UC Berkeley
UC Berkeley Electronic Theses and Dissertations

## Title

Photoprotective and Photosynthetic Mechanisms of Eustigmatophyte Microalga, Nannochloropsis oceanica

## Permalink

https://escholarship.org/uc/item/9vg3915z

## Author

Crisanto, Thien

## Publication Date

2023

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

# Photoprotective and Photosynthetic Mechanisms of Eustigmatophyte Microalga, *Nannochloropsis oceanica*

By

Thiên Erasma Nguyễn Crisanto

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Plant Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Krishna K. Niyogi, Chair
Professor Anastasios Melis
Professor Graham Fleming

Fall 2023

Abstract

Photoprotective and Photosynthetic Mechanisms of Eustigmatophyte Microalga, *Nannochloropsis oceanica*

By

Thiên Erasma Nguyễn Crisanto

Doctor of Philosophy in Plant Biology

University of California, Berkeley

Professor Krishna K. Niyogi, Chair

Photosynthetic stramenopiles (stramenochromes) are a very diverse group of algae. They range from microscopic phytoplankton that serve as primary producers in oceanic environments to macroscopic seaweeds that provide food worldwide. Stramenochromes originated from secondary endosymbiosis involving a red-algal ancestor and exhibit unique features that make them different from well-studied green algae and plants. Because they occupy such a wide range of ecological niches, they have evolved different strategies in photosynthesis and photoprotection to regulate light harvesting under varying environmental conditions. Chapter 1 highlights these unique features and strategies among stramenochromes while encompassing classes beyond well-known groups like Bacillariophyceae and Eustigmatophyceae. Subsequent chapters focus on a particular Eustigmatophyte microalga that has emerged as a model organism, *Nannochloropsis oceanica* CCMP1779.

*Nannochloropsis oceanica* is a stramenochrome that can produce high amounts of lipids including the valuable omega-3 fatty acid, eicosapentaenoic acid. This dissertation focuses on the mechanisms of photoprotection and photosynthesis in *N. oceanica*, holding potential applications in enhancing photosynthetic efficiency in mass cultures and algae farms. This begins with Chapter 2 which characterizes and investigates the mechanisms of a process known as non-photochemical quenching (NPQ), which dissipates excess absorbed light energy as heat. In this chapter we use chemical inhibitor treatments and mutant analysis to reveal that LHCX1 is crucial for qE induction, whereas LHCX2 and LHCX3 do not seem to play a role in NPQ in low-light grown cells. We also show that the *vde* mutant, deficient in the synthesis of the photoprotective pigment zeaxanthin, lacks both qE and qZ. These results show that photoprotection in *N. oceanica* is highly dependent on the formation of zeaxanthin and its collaboration with LHCX1.

Chapter 3 focuses on resolving the molecular function of LHCX1 by testing the hypothesis that LHCX1 is involved in sensing a decrease in thylakoid lumen pH in excess light. Knock-in mutations targeting putative pH-sensing sites were generated in the native LHCX1 gene, although they revealed no discernable impact on qE capacity.

This aligns with previous results in diatoms but is different from similar proteins in the green algae and plants, suggesting functional divergence in stramenochromes. Moreover, generation and analysis of the W143M mutant revealed that a mutation in this amino acid doesn't impact a putative pigment binding site involved in qE. This contrasts with previous results in diatoms, suggesting species-specific divergence in *N. oceanica*. This work prompts a reconsideration of ΔpH-sensing mechanisms in photosynthetic organisms. While the overall function of LHCX1 in qE remains elusive, strides are made towards resolving its structure in Chapter 4.

Chapter 4 focuses on developing methods towards unraveling the architecture and protein makeup of PSI-VCPI and PSII-VCPII supercomplexes that contain LHCX1. To do this, high-resolution single-particle cryogenic electron microscopy (EM) will be used. Developing the methods towards cryo-EM involves many steps, which were optimized in this chapter. Overall findings indicated that using maltose density gradients with gradient fixation (GraFix) improves sample quality and supercomplex isolation. These methods will be used for structural analysis to reveal the overall architecture, protein-protein interactions, and pigment locations in the photosynthetic supercomplexes, leading to inferences on energy-transfer and energy-dissipation pathways in *N. oceanica* and other diverse algae groups.

# Dedication

This dissertation is dedicated to:

my paternal grandparents Erasmo Crisanto and Montrose Hoyte Crisanto for immigrating from Honduras and Barbados to New York,

my maternal grandparents Nguyễn Huy Trúc and Bùi Thị Dung for leaving their home country, Vietnam, during the war to move to France before settling in California,

and my parents Oracio Crisanto and Mỹ-Thục Nguyễn Crisanto for raising me to be smart and kind.

Thank you for making the hard decisions that have provided me with the opportunity and the privilege to complete this Ph.D. and become a scientist.

Acknowledgements

This research wouldn't have been possible without my research advisor, Kris Niyogi, who has always been patient with me and provided me with this opportunity, amazing edits, and a space to grow as a scientist. Thank you to Marilyn Kobayashi for being the backbone to our lab, a wonderful lab manager, and a great person to talk to and share a birthday with. I couldn't have done much of my work without research mentorship from Masa Iwai or Setsuko Wakao; thank you for your guidance and your patience. Thank you to Dhruv Tupper-Patel and Tim Jeffers-Parish for being amazing grad school friends and lab mates. It's been so special to share my grad school experience with you, and get to watch you both fall in love and get married to your husbands. Thank you to my five wonderful undergraduates: Marley, Brittany, Shreya, Grant, and Marcus! You have all taught me so much about myself and have contributed so much to this dissertation. Thank you for being so patient with me as we ride the ups and downs of being students together. Thank you to Benjamin Endelman for your patience in helping me during the first few months of my graduate career and for being a friend.

I couldn't have done this PhD without the support of my animals, my lizard Moby and my dog Honey. They both remind me when it's time to take a break and have taken the meaning of "animal mom" and "solo camping" to a whole new level. Thank you for allowing me to transform into the adventurous van-camping, surfing, road-tripping, forest-exploring, lizard and dog mom I couldn't have dreamed of becoming.

For the past 6 years I have also leaned on the support of my closest friends. Thank you to my best friend PJ Ogden for 12+ years of the silliest friendship and for always letting me be myself. Thank you for letting me talk "at" you and vent about my stressors and meet up for bougie dinners during the school year, or watching my dog while I was out of town. Thank you, Nicholas Karavolias, for always letting me talk "at" you too and be 100% myself. For your loyalty and always having my back, and for your support and understanding throughout this crazy journey as scientists! This has been such a unique journey that we've gotten to experience together. Kimbrough Moore, thank you for being my rock during the final months of dissertation writing, and for providing me with endless snacks, dinner, understanding, patience, and support. Thank you to my hometown friends who have stuck by me throughout the decades, the friends I made pre-grad school, and friends that I have made during my time in the Bay Area. Thank you to my piano instructor, Larry Tasse, for 2.5 years of piano and song writing lessons during my Ph.D. Your patience and talent helped keep my creativity alive. Thank you mostly to my family for making me who I am, for always being down with my crazy ideas, and for always humbling me.

Table of Contents

Photoprotective and Photosynthetic Mechanisms of Eustigmatophyte Microalga, *Nannochloropsis oceanica* ..... 1
Abstract ..... 2
Photoprotective and Photosynthetic Mechanisms of Eustigmatophyte Microalga, *Nannochloropsis oceanica* ..... 2
Dedication ..... 1
Acknowledgements ..... 1
Table of Contents ..... 1
Chapter 1: Photoprotective Strategies of Photosynthetic Stramenopiles (Stramenochromes) ..... 1
- Abstract ..... 1
- Introduction ..... 1
- Bacillariophyceae ..... 5
- Chrysophyceae ..... 8
- Eustigmatophyceae ..... 9
- Phaeophyceae ..... 10
- Pinguiophyceae ..... 11
- Raphidophyceae ..... 12
- Xanthophyceae ..... 13
- Photoprotection and photosynthesis across minor classes ..... 14
- Conclusion ..... 15
- References ..... 17

Chapter 2: LHCX and Zeaxanthin-Dependent Induction of Non-Photochemical Quenching in *Nannochloropsis oceanica* ..... 42
- Abstract ..... 42
- Preface ..... 42
- Introduction ..... 42
- Materials and Methods ..... 45
- Results ..... 49
- Discussion ..... 57
- Supplemental Materials ..... 59
- References ..... 62

Chapter 3 : Investigating the Role of LHCX1 in Non-Photochemical Quenching Induction in *Nannochloropsis oceanica* ..... 87
Abstract ..... 87
Introduction ..... 87
Materials and Methods ..... 89
Algal strain and cultivation ..... 89
Algal Transformation ..... 89
Genotypic analysis by PCR and Sanger sequencing ..... 90
Phenotypic analysis by PAM fluorometry ..... 90
Results ..... 91
Discussion ..... 99
Supplemental Materials ..... 100
References ..... 105
Chapter 4: Towards the Structures of PSI-VCPI and PSII-VCPII Supercomplexes Containing LHCX1 in *Nannochloropsis oceanica* ..... 130
Abstract ..... 130
Preface ..... 130
Introduction ..... 130
Methods ..... 133
Results ..... 137
Discussion ..... 144
References ..... 145

# Chapter 1: Photoprotective Strategies of Photosynthetic Stramenopiles (Stramenochromes)


## Abstract

Photosynthetic stramenopiles (stramenochromes) are a very diverse group of algae, ranging from microscopic phytoplankton to macroscopic seaweeds. Stramenochromes originated from secondary endosymbiosis involving a red-algal ancestor and exhibit unique features that make them different from well-studied green algae and plants. Because they occupy such a wide range of ecological niches, they have evolved different strategies in photosynthesis and photoprotection to regulate light harvesting under varying environmental conditions. This introduction delves into the less-explored photosynthetic and photoprotective mechanisms in stramenochromes, encompassing classes beyond well-known groups like Bacillariophyceae and Eustigmatophyceae. Understanding these mechanisms in stramenochromes not only contributes to the knowledge of their biodiversity but also holds potential applications in enhancing photosynthetic efficiency in mass cultures and algae farms. This exploration may lead to valuable insights for both industrial and environmental advancements, providing innovative solutions to address climate change.


## Introduction

During oxygenic photosynthesis, light energy is absorbed by light-harvesting proteins in the peripheral antenna and transferred to the reaction centers of photosystem I and II (PSI, PSII). Though the core structure of each photosystem is highly conserved, the peripheral antenna proteins and associated pigments are very diverse, encompassing over a billion years of evolutionary divergence across the eukaryotic tree of life (see reviews in Büchel, 2015; Demmig-Adams & Adams, 2006; Keeling & Burki, 2019). The most abundant peripheral antenna systems found in plants and most eukaryotic algae are composed of light-harvesting complex (LHC) proteins. These LHC proteins interact with chlorophyll (Chl) molecules and carotenoid pigments that absorb and transfer light energy to the photosystems. It is typical for photosynthetic organisms to experience variations in light depending on environmental conditions, light niches, and habitats. Diversifying the LHCs and associated is one way that organisms cope with these environmental differences, allowing for differing light-absorption properties at different wavelengths. This can be seen in the variation of chlorophyll derivatives and carotenoids that are associated with antenna complexes across each evolutionary lineage.

Photosynthetic organisms must also cope with rapidly changing light conditions resulting from factors like cloud cover, transitions to full sunlight, or upwelling. These organisms have adapted to rapidly adjust to limiting and saturating light intensities by evolving regulatory mechanisms according to the environmental cues that they may encounter. For instance, exposure to high light can be harmful to the photosynthetic apparatus and damage essential proteins, cause the formation of disruptive radicals like reactive oxygen species (ROS), and decrease the amount of carbon fixation. One adaptive photoprotective mechanism is called non-photochemical quenching (NPQ),

which dissipates excess absorbed light energy as heat. The term NPQ refers to the way in which thermal dissipation is measured, through quenching of Chl fluorescence (Müller et al., 2001).

NPQ regulates the balance of input and utilization of light energy in photosynthesis to avoid photooxidative damage. The excess absorbed light energy is dissipated as heat and is a harmless process to the organism. The intricacies of NPQ in the green algae and plants, referred to as the green lineage, have been reviewed previously (Demmig-Adams & Adams 2006, Goss & Jakob 2010, Niyogi & Truong 2013, Goss & Lepetit 2015, Murchie & Ruban 2020, Bassi & Dall'osto, 2021). In summary, Chlorophyte algae, Charophytes, and Bryophytes utilize stress-related LHC proteins, which are ubiquitous among many other algal groups, while evolutionarily lost in some (see review in Giovagnetti & Ruban, 2018; Niyogi & Truong, 2013). Green algae utilize the LHCSR protein (Peers et al., 2009), while vascular plants have replaced LHCSR proteins with photosystem II subunit S (PSBS), an independently evolved member of the LHC protein superfamily (Li et al., 2000). Though much work has been done to investigate the key molecular players and mechanisms of NPQ in the green lineage, the past decade has shined a light on intriguing evolutionary differences among other groups of photosynthetic organisms, like the stramenopiles, formerly phylum Heterokonta (Yoon et al., 2009).

## NPQ in stramenochromes

NPQ in photosynthetic stramenopiles has been reviewed by Goss and Lepetit in 2012 and Giovagnetti and Ruban in 2018. However, this introduction seeks to dive deeper into classes beyond the well-studied Bacillariophyceae and Eustigmatophyceae and give an update on the new discoveries pertaining to the molecular players involved in NPQ in this phylum.

Stramenopiles are a large monophyletic eukaryotic group comprised of uni- and multi-cellular organisms ranging from microscopic picoplankton to macroscopic marine seaweeds (Figure 1). Despite their diversity, the unifying feature of stramenopiles is having unicells (and/or reproductive cells) with two "different flagella", a tinsel flagellum and a smooth flagellum. Stramenopiles can be photoautotrophic, mixotrophic, or heterotrophic, and include some colorless groups like Oomycetes ("aquatic fungi") and Labyrinthulomycetes. They are a part of the SAR supergroup, together with Rhizaria and Alveolata. Here we will focus on photosynthetic stramenopiles, phylum Ochrophyta, or hereafter called stramenochromes.

Stramenochromes originated from a secondary endosymbiotic event of a red alga and therefore have a chloroplast surrounded by four membranes. The high diversity in size, ecological niches, and evolutionary differences make stramenopiles interesting to study. For instance, some stramenopiles have silica scales like class Chrysophyceae, are keystone species in marine environments like seaweeds in class Phaeophyceae, can accumulate large amounts of lipids like Eustigmatophyceae, and can contribute to harmful algal blooms like class Raphidophyceae.

Many stramenochromes produce lipids and/or carbohydrates such as chrysolaminarin, cellulose, and alginates. These carbon-rich products of photosynthesis are valuable for industry and offer a sustainable approach to producing biomaterials, which is discussed later in this introduction. Stramenochromes have Chl a, but unlike the green lineage, they lack Chl b. Most stramenochromes instead utilize Chl c derivatives like Chl c1, Chl c2, or Chl c3, enabling them to harvest blue-green light under water (see review in (Büchel, 2020a). Stramenochromes usually contain other characteristic pigments like fucoxanthin (Fx), which gives them their brown color, and vaucheriaxanthin (Vax), which is yellow green (Table 1). These pigments are also known as xanthophylls, which are carotenoids that typically function in photosynthetic light harvesting.

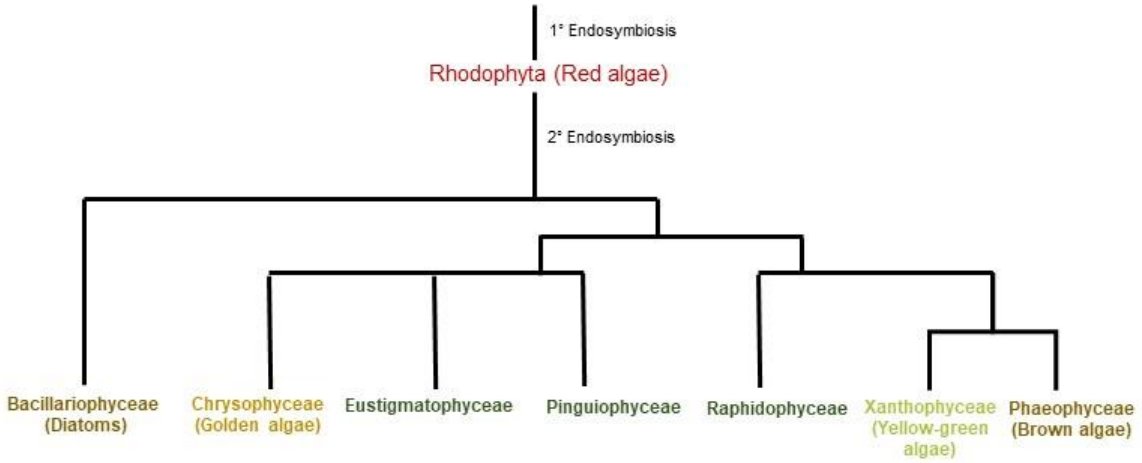



***Figure 1. Phylogenetic tree showing the relationships between major classes of stramenochromes.*** *The tree is adapted from Dorrel and Bowler 2017.*

Some xanthophylls, such as violaxanthin (Vx), antheraxanthin (Ax), and zeaxanthin (Zx), are interconverted in a cycle (i.e., the VAZ cycle), which is important for NPQ and is also present in green algae and land plants. Other photosynthetic organisms utilize xanthophylls like diadinoxanthin (Ddx) and diatoxanthin (Dtx) in the DD cycle for NPQ. Organisms displaying the DD cycle can also possess the VAZ cycle, like those in class Bacillariophyceae, Chrysophyceae, and Raphidophyceae; which cycle is more functional is determined by the pool size of the xanthophyll pigments (Lohr & Wilhelm, 1999a; Tanabe et al., 2009; Yuasa et al., 2020). The interconversion of xanthophylls in the VAZ cycle is catalyzed by violaxanthin de-epoxidase (VDE), which converts Vx to Ax and Zx upon exposure to high light, and zeaxanthin epoxidase (ZEP) which does the reverse epoxidation in low or limiting light. The DD cycle depends on the diadinoxanthin de-epoxidase (DDE) enzyme, which is closely related to VDE. DDE converts Ddx into Dtx, and the reverse reaction is catalyzed by the diatoxanthin epoxidase (DEP) enzyme. Like LHCSR and PSBS, VDE and DDE are activated by low pH in the thylakoid lumen in excess light to synthesize Zx and Dtx for NPQ. The regulation and function of the VAZ and DD cycle in algae were reviewed previously by Goss and Jakob (2010).

Differences in NPQ between stramenochromes and the green lineage

There are several components of NPQ in photosynthetic organisms called qE, qZ, qI, qT, and qH. The qE component is fast-acting, flexible, and controlled by three elements (with variability between evolutionary lineages): 1) the formation of a high ΔpH due to the acidification of the thylakoid lumen during high or excess light, 2) the presence of LHCSR or PSBS in the green lineage, and 3) the synthesis of Zx/Dtx, which is controlled by the xanthophyll cycle (see reviews in Goss & Jakob, 2010; Müller et al., 2001; Niyogi & Truong, 2013). In the green lineage, the carotenoid lutein (Lut) can also be involved in qE (Niyogi et al., 1997b). Protonatable amino acids facing the thylakoid lumen have been identified, examined, and shown to be involved in pH-sensing and qE induction in both *Chlamydomonas reinhardtiii* LHCSR (CrLHCSR3) and *Arabidopsis thaliana* PsbS (AtPSBS; Ballottari et al., 2016; Tian et al. 2019; Li et al., 2004). However, this is not the case in LHCSR-like LHCX proteins found in class Bacillariophyceae, although the presence of LHCX proteins is required for qE (Buck et al., 2021; Giovagnetti et al., 2022). LHCX proteins in stramenochromes are described further in subsequent sections, and examination of the role of LHCX proteins in class Eustigmatophyceae is discussed in Chapter 3 of this dissertation.

Another type of NPQ is called qZ, which is dependent on the enzymatic activity of VDE/DDE and the formation of Zx/Dtx. qZ relaxes at a slower rate than qE, within minutes versus seconds, respectively (Nilkens et al., 2010). The utilization of a xanthophyll cycle to produce Zx/Dt for qZ is nearly ubiquitous among photosynthetic eukaryotes. The green lineage has been shown to employ mainly ΔpH and lumen acidification to activate VDE for Zx formation, whereas some stramenochromes, like in

| Class | Common Name | Chlorophylls | Antenna system | Main Accessory Pigments | Xanthophyll Cycle Utilization | Species Examples | Experimentally Confirmed NPQ Mechanisms | References |
|---|---|---|---|---|---|---|---|---|
| **Bacillariophyceae** | Diatoms | *a, c* | FCP | Fx, Ddx | DD (main)<br>VAZ | Pennate: *Phaeodactylum tricornutum, Fragilariopsis cylindrus*<br>Centric: *Thalassiosira pseudonana, Chaetoceros sp.* | qE, qZ, qI | Taddei et al., 2018, Lacour et al., 2020, Büchel, 2020b |
| **Chrysophyceae** | Golden-brown algae, Chrysomonads, Chrysophytes | *a, c* | FCP | Fx, Vx, Ddx | VAZ (main)<br>DD | *Giraudyopsis stellifer, Ochromonas sp., Dinobryon sp.* | qZ, qT | Lichtlé et al., 1995, Tanabe et al., 2009 |
| **Eustigmatophyceae** | Eustigmatophytes | *a* | VCP, rVCP | Vx, Vax | VAZ | *Nannochloropsis sp. Trachydiscus minutus* | qE, qZ | Basso et al., 2014, Bina et al., 2018, Park et al., 2019, Perin et al., 2023 |
| **Phaeophyceae** | Brown algae, Phaeophyceans | *a, c* | FCP | Fx, Vx | VAZ | *Ectocarpus siliculosus, Macrocystis pyrifera, Saccharina sp., and Sargassum, Laminaria sp., Fucus sp.* | qZ, qI | García-Mendoza et al., 2011, Ocampo-Alvarez & García-Mendoza, 2013, Nan et al., 2022 |
| **Pinguiophyceae** | Pinguiophytes | *a, c* | FCP | Fx, Vx | VAZ | *Phaeomonas sp.,* | qZ | Giovagnetti el al., 2010, Berne et al., 2018 |
| **Raphidophyceae** | Chloromonads, Raphidophyceans | *a, c* | FCP | Fx, Vx, Ddx | VAZ (main)<br>DD | *Chattonella sp., Heterosigma akashiwo* | qE, qZ, qI | Hennige et al., 2013, Sun et al., 2020, Yuasa et al, 2020 |
| **Xanthophyceae** | Yellow-green algae, Xanthophytes | a | XLH | Dd, Vax | DD cycle | *Vaucheria litorea, Tribonema minus* | qE, qZ, qI | Büchel et al., 1988, Gardian et al., 2011, Christa et al., 2018 |

***Table 1: Photoprotective and Photosynthetic Features in Major Classes of Stramenochromes***

classes Phaeophyceae and Bacillariophyceae, can exhibit Zx/Dtx formation independent of the proton gradient (García-Mendoza & Colombo-Pallotta, 2007; Goss et al., 2006). Xanthophyll cycling has been shown to be directly correlated to NPQ in stramenochromes and is discussed more in later sections and Chapter 2. It is possible that this high importance of the xanthophyll cycle in stramenochromes is due to differences in their antenna structures, often made up of fucoxanthin chlorophyll *a/c* protein complexes (FCP), and the larger pool of xanthophylls in the thylakoid membranes when compared to the green lineage, as is the case in Bacillariophyceae (Goss et al., 2006; Goss & Jakob, 2010a).

