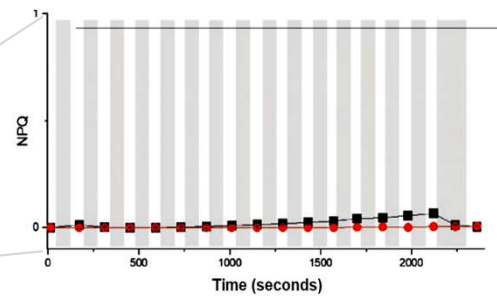
Nannochloropsis gaditana cells under fluctuating light showed extensive damage to PSI, with a reduction of active P700 per cell and a decrease in the PSI/PSII ratio, as in other organisms exposed to light fluctuations (Shikanai and Yamamoto 2017, Alboresi et al. 2019). The mechanistic explanation for PSI impairment under these conditions is that upon sudden increases in illumination, PSI becomes limited on the acceptor side, and excess electrons are accumulated at the level of iron–sulfur clusters, which are consequently damaged (Tiwari et al. 2016). (Bellan et al 2020)

And you can ask yourself «why light fluctuations are so important for microalgae?»

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(Bellan et al. 2020)
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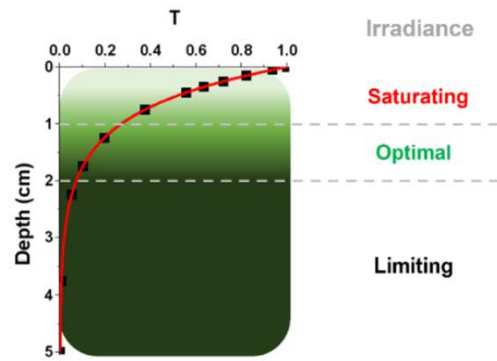
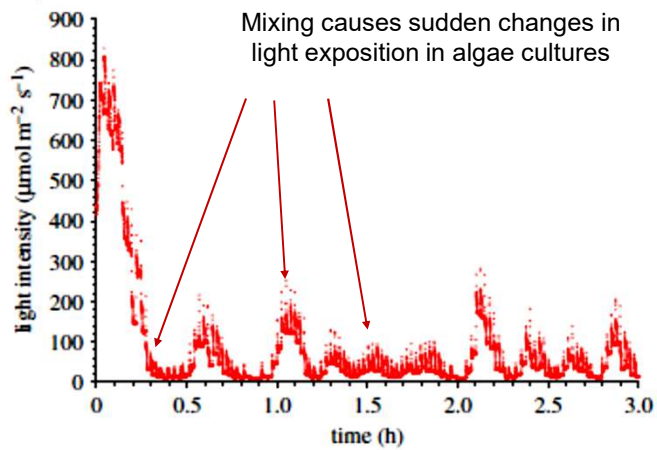
The most challenging conditions are, therefore, those in which strong light exposure lasts long enough to cause damage but not long enough for the activation of photoprotection mechanisms

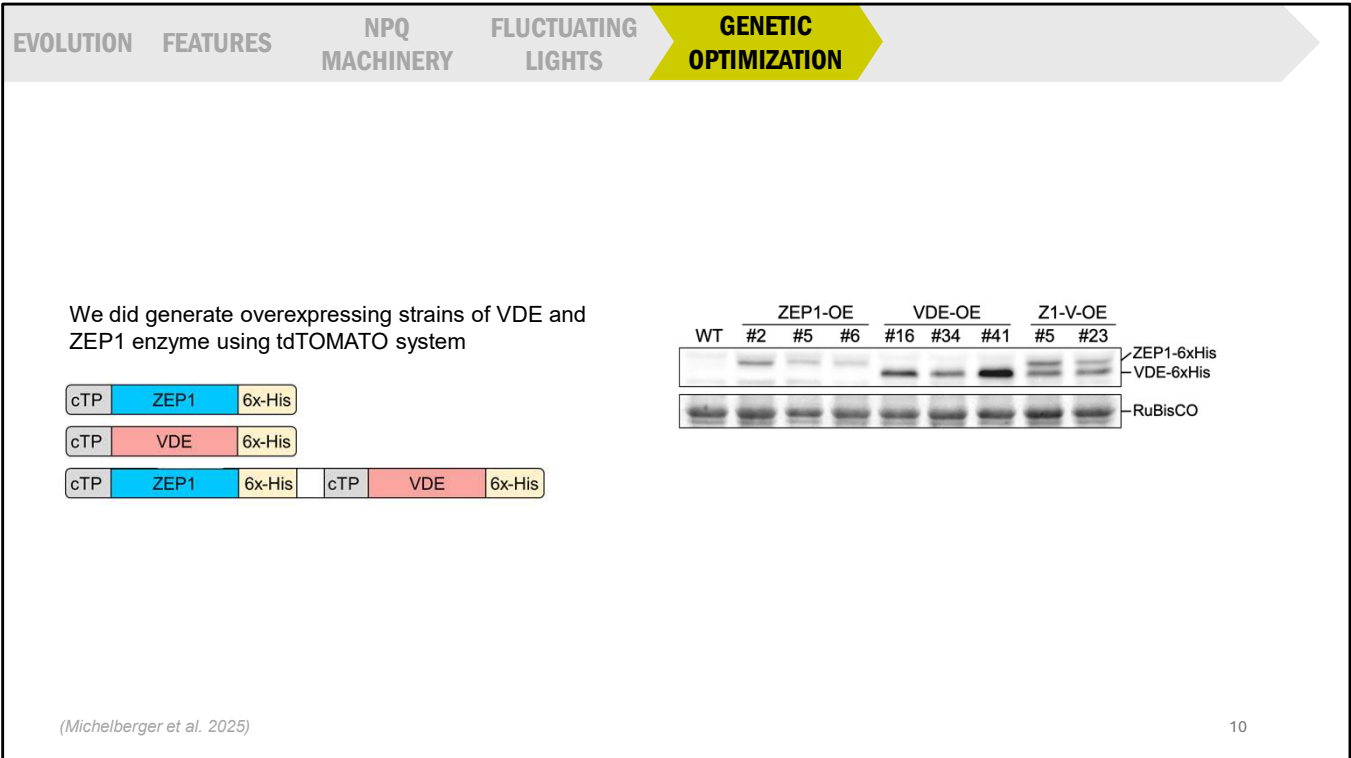
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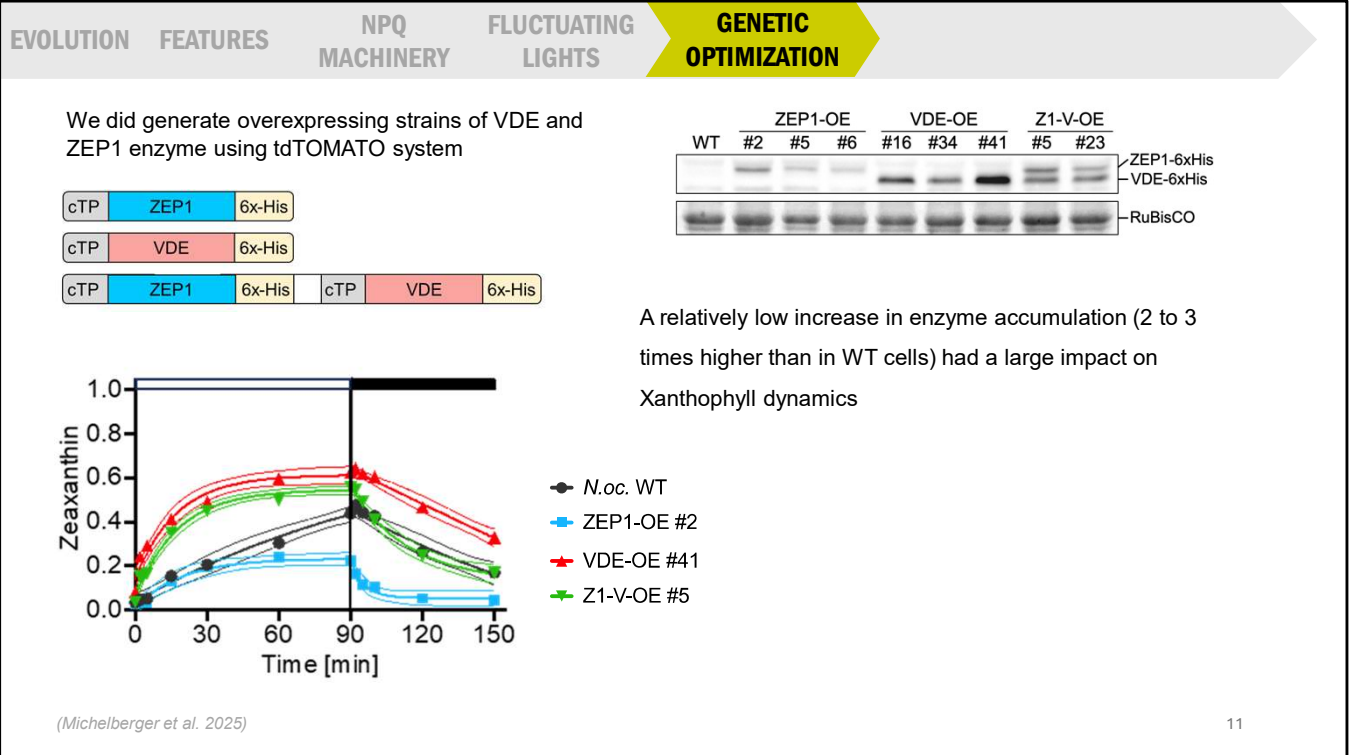
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