Other types of NPQ found in photosynthetic organisms include photoinhibitory quenching, qI, state transitions, qT, and sustained antenna quenching, qH (Goss & Lepetit, 2015; Malnoë et al., 2018; Müller et al., 2001). qI, as indicated by decreases in the maximum efficiency of PSII ($F_v/F_m$), is related to oxidative damage to the PSII reaction center and shows very slow relaxation kinetics in the form of hours (Krause & Krause, 1988; Nawrocki et al., 2021). qI is present in stramenochromes, like class Bacillariophyceae, Phaeophyceae, Raphidophyceae and Xanthophyceae, and is discussed in later sections (Hennige et al., 2013; Nan et al., 2022; Uhrmacher et al., 1995). qT is state-transition quenching in which LHC antenna complexes detach from PSII and attach to PSI, thereby adjusting the relative amounts of excitation energy available for PSII and and PSI. qT relaxes within tens of minutes (Nilkens et al., 2010). qT has been shown to exist in class Chrysophyceae (Gibbs & Biggins, 1989). qH is a photoadaptive strategy first described in overwintering evergreen trees (Demmig-Adams et al., 2014). There has been no experimental evidence so far showing that qH exists in stramenochromes, though some arctic Bacillariophytes and Chrysophytes can survive overwintering in arctic environments and rapidly restore photosynthetic capacity due to the availability of Zx/Dtx (Lacour et al., 2019; Tanabe et al., 2009).

This introduction will highlight the biodiversity of photoprotection in stramenochromes by describing the similarities and differences of 7 major classes, and a few important minor classes. The rich diversity of LHCs and the strong use of a xanthophyll cycle in stramenochromes has the potential to better our understanding of the evolution of NPQ and how these organisms differ from the green lineage. With modifications to green-lineage genes and proteins involved in photoprotection, researchers have been able to increase the efficiency of photosynthesis and crop productivity in plants (De Souza et al., 2022; Kromdijk et al., 2016). Understanding photosynthesis and photoprotection in stramenochromes could lead to similar improvements in the algal biotechnology industry and marine ecosystems. Further discussion on these topics is in the section “Stramenochromes are adapted for industry and environmental changes”.

## Bacillariophyceae

Class Bacillariophyceae is composed of diatoms, which are the most well studied class of stramenochromes in terms of photosynthesis and photoprotection. One main reason is that they are major contributors to global net primary productivity, and they also produce high-value compounds like eicosapentaenoic acid (EPA), making them targets for genetic engineering to improve photosynthetic efficiency (Yang et al., 2020).

Bacillariophyceae are separated into two main groups: (1) pennate, or bilaterally symmetrical, diatoms like *Phaeodactylum tricornutum* and *Fragilariopsis cylindrus* and (2) radially symmetrical centric diatoms like *Thalassiosira pseudonana*, *Chaetoceros gracilis*, and *Cyclotella meneghiniana.* Many of these species have sequenced genomes, facilitating the building of molecular tools and generation of knockout mutants in important NPQ genes and proteins. Most recently diatoms have had their photosynthetic apparatus analyzed using single-particle cryogenic electron microscopy (cryo-EM; Nagao et al., 2019; Xu et al., 2020); this will be discussed more in Chapter 4.

Diatoms have a unique antenna system that binds fucoxanthin (Fx) and Chl *a/c* molecules in the fucoxanthin chlorophyll binding protein (FCP) complex associated with each photosystem (Table 1; see review in Büchel, 2020b). Additionally, FCP in diatoms contains Ddx, which has been shown to serve two purposes: 1) synthesis of Dtx for NPQ, and 2) Fx biosynthesis for light harvesting under limiting light (Bai et al., 2022; Goss et al., 2006). NPQ in diatoms has been well studied and reviewed (Büchel, 2020c; Giovagnetti & Ruban, 2018; Lavaud et al., 2004; Lavaud & Goss, 2014; Lavaud & Kroth, 2006). Giovagnetti and Ruban (2018) outlines the evolution of LHCX proteins in different diatom species and compares them to CrLHCSR3 and AtPsbS proteins in the green lineage. Interestingly, there is a large range of the number of genes encoding LHCX proteins in diatoms. Among pennate diatoms, *P. tricornutum* contains genes encoding for four LHCX proteins (Bowler et al., 2008a), and *F. cylindrus* contains eleven (Mock et al., 2017). The centric diatom *T. pseudonana* contains five (Armbrust et al., 2004), whereas *C. meneghiniana* has three (Eppard et al., 2000). Among the different types of LHCX proteins, LHCX1 is the most important for NPQ in diatoms.

Differences between pennate and centric diatoms are also found in the supramolecular organization of their photosynthetic apparatuses. For instance, FCP in pennate *P. tricornutum* was shown to have similar oligomeric organizations as other stramenochromes, whereas centric *C. meneghiniana has a chain-like circular antenna* (Gardian et al., 2014)*. More recent studies probing the structure of PSII-FCPII supercomplexes show that P. tricornutum FCP exists in a dimeric form, T. pseudonana FCP exists in trimeric form, and C. gracilis has tetrameric FCPs* (Arshad et al., 2021; Wang et al., 2020)*.* Because of the organization of the FCP complexes and variation among LHCX proteins involved in NPQ, pennate and centric diatoms have different mechanisms for light-energy usage and photoprotection.

Most of the research on NPQ in diatoms has been done on the model species *P. tricornutum*. PtLHCX1 is found to be the main quencher involved in qE. Unlike *CrLHCSR3*, the *PtLHCX1* gene is constitutively expressed with mRNA levels remaining stable even in increasing light intensities (Bailleul et al., 2010). Knockouts (KOs) of *PtLHCX1* showed abolished qE, while cells grown under intermittent light showed very large qE due to PtLHCX1 constitutive upregulation (Buck et al., 2019; Giovagnetti et al., 2022). LHCX2/3 were found to modulate qE during dynamic light and prolonged HL (Bailleul et al., 2010; Hao et al., 2018; Lepetit et al., 2017; Nymark et al., 2009; Taddei et al., 2018) and during responses to nutrient stress (Buck et al., 2022). PtLHCX4 is induced by dark conditions and possibly involved in survival of cells during long periods of darkness (Taddei et al., 2016). Analysis of isolated thylakoid membranes from *P. tricornutum* showed that PtLHCX1 is distributed ubiquitously in PSI, PSII, and the FCPs,

whereas PtLHCX3 is associated with only PSI and FCP complexes (Taddei et al., 2018). In the same study, spectroscopic analysis showed that the PSII core is more effective in NPQ than the FCP antenna and may be more efficient at protecting diatoms against photoinhibition of PSII. Taken together, overall findings suggest that PtLHCX1 provides constitutive NPQ capacity near the PSII core in low-light growth conditions, with additional quenching in the FCPs after exposure to high light. PtLHCX3 is induced in high light and quenches in the FCPs, with possible help from PtLHCX2 (Lepetit et al., 2017; Taddei et al., 2016, 2018).

How PtLHCX proteins induce NPQ has been a topic of research for a few years. Conservation of two putative protonatable amino acid residues essential for CrLHCSR has been reported in PtLHCX1-3 proteins, whereas PtLHCX4 has only a single residue conserved (Buck et al., 2021). Targeted mutagenesis of these residues, D94 and E205, in PtLHCX1 showed no alteration in qE capacity (Buck et al., 2021; Giovagnetti et al., 2022). This indicates that PtLHCX1 is not involved in sensing ΔpH like CrLHCSR3 or AtPSBS and that these proteins may have functional divergence in this role. However, recent studies on sequence motifs by Buck et al. (2021) identified a transmembrane motif in PtLHCX1 that is involved in qE, with a tryptophan residue having major influence on qE establishment. While Giovagnetti et al. (2022) showed that PtLHCX1 might not bind pigments, Buck et al. (2021) predicts that there is a Ddx/Dtx binding site near the tryptophan residue that might directly participate in quenching absorbed energy from a nearby chlorophyll molecule.

Interestingly, pennate *F. cylindrus* has eleven *LHCX1* genes in its genome, but none were found to be related to the four *LHCX* genes in *P. tricornutum* (Mock et al., 2017). However, it does contain a gene similar to *LHCX6* in centric *T. pseudonana*, which is absent from the *P. tricornutum* genome. TpLHCX6 has been hypothesized to contain a Dtx binding site and play a direct role in NPQ (Zhu & Green, 2010). Another pennate diatom, *Seminavis robusta*, contains 17 putative *LHCX* sequences, although there is likely a degree of functional redundancy among them (Rico et al., 2020). Given that *S. robusta* is a benthic diatom, the abundance of *LHCX* genes may help with coping in highly variable light environments. Further analysis is needed to confirm the function of these SrLHCX proteins in relation to NPQ.

Analysis of LHCX proteins in centric *T. pseudonana*, which has five *LHCX* genes, revealed that TpLHCX1 and TpLHCX6 are important for NPQ. TpLHCX1 constitutively accumulated under low light and high light growth, whereas TpLHCX6 is rapidly induced under high light and may be involved in qI (Zhu & Green, 2010). Another centric diatom, *C. meneghiniana* contains three LHCX proteins (Eppard et al., 2000), and CmFCP6 has been found to be almost identical to LHCX1 in *T. pseudonana*, appearing to be involved in pigment interactions and qE (Ghazaryan et al., 2016a). The magnitude of NPQ in *C. meneghiana* also depends highly on the amount of Dtx available but can also be amplified by an increased proton gradient (Grouneva et al, 2008). Both *T. pseudonana* and *C. meneghiniana* contain LHCX6_1 which is constitutively expressed and may be involved in NPQ (Grouneva et al., 2011; Gundermann et al., 2019). Recently the structure of TpLHCX6_1 was elucidated using single-particle cryo-EM (Feng et al., 2023). The structure of monomeric TpLHCX6_1 was shown in complex with PSII-FCPII, binding Chl *a/c*, Fx, and Ddx molecules (Feng et al., 2023). TpLHCX6_1 also directly links homodimer LHCF7 to the PSII core. These new insights suggest that TpLHCX6_1

may be involved in quenching excess energy at the Ddx/Dtx site close to the core and may mediate aggregation of quenching from more flexible peripheral FCPs like LHCF7.

Bacillariophyceae contains both the VAZ and DD cycles, but mostly utilizes the DD cycle for NPQ, due to its abundance of Ddx over Vx (Lacour et al., 2020). The use of Dtx in NPQ is widely similar among diatom species, with Dtx accumulating under increased levels of high light (see review in Goss & Lepetit, 2015; Zhu & Green, 2010). The DD and VAZ cycles have been investigated mostly in mutants of *P. tricornutum*, with VDE/ZEP used interchangeably with DDE/DE in terms of names. Gene silencing of *PtVDE* showed a lower degree and slower kinetics of both Ddx de-epoxidation and NPQ induction (Lavaud et al., 2012). Recent research has examined the role of three genes encoding for PtZEP enzymes. Both PtZEP2/3 can epoxidize Vx into Zx (Eilers et al., 2016). Other experiments have shown that PtZEP2 may be involved in DD cycle mono-epoxidation of Dtx to Ddx (Bai et al., 2022). PtZEP1 was found to be nonfunctional in Zx epoxidation, but it can serve in mono-epoxidation of haptoxanthin (Hpx) towards Fx synthesis (Bai et al., 2022; Eilers et al., 2016)

## Chrysophyceae

Class Chrysophyceae, sometimes called chrysomonads, is a group of golden-brown algae that contain large amounts of Fx along with Chls *a a*nd *c* (Lichtlé et al., 1995). Their characteristic golden-brown color is due to Fx masking the green color of Chl. Like diatoms, the Chrysophyte antenna system is called FCP. Chrysophytes often have cell walls covered by mucilage or silica scales, including resting stages called stomatocysts covered in silica. Information about photoprotection in Chrysophyceae is limited, although it is known that *Giraudyopsis stellifer*, *Ochromonas smithii*, and *O. itoi* utilize only the VAZ cycle for NPQ, even though they possess the DD cycle (Table 1; Lichtlé et al., 1995; Tanabe et al., 2009). This contrasts with Bacillariophyceae, where the DD cycle is more functional in NPQ than the VAZ cycle (Lohr & Wilhelm, 1999a), and is due to a higher amount of Vx (Tanabe et al., 2009). Interestingly, *Ochromonas danica* is the only heterokont alga where wavelength-dependent state transitions, qT, have been observed (Gibbs & Biggins, 1989).

Chrysophytes can exhibit various nutritional modes, including photoautotrophy, osmotrophy, or phagotrophy, which make them interesting to study (Lie et al., 2018). Phagotrophy in Chrysophytes has been well studied in organisms like *Dinobryon* and *Ochromonas* (Bird & Kalff, 1987; Lie et al., 2018; Wilken et al., 2013, 2020). Recent studies have investigated contrasting modes of mixotrophic lifestyles in two closely related ecotypes of *Ochromonas*, revealing different adaptive ecological niches (Lie et al., 2018; Wilken et al., 2020). An oceanic isolate, CCMP1393, is an obligate mixotroph and requires light and prey for survival through a strong interdependence of photosynthesis and mitochondrial respiration. In contrast, the coastal isolate CCMP2951 is a facultative mixotroph that can substitute photosynthesis for phagotrophy and grow in complete darkness. CCMP2951 can exhibit NPQ and uses mitochondrial respiration as an electron sink, possibly allowing for adaptations to variable environments. Meanwhile, the obligate mixotroph CCMP1393 does not do NPQ in low or high light, and instead relies on mitochondrial respiration as an electron sink (Wilken et al., 2020).

Eustigmatophyceae

Eustigmatophytes are comprised of small coccoid-shaped unicells. They are a sister clade to Chrysophyceae and can exist in freshwater, marine, or terrestrial environments. What sets this class apart from other stramenochromes is that they have a red-pigmented body whose function is unknown but may act as a storage vesicle (cite Chris Gee bioRxiv here). Additionally, Eustigmatophytes do not contain Chl *c* or Fx unlike most other stramenochromes; this is a unifying feature between them and Xanthophyceae (Table 1). Eustigmatophytes utilize Vx as the main accessory pigment for light harvesting in what is called the violaxanthin chlorophyll *a*-binding protein (VCP) antenna (Amaral et al., 2021; Basso et al., 2014; Sukenik et al., 2000).

*Nannochloropsis* is a well-studied Eustigmatophyte for its ability to produce relatively high concentrations of lipids for biofuels (Ajjawi et al., 2017; Cancela et al., 2019; J. Liu et al., 2017a; Simionato et al., 2011a). Throughout the years with the sequencing of its genome and establishment of gene editing tools (Kilian et al., 2011; Vieler et al., 2012b), *Nannochloropsis* has been established as a model organism for genetic manipulation and increased bioproduct accumulation (Kurita et al., 2023). These technologies have also established *N. gaditana* and *N. oceanica* as model Eustigmatophytes for studying NPQ and ways to attenuate photoprotection to enhance light-use efficiencies.

Like diatoms, Eustigmatophytes contain stress-related LHCX proteins that are involved in NPQ (Litvín et al., 2016; Vieler et al., 2012b). Multiple studies have shown that NPQ in *Nannochloropsis sp.* is dependent on the ΔpH build-up, de-epoxidized xanthophylls, and LHCXs, which contribute to qE and qZ(Chapter 2; Cao et al., 2013; Chukhutsina et al., 2017). KO mutants have shown that LHCX1 is essential for qE, and VDE is essential for qE and qZ*,* with knockouts showing strongly diminished NPQ during high-light exposure (Chapter 2; Park et al., 2019; Perin et al., 2023), demonstrating that both qE and qZ are dependent on the presence of Zx (Bína, Bouda, et al., 2017; Park et al., 2019; Perin et al., 2023). Chapter 2 further characterizes NPQ in *N. oceanica* WT and KO lines affecting three LHCX proteins and VDE, while Chapter 3 looks further into the mechanism of how the LHCX1 protein functions in NPQ induction. Although it is unclear whether LHCX1 is actually the main site of quenching in *N. oceanica*, we make strides towards elucidating the structure of the protein in complex with other VCPs, PSI, and PSII as discussed in Chapter 4. We do know that Zx acts as the primary quencher, and Ax acts as short-term memory of HL stress and facilitates rapid responses to fluctuations in light (A. Short et al., 2023). Not included in this dissertation are knockouts of ZEP, which have recently been characterized by overexpression and RNAi lines (Liu et al., 2023; Perin et al., 2023).

*Nannochloropsis* harbors two ZEP genes, *NoZEP1* and *NoZEP2*. *NoZEP1* is phylogenetically closely related to ZEPs from red algae, whereas *NoZEP2* may have been acquired via lateral gene transfer from another organism and is distantly related (Liu et al., 2023). Unlike diatom PtZEPs, NoZEP proteins have similar functions in epoxidating Zx to Vx, although NoZEP1 is the major isoform. Two independent studies have shown the effects of overexpressing NoZEP in this species. Perin et al. (2023) showed that the NgZEP1 OX line can accelerate the reconversion of Zx to Vx, allowing for increases in biomass productivity in photobioreactors. Although NoZEP1 OX lines

created by Liu et al. (2023) resulted increases in Vx and Chl *a*, there was no significant difference observed with respect to maximum quantum efficiency of PSII and algal growth or biomass when compared to WT. Suppression of NoZEP1 led to increases in Zx and decreases in Vx, Chl *a,* and MGDG (a membrane lipid), with attenuated algal growth, indicating that the presence of Vx is important for photosynthesis and growth (Liu et al., 2023).

Though most work on NPQ in Eustigmatophytes has been done on *Nannochloropsis*, a few studies have been done on *Trachydiscus minutus* in recent years. Freshwater *T. minutus* is unique in that it contains a class of LHCs that contain red-shifted forms of Chl *a,* which absorb wavelengths above 690 nm, and it appears to be adapted to shaded habitats (Bína et al., 2018). The antenna here is called rVCP for red-Violaxanthin Chl *a* protein. Characterization of the photosynthetic apparatus of *T. minutus* shows that the thylakoid membranes contain rVCPs like that of the marine Alveolate alga *Chromera velia* (Litvín et al., 2019). The presence of rVCP antennas in Eustigmatophytes opens the door to use far-red light as an enhanced light-use strategy in mass cultures.

## Phaeophyceae

Phaeophyceans are a diverse group of multicellular brown algae. They inhabit marine ecosystems and exhibit remarkable cellular specialization unlike any other stramenochrome group, including photosynthetic blades, specialized blade-bearing stipes, and holdfast attachment structures. Like Bacillariophyceae and Chrysophyceae, the main accessory pigment in Phaeophycean antennas is Fx in the FCP (Table 1). *Ectocarpus siliculosus*, a small intertidal macroalgae, was the first brown alga to have its complete genome sequenced and has eleven different *LHCX* genes (Cock et al., 2010). While these *LHCX* genes were found to be similar to *LHCSR* genes in the green lineage, their function in photoprotection has yet to be examined (Dittami et al., 2010a). This abundance of LHCX proteins probably helps the organism cope with the extreme exposure of the intertidal zone to different light conditions, although experimental evidence suggests that the use of LHCXs in Phaeophyceans is minimal (Nan et al., 2022, Ocampo-Alvarez & García-Mendoza, 2013).

The extent of NPQ can vary significantly in brown algae due to differences in ecological niches and the position of blades of an organism at different depths in the water column. NPQ studies on Phaeophyceans like giant kelps *Macrocystis pyrifera, Saccharina japonica*, and *Sargassum thunbergii* have revealed that NPQ does not rely on ΔpH and is linked solely to the accumulation of Ax and Zx (Garcia-Mendoza et al., 2011; X. M. Li et al., 2014; Zhang et al., 2020), suggesting that they have qZ but lack qE. Even within a single organism like *M. pyrifera*, blades exposed to full sunlight near the eutrophic zone exhibited high NPQ levels, while blades at greater depths have a lower NPQ capacity (García-Mendoza & Colombo-Pallotta, 2007; Ocampo-Alvarez & García-Mendoza, 2013). This difference in NPQ levels is predominantly driven by the size of the xanthophyll cycle pigment pool, with top blades showing faster de-epoxidation from Vx to Zx than bottom blades (García-Mendoza & Colombo-Pallotta, 2007). NPQ variability in giant kelp was also linked to the minimal use of LHCX-like proteins, which may not be directly involved in qE since ΔpH-facilitated NPQ is absent

(Nan et al., 2022). In summary, it is possible that NPQ control in giant kelp, involves increase in quenching sites in the FCP antenna and LHCX proteins, accompanied by increases in Ax and Zx for a high NPQ induction (Nan et al., 2022; Ocampo-Alvarez & García-Mendoza, 2013). Lastly, qI has been shown in species like *Dictyota dichotoma* and *S. thunbergia*, with Zx content linearly correlated to the degree of photoinhibition and decreases in $F_v/F_m$ in both organisms (Nan et al., 2022; Uhrmacher et al., 1995).

Current research on photoprotection in Phaeophyceans is aimed at their strong resilience to ocean warming and environmental stressors. This work can be used to predict future impacts of climate change on seaweed communities. The NPQ strategies observed in kelp, particularly in species like *Sargassum fusiforme* and *Saccharina japonica*, hold promising implications for climate change mitigation efforts. *Sargassum fusiforme* displays a high tolerance of its holdfasts to elevated temperatures and nutrient-poor conditions, resulting in increased vegetative production (Endo, 2021). Additionally, the response of *S. japonica* to elevated $CO_2$ levels and high temperatures led to reductions in NPQ, increased growth, and enhanced photosynthetic performance (Zhang et al., 2020). These findings align with observations in other brown algae like *Padina pavonica, Cystoseira tamariscifolia*, and *Cystoseira humilis* (Betancor et al., 2015; Korbee et al., 2014). Interestingly, *Pelvetia canaliculate* is highly stress tolerant and exhibits ways to synthesize Zx from Vx in the dark when exposed to stressors like dehydration, submersion, anoxia, high UV-B radiation, and high heat (Fernández-Marín et al., 2011; Hupel et al., 2011).

However, some brown algae are more sensitive to the stressors of climate change, and ecological distribution is an important factor. Independent studies on *Laminaria digitata* and *Saccharina latissimi* growing in cold-temperature waters indicated that heat tolerance can be ecotype specific (Liesner et al., 2020; Nepper-Davidsen et al., 2019). In different ecotypes of intertidal *Fucus serratus* and *F. vesiculosus,* increases in temperature resulted in increased NPQ, resulting in yield loss and decreases in photosynthetic activity (Figueroa et al., 2019; Graiff et al., 2021).

## Pinguiophyceae

Pinguiophyceae is a more recently identified class of stramenopiles and is comprised of mostly coccoid shaped unicells, flagellated or nonmotile organisms, They are considered picoeukaryotes or picoplankton due to their small size. Pinguiophytes contain high percentages of PUFAs, especially EPA, which gives them their name from the Latin root “Pingue” meaning fat or grease (Kawachi et al., 2002). The major carotenoid pigments in the FCP antenna include Fx, and Vx, and they have *Chl c* (Table 1; Giovagnetti et al., 2010; Kawachi et al., 2002). Very little research has been done on photoprotection in Pinguiophytes, although two independent studies on *Phaeomonas sp.* indicate that this species exhibits unusual regulation of the xanthophyll cycle.

Like other phytoplankton, Pinguiophytes can find themselves having to adjust to rapid changes in light conditions due to turbulence in the water. *Phaeomonas sp.* is able to photoacclimate physiological and biochemical processes rapidly and grow under a broad range of photon flux densities (Berne et al., 2018; Giovagnetti et al., 2010). Interestingly, *Phaeomonas sp.* not only induces NPQ upon exposure to high light but can exhibit NPQ in the “dark”, or very low light. Giovagnetti et al., 2010 showed that

dark-acclimated cells exposed to “dark” conditions had an onset of NPQ and increases in Zx, followed by decreases in growth rate, cell size, and cell volume. The opposite happened upon exposure to medium light where NPQ levels decreased, and photosynthetic rates increased. When exposed to high light, the alga exhibited high NPQ and accumulation of Zx. This was further supported by Berne et al. (2018), who additionally showed that VDE is already active in the dark, due to low luminal pH, and ZEP exhibited maximal activity under moderate light conditions, being almost inactive in the dark and high light. This atypical regulation challenges what is known about VDE and ZEP activity. The authors hypothesize that NPQ formation in the dark can be developed from a trans-thylakoid proton gradient caused by chlororespiratory activity (Goss & Jakob, 2010a). The chlororespiration-induced generation of a proton gradient across the thylakoid membrane was first seen in Bacillariophyte, *P. tricornutum*, where DDE was activated in the dark and caused Dtx accumulation (Jakob et al., 1999). Other mechanisms of “dark NPQ” related to the xanthophyll cycle have recently been reviewed (Fernández-Marin 2021).

## Raphidophyceae

Raphidophyceae is a class made up of large, naked, unicellular flagellates. They can also occur as nonmotile masses embedded in mucilage and inhabit marine and freshwater environments. Few studies have investigated how Raphidophytes conduct photoprotection and NPQ, but it is known that these algae utilize the VAZ cycle for NPQ without the use of LHCXs (Sun et al., 2020). Most of the work on class Raphidophyceae focuses on the use of competitive light-utilization strategies that contribute to harmful algal blooms (HABs).

Raphidophytes like *Chantonella sp*. can accumulate high amounts of reactive oxygen species (ROS) like the superoxide anion radical ($O_2^-$) and hydrogen peroxide ($H_2O_2$). Both ROS can be secreted into waterways and contribute to ichthyotoxicity and mass fish mortality. *Chantonella sp*., exhibits reduced and inefficient NPQ even with an increased xanthophyll cycle pigment pool when exposed to high light. This low NPQ can be linked to higher ROS accumulation and toxin secretion, making it a critical factor for HAB dynamics (Ahumada-Fierro et al., 2021; Yuasa et al., 2020). To protect themselves from oxidative stress and maintain high photosynthetic activity, *Chantonella sp.* utilizes a peroxiredoxin (Prx) antioxidant enzyme called 2-CysPrx, to scavenge peroxides (Shimasaki et al., 2021). However, the strategic utilization of the xanthophyll cycle pigments, antioxidants, and ROS has been shown to vary among *Chantonella* ecotypes and can be dependent on environmental and global distributions (Marshall & Newman, 2002).

Another Raphidophyte that is associated with HABs is *Heterosigma akashiwo*. This species displays a competitive advantage over other phytoplankton in that it can capitalize on changes in light environments by surface-level migration and short-term surges in growth induced by light (Hennige et al., 2013). Cells grown in long-term low-light conditions can fully acclimate to high light environments within 48 h, with photochemistry resembling that of long-term high-light acclimated cells in that time. While shifts to high light did negatively affect $F_v/F_m$, this rapid acclimation to HL was facilitated by short-term increases in NPQ and de-epoxidized xanthophylls, reduced

PSII reaction center connectivity, and electron transport (Hennige et al., 2013). However, these short increases subsided within 10 min. Recent work has explored the VAZ cycle in *H. akashiwo*, suggesting that Ax and Zx play a role in qE (Sun et al., 2020). Probing second to minute time scales, their findings showed that Ax and Zx are generated in *H. akashiwo* during 15 s of high-light exposure. They suggest that Ax may play a role at the stromal and luminal sides of the thylakoid membrane and participate in the induction of qE without the use of LHCXs. It has also been suggested that not all NPQ in *H. akashiwo* is dependent on the VAZ cycle, because adding VDE inhibitors, DTT and $NH_4Cl$, did not influence the induction of NPQ (Sun et al., 2020). Future research probing the utilization of the VAZ cycle pigments, and molecular components involved, is needed here.

## Xanthophyceae

Xanthophyceae comprises yellow-green algae. These macro- and microscopic algae are physiologically diverse and can exist as amoeba-like forms, filaments, multinucleate coenocytes, non-flagellated unicells, or flagellates. Their cell walls are composed of mostly cellulose and pectin, although some contain silica like Bacillariophyceae and Chrysophyceae. Their main storage product is lipids, which make species like *Tribonema minus* valuable for biofuel precursors (Wang et al., 2014).

Xanthophytes contain no Chl *c* or Fx in the reaction centers or the Xanthophyceae light-harvesting (XLH) antenna, like Eustigmatophyceae (Table 1; Gardian et al., 2011; Khoroshyy et al., 2018). Unlike the Eustigmatophytes, Xanthophytes utilize the DD cycle for NPQ (Büchel et al., 1988; Cruz et al., 2015; Streckaite et al., 2018). Negative-stain electron microscopy and single-particle analysis revealed that the overall structures of the PSI and PSII reaction centers in *Xanthonema debile* were like those of plants and algae (Gardian et al., 2011). However, samples containing the XLH antenna bound to PSI or PSII could not be obtained, and their associations are still unknown and need further investigation. Results did reveal that the XLH alone formed trimeric and oligomeric associations, and that the XLH contains Chl *a*, Ddx, Dtx, Vax, and heteroxanthin (Hx), with Ddx and Hx as the main light-harvesting carotenoids (Büchel & Garab, 1995; Gardian et al., 2011; Streckaite et al., 2018).

*Vaucheria sp.* is a mat-forming macroalga that inhabits muddy intertidal environments. It is adapted to survive hypervariable changes in emersion and immersion periods, temperature, irradiance, and water availability. In-situ experiments showed that *Vaucheria sp.* has no apparent limitation of photosynthetic capacity due to temperature or light and that the organisms did not show typical decreases in photosynthetic capacity due to high light exposure (Kvíderová et al., 2018). Further probing with ex-situ experiments showed that the main driver of photosynthetic activity was irradiance levels, and that *Vaucheria sp*. can be tolerant to high-light conditions. (Kvíderová et al., 2018). NPQ experiments have been conducted to test photoprotective mechanisms in *V. litorea,* specifically in its role in photosymbiotic systems.

Some Xanthophytes can exist as multinucleate coenocytic forms, like *Vaucheria sp*., making them easy prey for sacoglossan sea slugs. The sea slugs can steal the chloroplasts of the alga through a process called kleptoplasty and form a short- to- long-term symbiotic relationship. Sacoglossan sea slugs, such as *Elysia timida* and *E.*

*chlorotica* (Mollusca, Gastropoda), are adapted to incorporate plastids into their digestive systems while the organelles maintain functional photosynthesis and photoprotective mechanisms. Though photosynthetic gene expression is lowered in the photosymbiotic system, the algal plastid-encoded genes are actively transcribed in *Elysia sp.* even after months of starvation (Soule & Rumpho, 2012). The kleptoplasts have been found to utilize the qE, qZ, and qI types of NPQ for their longevity in *Elysia sp*. (Christa et al., 2018), while the sea slugs utilize behavioral regulation like closing their parapodia to shade the kelptoplasts from high light intensities (Jesus et al., 2010) . Studies have shown that *V. litorea* kelptoplasts have active qE and qZ and higher amounts of Dtx than the alga alone (Cruz et al., 2015).

In addition to photosymbiotic relationships, Xanthophytes can survive prolonged dormancy in arctic environments. Studies on artic-dwelling *Tribonema minus* shows that this species can also undergo morphological changes from vegetative cells to dormant akinetes to survive the wintering season (Kvernvik et al., 2018). Like other artic phytoplankton, *T. minus* has been shown to rapidly reactivate photosynthesis after periods of wintering and dormancy, low temperature, and low-light conditions (Jimel et al., 2020; Kvernvik et al., 2018). However, NPQ values of *T. minus* coming out of dormancy were lower than other marine phytoplankton such as diatoms and dinoflagellates, which can be seen as a success strategy to out-compete other artic phytoplankton at the start of the vegetative season (Jimel et al., 2020).

## Photoprotection and photosynthesis across minor classes

There are several minor classes of stramenochromes, including Bolidophyceae, Chrysomerophyceae, Chrysoparadoxophycae, Dictyochophyceae, Olisthodiscophycae, Pelagophyceae, Phaeothamniophyceae, Schizocladiophyceae, and Synchromophyceae (Derelle et al., 2016; Dorrell & Bowler, 2017; Yoon et al., 2009). Several new classes including Aurearenophycea, Picophagophycae and Phaeosacciophyceae have also been proposed (Eliáš, 2021; Graf et al., 2020; Horn et al., 2007; Kai et al., 2008). Very few studies have been conducted on these minor classes in terms of photoprotection or photosynthesis, leaving room for new discoveries and insights on how these organisms may be similar to or different from other stramenochromes.

Here is a summary of what is known so far. Bolidophyceae picoplankton are considered to be the closest sister group to the diatoms, and pigment analysis confirms that Fx is the major accessory carotenoid (Guillou et al., 1999; Ichinomiya et al., 2016). Chrysoparadoxophyceae contains golden-brown alga Chrysoparadoxa australica, which is considered the “golden paradox” due to having an atypical plastid surrounded by two membranes instead of four (Wetherbee et al., 2019). Another unique feature of this alga is that it is sand-dwelling and briefly amoeboid during vegetative reproduction. Class Pelagophyceae includes Pelagomonas calceolate, which contains an FCP antenna system with an atypical accumulation of xanthophyll cycle pigments for NPQ. While P. calceolate contains DD cycle pigments, Vx and Ax are also present, though no Zx is synthesized during high light exposure (Dimier et al., 2009). Similar absence of Zx has been found in a Prasinophyte alga, Ostreococcus tauri, which is in the green lineage (Six et al., 2008). Additionally, increases in Dtx coincided with increases in Ax in high light, but not Ddx; the authors hypothesize that Ax is a precursor to Dtx. Class

Phaeothamniophyceae contains olive-brown algae that are considered sister taxa to Xanthophyceae and Pinguiophyceae. HPLC analysis on pigments of Phaeothamnion confervicolum showed the presence of Fx, Chl a/c, Hx, and DD cycle pigments (Bailey et al., 1998).

## Conclusion

### Unique photoprotective strategies among stramenochromes

Stramenochromes are a highly diverse group of photosynthetic organisms that occupy a myriad of ecological niches. Intertidal zones, benthic zones, arctic snow/seas, and sand are just some environments in which stramenochromes can be found, contributing to the diversity of photoprotective strategies among this group.

The mechanisms of NPQ in stramenochromes are highly diverse. Stramenochromes have a large variety in the number of different LHCX proteins which may allow them to cope with dynamic changes in light environments, oceanic depths, and nutrient stressors. LHCX proteins are well studied in classes Bacillariophyceae and Eustigmatophyceae, with sequenced genomes and molecular tools paving the way for the generation of mutants. Both classes utilize LHCX proteins for qE, with PtLHCX1/NoLHCX1 being the most important for qE induction (Buck et al., 2021; Giovagnetti et al., 2022; Park et al., 2019; Perin et al., 2023), and PtLHCX2/3 are involved in photoprotection during dynamic light, prolonged high light, and nutrient stress (Bailleul et al., 2010; Buck et al., 2019, 2022; Hao et al., 2018; Lepetit et al., 2017; Nymark et al., 2009; Taddei et al., 2018). Unlike CrLHCSR3 and AtPSBS of the green lineage, stramenochrome LHCX proteins have functional divergence in sensing ΔpH, and may be involved in increasing the number of pigment binding sites for NPQ (Chapter 3, Buck et al., 2021; Giovagnetti et al., 2022). However, recent studies identified a tryptophan residue in PtLHCX1 having major influence on qE establishment (Buck et al., 2021). Other LHCX proteins shown to be important for NPQ induction include TpLHCX1, TpLHCX6, and CmFCP6 from class Bacillariophyceae (Ghazaryan et al., 2016b; Zhu & Green, 2010). Other classes of stramenochromes contain *LHCX* genes, like Phaeophyceae and Raphidophyceae, although studies have shown that these proteins may not have major roles in photoprotection (Dittami et al., 2010a; Nan et al., 2022; Sun et al., 2020). Instead, organisms were shown to heavily rely on the VAZ cycle for NPQ. The abundance of *LHCX* genes in stramenochromes may indicate that these proteins can help the organism cope with exposure to varying light conditions and environmental stressors, and closer examination or mutant lines may help elucidate the function of LHCX proteins other stramenochromes.

The utilization of the xanthophyll cycle for NPQ is ubiquitous among stramenochromes, with all classes containing the DD and/or VAZ cycle. However, the functional utilization of each cycle in qE and qZ can vary. For instance, Bacillariophyceae, Chrysophyceae, and Raphidophyceae contain both the DD and VAZ cycles, while only Bacillariophyceae favors the DD cycle due to a larger pool of Ddx (Lohr & Wilhelm, 1999a; Tanabe et al., 2009; Yuasa et al., 2020). Of all the stramenochromes, only class Xanthophyceae utilizes solely the DD cycle for NPQ, while all others utilize only the VAZ cycle. One reason for this is that VAZ cycle

pigments are obligatory precursors for the formation of DD cycle pigments and Fx, and this mechanism most likely evolved first (Goss & Jakob, 2010a).

In addition to qE and qZ, some stramenochromes have been shown to exhibit qI. This includes class Bacillariophyceae, Phaeophyceae, Raphidophyceae, and Xanthophyceae (Christa et al., 2018; Hennige et al., 2013; Nan et al., 2022; Uhrmacher et al., 1995; Zhu & Green, 2010). qT has been experimentally shown to exist in class Chrysophyceae, *Ochromonas danica,* where wavelength-dependent state transitions were observed (Gibbs & Biggins, 1989). Although no experimental evidence shows that qH exists in stramenochromes, arctic Bacillariophytes and Chrysophytes can rapidly reactivate photosynthesis after overwintering, which may be attributed to a readily available pool for de-epoxidized xanthophylls (Lacour et al., 2019; Tanabe et al., 2009).

Some species of stramenochromes can conduct NPQ in the dark. This has also been observed across the green lineage, with possible mechanisms of "dark NPQ" reviewed by Fernández-Marín et al. (2021). In class Phaeophyceae, *Pelvetia canaliculate* can induce Zx synthesis in the dark when exposed to environmental stressors like dehydration, submersion, anoxia, high UV-B radiations, and high heat (Fernández-Marín et al., 2011; Hupel et al., 2011). Additionally, Pinguiophyte *Phaeomonas sp.,* exhibits a "dark NPQ" that may be linked to chlororespiration (Berne et al., 2018; Giovagnetti et al., 2010). This intriguing phenomenon challenges the conventional view of a high light regulated xanthophyll cycle, and these species above may be well suited for further molecular studies. Other interesting strategies of light-utilization in stramenochromes include the high formation of ROS in Raphidophyceae that contribute to their success in HABs (Ahumada-Fierro et al., 2021; Hennige et al., 2013; Yuasa et al., 2020) and an atypical XC in Pelagophycean, *Pelagomonas calceolate* which only has Vx, and Ax, but no Zx (Dimier et al., 2009).

Stramenochromes are well suited for metabolite production and environmental changes.

Many stramenochromes produce high amounts of biomass, sequester carbon, and convert solar energy into high-value metabolites like pigments, lipids, polyunsaturated fatty acids (PUFAs), polysaccharides, and pharmaceuticals (Borowitzka, 2013). For instance, class Bacillariophyceae can produce silica for industrial applications, pigments, lipids for renewable biodiesel, and chrysolaminarin as a carbon storage product (Yang et al., 2020). Because stramenochromes are photosynthetic and have adapted to grow in a wide range of environmental conditions, they can be used as a renewable energy source and for carbon dioxide removal.

Realizing algae as sustainable biofactories requires new innovations to decrease production costs and increase yields. Because there is a trade-off between photoprotection and light-use efficiency, photoprotective mechanisms can be altered to achieve higher yields of biomass and bio-products (see reviews in Benedetti et al., 2018; Walter & Kromdijk, 2022). Accelerating the reconversion back to light-harvesting and Vx formation is one way to provide an advantage for light-to-biomass productivity (De Souza et al., 2022; Kromdijk et al., 2016). Recently Perin et al. (2023) showed that overexpressing ZEP in Eustigmatophyte, *Nannochloropsis oceanica*, accelerated Vx formation and light harvesting capacity, resulting in increases in biomass productivity.

Another method is to decrease the LHC antenna size, which has been shown to improve productivity in crops and cyanobacteria in high light but not yet in stramenochromes (Kirst et al., 2014, 2017). Many other strain selection and bioengineering techniques have been used on stramenochromes, such as nutrient depletion, differing light regimes, altering wavelengths, and inducing mutations (Fattore et al., 2021; Perin et al., 2015; Simionato, Basso, et al., 2013; Simionato et al., 2011a; Teo et al., 2014).

Another strategy to increase the production of high-value metabolites in stramenochromes is to take advantage of mixotrophic growth strategies. For instance, some Chrysophytes and Xanthophytes can grow mixotrophically, with *Tribonema minus* accumulating high amounts of lipids for biofuel precursors (Lie et al., 2018; H. Wang et al., 2014). Because stramenochromes are photosynthetic, some industrial growth conditions may be limited by light availability in large-scale open ponds, causing decreases in biomass and metabolite production. Taking advantage of mixotrophic growth strategies could be used to increase growth rates, biomass, and compound production (see review in Castillo et al., 2021).

Many marine ecosystems are affected by climate change such as increasing ocean acidification, land-based pollution, sea-surface temperature rise, and Arctic ice thinning. Some stramenochromes are resilient to challenges posed by climate change. Many species in class Phaeophyceae are prime examples of this, like *Saccharina japonica* which can decrease NPQ and increase growth in elevated $CO_2$ levels and high temperatures (Zhang et al., 2020). Brown algae can also be used for bioremediation and ocean alkalinization, like *Fucus vesiculosus* and *Ectocarpus siliculosus,* which sequester carbon from the ocean and store it as stable polysaccharides (Buck-Wiese et al., 2023; Michel et al., 2010). Other stramenochromes are resilient to environmental stressors like high light, $CO_2$ levels, salinity, UV radiation, and temperature. For instance Chrysophytes, *Ochromonas smithii* and *O .itoi,* utilize VAZ cycle pigments for photoprotection, allowing them to bloom in environments with low temperature, low nutrients, and high light (Tanabe et al., 2009). Raphidophyte *Heterosigma akashiwo* is also resilient to challenges posed by climate change and can increase growth in response to elevated $CO_2$ and temperature (Fu et al., 2008). Overall, the ability of stramenochromes to produce highly valuable metabolites and thrive in dynamic environments makes them well suited for industrial purposes and the challenges of climate change.