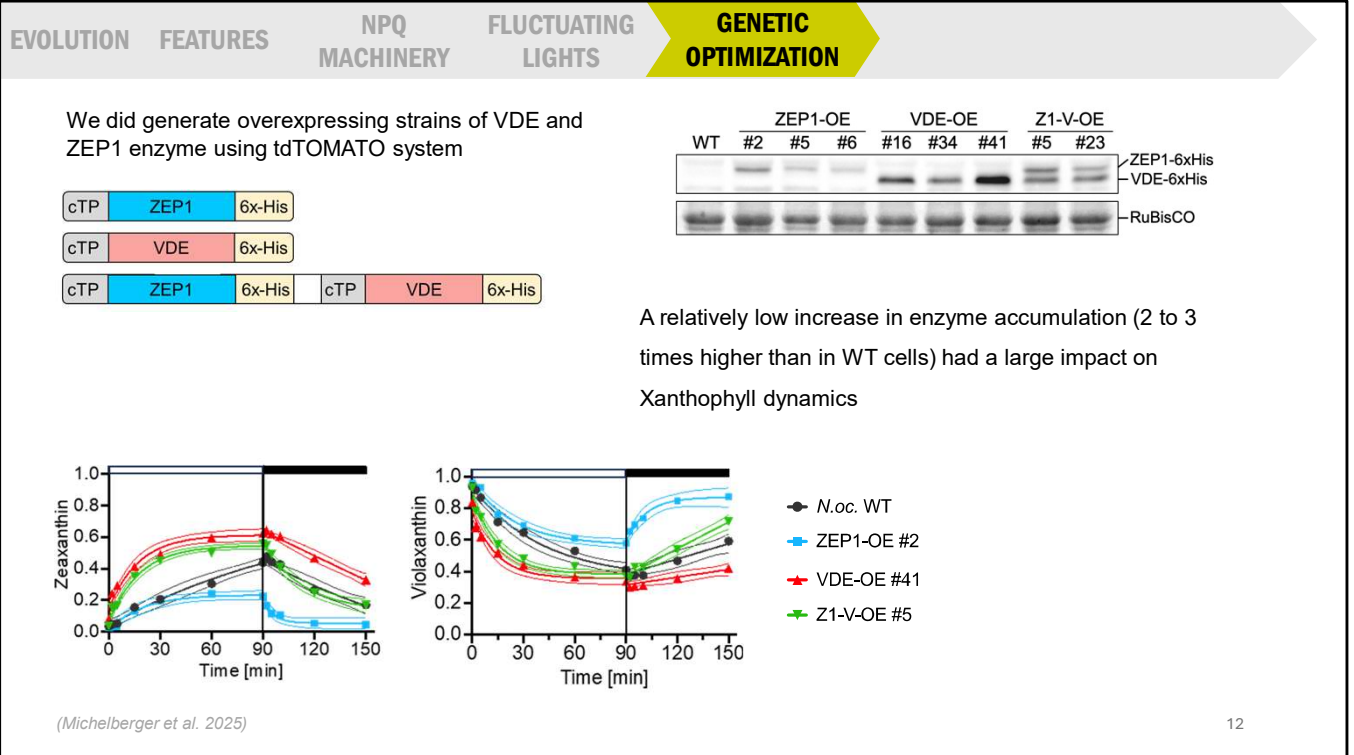
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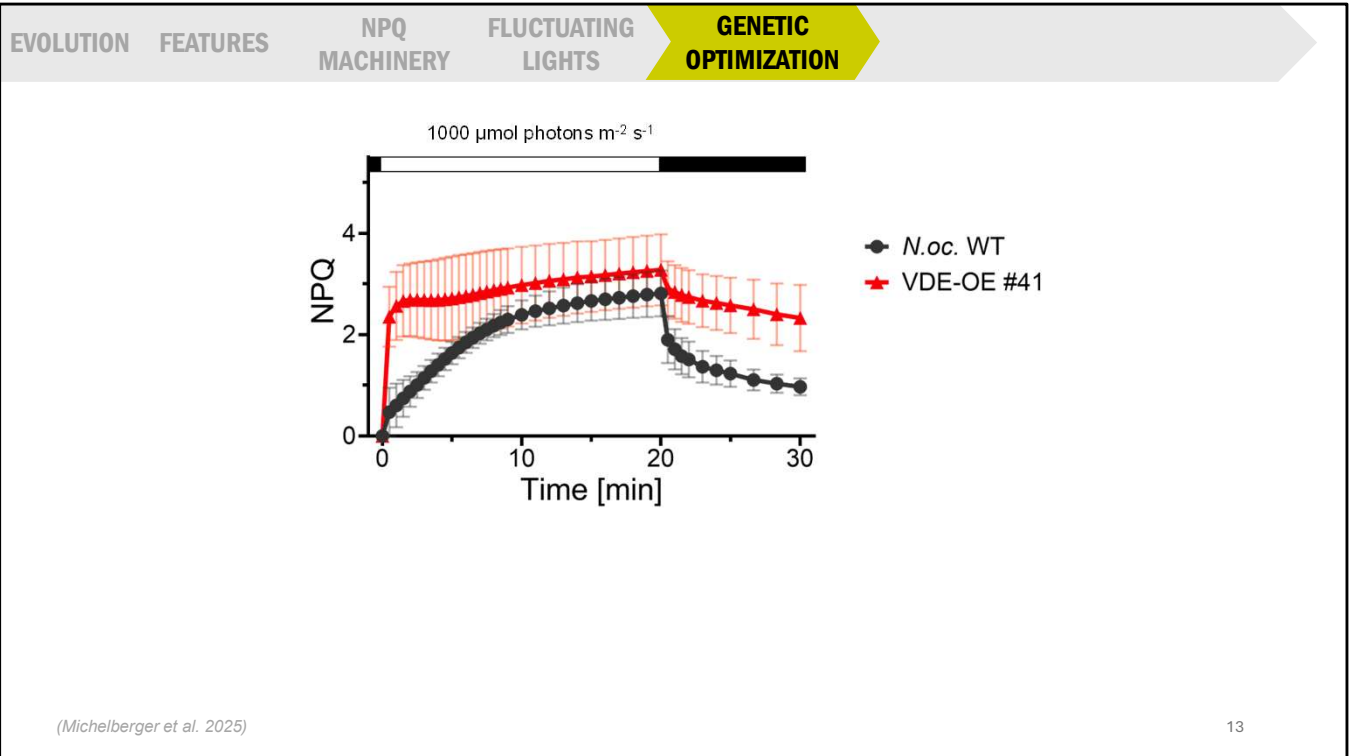
Photobioreactor environment



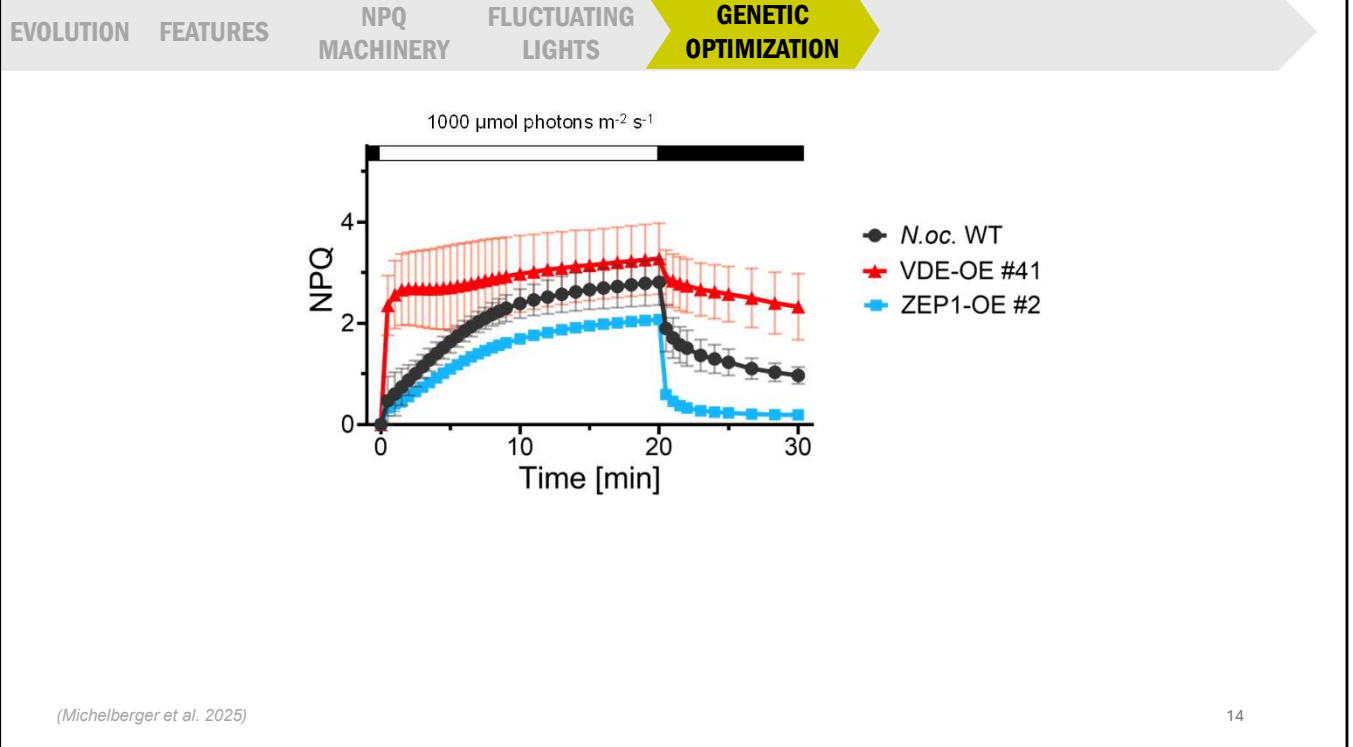




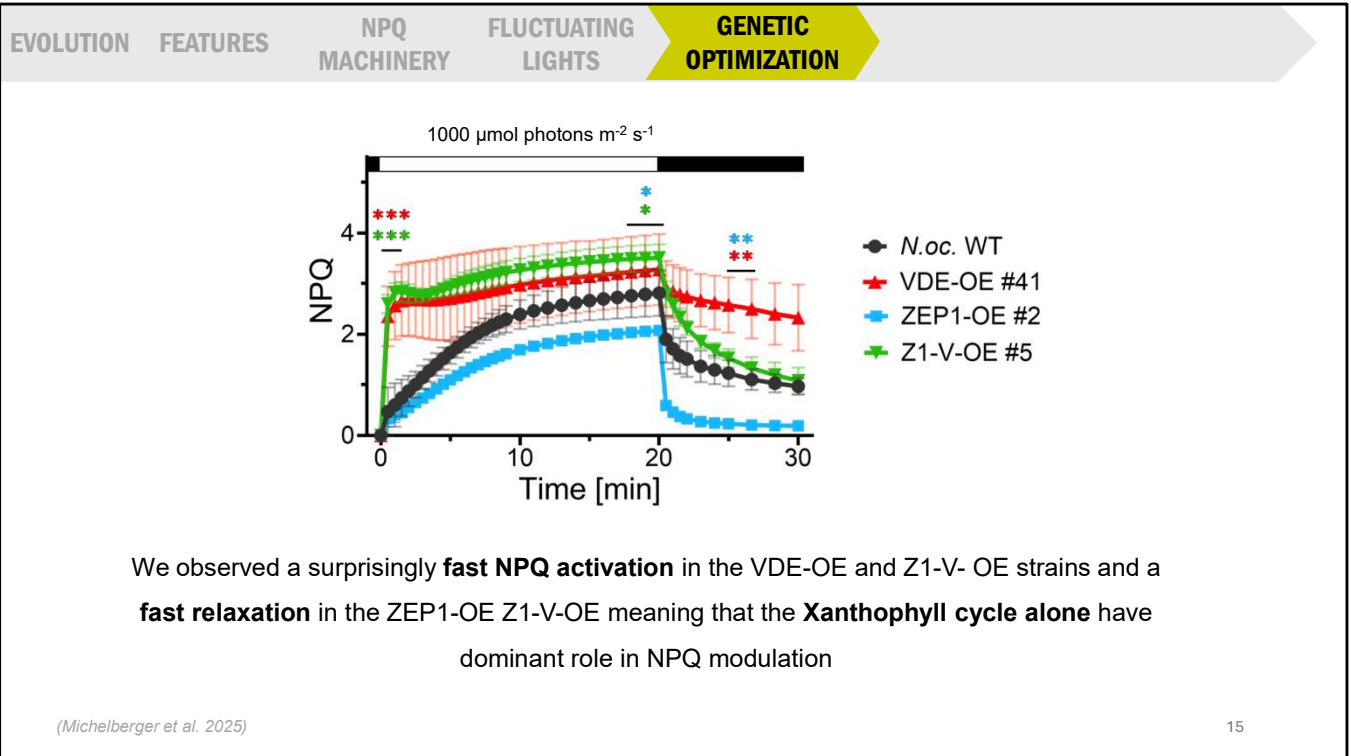




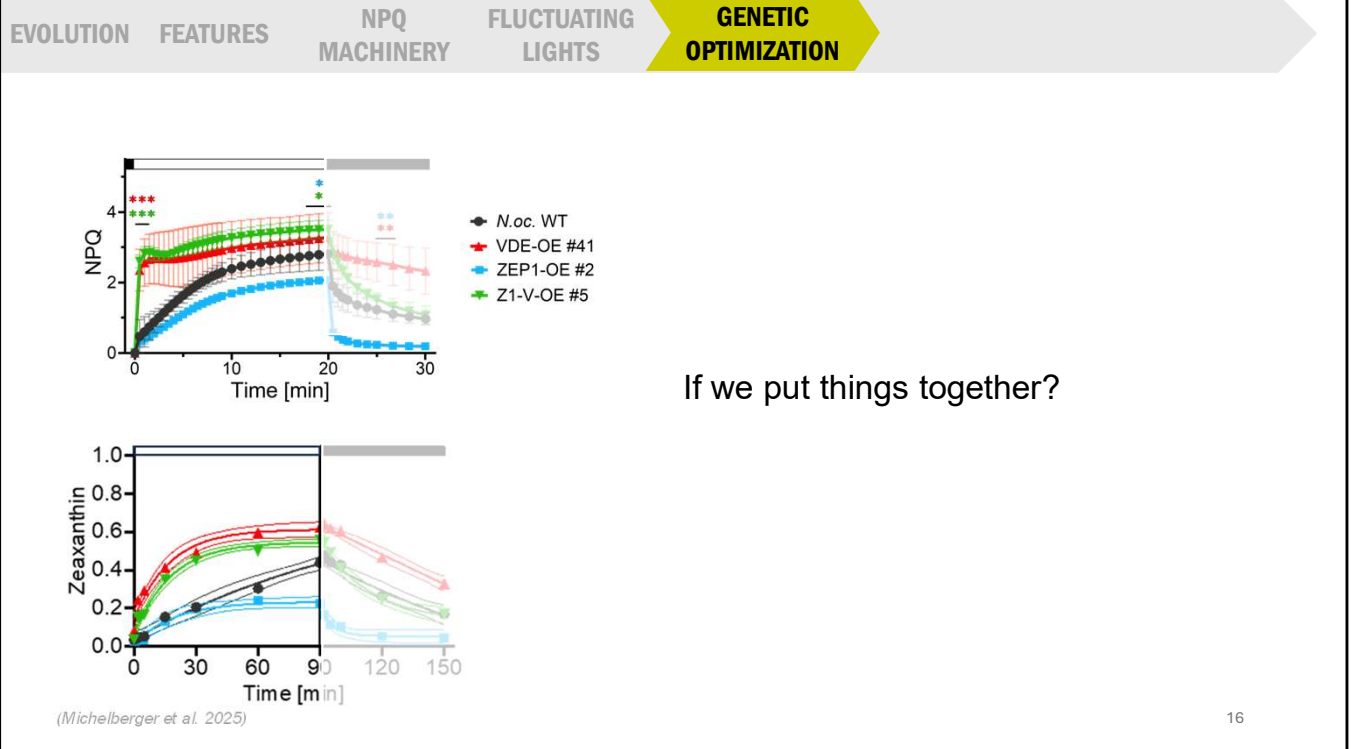
n 30 s of low illumination, NPQ reached values close to those observed with much stronger illumination (approximately NPQ=2.5), while in WT cells NPQ was almost not activated (NPQ=0.14). However, after initial activation we observed a strong drop in the level of NPQ, which can be explained by the activation of the Calvin-Benson cycle after a few minutes, acting as an electron sink and dissipating a part of .pH, the lumenal relaxing the NPQ