## References


Ahumada-Fierro, N. V., García-Mendoza, E., Sandoval-Gil, J. M., & Band-Schmidt, C. J. (2021). Photosynthesis and photoprotection characteristics related to ROS production in three Chattonella (Raphidophyceae) species. *Journal of Phycology*, *57*(3), 941–954. https://doi.org/10.1111/JPY.13138

Ajjawi, I., Verruto, J., Aqui, M., Soriaga, L. B., Coppersmith, J., Kwok, K., Peach, L., Orchard, E., Kalb, R., Xu, W., Carlson, T. J., Francis, K., Konigsfeld, K., Bartalis, J., Schultz, A., Lambert, W., Schwartz, A. S., Brown, R., & Moellering, E. R. (2017). Lipid production in Nannochloropsis gaditana is doubled by decreasing expression

of a single transcriptional regulator. *Nature Biotechnology*, *35*(7), 647–652. https://doi.org/10.1038/NBT.3865

Alboresi, A., Le Quiniou, C., Yadav, S. K. N., Scholz, M., Meneghesso, A., Gerotto, C., Simionato, D., Hippler, M., Boekema, E. J., Croce, R., & Morosinotto, T. (2017). Conservation of core complex subunits shaped the structure and function of photosystem I in the secondary endosymbiont alga Nannochloropsis gaditana. *New Phytologist*, *213*, 714–726. https://doi.org/10.1111/nph.14156

Alboresi, A., Perin, G., Vitulo, N., Diretto, G., Block, M., Jouhet, J., Meneghesso, A., Valle, G., Giuliano, G., Maréchal, E., & Morosinotto, T. (2016). Light Remodels Lipid Biosynthesis in Nannochloropsis gaditana by Modulating Carbon Partitioning between Organelles. *Plant Physiology*, *171*(4), 2468–2482. https://doi.org/10.1104/PP.16.00599

Alboresia, A., Gerottob, C., Giacomettib, G. M., Bassia, R., & Morosinotto, T. (2010). Physcomitrella patens mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(24), 11128–11133. https://doi.org/10.1073/PNAS.1002873107

Amaral, R., Sérgio Seixas de Melo, J., & Maria Antunes dos Santos, L. (2021). Pigments from Eustigmatophyceae: an interesting class of microalgae for carotenoid production. *Journal of Applied Phycology*, *33*, 371–384. https://doi.org/10.1007/s10811-020-02312-z/Published

Antoshvili, M., Caspy, I., Hippler, · Michael, & Nelson, · Nathan. (2019). Structure and function of photosystem I in Cyanidioschyzon merolae. *Photosynthesis Research*, *139*, 499–508. https://doi.org/10.1007/s11120-018-0501-4

Armbrust, E. V., Berges, J. A., Bowler, C., Green, B. R., Martinez, D., Putnam, N. H., Zhou, S., Allen, A. E., Apt, K. E., Bechner, M., Brzezinski, M. A., Chaal, B. K., Chiovitti, A., Davis, A. K., Demarest, M. S., Detter, J. C., Glavina, T., Goodstein, D., Hadi, M. Z., … Rokhsar, D. S. (2004). The genome of the diatom Thalassiosira Pseudonana: Ecology, evolution, and metabolism. *Science*, *306*(5693), 79–86. https://doi.org/10.1126/science.1101156

Arsalane, W., Rousseau, B., & Duval, J. -C. (1994). INFLUENCE OF THE POOL SIZE OF THE XANTHOPHYLL CYCLE ON THE EFFECTS OF LIGHT STRESS IN A DIATOM: COMPETITION BETWEEN PHOTOPROTECI'ION AND PHOTOINHIBITION. *Photochemistry and Photobiology*, *60*(3), 237–243. https://doi.org/10.1111/J.1751-1097.1994.TB05097.X

Arshad, R., Calvaruso, C., Boekema, E. J., Buchel, C., & Kouril, R. (2021). Revealing the architecture of the photosynthetic apparatus in the diatom Thalassiosira pseudonana. *Plant Physiology*, *186*(4), 2124–2136. https://doi.org/10.1093/PLPHYS/KIAB208

Bai, Y., Cao, T., Dautermann, O., Buschbeck, P., Cantrell, M. B., Chen, Y., Lein, C. D., Shi, X., Ware, M. A., Yang, F., Zhang, H., Zhang, L., Peers, G., Li, X., & Lohr, M. (2022). Green diatom mutants reveal an intricate biosynthetic pathway of fucoxanthin. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(38). https://doi.org/10.1073/PNAS.2203708119/-/DCSUPPLEMENTAL

Bailey, J. C., Bidigare, R. R., Christensen, S. J., & Andersen, R. A. (1998). Phaeothamniophyceae Classis Nova: A New Lineage of Chromophytes Based upon Photosynthetic Pigments, rbcL Sequence Analysis and Ultrastructure. *Protist*, *149*(3), 245–263. https://doi.org/10.1016/S1434-4610(98)70032-X

Bailleul, B., Rogato, A., De Martino, A., Coesel, S., Cardol, P., Bowler, C., Falciatore, A., & Finazzi, G. (2010). An atypical member of the light-harvesting complex stress-related protein family modulates diatom responses to light. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(42), 18214–18219. https://doi.org/10.1073/pnas.1007703107

Ballottari, M., Truong, T. B., De Re, E., Erickson, E., Stella, G. R., Fleming, G. R., Bassi, R., & Niyogi, K. K. (2016). Identification of pH-sensing Sites in the Light Harvesting Complex Stress-related 3 Protein Essential for Triggering Non-photochemical Quenching in Chlamydomonas reinhardtii. *The Journal of Biological Chemistry*, *291*(14), 7334–7346. https://doi.org/10.1074/jbc.M115.704601

Baroli, I., Do, A. D., Yamane, T., & Niyogi, K. K. (2003). Zeaxanthin Accumulation in the Absence of a Functional Xanthophyll Cycle Protects Chlamydomonas reinhardtii from Photooxidative Stress. *The Plant Cell*, *15*(4), 992–1008. https://doi.org/10.1105/TPC.010405

Bassi, R., & Dall'osto, L. (2021). *Dissipation of Light Energy Absorbed in Excess: The Molecular Mechanisms*. https://doi.org/10.1146/annurev-arplant-071720

Basso, S., Simionato, D., Gerotto, C., Segalla, A., Giacometti, G. M., & Morosinotto, T. (2014). Characterization of the photosynthetic apparatus of the Eustigmatophycean Nannochloropsis gaditana: Evidence of convergent evolution in the supramolecular organization of photosystem I. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(2), 306–314. https://doi.org/10.1016/J.BBABIO.2013.11.019

Benedetti, M., Vecchi, V., Barera, S., & Dall'Osto, L. (2018). Biomass from microalgae: The potential of domestication towards sustainable biofactories. *Microbial Cell Factories*, *17*(1). https://doi.org/10.1186/S12934-018-1019-3

Berne, N., Fabryova, T., Istaz, B., Cardol, P., & Bailleul, B. (2018). The peculiar NPQ regulation in the stramenopile Phaeomonas sp. challenges the xanthophyll cycle dogma. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1859*(7), 491–500. https://doi.org/10.1016/J.BBABIO.2018.03.013

Betancor, S., Domínguez, B., Tuya, F., Figueroa, F. L., & Haroun, R. (2015). Photosynthetic performance and photoprotection of Cystoseira humilis

(Phaeophyceae) and Digenea simplex (Rhodophyceae) in an intertidal rock pool. *Aquatic Botany*, *121*, 16–25. https://doi.org/10.1016/J.AQUABOT.2014.10.008

Bína, D., Bouda, K., & Litvín, R. (2017). A two-component nonphotochemical fluorescence quenching in eustigmatophyte algae. *Photosynthesis Research*, *131*(1), 65–77. https://doi.org/10.1007/S11120-016-0299-X/FIGURES/9

Bína, D., Durchan, M., Kuznetsova, V., Vácha, F., Litvín, R., & Polívka, T. (2018). *Energy transfer dynamics in a red-shifted violaxanthin-chlorophyll a light-harvesting complex*. https://doi.org/10.1016/j.bbabio.2018.11.006

Bína, D., Gardian, Z., Herbstová, M., & Litvín, R. (2017). Modular antenna of photosystem I in secondary plastids of red algal origin: a Nannochloropsis oceanica case study. *Photosynthesis Research*, *131*(3), 255–266. https://doi.org/10.1007/s11120-016-0315-1

Bird, D. F., & Kalff, J. (1987). Algal phagotrophy: Regulating factors and importance relative to photosynthesis in Dinobryon (Chrysophyceae). *Limnology and Oceanography*, *32*(2), 277–284. https://doi.org/10.4319/LO.1987.32.2.0277

Bonente, G., Ballottari, M., Truong, T. B., Morosinotto, T., Ahn, T. K., Fleming, G. R., Niyogi, K. K., & Bassi, R. (2011). Analysis of LHcSR3, a protein essential for feedback de-excitation in the green alga Chlamydomonas reinhardtii. *PLoS Biology*, *9*(1). https://doi.org/10.1371/JOURNAL.PBIO.1000577

Borowitzka, M. A. (2013). High-value products from microalgae—their development and commercialisation. *Journal of Applied Phycology 2013 25:3*, *25*(3), 743–756. https://doi.org/10.1007/S10811-013-9983-9

Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., Otillar, R. P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde, M., Katinka, M., Mock, T., Valentin, K., … Grigoriev, I. V. (2008a). The Phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature 2008 456:7219*, *456*(7219), 239–244. https://doi.org/10.1038/nature07410

Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., Otillar, R. P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde, M., Katinka, M., Mock, T., Valentin, K., … Grigoriev, I. V. (2008b). The Phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature 2008 456:7219*, *456*(7219), 239–244. https://doi.org/10.1038/nature07410

Brenner, S., & Horne, R. W. (1959). A NEGATIVE STAINING METHOD FOR HIGH RESOLUTION ELECTRON MICROSCOPY OF VIRUSES. *Biochimica Et Biophysica Acta* , *34*, 103–110.

Büchel, C. (2015a). Evolution and function of light harvesting proteins. *Journal of Plant Physiology*, *172*, 62–75. https://doi.org/10.1016/J.JPLPH.2014.04.018

Büchel, C. (2015b). Evolution and function of light harvesting proteins. *Journal of Plant Physiology*, *172*, 62–75. https://doi.org/10.1016/J.JPLPH.2014.04.018

Büchel, C. (2020a). Light harvesting complexes in chlorophyll c-containing algae. *Biochimica et Biophysica Acta - Bioenergetics*, *1861*(4). https://doi.org/10.1016/j.bbabio.2019.05.003

Büchel, C. (2020b). Light harvesting complexes in chlorophyll c-containing algae. *Biochimica et Biophysica Acta - Bioenergetics*, *1861*(4). https://doi.org/10.1016/J.BBABIO.2019.05.003

Büchel, C. (2020c). *Light-Harvesting Complexes of Diatoms: Fucoxanthin-Chlorophyll Proteins*. 441–457. https://doi.org/10.1007/978-3-030-33397-3_16

Büchel, C., & Garab, G. (1995). Electrochromic absorbance changes in the chlorophyll-c-containing alga Pleurochloris meiringensis (Xanthophyceae). *Photosynthesis Research*, *43*, 49–56.

Büchel, C., Wilhelm, C., & Lenartz-Weiler, I. (1988). The Molecular Analysis of the Light Adaptation Reactions in the Yellow-green Alga Pleurochloris meiringensis (Xanthophyceae). *Botanica Acta*, *101*(4), 306–310. https://doi.org/10.1111/J.1438-8677.1988.TB00049.X

Buck, J. M., Kroth, P. G., & Lepetit, B. (2021). Identification of sequence motifs in Lhcx proteins that confer qE-based photoprotection in the diatom Phaeodactylum tricornutum. *The Plant Journal*, *108*(6), 1721–1734. https://doi.org/10.1111/TPJ.15539

Buck, J. M., Sherman, J., Bártulos, C. R., Serif, M., Halder, M., Henkel, J., Falciatore, A., Lavaud, J., Gorbunov, M. Y., Kroth, P. G., Falkowski, P. G., & Lepetit, B. (2019). Lhcx proteins provide photoprotection via thermal dissipation of absorbed light in the diatom Phaeodactylum tricornutum. *Nature Communications 2019 10:1*, *10*(1), 1–12. https://doi.org/10.1038/s41467-019-12043-6

Buck, J. M., Wünsch, M., Schober, A. F., Kroth, P. G., & Lepetit, B. (2022). Impact of Lhcx2 on Acclimation to Low Iron Conditions in the Diatom Phaeodactylum tricornutum. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/FPLS.2022.841058

Buck-Wiese, H., Andskog, M. A., Nguyen, N. P., Bligh, M., Asmala, E., Vidal-Melgosa, S., Liebeke, M., Gustafsson, C., & Hehemann, J. H. (2023). Fucoid brown algae inject fucoidan carbon into the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(1), e2210561119. https://doi.org/10.1073/PNAS.2210561119/SUPPL_FILE/PNAS.2210561119.SAPP.PDF

Burton-Smith, R. N., Akimasa Watanabe, X., Ryutaro Tokutsu, X., Song, C., Kazuyoshi Murata, X., & Jun Minagawa, X. (2019). Structural determination of the large photosystem IIâ€“light-harvesting complex II supercomplex of Chlamydomonas

reinhardtii using nonionic amphipol. *J. Biol. Chem*, *294*(41), 15003. https://doi.org/10.1074/jbc.RA119.009341

Cancela, A., Pérez, L., Febrero, A., Sánchez, A., Salgueiro, J. L., & Ortiz, L. (2019). Exploitation of Nannochloropsis gaditana biomass for biodiesel and pellet production. *Renewable Energy*, *133*, 725–730. https://doi.org/10.1016/j.renene.2018.10.075

Cao, S., Zhang, X., Fan, X., Qiao, H., Liang, C., Xu, D., Mou, S., Wang, W., & Ye, N. (2013). Phylogeny and characterisation of Nannochloropsis oceanica var. sinensis var. nov. (Eustigmatophyceae), a new oleaginous alga from China. *Phycologia*, *52*(6), 573–577. https://doi.org/10.2216/13-164.1

Cao, S., Zhang, X., Xu, D., Fan, X., Mou, S., Wang, Y., Ye, N., & Wang, W. (2013). A transthylakoid proton gradient and inhibitors induce a non-photochemical fluorescence quenching in unicellular algae Nannochloropsis sp. *FEBS Letters*, *587*(9), 1310–1315. https://doi.org/10.1016/J.FEBSLET.2012.12.031

Carbonera, D., Agostini, A., Di Valentin, M., Gerotto, C., Basso, S., Giacometti, G. M., & Morosinotto, T. (2014). Photoprotective sites in the violaxanthin-chlorophyll a binding Protein (VCP) from Nannochloropsis gaditana. *Biochimica et Biophysica Acta - Bioenergetics*, *1837*(8), 1235–1246. https://doi.org/10.1016/J.BBABIO.2014.03.014

Castillo, T., Ramos, D., García-Beltrán, T., Brito-Bazan, M., & Galindo, E. (2021). Mixotrophic cultivation of microalgae: An alternative to produce high-value metabolites. *Biochemical Engineering Journal*, *176*, 108183. https://doi.org/10.1016/j.bej.2021.108183

Christa, G., Pütz, L., Sickinger, C., Clavijo, J. M., Laetz, E. M. J., Greve, C., & Serôdio, J. (2018). Photoprotective Non-photochemical Quenching Does Not Prevent Kleptoplasts From Net Photoinactivation. *Frontiers in Ecology and Evolution | Www.Frontiersin.Org*, *1*, 121. https://doi.org/10.3389/fevo.2018.00121

Chukhutsina, V. U., Fristedt, R., Morosinotto, T., & Croce, R. (2017). Photoprotection strategies of the alga Nannochloropsis gaditana. *Biochimica et Biophysica Acta - Bioenergetics*, *1858*(7), 544–552. https://doi.org/10.1016/J.BBABIO.2017.05.003

Cock, J. M., Sterck, L., Rouzé, P., Scornet, D., Allen, A. E., Amoutzias, G., Anthouard, V., Artiguenave, F., Aury, J.-M., Badger, J. H., Beszteri, B., Billiau, K., Bonnet, E., Bothwell, J. H., Bowler, C., Boyen, C., Brownlee, C., Carrano, C. J., Charrier, B., … Wincker, P. (2010). *The Ectocarpus genome and the independent evolution of multicellularity in brown algae*. https://doi.org/10.1038/nature09016

Correa-Galvis, V., Redekop, P., Guan, K., Griess, A., Truong, T. B., Wakao, S., Niyogi, K. K., & Jahns, P. (2016). Photosystem II Subunit PsbS Is Involved in the Induction of LHCSR Protein-dependent Energy Dissipation in Chlamydomonas reinhardtii*. *Journal of Biological Chemistry*, *291*(33), 17478–17487. https://doi.org/10.1074/JBC.M116.737312

Cruz, S., Cartaxana, P., Newcomer, R., Dionísio, G., Calado, R., Serôdio, J., Pelletreau, K. N., Rumpho, M. E., & Biological, M. (2015). *Photoprotection in sequestered plastids of sea slugs and respective algal sources*. https://doi.org/10.1038/srep07904

D'Amici, G. M., Huber, C. G., & Zolla, L. (2009). Separation of thylakoid membrane proteins by sucrose gradient ultracentrifugation or blue native-SDS-PAGE two-dimensional electrophoresis. *Methods in Molecular Biology (Clifton, N.J.)*, *528*, 61–70. https://doi.org/10.1007/978-1-60327-310-7_4/COVER

Daskalakis, V., Papadatos, S., & Kleinekathöfer, U. (2019). Fine tuning of the photosystem II major antenna mobility within the thylakoid membrane of higher plants. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1861*(12), 183059. https://doi.org/10.1016/J.BBAMEM.2019.183059

De Meyer, F., Maddalena Venturoli, & Berend Smit. (2008). Hydrophobic Mismatch Can Induce Specific and Selective Membrane Protein Interactions. *The 2008 Spring National Meeting*.

De Souza, A. P., Burgess, S. J., Doran, L., Hansen, J., Manukyan, L., Maryn, N., Gotarkar, D., Leonelli, L., Niyogi, K. K., & Long, S. P. (2022). Soybean photosynthesis and crop yield are improved by accelerating recovery from photoprotection. *Science*, *377*(6608), 851–854. https://doi.org/10.1126/SCIENCE.ADC9831/SUPPL_FILE/SCIENCE.ADC9831_MDAR_REPRODUCIBILITY_CHECKLIST.PDF

Demmig-Adams, B. (1990). Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1020*(1), 1–24. https://doi.org/10.1016/0005-2728(90)90088-L

Demmig-Adams, B., & Adams, W. W. (1996). The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant Science*, *1*(1), 21–26. https://doi.org/10.1016/S1360-1385(96)80019-7

Demmig-Adams, B., & Adams, W. W. (2006a). Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New Phytol.*, *172*(1), 11–21. https://doi.org/10.1111/j.1469-8137.2006.01835.x

Demmig-Adams, B., & Adams, W. W. (2006b). Photoprotection in an ecological context: The remarkable complexity of thermal energy dissipation. *New Phytologist*, *172*(1), 11–21. https://doi.org/10.1111/J.1469-8137.2006.01835.X

Demmig-Adams, B., Koh, S.-C., Cohu, C. M., Muller, O., Stewart, J. J., & Iii, W. W. A. (2014). Non-Photochemical Fluorescence Quenching in Contrasting Plant Species and Environments. *Advances in Photosynthesis and Respiration*, *40*, 531–552. https://doi.org/10.1007/978-94-017-9032-1_24

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., & Gascuel, O. (2008).

Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, *36*(Web Server issue). https://doi.org/10.1093/NAR/GKN180

Derelle, R., López-García, P., Timpano, H., & Moreira, D. (2016). A Phylogenomic Framework to Study the Diversity and Evolution of Stramenopiles (=Heterokonts). *Molecular Biology and Evolution*, *33*(11), 2890–2898. https://doi.org/10.1093/MOLBEV/MSW168

Dimier, C., Brunet, C., Geider, R., & Raven, J. (2009). Growth and photoregulation dynamics of the picoeukaryote Pelagomonas calceolata in fluctuating light. *Limnology and Oceanography*, *54*(3), 823–836. https://doi.org/10.4319/LO.2009.54.3.0823

Dittami, S. M., Michel, G., Collén, J., Boyen, C., & Tonon, T. (2010a). Chlorophyll-binding proteins revisited - A multigenic family of light-harvesting and stress proteins from a brown algal perspective. *BMC Evolutionary Biology*, *10*(1), 1–14. https://doi.org/10.1186/1471-2148-10-365/FIGURES/5

Dittami, S. M., Michel, G., Collén, J., Boyen, C., & Tonon, T. (2010b). Chlorophyll-binding proteins revisited - A multigenic family of light-harvesting and stress proteins from a brown algal perspective. *BMC Evolutionary Biology*, *10*(1), 1–14. https://doi.org/10.1186/1471-2148-10-365/FIGURES/5

Dorrell, R. G., & Bowler, C. (2017). Secondary Plastids of Stramenopiles. *Advances in Botanical Research*, *84*, 57–103. https://doi.org/10.1016/BS.ABR.2017.06.003

Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, *346*(6213). https://doi.org/10.1126/SCIENCE.1258096/ASSET/2313E70A-5C58-4755-A0E6-2E64EE240A09/ASSETS/GRAPHIC/346_1258096_F6.JPEG

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797. https://doi.org/10.1093/NAR/GKH340

Eilers, U., Dietzel, L., Breitenbach, J., Büchel, C., & Sandmann, G. (2016). Identification of genes coding for functional zeaxanthin epoxidases in the diatom Phaeodactylum tricornutum. *Journal of Plant Physiology*, *192*, 64–70. https://doi.org/10.1016/j.jplph.2016.01.006

Eliáš, M. (2021). Protist diversity: Novel groups enrich the algal tree of life. *Current Biology*, *31*(11), R733–R735. https://doi.org/10.1016/J.CUB.2021.04.025

Eppard, M., Krumbein, W. E., Von Haeseler, A., & Rhiel, E. (2000). Characterization of fcp4 and fcp12, two additional genes encoding light harvesting proteins of Cyclotella cryptica (Bacillariophyceae) and phylogenetic analysis of this complex gene family. *Plant Biology*, *2*(3), 283–289. https://doi.org/10.1055/S-2000-3702/ID/36/BIB

Fan, M., Li, M., Liu, Z., Cao, P., Pan, X., Zhang, H., Zhao, X., Zhang, J., & Chang, W. (2015). Crystal structures of the PsbS protein essential for photoprotection in

plants. *Nature Structural & Molecular Biology*, *22*. https://doi.org/10.1038/nsmb.3068

Fattore, N., Bellan, A., Pedroletti, L., Vitulo, N., & Morosinotto, T. (2021). Acclimation of photosynthesis and lipids biosynthesis to prolonged nitrogen and phosphorus limitation in Nannochloropsis gaditana. *Algal Research*, *58*, 102368. https://doi.org/10.1016/J.ALGAL.2021.102368

Feng, Y., Li, Z., Li, X., Shen, L., Liu, X., Zhou, C., Zhang, J., Sang, M., Han, G., Yang, W., Kuang, T., Wang, W., & Shen, J.-R. (2023). Structure of a diatom photosystem II supercomplex containing a member of Lhcx family and dimeric FCPII. *Science Advances*.

Fernández-Marín, B., Míguez, F., Becerril, J. M., & García-Plazaola, J. I. (2011). Activation of violaxanthin cycle in darkness is a common response to different abiotic stresses: A case study in Pelvetia canaliculata. *BMC Plant Biology*, *11*(1), 1–11. https://doi.org/10.1186/1471-2229-11-181/FIGURES/8

Figueroa, F. L., Celis-Plá, P. S. M., Martínez, B., Korbee, N., Trilla, A., & Arenas, F. (2019). Yield losses and electron transport rate as indicators of thermal stress in Fucus serratus (Ochrophyta). *Algal Research*, *41*, 101560. https://doi.org/10.1016/J.ALGAL.2019.101560

Fu, F. X., Zhang, Y., Warner, M. E., Feng, Y., Sun, J., & Hutchins, D. A. (2008). A comparison of future increased CO2 and temperature effects on sympatric Heterosigma akashiwo and Prorocentrum minimum. *Harmful Algae*, *7*(1), 76–90. https://doi.org/10.1016/J.HAL.2007.05.006

García-Mendoza, E., & Colombo-Pallotta, M. F. (2007). The giant kelp Macrocystis pyrifera presents a different nonphotochemical quenching control than higher plants. *New Phytologist*, *173*(3), 526–536. https://doi.org/10.1111/J.1469-8137.2006.01951.X

Garcia-Mendoza, E., Ocampo-Alvarez, H., & Govindjee. (2011). Photoprotection in the brown alga Macrocystis pyrifera: Evolutionary implications. *Journal of Photochemistry and Photobiology B: Biology*, *104*(1–2), 377–385. https://doi.org/10.1016/j.jphotobiol.2011.04.004

Gardian, Z., Litvín, R., Bína, D., & Vácha, F. (2014). Supramolecular organization of fucoxanthin-chlorophyll proteins in centric and pennate diatoms. *Photosynthesis Research*, *121*, 79–86. https://doi.org/10.1007/s11120-014-9998-3

Gardian, Z., Tichy, J., & Vácha, F. (2011). Structure of PSI, PSII and antennae complexes from yellow-green alga Xanthonema debile. *Photosynthesis Research*, *108*, 25–32. https://doi.org/10.1007/s11120-011-9647-z

Ghazaryan, A., Akhtar, P., Garab, G., Lambrev, P. H., & Büchel, C. (2016a). *Involvement of the Lhcx protein Fcp6 of the diatom Cyclotella meneghiniana in the macro-organisation and structural flexibility of thylakoid membranes*. https://doi.org/10.1016/j.bbabio.2016.04.288

Ghazaryan, A., Akhtar, P., Garab, G., Lambrev, P. H., & Büchel, C. (2016b). Involvement of the Lhcx protein Fcp6 of the diatom Cyclotella meneghiniana in the macro-organisation and structural flexibility of thylakoid membranes. *Biochimica et Biophysica Acta - Bioenergetics*, *1857*(9), 1373–1379. https://doi.org/10.1016/j.bbabio.2016.04.288

Gibbs, P. B., & Biggins, J. (1989). Regulation of the distribution of excitation energy in Ochromonas danica, an organism containing a chlorophyll-A/C/carotenoid light harvesting antenna. *Photosynthesis Research*, *21*, 81–91.

Giovagnetti, V., Cataldo, M. L., Conversano, F., & Brunet, C. (2010). Functional relation between growth, photosynthetic rate and regulation in the coastal picoeukaryote Phaeomonas sp. RCC 503 (Pinguiophyceae, Stramenopiles). *Journal of Plankton Research*, *32*(11), 1501–1511. https://doi.org/10.1093/plankt/fbq074

Giovagnetti, V., Jaubert, M., Shukla, M. K., Ungerer, P., Bouly, J. P., Falciatore, A., & Ruban, A. V. (2022). Biochemical and molecular properties of LHCX1, the essential regulator of dynamic photoprotection in diatoms. *Plant Physiology*, *188*(1), 509–525. https://doi.org/10.1093/PLPHYS/KIAB425

Giovagnetti, V., & Ruban, A. V. (2018). The evolution of the photoprotective antenna proteins in oxygenic photosynthetic eukaryotes. *Biochemical Society Transactions*, *46*(5), 1263–1277. https://doi.org/10.1042/BST20170304

Goss, R., Ann Pinto, E., Wilhelm, C., & Richter, M. (2006). The importance of a highly active and ΔpH-regulated diatoxanthin epoxidase for the regulation of the PS II antenna function in diadinoxanthin cycle containing algae. *Journal of Plant Physiology*, *163*(10), 1008–1021. https://doi.org/10.1016/J.JPLPH.2005.09.008

Goss, R., & Jakob, T. (2010a). Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynthesis Research 2010 106:1*, *106*(1), 103–122. https://doi.org/10.1007/S11120-010-9536-X

Goss, R., & Jakob, T. (2010b). Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynthesis Research 2010 106:1*, *106*(1), 103–122. https://doi.org/10.1007/S11120-010-9536-X

Goss, R., & Lepetit, B. (2015). Biodiversity of NPQ. *Journal of Plant Physiology*, *172*, 13–32. https://doi.org/10.1016/j.jplph.2014.03.004

Graf, L., Yang, E. C., Han, K. Y., Küpper, F. C., Benes, K. M., Oyadomari, J. K., Herbert, R. J. H., Verbruggen, H., Wetherbee, R., Andersen, R. A., & Yoon, H. S. (2020). Multigene Phylogeny, Morphological Observation and Re-examination of the Literature Lead to the Description of the Phaeosacciophyceae Classis Nova and Four New Species of the Heterokontophyta SI Clade. *Protist*, *171*(6), 125781. https://doi.org/10.1016/J.PROTIS.2020.125781

Graiff, A., Bartsch, I., Glaser, K., & Karsten, U. (2021). Seasonal Photophysiological Performance of Adult Western Baltic Fucus vesiculosus (Phaeophyceae) Under

Ocean Warming and Acidification. *Frontiers in Marine Science*, *8*, 666493. https://doi.org/10.3389/FMARS.2021.666493/BIBTEX

Grossman, A. R., Bhaya, D., Apt, K. E., & Kehoe, D. M. (1995). LIGHT-HARVESTING COMPLEXES IN OXYGENIC PHOTOSYNTHESIS: Diversity, Control, and Evolution. *Https://Doi.Org/10.1146/Annurev.Ge.29.120195.001311*, *29*, 231–288. https://doi.org/10.1146/ANNUREV.GE.29.120195.001311

Grouneva, I., Rokka, A., & Aro, E. M. (2011). The thylakoid membrane proteome of two marine diatoms outlines both diatom-specific and species-specific features of the photosynthetic machinery. *Journal of Proteome Research*, *10*(12), 5338–5353. https://doi.org/10.1021/PR200600F/SUPPL_FILE/PR200600F_SI_015.PDF

Guillou, L., Chrétiennot-Dinet, M. J., Medlin, L. K., Claustre, H., Loiseaux-de Goër, S., & Vaulot, D. (1999). BOLIDOMONAS: A NEW GENUS WITH TWO SPECIES BELONGING TO A NEW ALGAL CLASS, THE BOLIDOPHYCEAE (HETEROKONTA). *Journal of Phycology*, *35*(2), 368–381. https://doi.org/10.1046/J.1529-8817.1999.3520368.X

Gundermann, K., Wagner, V., Mittag, M., & Büchel, C. (2019). Fucoxanthin-Chlorophyll Protein Complexes of the Centric Diatom Cyclotella Meneghiniana Differ in Lhcx1 and Lhcx6_1 Content. *Plant Physiology*, *179*(4), 1779–1795. https://doi.org/10.1104/PP.18.01363

Hao, T. Bin, Jiang, T., Dong, H. P., Ou, L. jian, He, X., & Yang, Y. F. (2018). Light-harvesting protein Lhcx3 is essential for high light acclimation of Phaeodactylum tricornutum. *AMB Express*, *8*(1), 1–11. https://doi.org/10.1186/S13568-018-0703-3/TABLES/1

Havaux, M., & Niyogi, K. K. (1999). The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proc. Natl Acad. Sci. USA*, *96*(15), 8762–8767. https://doi.org/10.1073/pnas.96.15.8762

Hennige, S. J., Coyne, K. J., Macintyre, H., Liefer, J., & Warner, M. E. (2013). *THE PHOTOBIOLOGY OF HETEROSIGMA AKASHIWO. PHOTOACCLIMATION, DIURNAL PERIODICITY, AND ITS ABILITY TO RAPIDLY EXPLOIT EXPOSURE TO HIGH LIGHT 1*. https://doi.org/10.1111/jpy.12043

Horn, S., Ehlers, K., Fritzsch, G., Gil-Rodríguez, C., Wilhelm, C., & Schnetter, R. (2007). Synchroma grande spec. nov. (Synchromophyceae class. nov., Heterokontophyta): An Amoeboid Marine Alga with Unique Plastid Complexes. *Protist*, *158*, 277–293. https://doi.org/10.1016/j.protis.2007.02.004

Hupel, M., Lecointre, C., Meudec, A., Poupart, N., & Gall, E. A. (2011). Comparison of photoprotective responses to UV radiation in the brown seaweed Pelvetia canaliculata and the marine angiosperm Salicornia ramosissima. *Journal of Experimental Marine Biology and Ecology*, *401*(1–2), 36–47. https://doi.org/10.1016/J.JEMBE.2011.03.004

Ichinomiya, M., Dos Santos, A. L., Gourvil, P., Yoshikawa, S., Kamiya, M., Ohki, K., Audic, S., De Vargas, C., Noël, M. H., Vaulot, D., & Kuwata, A. (2016). Diversity and oceanic distribution of the Parmales (Bolidophyceae), a picoplanktonic group closely related to diatoms. *The ISME Journal 2016 10:10*, *10*(10), 2419–2434. https://doi.org/10.1038/ismej.2016.38

Iwai, M., Grob, P., Iavarone, A. T., Nogales, E., & Niyogi, K. K. (2018). A unique supramolecular organization of photosystem I in the moss Physcomitrella patens. *Nature Plants*, *4*(11), 904–909. https://doi.org/10.1038/s41477-018-0271-1

Jakob, T., Goss, R., & Wilhelm, C. (1999). Activation of Diadinoxanthin De-Epoxidase Due to a Chiororespiratory Proton Gradient in the Dark in the Diatom Phaeodactylum tricornutum. *Plant Biology*, *1*(1), 76–82. https://doi.org/10.1111/J.1438-8677.1999.TB00711.X

Jesus, B., Ventura, P., & Calado, G. (2010). Behaviour and a functional xanthophyll cycle enhance photo-regulation mechanisms in the solar-powered sea slug Elysia timida (Risso, 1818). *Journal of Experimental Marine Biology and Ecology*, *395*(1–2), 98–105. https://doi.org/10.1016/J.JEMBE.2010.08.021

Jimel, M. S., Kv Iderov, J., & Elster, J. (2020). *Annual Cycle of Mat-FOrming Filamentous Alga Tribonema CF. Minus (Stramenopiles, Xanthophyceae) in Hydro-terrestrial habitats In the High Arctic Revealed By Multiparameter Fluorescence Staining*. https://doi.org/10.1111/jpy.13109-20-203

Johnson, M. P., & Ruban, A. v. (2011). Restoration of Rapidly Reversible Photoprotective Energy Dissipation in the Absence of PsbS Protein by Enhanced ΔpH *. *Journal of Biological Chemistry*, *286*(22), 19973–19981. https://doi.org/10.1074/JBC.M111.237255

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. A. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., … Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature 2021 596:7873*, *596*(7873), 583–589. https://doi.org/10.1038/s41586-021-03819-2

Kai, A., Yoshii, Y., Nakayama, T., & Inouye, I. (2008). Aurearenophyceae classis nova, a new class of Heterokontophyta based on a new marine unicellular alga Aurearena cruciata gen. et sp. nov. inhabiting sandy beaches. *Protist*, *159*(3), 435–457. https://doi.org/10.1016/J.PROTIS.2007.12.003

Kastner, B., Fischer, N., Golas, M. M., Sander, B., Dube, P., Boehringer, D., Hartmuth, K., Deckert, J., Hauer, F., Wolf, E., Uchtenhagen, H., Urlaub, H., Herzog, F., Peters, J. M., Poerschke, D., Lührmann, R., & Stark, H. (2007). GraFix: sample preparation for single-particle electron cryomicroscopy. *Nature Methods 2008 5:1*, *5*(1), 53–55. https://doi.org/10.1038/nmeth1139

Kawachi, M., Inouye, I., Honda, D., Charles, ‡, O'kelly, J., Bailey, J. C., Robert, §, Bidigare, R., & Andersen, R. A. (2002). The Pinguiophyceae classis nova , a new class of photosynthetic stramenopiles whose members produce large amounts of omega-3 fatty acids. *Phycological Research*, *50*, 31–47. https://doi.org/10.1046/j.1440-1835.2002.00260.x

Keeling, P. J., & Burki, F. (2019). Progress towards the Tree of Eukaryotes. *Current Biology*, *29*, R808–R817. https://doi.org/10.1016/j.cub.2019.07.031

Khoroshyy, P., David Bína, ·, Gardian, Z., Litvín, R., Alster, J., & Pšenčík, J. (2018). Quenching of chlorophyll triplet states by carotenoids in algal light-harvesting complexes related to fucoxanthin-chlorophyll protein. *Photosynthesis Research*, *135*, 213–225. https://doi.org/10.1007/s11120-017-0416-5

Kilian, O., Benemann, C. S. E., Niyogi, K. K., & Vick, B. (2011). High-efficiency homologous recombination in the oil-producing alga Nannochloropsis sp. *Proceedings of the National Academy of Sciences*, *108*(52), 21265–21269. https://doi.org/10.1073/pnas.1105861108

Kirst, H., Formighieri, C., & Melis, A. (2014). Maximizing photosynthetic efficiency and culture productivity in cyanobacteria upon minimizing the phycobilisome light-harvesting antenna size. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(10), 1653–1664. https://doi.org/10.1016/J.BBABIO.2014.07.009

Kirst, H., Gabilly, S. T., Niyogi, K. K., Lemaux, P. G., & Melis, A. (2017). Photosynthetic antenna engineering to improve crop yields. *Planta*, *245*(5), 1009–1020. https://doi.org/10.1007/s00425-017-2659-y

Korbee, N., Navarro, N. P., García-Sánchez, M., Celis-Plá, P. S., Quintano, E., Copertino, M. S., Pedersen, A., Mariath, R., Mangaiyarkarasi, N., Pérez-Ruzafa, Figueroa, F. L., & Martínez, B. (2014). A novel in situ system to evaluate the effect of high CO2 on photosynthesis and biochemistry of seaweeds. *Aquatic Biology*, *22*, 245–259. https://doi.org/10.3354/AB00594

Kouřil, R., Dekker, J. P., & Boekema, E. J. (2012). Supramolecular organization of photosystem II in green plants. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1817*(1), 2–12. https://doi.org/10.1016/J.BBABIO.2011.05.024

Krause, G. H., & Krause, G. H. (1988). Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiologia Plantarum*, *74*(3), 566–574. https://doi.org/10.1111/J.1399-3054.1988.TB02020.X

Krogmann, D. W., Jagendorf, A. T., & Avron, M. (1959). Uncouplers of Spinach Chloroplast Photosynthetic Phosphorylation. *Plant Physiology*, *34*(3), 272. https://doi.org/10.1104/PP.34.3.272

Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S. T., Iwai, M., Niyogi, K. K., & Long, S. P. (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science*, *354*(6314). https://doi.org/10.1126/science.aai8878

Kuczynska, P., Jemiola-Rzeminska, M., Nowicka, B., Jakubowska, A., Strzalka, W., Burda, K., & Strzalka, K. (2020). The xanthophyll cycle in diatom Phaeodactylum tricornutum in response to light stress. *Plant Physiology and Biochemistry*, *152*, 125–137. https://doi.org/10.1016/J.PLAPHY.2020.04.043

Kumazawa, M., Nishide, H., Nagao, | Ryo, Inoue-Kashino, N., Shen, J.-R., Nakano, T., Uchiyama, I., Kashino, Y., & Ifuku, K. (2021). *Molecular phylogeny of fucoxanthin-chlorophyll a/c proteins from Chaetoceros gracilis and Lhcq/Lhcf diversity*. https://doi.org/10.1111/ppl.13598

Kurita, T., Iwai, M., Ohta, H., Sakuma, T., & Yamamoto, T. (2023). Genome editing for biodiesel production in oleaginous microalga, Nannochloropsis species. *Gene and Genome Editing*, *6*, 100027. https://doi.org/10.1016/J.GGEDIT.2023.100027

Kvernvik, A. C., Hoppe, C. J. M., Lawrenz, E., Prášil, O., Greenacre, M., Wiktor, J. M., & Leu, E. (2018). Fast reactivation of photosynthesis in arctic phytoplankton during the polar night. *Journal of Phycology*, *54*(4), 461–470. https://doi.org/10.1111/JPY.12750

Kvíderová, J., Souquieres, C.-E., & Elster, J. (2018). *Ecophysiology of photosynthesis of Vaucheria sp. mats in a Svalbard tidal flat*. https://doi.org/10.1016/j.polar.2018.11.006

Lacour, T., Babin, M., & Lavaud, J. (2020). Diversity in Xanthophyll Cycle Pigments Content and Related Nonphotochemical Quenching (NPQ) Among Microalgae: Implications for Growth Strategy and Ecology. *Journal of Phycology*, *56*(2), 245–263. https://doi.org/10.1111/JPY.12944

Lacour, T., Morin, P. I., Sciandra, T., Donaher, N., Campbell, D. A., Ferland, J., & Babin, M. (2019). Decoupling light harvesting, electron transport and carbon fixation during prolonged darkness supports rapid recovery upon re-illumination in the Arctic diatom Chaetoceros neogracilis. *Polar Biology*, *42*(10), 1787–1799. https://doi.org/10.1007/S00300-019-02507-2/FIGURES/7

Lavaud, J., & Goss, R. (2014). The Peculiar Features of Non-Photochemical Fluorescence Quenching in Diatoms and Brown Algae. *Advances in Photosynthesis and Respiration*, *40*, 421–443. https://doi.org/10.1007/978-94-017-9032-1_20

Lavaud, J., & Kroth, P. G. (2006). In diatoms, the transthylakoid proton gradient regulates the photoprotective non-photochemical fluorescence quenching beyond its control on the xanthophyll cycle. *Plant and Cell Physiology*, *47*(7), 1010–1016. https://doi.org/10.1093/PCP/PCJ058

Lavaud, J., Materna, A. C., Sturm, S., Vugrinec, S., & Kroth, P. G. (2012). Silencing of the Violaxanthin De-Epoxidase Gene in the Diatom Phaeodactylum tricornutum Reduces Diatoxanthin Synthesis and Non-Photochemical Quenching. *PLOS ONE*, *7*(5), e36806. https://doi.org/10.1371/JOURNAL.PONE.0036806

Lavaud, J., Rousseau, B., & Etienne, A. (2004). General features of photoprotection by energy dissipation in planktonic diatoms (Bacillariophyceae). *J Phycol*, *40*.