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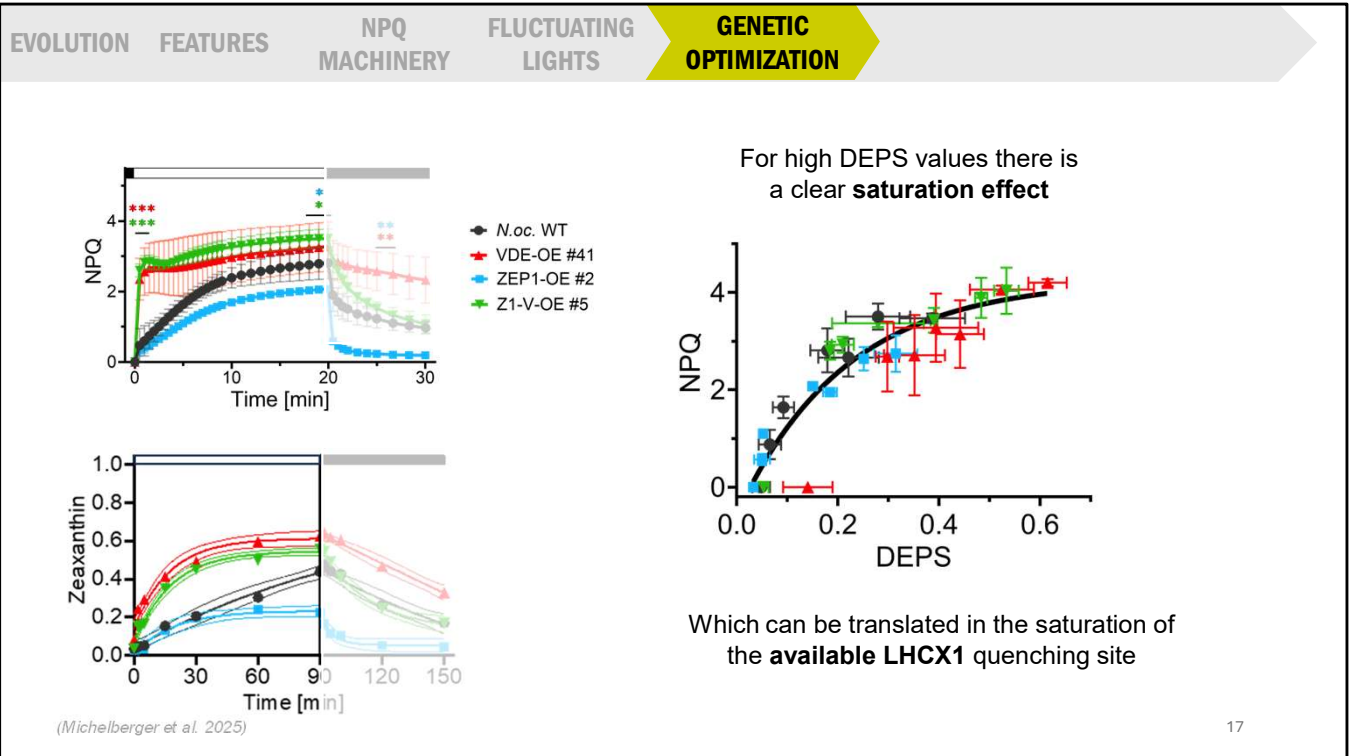


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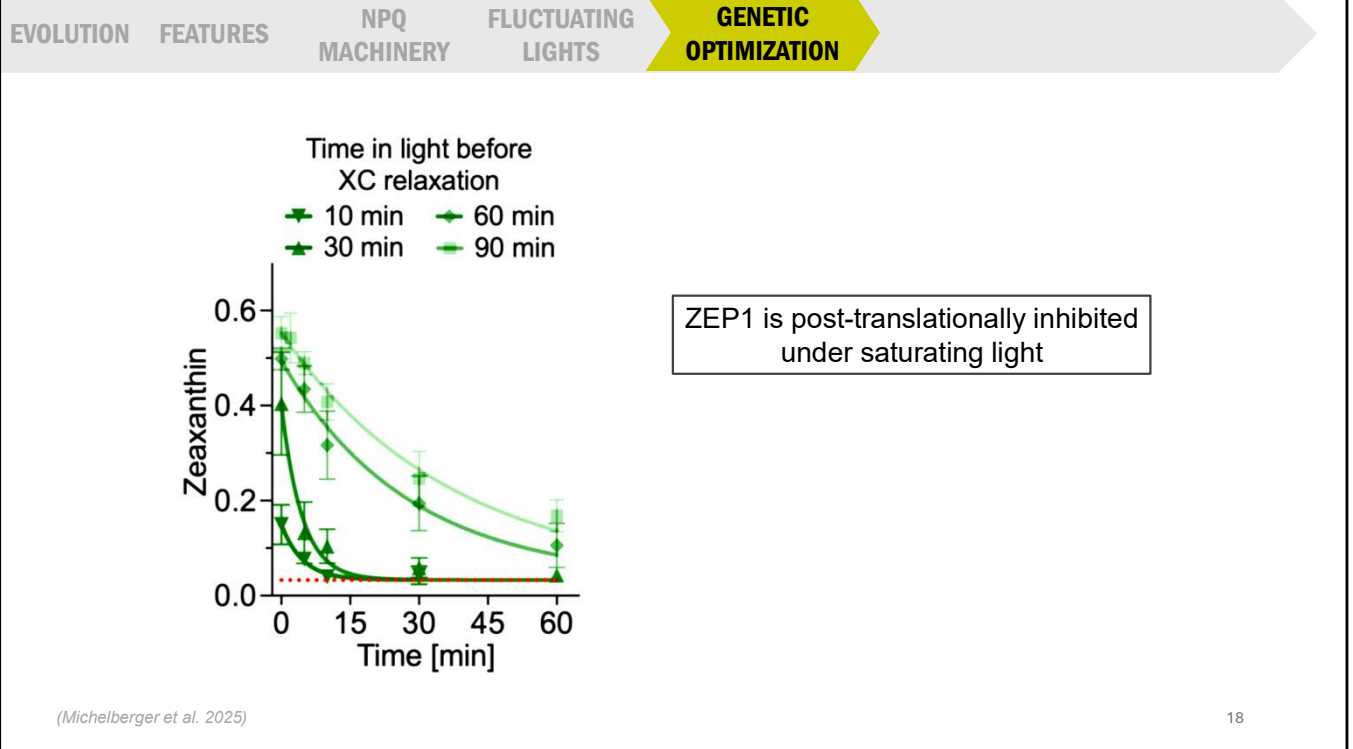


If we put things together?

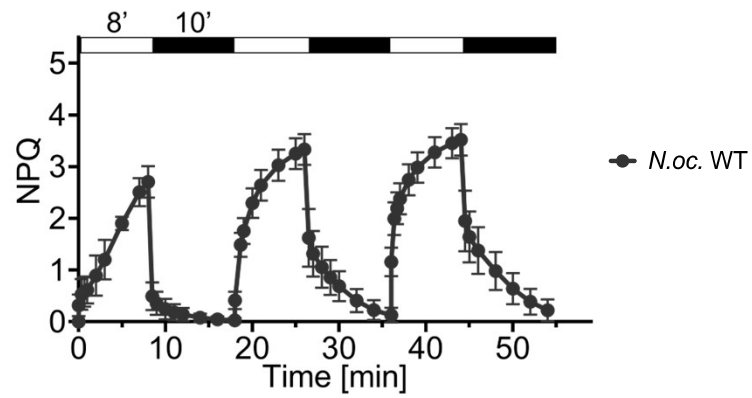
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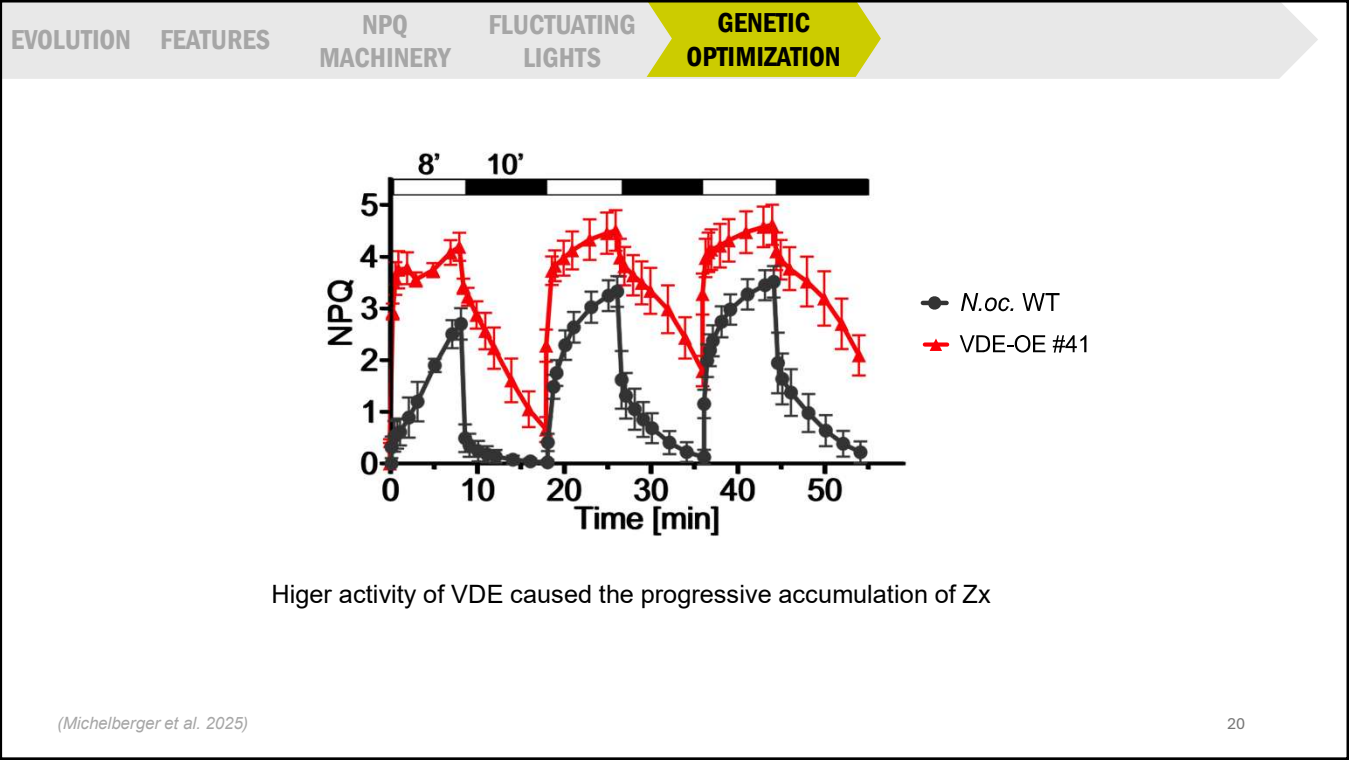
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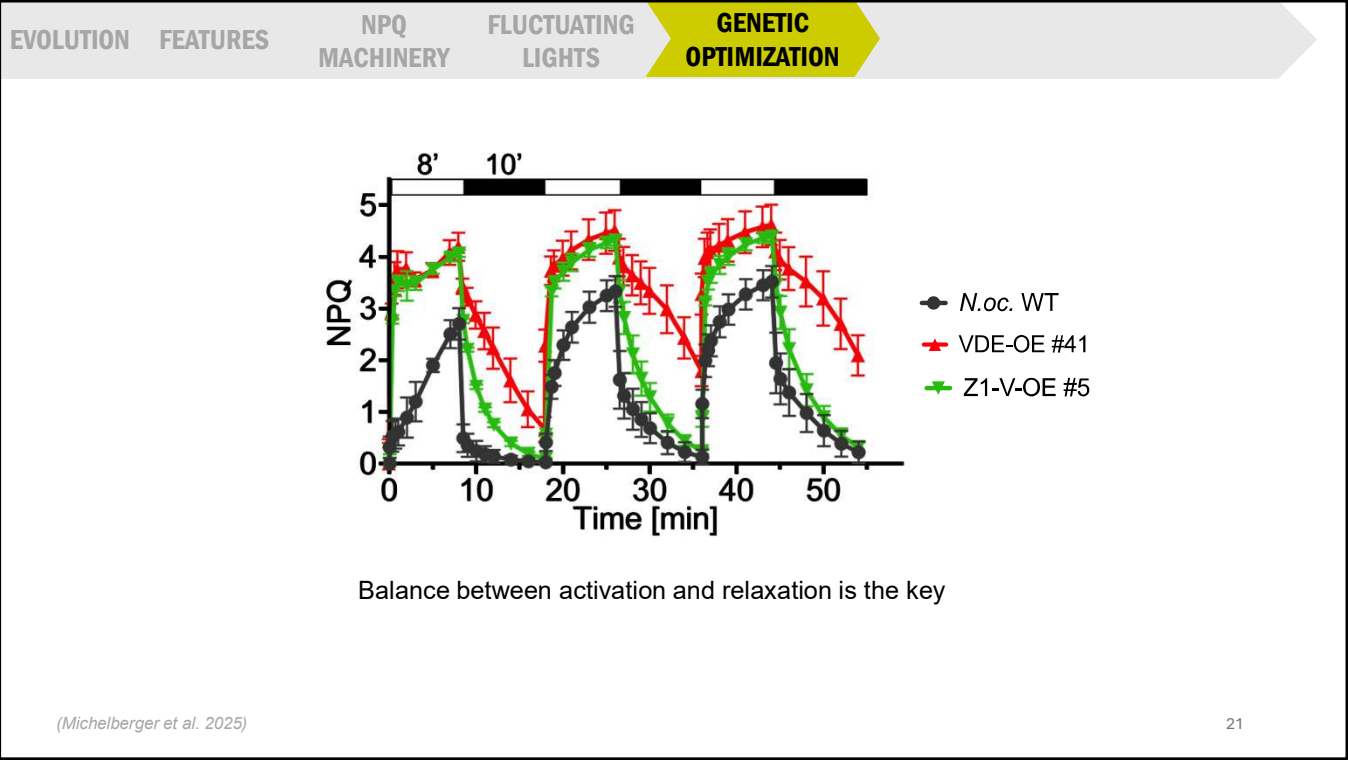