Lavaud, J., Rousseau, B., Van Gorkom, H. J., & Etienne, A. L. (2002). Influence of the Diadinoxanthin Pool Size on Photoprotection in the Marine Planktonic Diatom Phaeodactylum tricornutum. *Plant Physiology*, *129*(3), 1398–1406. https://doi.org/10.1104/PP.002014

Leonelli, L., Erickson, E., Lyska, D., & Niyogi, K. K. (2016). Transient expression in Nicotiana benthamiana for rapid functional analysis of genes involved in non-photochemical quenching and carotenoid biosynthesis. *The Plant Journal*, *88*(3), 375–386. https://doi.org/10.1111/TPJ.13268

Lepetit, B., Gélin, G., Lepetit, M., Sturm, S., Vugrinec, S., Rogato, A., Kroth, P. G., Falciatore, A., & Lavaud, J. (2017). The diatom Phaeodactylum tricornutum adjusts nonphotochemical fluorescence quenching capacity in response to dynamic light via fine-tuned Lhcx and xanthophyll cycle pigment synthesis. *New Phytologist*, *214*(1), 205–218. https://doi.org/10.1111/NPH.14337

Li, D. H., Wilson, S., Mastroianni, G., & Ruban, A. V. (2023). Altered lipid acyl chain length controls energy dissipation in light-harvesting complex II proteoliposomes by hydrophobic mismatch. *Journal of Photochemistry and Photobiology B: Biology*, *246*, 112758. https://doi.org/10.1016/J.JPHOTOBIOL.2023.112758

Li, X. M., Zhang, Q. S., Tang, Y. Z., Yu, Y. Q., Liu, H. L., & Li, L. X. (2014). Highly efficient photoprotective responses to high light stress in Sargassum thunbergii germlings, a representative brown macroalga of intertidal zone. *Journal of Sea Research*, *85*, 491–498. https://doi.org/10.1016/J.SEARES.2013.08.004

Li, X. P., Björkman, O., Shih, C., Grossman, A. R., Rosenquist, M., Jansson, S., & Niyogi, K. K. (2000). A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature 2000 403:6768*, *403*(6768), 391–395. https://doi.org/10.1038/35000131

Li, X. P., Gilmore, A. M., Caffarri, S., Bassi, R., Golan, T., Kramer, D., & Niyogi, K. K. (2004). Regulation of Photosynthetic Light Harvesting Involves Intrathylakoid Lumen pH Sensing by the PsbS Protein. *Journal of Biological Chemistry*, *279*(22), 22866–22874. https://doi.org/10.1074/JBC.M402461200

Li, Z., Peers, G., Dent, R. M., Bai, Y., Yang, S. Y., Apel, W., Leonelli, L., & Niyogi, K. K. (2016). Evolution of an atypical de-epoxidase for photoprotection in the green lineage. *Nature Plants 2016 2:10*, *2*(10), 1–6. https://doi.org/10.1038/nplants.2016.140

Lichtlé, C., Arsalane, W., Duval, J. C., & Passaquet, C. (1995). CHARACTERIZATION OF THE LIGHT-HARVESTING COMPLEX OF GIRAUDYOPSIS STELLIFER (CHRYSOPHYCEAE) AND EFFECTS OF LIGHT STRESS1. *Journal of Phycology*, *31*(3), 380–387. https://doi.org/10.1111/J.0022-3646.1995.00380.X

Lie, A. A. Y., Liu, Z., Terrado, R., Tatters, A. O., Heidelberg, K. B., & Caron, D. A. (2018). *A tale of two mixotrophic chrysophytes: Insights into the metabolisms of two Ochromonas species (Chrysophyceae) through a comparison of gene expression*. https://doi.org/10.1371/journal.pone.0192439

Liesner, D., Fouqueau, L., Valero, M., Roleda, M. Y., Pearson, G. A., Bischof, K., Valentin, K., & Bartsch, I. (2020). Heat stress responses and population genetics of the kelp Laminaria digitata (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations. *Ecology and Evolution*, *10*(17), 9144–9177. https://doi.org/10.1002/ECE3.6569

Litvín, R., Bína, D., Herbstová, M., & Gardian, Z. (2016). Architecture of the light-harvesting apparatus of the eustigmatophyte alga Nannochloropsis oceanica. *Photosynthesis Research*, *130*(1–3), 137–150. https://doi.org/10.1007/S11120-016-0234-1

Litvín, R., David Bína, ·, Miroslava Herbstová, ·, Pazderník, M., Kotabová, E., Gardian, Z., Trtílek, M., Prášil, · Ondřej, & Vácha, F. (2019). *Red-shifted light-harvesting system of freshwater eukaryotic alga Trachydiscus minutus (Eustigmatophyta, Stramenopila)*. *142*, 137–151. https://doi.org/10.1007/s11120-019-00662-5

Liu, J., Song, Y., & Qiu, W. (2017a). Oleaginous microalgae Nannochloropsis as a new model for biofuel production: Review & analysis. *Renewable and Sustainable Energy Reviews*, *72*, 154–162. https://doi.org/10.1016/J.RSER.2016.12.120

Liu, J., Song, Y., & Qiu, W. (2017b). Oleaginous microalgae Nannochloropsis as a new model for biofuel production: Review & analysis. *Renewable and Sustainable Energy Reviews*, *72*, 154–162. https://doi.org/10.1016/J.RSER.2016.12.120

Liu, M., Ding, W., Pan, Y., Hu, H., & Liu, J. (2023). Zeaxanthin epoxidase is involved in the carotenoid biosynthesis and light-dependent growth of the marine alga Nannochloropsis oceanica. *Biotechnology for Biofuels and Bioproducts*, *16*(1), 74. https://doi.org/10.1186/S13068-023-02326-Y

Lohr, M., & Wilhelm, C. (1999a). Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(15), 8784–8789. https://doi.org/10.1073/PNAS.96.15.8784

Lohr, M., & Wilhelm, C. (1999b). Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(15), 8784–8789. https://doi.org/10.1073/PNAS.96.15.8784

Malnoë, A., Schultink, A., Shahrasbi, S., Rumeau, D., Havaux, M., & Niyogi, K. K. (2018). The Plastid Lipocalin LCNP Is Required for Sustained Photoprotective Energy Dissipation in Arabidopsis. *The Plant Cell*, *30*(1), 196–208. https://doi.org/10.1105/tpc.17.00536

Marshall, J. A., & Newman, S. (2002). Differences in photoprotective pigment production between Japanese and Australian strains of Chattonella marina (Raphidophyceae). *Journal of Experimental Marine Biology and Ecology*, *272*, 13–27. www.elsevier.com/locate/jembe

Mazor, Y., Borovikova, A., Caspy, I., & Nelson, N. (2017). *Structure of the plant photosystem I supercomplex at 2.6 Å resolution*. https://doi.org/10.1038/nplants.2017.14

Meng, E. C., Goddard, T. D., Pettersen, E. F., Couch, G. S., Pearson, Z. J., Morris, J. H., Ferrin, T. E., & Thomas Ferrin, C. E. (2023). UCSF ChimeraX: Tools for structure building and analysis. *Protein Science*, *32*(11), e4792. https://doi.org/10.1002/PRO.4792

Michel, G., Tonon, T., Scornet, D., Cock, J. M., & Kloareg, B. (2010). Central and storage carbon metabolism of the brown alga Ectocarpus siliculosus: Insights into the origin and evolution of storage carbohydrates in Eukaryotes. *New Phytologist*, *188*(1), 67–81. https://doi.org/10.1111/J.1469-8137.2010.03345.X

Mock, T., Otillar, R. P., Strauss, J., McMullan, M., Paajanen, P., Schmutz, J., Salamov, A., Sanges, R., Toseland, A., Ward, B. J., Allen, A. E., Dupont, C. L., Frickenhaus, S., Maumus, F., Veluchamy, A., Wu, T., Barry, K. W., Falciatore, A., Ferrante, M. I., … Grigoriev, I. V. (2017). Evolutionary genomics of the cold-adapted diatom Fragilariopsis cylindrus. *Nature 2017 541:7638*, *541*(7638), 536–540. https://doi.org/10.1038/nature20803

Müller, P., Li, X.-P., & Niyogi, K. K. (2001). *Update on Photosynthesis Non-Photochemical Quenching. A Response to Excess Light Energy*. www.plantphysiol.org

Müller-Moulé, P., Conklin, P. L., & Niyogi, K. K. (2002). Ascorbate Deficiency Can Limit Violaxanthin De-Epoxidase Activity in Vivo. *Plant Physiology*, *128*(3), 970–977. https://doi.org/10.1104/PP.010924

Murakami', S., & Packer, L. (1970). Light-induced Changes in the Conformation and Configuration of the Thylakoid Membrane of Ulva and Porphyra Chloroplasts in Vivo. *Plant Physiol*, *45*, 289–299. https://academic.oup.com/plphys/article/45/3/289/6090237

Murchie, E. H., & Ruban, A. V. (2020). Dynamic non-photochemical quenching in plants: from molecular mechanism to productivity. *The Plant Journal*, *101*(4), 885–896. https://doi.org/10.1111/TPJ.14601

Nagao, R., Kato, K., Ifuku, K., Suzuki, T., Kumazawa, M., Uchiyama, I., Kashino, Y., Dohmae, N., Akimoto, S., Shen, J.-R., Miyazaki, N., & Akita, F. (2020). Structural basis for assembly and function of a diatom photosystem I-light-harvesting supercomplex. *Nature Communications*. https://doi.org/10.1038/s41467-020-16324-3

Nagao, R., Kato, K., Suzuki, T., Ifuku, K., Uchiyama, I., Kashino, Y., Dohmae, N., Akimoto, S., Shen, J. R., Miyazaki, N., & Akita, F. (2019). Structural basis for energy harvesting and dissipation in a diatom PSII–FCPII supercomplex. *Nature Plants*, *5*(8), 890–901. https://doi.org/10.1038/s41477-019-0477-x

Nan, G. N., Zhou, X. Q., Zhang, X. M., Zhang, Q. S., Hu, Z. M., Huang, R. P., & Zhang, D. (2022). Xanthophyll cycle-related non-photochemical quenching protects Sargassum thunbergii from high light-induced photoinhibition. *Frontiers in Marine Science*, *9*, 1067596. https://doi.org/10.3389/FMARS.2022.1067596/BIBTEX

Nawrocki, W. J., Liu, X., Raber, B., Hu, C., De Vitry, C., Bennett, D. I. G., & Croce, R. (2021). Molecular origins of induction and loss of photoinhibition-related energy dissipation qI. *Science Advances*, *7*(52), 55. https://doi.org/10.1126/SCIADV.ABJ0055/SUPPL_FILE/SCIADV.ABJ0055_SM.PDF

Nepper-Davidsen, J. N., Andersen, D. T., & Foldager, M. F. (2019). Exposure to simulated heatwave scenarios causes long-term reductions in performance in Saccharina latissima. *Marine Ecology Progress Series*, *630*(630), 25–39. https://doi.org/10.3354/meps13133

Neubauer, C. (1993). Multiple Effects of Dithiothreitol on Nonphotochemical Fluorescence Quenching in Intact Chloroplasts (Influence on Violaxanthin De-epoxidase and Ascorbate Peroxidase Activity). *Plant Physiology*, *103*(2), 575–583. https://doi.org/10.1104/PP.103.2.575

Nilkens, M., Kress, E., Lambrev, P., Miloslavina, Y., Müller, M., Holzwarth, A. R., & Jahns, P. (2010). Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in Arabidopsis. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1797*(4), 466–475. https://doi.org/10.1016/J.BBABIO.2010.01.001

Niyogi, K. K., Björkman, O., & Grossman, A. R. (1997a). Chlamydomonas Xanthophyll Cycle Mutants Identified by Video Imaging of Chlorophyll Fluorescence Quenching. *The Plant Cell*, *9*(8), 1369–1380. https://doi.org/10.1105/TPC.9.8.1369

Niyogi, K. K., Björkman, O., & Grossman, A. R. (1997b). The roles of specific xanthophylls in photoprotection. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(25), 14162–14167. https://doi.org/10.1073/PNAS.94.25.14162

Niyogi, K. K., Grossman, A. R., & Björkman, O. (1998). Arabidopsis mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. *The Plant Cell*, *10*(7), 1121–1134. https://doi.org/10.1105/TPC.10.7.1121

Niyogi, K. K., Shih, C., Soon Chow, W., Pogson, B. J., DellaPenna, D., & Björkman, O. (2001). Photoprotection in a zeaxanthin- and lutein-deficient double mutant of

Arabidopsis. *Photosynthesis Research 2001 67:1*, *67*(1), 139–145. https://doi.org/10.1023/A:1010661102365

Niyogi, K. K., & Truong, T. B. (2013). Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Current Opinion in Plant Biology*, *16*(3), 307–314. https://doi.org/10.1016/J.PBI.2013.03.011

Nymark, M., Valle, K. C., Brembu, T., Hancke, K., Winge, P., Andresen, K., Johnsen, G., & Bones, A. M. (2009). An Integrated Analysis of Molecular Acclimation to High Light in the Marine Diatom Phaeodactylum tricornutum. *PLOS ONE*, *4*(11), e7743. https://doi.org/10.1371/JOURNAL.PONE.0007743

Nymark, M., Valle, K. C., Hancke, K., Winge, P., Andresen, K., Johnsen, G., Bones, A. M., & Brembu, T. (2013). Molecular and Photosynthetic Responses to Prolonged Darkness and Subsequent Acclimation to Re-Illumination in the Diatom Phaeodactylum tricornutum. *PLOS ONE*, *8*(3), e58722. https://doi.org/10.1371/JOURNAL.PONE.0058722

Ocampo-Alvarez, H., & García-Mendoza, E. (2013). Antagonist effect between violaxanthin and de-epoxidated pigments in nonphotochemical quenching induction in the qE deficient brown alga Macrocystis pyrifera. *Biochimica et Biophysica Acta*, *1827*, 427–437. https://doi.org/10.1016/j.bbabio.2012.12.006

Ohi, M., Li, Y., Cheng, Y., & Walz, T. (2004). Negative Staining and Image Classification - Powerful Tools in Modern Electron Microscopy. *Biological Procedures Online*, *6*(1), 23–34. https://doi.org/10.1251/BPO70

Olaizola, M., La Roche, J., Kolber, Z., & Falkowski, P. G. (1994). Non-photochemical fluorescence quenching and the diadinoxanthin cycle in a marine diatom. *Photosynthesis Research 1994 41:2*, *41*(2), 357–370. https://doi.org/10.1007/BF00019413

Owens, T. G., Gallagher, J. C., & Alberte, R. S. (1987). PHOTOSYNTHETIC LIGHT-HARVESTING FUNCTION OF VIOLAXANTHIN IN NANNOCHLOROPSIS SPP. (EUSTIGMATOPHYCEAE). *Journal of Phycology*, *23*(2), 79–85. https://doi.org/10.1111/J.1529-8817.1987.TB04429.X

Park, S., Steen, C. J., Lyska, D., Fischer, A. L., Endelman, B., Iwai, M., Niyogi, K. K., & Fleming, G. R. (2019). Chlorophyll-carotenoid excitation energy transfer and charge transfer in Nannochloropsis oceanica for the regulation of photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(9), 3385–3390. https://doi.org/10.1073/pnas.1819011116

Peers, G., Truong, T. B., Ostendorf, E., Busch, A., Elrad, D., Grossman, A. R., Hippler, M., & Niyogi, K. K. (2009). An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. *Nature*, *462*(7272), 518–521. https://doi.org/10.1038/NATURE08587

Perin, G., Bellan, A., Lyska, D., Niyogi, K. K., & Morosinotto, T. (2022). *Modulation of xanthophyll cycle impacts biomass productivity in the marine microalga Nannochloropsis 2 3*. https://doi.org/10.1101/2022.08.16.504082

Perin, G., Bellan, A., Michelberger, T., Lyska, D., Wakao, S., Niyogi, K. K., & Morosinotto, T. (2023). Modulation of xanthophyll cycle impacts biomass productivity in the marine microalga Nannochloropsis . *Proceedings of the National Academy of Sciences*, *120*(25), e2214119120. https://doi.org/10.1073/PNAS.2214119120/SUPPL_FILE/PNAS.2214119120.SAPP.PDF

Perin, G., Bellan, A., Segalla, A., Meneghesso, A., Alboresi, A., & Morosinotto, T. (2015). Generation of random mutants to improve light-use efficiency of Nannochloropsis gaditana cultures for biofuel production. *Biotechnology for Biofuels*, *8*(1), 1–13. https://doi.org/10.1186/S13068-015-0337-5/FIGURES/7

Pi, X., Tian, L., Dai, H. E., Qin, X., Cheng, L., Kuang, T., Sui, S. F., & Shen, J. R. (2018). Unique organization of photosystem I-light-harvesting supercomplex revealed by cryo-EM from a red alga. *Proc. Natl Acad. Sci. USA*, *115*(17), 4423–4428. https://doi.org/10.1073/pnas.1722482115

Pi, X., Zhao, S., Wang, W., Liu, D., Xu, C., Han, G., Kuang, T., Sui, S.-F., & Shen, J.-R. (2019). The pigment-protein network of a diatom photosystem II-light-harvesting antenna supercomplex. *Science (New York, N.Y.)*, *365*(6452), eaax4406. https://doi.org/10.1126/science.aax4406

Pinnola, A., Dall'Osto, L., Gerotto, C., Morosinotto, T., Bassi, R., & Alboresi, A. (2013). Zeaxanthin Binds to Light-Harvesting Complex Stress-Related Protein to Enhance Nonphotochemical Quenching in Physcomitrella patens. *The Plant Cell*, *25*(9), 3519–3534. https://doi.org/10.1105/TPC.113.114538

Porra, R. J., Thompson, W. A., & Kriedemann, P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *975*(3), 384–394. https://doi.org/10.1016/S0005-2728(89)80347-0

Qin, X., Pi, X., Wang, W., Han, G., Zhu, L., Liu, M., Cheng, L., Shen, J. R., Kuang, T., & Sui, S. F. (2019). Structure of a green algal photosystem I in complex with a large number of light-harvesting complex I subunits. *Nat. Plants*, *5*(3), 263–272. https://doi.org/10.1038/s41477-019-0379-y

Qin, X., Suga, M., Kuang, T., & Shen, J. R. (2015). Photosynthesis. Structural basis for energy transfer pathways in the plant PSI-LHCI supercomplex. *Science*, *348*(6238), 989–995. https://doi.org/10.1126/science.aab0214

Quaas, T., Berteotti, S., Ballottari, M., Flieger, K., Bassi, R., Wilhelm, C., & Goss, R. (2015). Non-photochemical quenching and xanthophyll cycle activities in six green

algal species suggest mechanistic differences in the process of excess energy dissipation. *Journal of Plant Physiology*, *172*, 92–103. https://doi.org/10.1016/J.JPLPH.2014.07.023

Rico, C., Matsuda, Y., Lin, X., Litvin, R., Blommaert, L., Vancaester, E., J Huysman, M. J., Osuna-Cruz, C. M., Lavaud, J., Lepetit, B., Winge, P., Bones, A. M., Vandepoele, K., Vyverman, W., & Sabbe, K. (2020). *Light Regulation of LHCX Genes in the Benthic Diatom Seminavis robusta*. https://doi.org/10.3389/fmars.2020.00192

Semchonok, D. A., Sathish Yadav, K. N., Xu, P., Drop, B., Croce, R., & Boekema, E. J. (2017). Interaction between the photoprotective protein LHCSR3 and C2S2 Photosystem II supercomplex in Chlamydomonas reinhardtii. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1858*(5), 379–385. https://doi.org/10.1016/J.BBABIO.2017.02.015

Shen, L., Huang, Z., Chang, S., Wang, W., Wang, J., Kuang, T., Han, G., Shen, J. R., & Zhang, X. (2019). Structure of a C2S2M2N2-type PSII–LHCII supercomplex from the green alga Chlamydomonas reinhardtii. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(42), 21246–21255. https://doi.org/10.1073/pnas.1912462116

Shimasaki, Y., Mukai, K., Takai, Y., Qiu, X., & Oshima, Y. (2021). *antioxidants Recent Progress in the Study of Peroxiredoxin in the Harmful Algal Bloom Species Chattonella marina*. https://doi.org/10.3390/antiox10020162

Short, A., Fay, T. P., Crisanto, T., Mangal, R., Niyogi, K. K., Limmer, D. T., & Fleming, G. R. (2023). Kinetics of the xanthophyll cycle and its role in photoprotective memory and response. *Nature Communications 2023 14:1*, *14*(1), 1–10. https://doi.org/10.1038/s41467-023-42281-8

Short, A. H., Fay, T. P., Crisanto, T., Hall, J., Steen, C. J., Niyogi, K. K., Limmer, D. T., & Fleming, G. R. (2022). Xanthophyll-cycle based model of the rapid photoprotection of Nannochloropsis in response to regular and irregular light/dark sequences. *The Journal of Chemical Physics*, *156*(20), 205102. https://doi.org/10.1063/5.0089335

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology*, *7*. https://doi.org/10.1038/MSB.2011.75

Simionato, D., Basso, S., Giacometti, G. M., & Morosinotto, T. (2013). Optimization of light use efficiency for biofuel production in algae. *Biophys Chem*, *182*, 71–78. https://doi.org/10.1016/j.bpc.2013.06.017

Simionato, D., Block, M. A., la Rocca, N., Jouhet, J., Maréchal, E., Finazzi, G., & Morosinotto, T. (2013). The response of Nannochloropsis gaditana to nitrogen

starvation includes de novo biosynthesis of triacylglycerols, a decrease of chloroplast galactolipids, and reorganization of the photosynthetic apparatus. *Eukaryot Cell*, *12*(5), 665–676. https://doi.org/10.1128/ec.00363-12

Simionato, D., Sforza, E., Corteggiani Carpinelli, E., Bertucco, A., Giacometti, G. M., & Morosinotto, T. (2011a). Acclimation of Nannochloropsis gaditana to different illumination regimes: Effects on lipids accumulation. *Bioresource Technology*, *102*(10), 6026–6032. https://doi.org/10.1016/J.BIORTECH.2011.02.100

Simionato, D., Sforza, E., Corteggiani Carpinelli, E., Bertucco, A., Giacometti, G. M., & Morosinotto, T. (2011b). Acclimation of Nannochloropsis gaditana to different illumination regimes: Effects on lipids accumulation. *Bioresource Technology*, *102*(10), 6026–6032. https://doi.org/10.1016/J.BIORTECH.2011.02.100

Six, C., Finkel, Z. V., Rodriguez, F., Marie, D., Partensky, F., & Campbell, D. A. (2008). Contrasting photoacclimation costs in ecotypes of the marine eukaryotic picoplankter Ostreococcus. *Limnology and Oceanography*, *53*(1), 255–265. https://doi.org/10.4319/LO.2008.53.1.0255

Soule, K. M., & Rumpho, M. E. (2012). Light-regulated photosynthetic gene expression and phosphoribulokinase enzyme activity in the heterokont alga vaucheria litorea (xanthophyceae) and its symbiotic molluskan partner elysia chlorotica (gastropoda). *Journal of Phycology*, *48*(2), 373–383. https://doi.org/10.1111/J.1529-8817.2012.01111.X

Streckaite, S., Gardian, Z., Li, F., Pascal, A. A., Litvin, R., Robert, B., & Llansola-Portoles, M. J. (2018). Pigment configuration in the light-harvesting protein of the xanthophyte alga Xanthonema debile. *Photosynthesis Research*, *138*, 139–148. https://doi.org/10.1007/s11120-018-0557-1

Suga, M., Ozawa, S. I., Yoshida-Motomura, K., Akita, F., Miyazaki, N., & Takahashi, Y. (2019). Structure of the green algal photosystem I supercomplex with a decameric light-harvesting complex I. *Nature Plants 2019 5:6*, *5*(6), 626–636. https://doi.org/10.1038/s41477-019-0438-4

Sukenik, A., Livne, A., Apt, K. E., & Grossman, A. R. (2000). CHARACTERIZATION OF A GENE ENCODING THE LIGHT-HARVESTING VIOLAXANTHIN-CHLOROPHYLL PROTEIN OF NANNOCHLOROPSIS SP. (EUSTIGMATOPHYCEAE). *Journal of Phycology*, *36*(3), 563–570. https://doi.org/10.1046/J.1529-8817.2000.99115.X

Sun, K.-M., Gao, C., Zhang, J., Tang, · Xuexi, Wang, Z., Zhang, X., & Li, Y. (2020). Rapid formation of antheraxanthin and zeaxanthin in seconds in microalgae and its relation to non-photochemical quenching. *Photosynthesis Research*, *144*, 317–326. https://doi.org/10.1007/s11120-020-00739-6

Sylak-Glassman, E. J., Malnoe, A., De Re, E., Brooks, M. D., Fischer, A. L., Niyogi, K. K., & Fleming, G. R. (2014). Distinct roles of the photosystem II protein PsbS and zeaxanthin in the regulation of light harvesting in plants revealed by fluorescence

lifetime snapshots. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(49), 17498–17503. https://doi.org/10.1073/PNAS.1418317111

Taddei, L., Chukhutsina, V. U., Lepetit, B., Stella, G. R., Bassi, R., van Amerongen, H., Bouly, J. P., Jaubert, M., Finazzi, G., & Falciatore, A. (2018). Dynamic Changes between Two LHCX-Related Energy Quenching Sites Control Diatom Photoacclimation. *Plant Physiology*, *177*(3), 953–965. https://doi.org/10.1104/PP.18.00448

Taddei, L., Stella, G. R., Rogato, A., Bailleul, B., Fortunato, A. E., Annunziata, R., Sanges, R., Thaler, M., Lepetit, B., Lavaud, J., Jaubert, M., Finazzi, G., Bouly, J. P., & Falciatore, A. (2016). Multisignal control of expression of the LHCX protein family in the marine diatom Phaeodactylum tricornutum. *Journal of Experimental Botany*, *67*(13), 3939–3951. https://doi.org/10.1093/JXB/ERW198

Takahashi, H., Iwai, M., Takahashi, Y., & Minagawa, J. (2006). Identification of the mobile light-harvesting complex II polypeptides for state transitions in Chlamydomonas reinhardtii. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(2), 477–482. https://doi.org/10.1073/PNAS.0509952103

Tanabe, Y., Shitara, T., Kashino, Y., Hara, Y., & Kudoh, S. (2009). Utilizing the Effective Xanthophyll Cycle for Blooming of Ochromonas smithii and O. itoi (Chrysophyceae) on the Snow Surface. *Plos One*. https://doi.org/10.1371/journal.pone.0014690

Teo, C. L., Atta, M., Bukhari, A., Taisir, M., Yusuf, A. M., & Idris, A. (2014). Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths. *Bioresource Technology*, *162*, 38–44. https://doi.org/10.1016/J.BIORTECH.2014.03.113

Tian, L., Nawrocki, W. J., Liu, X., Polukhina, I., Van Stokkum, I. H. M., & Croce, R. (2019). PH dependence, kinetics and light-harvesting regulation of nonphotochemical quenching in Chlamydomonas. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(17), 8320–8325. https://doi.org/10.1073/PNAS.1817796116/SUPPL_FILE/PNAS.1817796116.SAPP.PDF

Tibiletti, T., Auroy, P., Peltier, G., & Caffarri, S. (2016). Chlamydomonas reinhardtii PsbS Protein Is Functional and Accumulates Rapidly and Transiently under High Light. *Plant Physiology*, *171*(4), 2717–2730. https://doi.org/10.1104/PP.16.00572

Uhrmacher, S., Hanelt, D., & Nultsch, W. (1995). Zeaxanthin content and the degree of photoinhibition are linearly correlated in the brown alga Dictyota dichotoma. *Marine Biology*, *123*, 159–165.

Umetani, I., Kunugi, M., Yokono, M., Takabayashi, A., & Tanaka, A. (2018). Evidence of the supercomplex organization of photosystem II and light-harvesting complexes in

Nannochloropsis granulata. *Photosynthesis Research*, *136*(1), 49–61. https://doi.org/10.1007/s11120-017-0438-z

Vieler, A., Wu, G., Tsai, C. H., Bullard, B., Cornish, A. J., Harvey, C., Reca, I. B., Thornburg, C., Achawanantakun, R., Buehl, C. J., Campbell, M. S., Cavalier, D., Childs, K. L., Clark, T. J., Deshpande, R., Erickson, E., Armenia Ferguson, A., Handee, W., Kong, Q., … Benning, C. (2012a). Genome, Functional Gene Annotation, and Nuclear Transformation of the Heterokont Oleaginous Alga Nannochloropsis oceanica CCMP1779. *PLoS Genetics*, *8*(11). https://doi.org/10.1371/journal.pgen.1003064

Vieler, A., Wu, G., Tsai, C.-H., Bullard, B., Cornish, A. J., Harvey, C., Reca, I.-B., Thornburg, C., Achawanantakun, R., Buehl, C. J., Campbell, M. S., Cavalier, D., Childs, K. L., Clark, T. J., Deshpande, R., Erickson, E., Armenia Ferguson, A., Handee, W., Kong, Q., … Benning, C. (2012b). Genome, Functional Gene Annotation, and Nuclear Transformation of the Heterokont Oleaginous Alga Nannochloropsis oceanica CCMP1779. *PLoS Genetics*, *8*(11), e1003064. https://doi.org/10.1371/journal.pgen.1003064

Walter, J., & Kromdijk, J. (2022). Here comes the sun: How optimization of photosynthetic light reactions can boost crop yields. *Journal of Integrative Plant Biology*, *64*(2), 564–591. https://doi.org/10.1111/JIPB.13206

Walters, R. G., Ruban, A. v., & Horton, P. (1996). Identification of proton-active residues in a higher plant light-harvesting complex. *Proceedings of the National Academy of Sciences*, *93*(24), 14204–14209. https://doi.org/10.1073/PNAS.93.24.14204

Wang, H., Ji, B., Wang, J., Guo, F., Zhou, W., Gao, L., & Liu, T. Z. (2014). Growth and biochemical composition of filamentous microalgae Tribonema sp. as potential biofuel feedstock. *Bioprocess and Biosystems Engineering*, *37*(12), 2607–2613. https://doi.org/10.1007/S00449-014-1238-X/TABLES/2

Wang, W., Yu, L. J., Xu, C., Tomizaki, T., Zhao, S., Umena, Y., Chen, X., Qin, X., Xin, Y., Suga, M., Han, G., Kuang, T., & Shen, J. R. (2019). Structural basis for blue-green light harvesting and energy dissipation in diatoms. *Science*, *363*(6427). https://doi.org/10.1126/SCIENCE.AAV0365/SUPPL_FILE/AAV0365-WANG-SM.PDF

Wang, W., Zhao, S., Pi, X., Kuang, T., Sui, S. F., & Shen, J. R. (2020). Structural features of the diatom photosystem II–light-harvesting antenna complex. *The FEBS Journal*, *287*(11), 2191–2200. https://doi.org/10.1111/FEBS.15183

Wang, W., Zhao, S., Shen, L., Li, X., Tao, Q., Li, Z., Xu, C., Zhou, C., Yang, Y., Sang, M., & Han, G. (2023). Structural insights into photosystem II supercomplex and trimeric FCP antennae of a centric diatom Cyclotella meneghiniana. *Preprint*. https://doi.org/10.21203/rs.3.rs-3094926/v1

Wetherbee, R., Jackson, C. J., Repetti, S. I., Clementson, L. A., Costa, J. F., van de Meene, A., Crawford, S., & Verbruggen, H. (2019). The golden paradox - a new

heterokont lineage with chloroplasts surrounded by two membranes. *Journal of Phycology*, *55*(2), 257–278. https://doi.org/10.1111/JPY.12822

Wilken, S., Choi, C. J., & Worden, A. Z. (2020). Contrasting Mixotrophic Lifestyles Reveal Different Ecological Niches in Two Closely Related Marine Protists. *Journal of Phycology*, *56*(1), 52–67. https://doi.org/10.1111/JPY.12920

Wilken, S., Huisman, J., Naus-Wiezer, S., & Van Donk, E. (2013). Mixotrophic organisms become more heterotrophic with rising temperature. *Ecology Letters*, *16*(2), 225–233. https://doi.org/10.1111/ELE.12033

Xu, C., Pi, X., Huang, Y., Han, G., Chen, X., Qin, X., Huang, G., Zhao, S., Yang, Y., Kuang, T., Wang, W., Sui, S. F., & Shen, J. R. (2020). Structural basis for energy transfer in a huge diatom PSI-FCPI supercomplex. *Nature Communications 2020 11:1*, *11*(1), 1–12. https://doi.org/10.1038/s41467-020-18867-x

Yang, R., Wei, D., & Xie, J. (2020). Diatoms as cell factories for high-value products: chrysolaminarin, eicosapentaenoic acid, and fucoxanthin. *Critical Reviews in Biotechnology*, *40*(7), 993–1009. https://doi.org/10.1080/07388551.2020.1805402

Yoon, H. S., Andersen, R. A., Boo, S. M., & Bhattacharya, D. (2009). Stramenopiles. *Encyclopedia of Microbiology, Third Edition*, 721–731. https://doi.org/10.1016/B978-012373944-5.00253-4

You, X., Zhang, X., Cheng, J., Xiao, Y., Ma, J., Sun, S., Zhang, X., Wang, H.-W., & Sui, S.-F. (2023). In situ structure of the red algal phycobilisome-PSII-PSI-LHC megacomplex. *Nature*, *616*. https://doi.org/10.1038/s41586-023-05831-0

Yuasa, K., Shikata, T., Kitatsuji, S., Yamasaki, Y., & Nishiyama, Y. (2020). *Extracellular secretion of superoxide is regulated by photosynthetic electron transport in the noxious red-tide-forming raphidophyte Chattonella antiqua*. https://doi.org/10.1016/j.jphotobiol.2020.111839

Zhang, X., Xu, D., Guan, Z., Wang, S., Zhang, Y., Wang, W., Zhang, X., Fan, X., Li, F., & Ye, N. (2020). Elevated CO 2 concentrations promote growth and photosynthesis of the brown alga Saccharina japonica. *Journal of Applied Phycology*, *32*, 1949–1959. https://doi.org/10.1007/s10811-020-02108-1

Zhao, L.-S., Wang, P., Li, K., Zhang, Q.-B., He, F.-Y., Li, C.-Y., Su, H.-N., Chen, X.-L., Liu, L.-N., & Zhang, Y.-Z. (2023). *Structural basis and evolution of the photosystem I-light-harvesting supercomplex of cryptophyte algae*. https://doi.org/10.1093/plcell/koad087

Zhu, S. H., & Green, B. R. (2010). Photoprotection in the diatom Thalassiosira pseudonana: Role of LI818-like proteins in response to high light stress. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1797*(8), 1449–1457. https://doi.org/10.1016/J.BBABIO.2010.04.003

# Chapter 2: LHCX and Zeaxanthin-Dependent Induction of Non-Photochemical Quenching in *Nannochloropsis oceanica*


## Abstract

This chapter investigates the photoprotective processes known as non-photochemical quenching (NPQ) the photosynthetic stramenopile alga *Nannochloropsis oceanica* CCMP1779 (Eustigmatophyceae). Wild-type cells exhibit a substantial NPQ capacity, composed of rapidly relaxing qE and slower-relaxing qZ. Through inhibitor treatments and mutant analyses, it is revealed that LHCX1 is crucial for qE induction, while LHCX2 and LHCX3 do not seem to play a role in NPQ in low-light grown cells. DTT inhibition of violaxanthin de-epoxidase (VDE) indicates that the residual NPQ in the *lhcx1* mutant is solely qZ, confirmed by the *vde* mutant lacking both qE and qZ. Experimental results suggest a strong dependence of LHCX1-dependent qE on zeaxanthin (Zx), emphasizing its significance in photoprotection. This relationship is hypothesized to involve direct interaction within a pigment-protein complex or induced conformational changes during Zx binding. The study proposes the application of this knowledge to optimize light-energy utilization, enhance biomass productivity, and improve NPQ dynamics in *N. oceanica*, drawing inspiration from successful strategies in other organisms.


## Preface

Dagmar Lyska generated the *vde*, *lhcx2*, and *lhcx3* KO mutants, characterized the WT, created the phylogenetic tree and protein sequence alignments, and contributed to the HPLC, culture growth, and genotyping. Benjamin Endelman generated the *lhcx1* KO mutant. Collin Steen, Soomin Park, and Lam Lam contributed to the TCSPC measurements. Thien Crisanto wrote the manuscript/chapter, performed the western blotting, performed the phenotyping, and contributed to the HPLC, culture growth, and genotyping.

## Introduction

Photosynthesis is a highly dynamic process that requires constant adjustment to changing light intensities for optimum solar energy to biomass conversion. Plants and algae have adapted to different environments by evolving multiple strategies for efficient light harvesting (Büchel, 2015b; Grossman et al., 1995), and although these adaptations may ensure optimal growth in their natural habitats, they may limit maximum photosynthetic productivity in large-scale cultivation of crops or industrial microalgae (Perin et al., 2023). In recent years, it has been experimentally demonstrated that light-energy utilization can be improved by reducing the size of light-harvesting antennae or by modifying the mechanisms regulating thermal dissipation of excess light energy, also referred to as non-photochemical quenching (NPQ; Kirst & Melis, 2014; Kromdijk et al., 2016; Murchie & Niyogi, 2011; Simionato et al., 2011; Walter & Kromdijk, 2022; Work et al., 2012)

NPQ prevents the over-excitation of photosystem II and subsequent formation of reactive oxygen species (ROS) that otherwise would cause oxidative damage to the cell in excess light conditions. A fast-acting mechanism, energy-dependent quenching (qE), responds directly to the trans-thylakoid proton gradient and is induced and relaxed within seconds. The induction of qE requires protonation of the membrane proteins Photosystem II Subunit S (PsbS) or Light-Harvesting Complex Stress Related proteins (LHCSRs) in plants and green algae, respectively, and is facilitated by the presence of a photoprotective xanthophyll, zeaxanthin (Zx) (Bonente et al., 2011; Demmig-Adams & Adams, 1996; Li et al., 2000; Niyogi et al., 1998; Peers et al., 2009). Zx is formed by the de-epoxidation of violaxanthin (Vx) via the intermediate antheraxanthin (Ax) by the enzyme violaxanthing de-epoxidase (VDE) in the VAZ cycle. In addition to qE, Zx is also involved in a slowly relaxing mechanism of Zx-dependent NPQ, qZ, which is turned on/off in minutes (Demmig-Adams, 1990; Demmig-Adams & Adams, 1996, 2006; Nilkens et al., 2010). Analysis of key proteins and xanthophylls involved in qE and qZ have shed light on the molecular mechanisms involved in photoprotection, differences among evolutionary lineages, and adaptations to ecological niches.

Multiple NPQ mechanisms have been identified through mutant analysis in genetically tractable organisms descending from primary plastids, in particular the model plant *Arabidopsis thaliana* and the green alga *Chlamydomonas reinhardtii*. In *A. thaliana*, PsbS senses changes in pH through lumen-exposed glutamate residues (Li et al., 2004) and induces the formation of one or multiple quenching sites within the LHCs, but it is most likely not directly involved in quenching, because it does not bind pigments (Bonente et al., 2011; Sylak-Glassman et al., 2014). However, for full activation, PsbS requires Zx and/or the α-carotenoid-derived xanthophyll, lutein, which can replace Zx in *npq1* mutants lacking VDE activity (Demmig-Adams, 1990; Havaux & Niyogi, 1999; Johnson & Ruban, 2011; X. P. Li et al., 2000; Niyogi et al., 1998, 2001; Sylak-Glassman et al., 2014). *C. reinhardtii npq1* mutants lacking de-epoxidase activity show only a slightly reduced quenching capacity, whereas qE is almost completely abolished in the simultaneous absence of lutein (Baroli et al., 2003; Niyogi et al., 1997a, 1997b). The plant VDE is a lumenal lipocalin family protein and is probably activated through oligomerization induced by a low pH (Arnoux et al., 2009; Hallin et al., 2016a, 2016b), whereas *C. reinhardtii* employs an enzyme related to a lycopene cyclase from photosynthetic bacteria that is located at the stromal side of the thylakoid membrane (Li et al., 2016).

While vascular plants rely on PsbS as the only stress-related LHC protein for qE induction, more basal groups within the green lineage also utilize a second stress-related LHC protein, LHCSR (also termed LI818 or LHCX; Correa-Galvis, Redekop, et al., 2016; Peers et al., 2009). Like PsbS, LHCSR proteins sense changes in lumen pH, but they also bind multiple chlorophylls and xanthophylls and therefore act as direct quenchers of chlorophyll excited states (Liguori et al., 2013; Ballottari et al., 2016; Bonente et al., 2011; Pinnola et al., 2013). In the moss *Physcomitrella patens,* LHCSR and PsbS act concomitantly, and accumulate constitutively (Alboresia et al., 2010), and LHCSR-dependent quenching is enhanced by binding of Zx (Pinnola et al., 2013). In the green alga *C. reinhardtii*, the main isoform of LHCSR, LHCSR3, only accumulates in high light-acclimated cells, and thus cells only exhibit qE capacity in these conditions (Peers et al., 2009). PsbS in *C. reinhardtii* accumulates only transiently under high-light

conditions, and like in vascular plants, it seems to be involved in the initiation of quenching sites, but not directly in quenching (Correa-Galvis et al., 2016; Tibiletti et al., 2016).

Phylogenetic analyses of LHCSR/LI8181/LHCX proteins suggest that this group of proteins evolved first in algae containing secondary red plastids, although they are absent in red algae and cryptophytes, and were acquired by the green lineage through horizontal gene transfer (Dittami et al., 2010b; Giovagnetti & Ruban, 2018; Niyogi & Truong, 2013). The genomes of algae belonging to the phylum Heterokontophyta encode multiple LHCX proteins, which are differentially expressed in response to different environmental stresses, and they do not possess a gene encoding PsbS (Alboresi et al., 2016; Bailleul et al., 2010; Bowler et al., 2008b; Lepetit et al., 2017; Nymark et al., 2009; Taddei et al., 2016; Vieler et al., 2012b; Zhu & Green, 2010). In the diatom *Phaeodactylum tricornutum*, the constitutively expressed LHCX1 protein seems to be the main factor for qE in low light-acclimated cells, as downregulation of *LHCX1* and knockouts of the protein prevent wild type-like NPQ capacity (Bailleul et al., 2010; Buck et al., 2019, 2021; Giovagnetti et al., 2022; Taddei et al., 2018). Like most Chromalveolate/Heterokont algae, diatoms use a xanthophyll cycle for photoprotection in which diadinoxanthin (Ddx) is converted to diatoxanthin (Dtx), and this has been proposed to be the basis for their outstanding capability to deal with light stress (Arsalane et al., 1994; Goss & Jakob, 2010b; Kuczynska et al., 2020; Lavaud et al., 2002; Lohr & Wilhelm, 1999b; Olaizola et al., 1994). Silencing of the diadinoxanthin de-epoxidase (*DDE*) gene in *P. tricornutum*, which is functionally related to VDE from higher plants, inhibits NPQ induction by reducing diadinoxanthin de-epoxidation rates (Lavaud et al., 2012). Other heterokont algae in the class Eustigmatophyceae, such as *Nannochloropsis*, exhibit NPQ which relies on the VAZ cycle and shows similar characteristics to NPQ in diatoms (Cao et al., 2013; Goss & Lepetit, 2015).

With the ability of *Nannochloropsis* to grow fast and accumulate large amounts of lipids when exposed to nutrient limitation and different light regimes (Alboresi et al., 2016; Perin et al., 2015; Simionato, Block, et al., 2013; Simionato et al., 2011b), improving light-energy utilization by modifying NPQ could be essential for higher productivity in this organism. *Nannochloropsis* has been shown to have a higher capacity to dissipate excess light energy than diatoms (Park et al., 2019; A. H. Short et al., 2022; Simionato et al., 2011b; Taddei et al., 2016; Zhu & Green, 2010). While diatoms mainly employ chlorophylls *a* and *c* and fucoxanthin (Fx) as the major light-harvesting pigments, *Nannochloropsis* utilizes chlorophyll *a*, Vx, and vaucheriaxanthin (Vau) in a violaxanthin-chlorophyll *a* protein (VCP) antenna complex (Chukhutsina et al., 2017; Owens et al., 1987). NPQ in *N. oceanica* has been shown to occur in the VCP antennas of both photosystems, involving both qE and qZ components of NPQ, which correlate to the formation of Zx and the presence of LHCX proteins (Bína, Bouda, et al., 2017; Chukhutsina et al., 2017; A. H. Short et al., 2022). Here, we investigate mechanisms underlying NPQ in *Nannochloropsis oceanica* CCMP1779 by CRISPR/Cas9-mediated homologous recombination and knockout of *LHCX* and *VDE* genes.