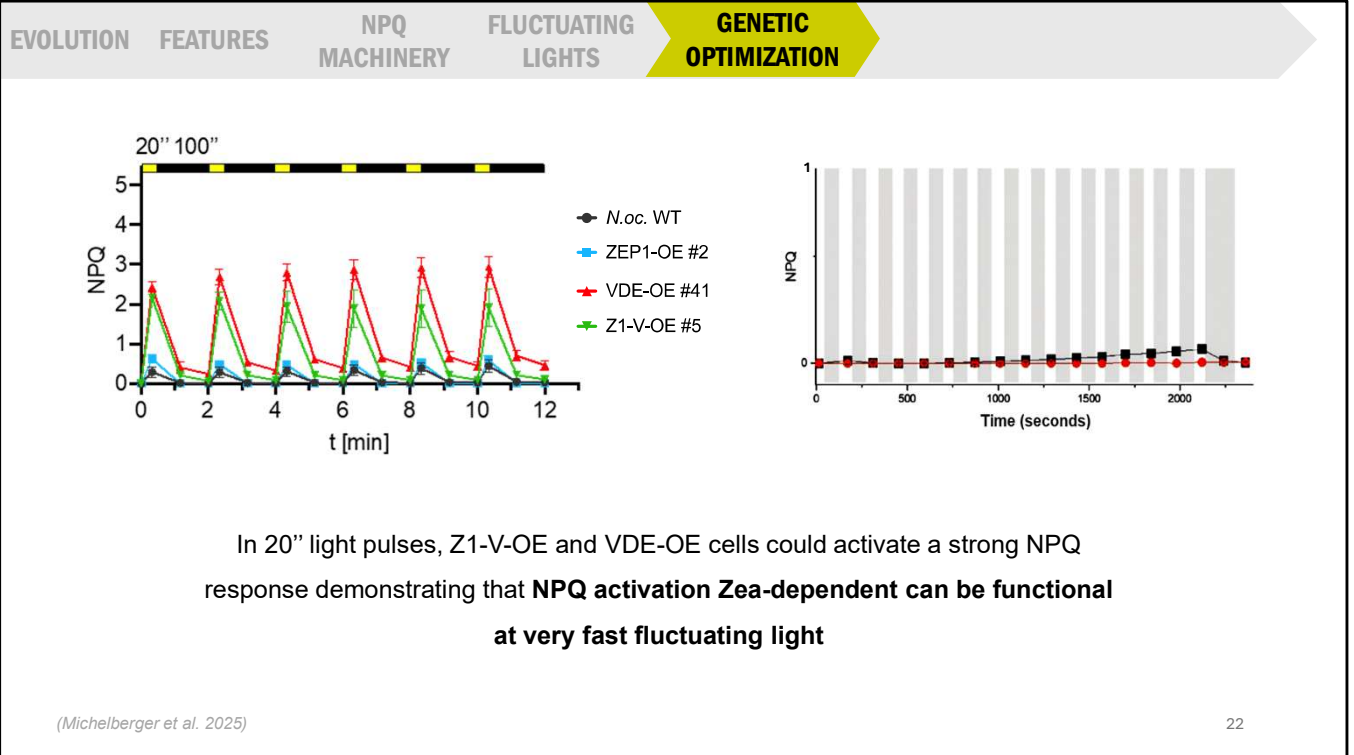
relaxation times are longer after prolonged illumination periods, suggesting a light-dependent downregulation of ZEP activity

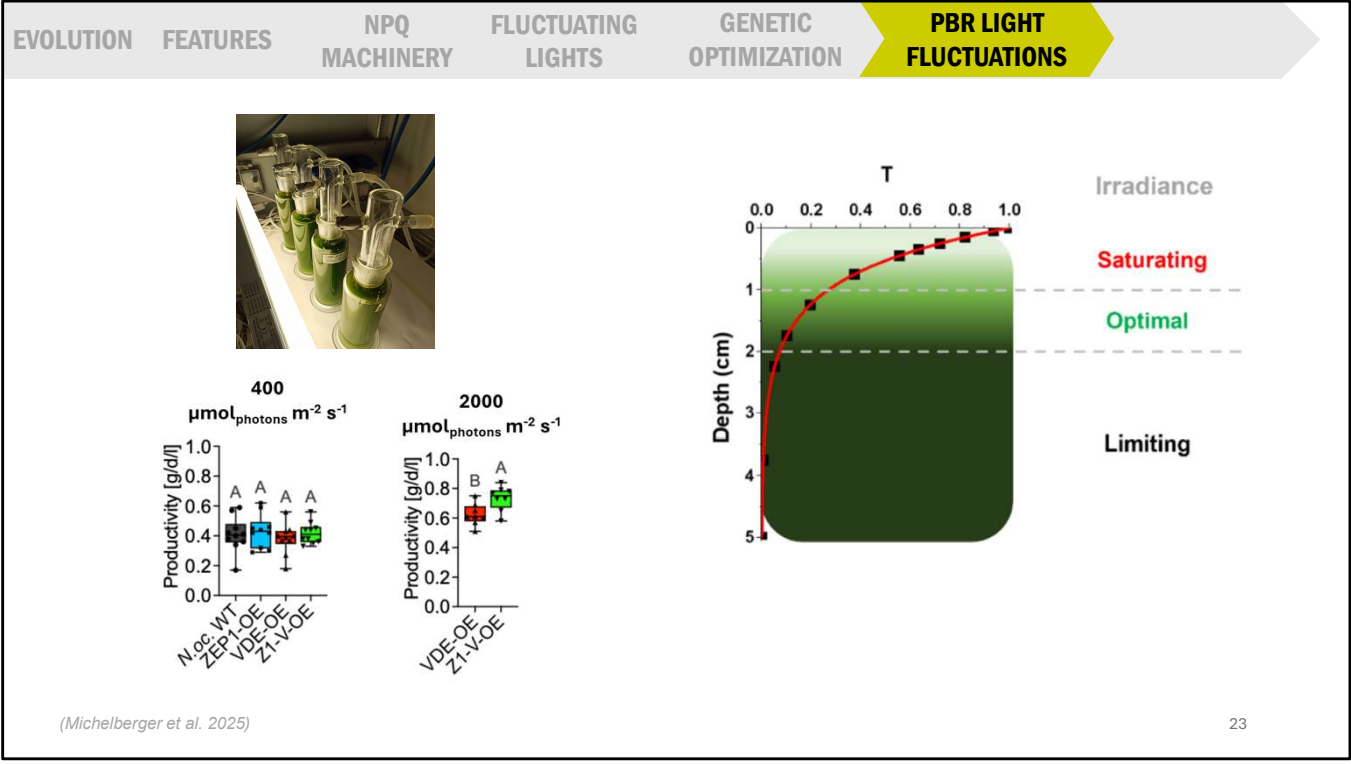


In the WT cells is evident the **photoprotective memory**



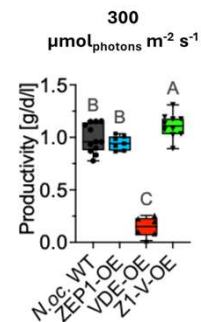
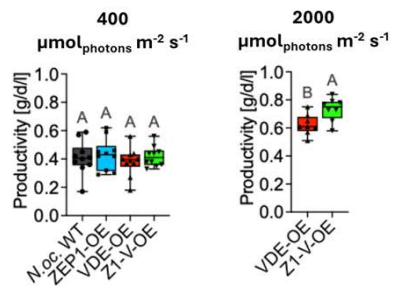








Optimization of the xanthophyll cycle leads to biomass productivity benefits depending on the cultivation conditions



(Michelberger et al. 2025)

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AKNOWLEDGMENTS

I would like to thank my colleagues from Plant Lab
Thank you for your attention



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Prof Tomas Morosinotto
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Prof Nicoletta La Rocca

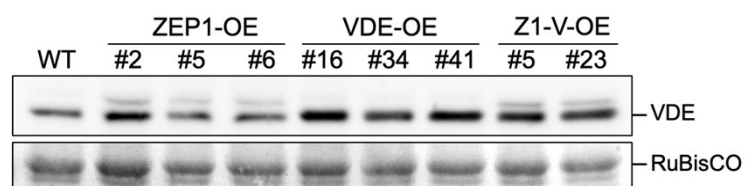
PhD Tim Michelberger
PhD Alessandra Bellan

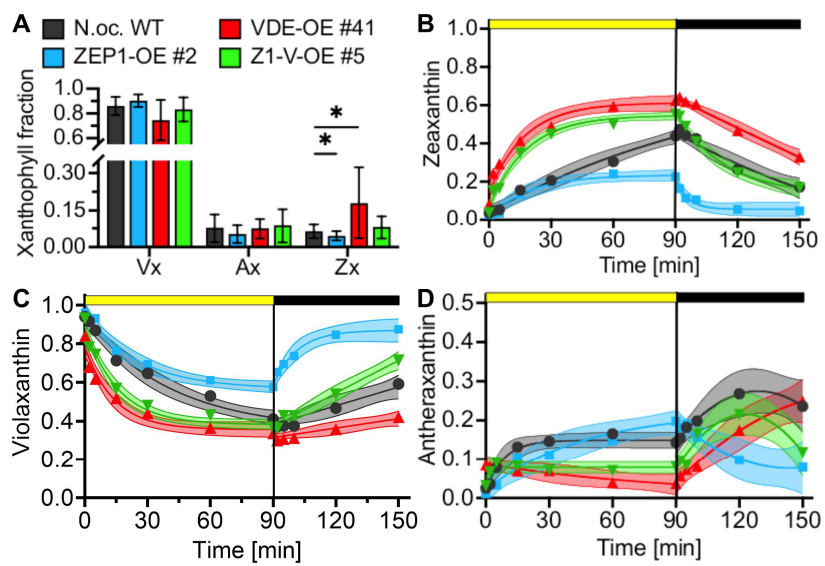
MS Britt Rietbroek
Prof Sarah D'Adamo
Prof Marcel Janssen

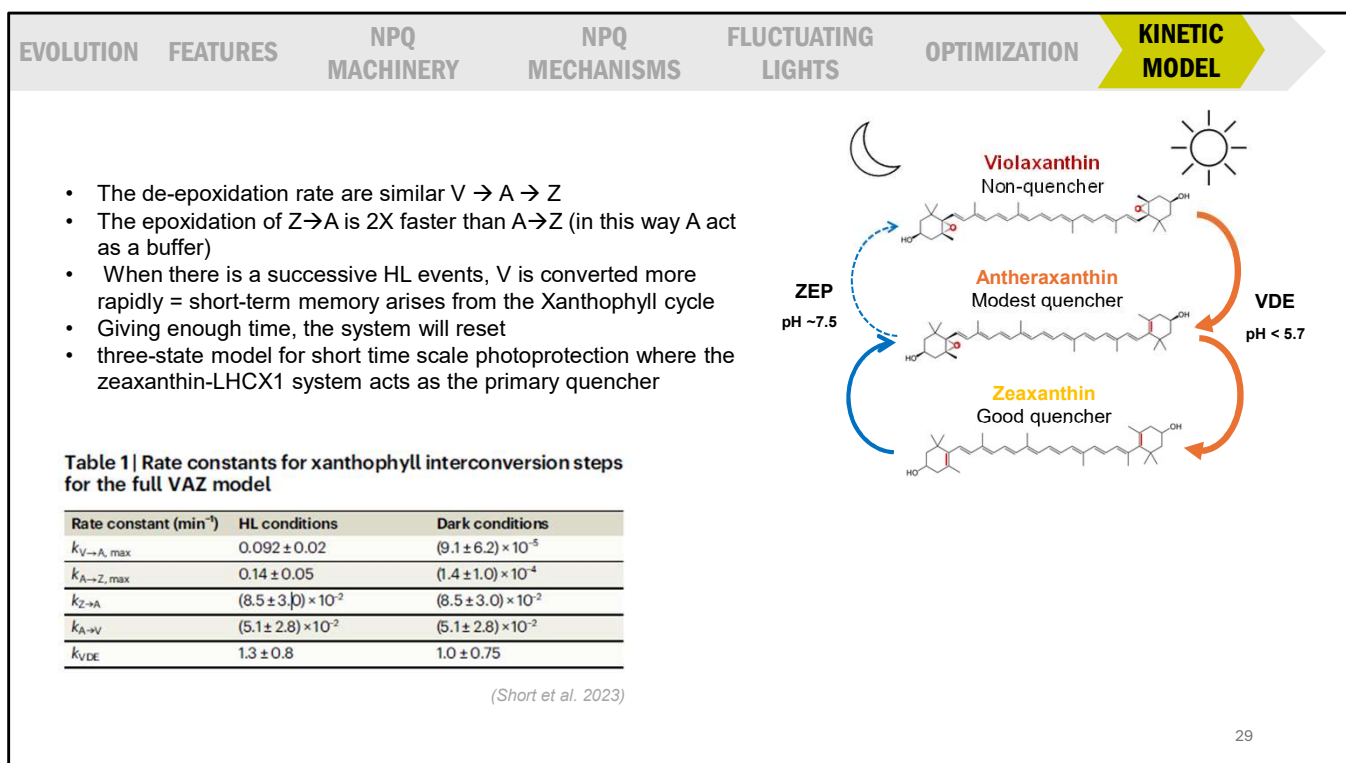
Are there any implications for applications involving aquatic organisms? What about the physics of oscillating light interacting with **mixed water /moving cells**?

Can frequency-domain signals provide information on NPQ kinetics at time scales of seconds or milliseconds?

Is it possible to use frequency-domain signals as a fingerprint to identify the species or physiological status of cells present in a **heterogeneous system**?







Assumption of single rate constant for individual reaction step
 presence of A in a system could keep plants and algae primed to respond to further HL stress, due to the slow rates of transforming A back to V.
 zeaxanthin-dependent but LHCX1-independent “qZ” quenching has also been found, although its contribution to NPQ appears to be much smaller than that of LHCX1-dependent “qE” quenching