## Materials and Methods

### Strains and culture growth conditions

*Nannochloropsis oceanica* CCMP1779 was obtained from The Provasoli-Guillard National Center for Culture of Marine Phytoplankton (https://ncma.bigelow.org/) and cultivated in F2N medium (Kilian et al., 2011). For chlorophyll fluorescence induction measurements, protein extraction, and pigment analyses by HPLC, liquid cultures were grown in continuous light at a photon flux density of 30 µmol photons $m^{-2}$ $s^{-1}$ at 23°C. For transformation, cultures were grown to 2-5 x $10^6$ cells/ml in continuous light at 50 µmol photons $m^{-2}$ $s^{-1}$. Agar plates containing 0.9% Bacto agar were maintained at 100 µmol photons $m^{-2}$ $s^{-1}$ and 25°C. 2 µg/ml Zeocin or 50 µg/ml Hygromycin were added for selection of transformants.

### Mutant generation and *Nannochloropsis* transformation

Nannochloropsis oceanica CCMP1779 genome v2.0
(https://genome.jgi.doe.gov/Nanoce1779_2):
VDE: transcript ID 598302; protein ID 598216
LHCX1: transcript ID 575769; protein ID 575683
LHCX2: transcript ID 581108; protein ID 581022
LHCX3: transcript ID 262385; protein ID 262299

A hygromycin-resistance ($Hyg^R$) cassette containing the *N. oceanica* CCMP1779 LDSP promoter and a 35S terminator (Vieler et al., 2012) was obtained from the laboratory of Christoph Benning (Michigan State University). The $Hyg^R$ cassette was amplified with primers Hyg_F and Hyg_R.

For *vde*, primer combinations 5'HR_VDE_F/ 5'HR_VDE_R and 3'HR_VDE_F/ 3'HR_VDE_R were used to amplify the 5' and 3' homologous arms flanking the first 254 bp of the *VDE* (NannoCCMP1779_11475/11477) coding region. The $Hyg^R$ cassette was amplified with primers VDE_HygR_HR_F and VDE_HygR_HR_R, and the three fragments were combined by MultiSite Gateway Pro cloning (Thermo Fisher Scientific, Waltham, MA, USA). For algal transformation, the VDE HR repair template was linearized with primers VDE_HR_F and VDE_HR_R.

For *lhcx1*, 1 kb sequences flanking the target sequence were amplified using primers LHCX1_5arm_F and LHCX1_5arm_R and LHCX1_3arm_F, LHCX1_3arm_R (Supplementary Table 1) and fused to the 5' and 3' end of the $Hyg^R$ cassette amplified with primer LHCX1_HygR_F and LHCX1_HygR_R for Gibson cloning. For transformations, the LHCX1 repair template was amplified using primers LHCX1_HR_F and LHCX1_HR_R. The *lhcx1* mutant was generated by CRISPR-Cas9 RNP-mediated HR. RNP assembly was based on the Alt-R™ CRISPR-Cas9 System from IDT (Coralville, IA). *Streptococcus pyogenes* Cas9 nuclease (SpCas9; 40 µM, stored in 25 mM HEPES (pH 7.5), 150 mM KCl, 10% glycerol, and 1 mM DTT) containing a double nuclear-localization tag at the C-terminus was obtained from the QB3 MacroLab (University of California, Berkeley). To anneal the gRNA duplex, equimolar amounts (10

µM) of crRNA and tracrRNA were incubated at 98°C for 5 min and allowed to cool to room temperature. Per transformation, 1 µmol of the annealed RNA duplex and 1 µmol of SpCas9 were mixed in $ddH_2O$ in a final volume of 10 µl and incubated at room temperature for 5-30 min to allow assembly of the RNP complexes.

For the *lhcx2* knockout mutant, a construct containing a zeocin-resistance cassette (ZeoR) flanked by sequences corresponding to the 1 kb upstream and 1 kb downstream of coding region of NoLHCX2 was transformed into wild type.

The *lhcx3* knockout mutant was generated by stable transformation of the CRISPR-Cas9 system to induce a 207-211 bp deletion and cloning of gRNAs into the gRNA expression vector. All transformations were conducted by electroporation and followed the protocol described by Kilian et al. (2011). All primer and gRNA sequences are listed in Supplementary Table 1. For the single guide RNA expression vector, the *N. oceanica* U6 promoter (nanno_639:2467-2866) was amplified from genomic DNA with primers U6-F and U6-R. The CRISPR gRNA scaffold was synthesized as a gBlocks fragment from IDT (Coralville, IA, USA) and combined with the U6 promoter by fusion PCR using the primers sets Fus1_F/ Fus1_R and Fus2_F/ Fus2_R. This fragment was digested with *Aat*II and *Not*I and cloned into a spectinomycin resistance vector backbone.

Cultures for transformations were grown to 2-5 x $10^6$ cells/ml in F2N. In continuous light at 100 µmol photons $m^{-2}$ $s^{-1}$ and in $CO_2$-enriched air (3%). Transformations by electroporation followed the protocol described by Kilian et al., (2011). Generally, 1 µg of linear DNA was transformed, except the Cas9 and gRNA expression constructs were co-transformed in a 1:2 ratio (0.5 µg Cas9 construct, 1 µg gRNA construct) to ensure co-transformation of the gRNA expression construct that does not harbor a selection marker with the Cas9 expression construct with the ZeoR cassette. The transformed cells were plated onto F2N plates containing either 2 µg/ml zeocin (Cas9 expression) or 50 µg/ml hygromycin, and colonies were processed after 3 weeks.

## Screening and analysis of mutants

Hygromycin- or zeocin-resistant colonies were restreaked in larger patches onto fresh F2N plates without selection, grown for 1 week, and screened by video imaging of chlorophyll fluorescence using the IMAGING-PAM M-Series (Heinz Walz, Effeltrich, Germany). For PCR screening for HR events at the *VDE* locus, colony PCRs with primers HRgeno-F and HRgeno-R were performed, and the products were analyzed based on a 1.75-kb size shift.

## Sequence analysis

Sequences used for phylogenetic analysis were derived from the NCBI database. *N. oceanica* CCMP1779 sequences are available at JGI (https://genome.jgi.doe.gov/Nanoce1779_2). Amino acid sequences were aligned using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm (Edgar, 2004). Phylogenetic trees were constructed using the Maximum Likelihood (ML) method implemented in PhyML (http://www.phylogeny.fr; Dereeper et al., 2008).

## Chlorophyll fluorescence measurements

Chl *a* fluorescence imaging of cells grown on plates at 100 µmol photons $m^{-2}$ $s^{-1}$ was performed with an IMAGING_PAM M-Series fluorometer (Heinz Walz, Effeltrich, Germany). Cells were dark-acclimated for 30 min, then exposed to 800 µmol photons $m^{-2}$ $sec^{-1}$ of blue actinic light, followed by recovery in the dark for 10 min. NPQ was calculated as $(F_m-F_m')/F_m'$.

To measure Chl *a* fluorescence of liquid cultures, cells were grown at 30 µmol photons $m^{-2}$ $sec^{-1}$ to a cell density of 1-2 x $10^7$ cells/ml. Samples were dark-acclimated for 30 min, and measurements were performed using a Hansatech FMS2+ PAM fluorometer, treating samples with 10 min of white actinic light (800 µmol photons $m^{-2}$ $sec^{-1}$; saturating light pulses: 1800 µmol photons $m^{-2}$ $sec^{-1}$) followed by a 10 min dark recovery. For treatment with NPQ inhibitors, dithiothreitol (DTT) or $NH_4Cl$ were added before dark acclimation to final concentrations of 10 mM or 50 mM, respectively.

## Protein extraction, SDS-PAGE and Immunoblotting

Cells were grown to a density of 1-2 x $10^7$ cells/ml. 1 x $10^9$ cells were collected by centrifugation at 3,000 x *RPM* for 10 min at room temperature. The pellet was resuspended in a 0.33 MS buffer (25 mM MES-NaOH pH 6.5, 0.33 M sucrose, 1.5 mM NaCl, 0.2 mM benzamidine, 1 mM aminocaproic acid) before being frozen in liquid nitrogen. The cells were thawed and disrupted using a Multi Shot Cell Disruptor (Pressure BioSciences Inc., South Easton, MA, USA) at 10 kPSI. Disrupted cells were then frozen in liquid nitrogen, thawed, and disrupted for a second time. Sucrose density gradient centrifugation was performed to isolate chloroplasts as described previously in Takahashi et al. (2006).

The isolated chloroplasts were loaded into a sucrose gradient of 1.8 MS buffer (25 mM MES-NaOH, pH 6.5, 1.8 M sucrose, 1.5 mM NaCl), and 1.3 MS buffer (25 mM MES-NaOH, pH 6.5, 1.3 M sucrose, 1.5 mM NaCl), and centrifuged at 27,000 RPM (SW 32 Ti rotor, Beckman Coulter) for 30 min at 4 °C. The layer of crude thylakoids was separated from cell debris by pipetting, and centrifuged at 15,000 RPM (JA-20 rotor, Beckman Coulter) for 15 min at 4 °C. The resulting crude chloroplast pellet was resuspended in 100 µL of protein extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% (v/v) Ipegal CA-40, 0.5% (w/v) SDS).

The protein concentration was determined with the Pierce BCA Protein Assay kit (Thermo Fischer Scientific, Waltham, Massachusetts, USA), extracts were then mixed with 5x sample buffer (0.31 M Tris-HCl, pH 7.8; 10% (w/v) SDS; 50% (w/v) glycerol; 0.5% (w/v) bromophenol blue; 0.1 M dithiothreitol) and incubated at room temperature for 30 min. 11 µg of protein was loaded onto Mini-PROTREAN TGX precast gels (Bio-Rad, Hercules, California, USA) for SDS-PAGE analysis and blotted onto a PVDF membrane. Antibodies against LHCX1 were used at a 1:40,000 dilution and ATP synthase beta-subunit (ATPB) (Agrisera) at a 1:5000 dilution in TBS with 0.1% Tween. The LHCX1 antibody was a gift from Tomas Morosinotto (University of Padova, Italy). The blots were developed using SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, Waltham, MA, USA) and imaged using the ChemiDoc MP Imaging System. Blots were analyzed using Image Lab 5.2.1 (Bio-Rad).

HPLC analyses of pigments

Cells were grown to a cell density of 1-2 x $10^7$ cells/ml, and a volume equal to 2 x $10^8$ cells was spun down for 3 min at 14,000 RPM. The supernatant was removed, and the pellet was frozen in liquid nitrogen. The cells were thawed at room temperature and frozen in liquid nitrogen a second time. The frozen material was powdered (6.5 m/sec for 60 s) on dry ice using Fastprep-24 (MP Bio), resuspended in 100 µl acetone, and vortexed for 15 s. After centrifugation at 14,000 RPM for 3 min, the supernatant was collected, and the pellet was extracted a second time as described above. The supernatants were pooled, filtered through a 0.45 µm nylon filter (Thermo Fisher Scientific, Waltham, MA, USA) and separated on a Spherisorb S5 column (Waters, Milford, MA) as described by Müller-Moulé et al. (2002).

Time-resolved spectroscopy

Fluorescence lifetime and transient absorption spectroscopy measurements were performed as previously described in Park et al., 2019. The sample for TCSPC and TA measurements consisted of intact *N. oceanica* cells in Ficoll at a concentration of 80 µg Chl *a* $mL^{-1}$. Cells were kept in low light (30-40 µmol photons $m^{-2}$ $s^{-1}$) throughout the day. Prior to all measurements, the sample was dark acclimated in a cuvette for 30 min to relax NPQ. For activation of NPQ, the samples were exposed to an actinic light (850 µmol photons $m^{-2}$ $s^{-1}$) for 15 min of high-light exposure.

For fluorescence lifetime snapshots, an ultrafast Ti:sapphire oscillator (Coherent Mira 900f) pumped by a diode laser (Verdi G10, Coherent) generated 840 nm pulses (9 nm fwhm) with a repetition rate of 76 MHz. The output was frequency doubled using a BBO crystal to generate 420 nm pulses for excitation of Chl *a* inside the *N. oceanica* cells. The excitation power was set to 1.7 mW, and exposure of the sample to the laser was controlled by three shutters that were synchronized using LabView. To monitor the changes in the fluorescence lifetime during induction and relaxation of NPQ, the sample was illuminated with a white actinic light (Leica KL 1500 LCD, peak 648 nm, FWHM 220 nm) at an intensity of 850 µmol $m^{-2}$ $s^{-1}$ for 10 min followed by 5 min of relaxation of NPQ in the dark. Every 30 s, the sample was exposed to the saturating laser for a 1 s interval and Chl *a* fluorescence emission at 680 nm was detected via time-correlated single photon counting (TCSPC) using a sync photodiode for reference and a MCP-PMT (Hamamatsu R3809U). The average lifetime for each fluorescence decay was calculated using the 0.2-s segment with the longest lifetime to ensure that reaction centers were saturated. The quantification of quenching was defined analogously to the NPQ parameter derived from PAM measurements: NPQ(t)=(dark(0)-light(t))/light(t), where t is the amplitude-weighted average fluorescence lifetime obtained from a bi-exponential fit for each snapshot.

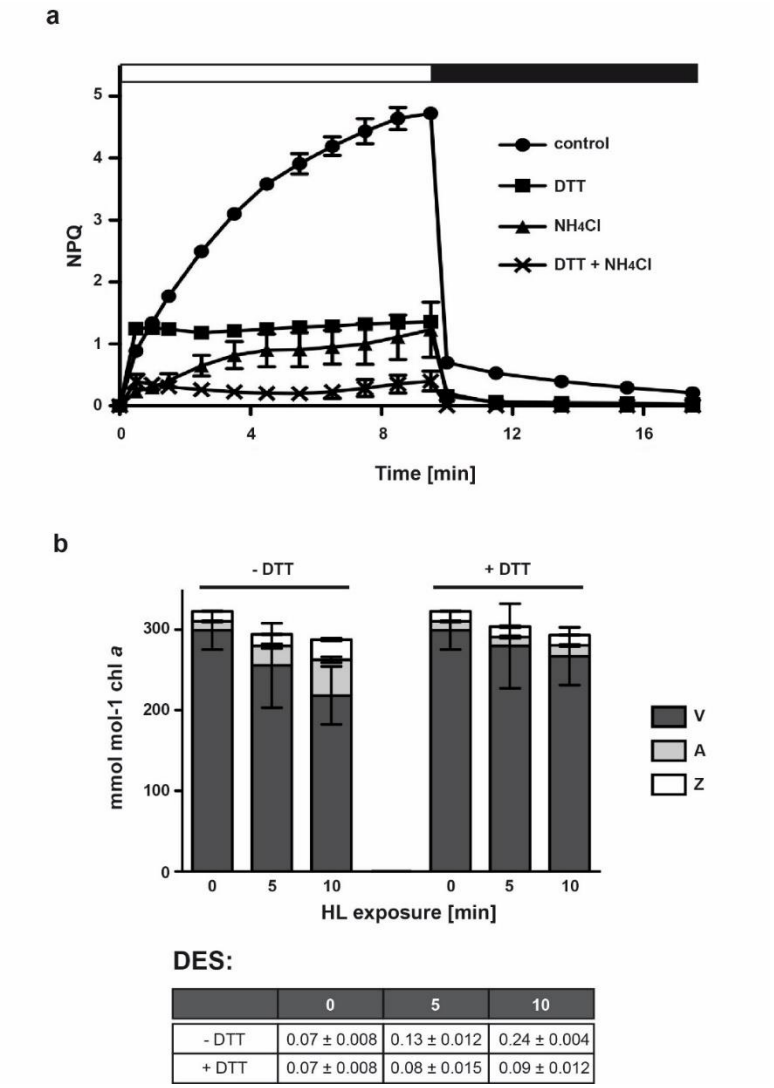
For transient absorption (TA) spectroscopy, a Verdi-V18 (Coherent) pumped an ultrafast oscillator (Coherent Mira Seed) generating 800 nm pulses with a repetition rate of 250 kHz and amplified using a Ti:sapphire regenerative amplifier (Coherent RegA 9050). The beam was split into pump and probe lines. For excitation of the Chl Qy transition, the wavelength of the pump line was adjusted to 665 nm using an optical parametric amplifier (Coherent OPA 9450). The pump pulses were compressed to 42 fs and adjusted to have an energy of 40 nJ per pulse at the sample position. The probe

line consisted of a white light continuum generated by focusing the 800 nm laser into a 1 mm sapphire crystal and passing the output through a 700 nm short pass filter. The pump and probe overlapped at the sample at the magic-angle polarization with diameters of 140 and 65 µm, respectively. The cross-correlation time of the pump and probe was 120 fs. During TA measurements, the sample cuvette was continuously shaken to minimize sample damage. Absorption of the probe was detected using a Si-based photodiode (Thorlabs, DET10A) after passing through a monochromator (Acton Research, SpectraPro 300i). The detection was synchronized to the chopping frequency of the pump pulse via a lock-in amplifier (Stanford Research, SR830).

## Results

### Characterization of NPQ in wild-type *N. oceanica* CCMP1779

Characterization of NPQ has previously been performed in different wild-type (WT) strains of *Nannochloropsis/Microchloropsis* (Bína et al., 2017; Cao et al., 2013;




DES:

| | 0 | 5 | 10 |
|---|---|---|---|
| - DTT | 0.07 ± 0.008 | 0.13 ± 0.012 | 0.24 ± 0.004 |
| + DTT | 0.07 ± 0.008 | 0.08 ± 0.015 | 0.09 ± 0.012 |

***Figure 1. Effect of inhibitors on NPQ induction in wild-type N. oceanica CCMP1779.*** *(a) NPQ measurements of wild-type (WT) N. oceanica cells without inhibitors (control) or treated with 50 mM NH4Cl or 10 mM DTT during exposure to 800 µmol photons m-2 s-1 white actinic light and darkness, indicated by the white and black bar respectively. (b) Quantification of VAZ cycle pigments by HPLC and table of de-epoxidation states (DES) of cells after 0, 5 and 10 min of exposure to 800 µmol photons m-2 s-1 white actinic light. V = violaxanthin; A = antheraxanthin; Z = zeaxanthin; DES = de-epoxidation state ((A+Z)/ (V+A+Z))*

Chukhutsina et al., 2017; Perin et al., 2023). We tested NPQ capacity in WT *N. oceanica* and the effect of inhibitors to estimate how much the ΔpH and the VAZ cycle contribute to NPQ in this species. Cells were grown in LL conditions (50 µmol photons m-2 s-1), dark-acclimated for 30 min, and NPQ was measured at a white actinic light of 800 µmol photons $m^{-}2$ $s^{-}1$. This actinic light intensity was sufficient to saturate NPQ capacity as seen previously (Cao et al., 2013), which reached a maximum NPQ of around 5 after 10 min of illumination (Figure 1a). To determine to which extent ΔpH formation triggers NPQ induction in *N. oceanica* CCMP1779, cells were treated with $NH_4Cl$, an uncoupler that dissipates the pH gradient in photosynthesis (Krogmann et al., 1959). At 50 mM $NH_4Cl$, the quick rise associated with the fast-acting qE component of NPQ was abolished, and only a more slowly increasing component remained (Figure 1a). To determine how much the VAZ cycle contributes to NPQ induction, cells were treated with 10 mM DTT, which inhibits VDE (Neubauer, 1993). After a sharp initial rise in qE to about 1.2 within the first few seconds, NPQ did not increase any further, suggesting that the rise of NPQ in WT cells requires the de-epoxidation of Vx to Zx (Figure 1a). Treatment with both $NH_4Cl$ and DTT almost eliminated NPQ induction.

We checked accumulation levels of the VAZ cycle pigments in WT cells with and without the addition of DTT in dark-acclimated cells and compared that to cells illuminated with 5 and 10 min of 800 µmol photons m-2 s-1 actinic light. In cells that were not treated with DTT, the de-epoxidation state (DES, ratio of de-epoxidized to total xanthophyll pool, (A+Z)/(V+A+Z), increased by more than three-fold, which was due to an increase in Z and A levels and decrease of V (Figure 1b, DES table) However, in dark-acclimated samples at time 0, some Zx was already present (12.3 $\pm$ 0.12 mmol mol-1 Chl *a*), and may contribute to the sharp rise in NPQ within the first seconds of illumination in DTT-treated cells. No Zx was formed upon illumination in DTT-treated cells, and the DES remained constant.

Identification and knockout of candidate genes involved in qE induction.

Like diatoms, *Nannochloropsis* species have multiple genes encoding LHCX proteins (Chukhutsina et al., 2017; Litvín et al., 2016; Vieler et al., 2012).To identify candidates for targeted mutagenesis, we revisited the updated *N. oceanica* CCMP1779 genome and identified all proteins containing a chlorophyll *a/b* binding domain. Together with protein sequences from *Phaeodactylum tricornutum, Thalassiosira pseudonana, Ectocarpus siliculosus, Cyanidioschyzon merolae, Galdieria sulphuraria, Chlamydomonas reinhardtii,* and *Physcomitrium patens,* we generated a phylogenetic tree (Figure 2) to group the proteins into three subclasses of LHC-like proteins, LHCR, LHCF, and LHCX/LHCSR. LHCA/LHCB proteins from *Arabidopsis thaliana,* a land plant, were used as an outgroup. Three proteins from *N. oceanica* CCMP1779 clustered with the LHCX/LHCSR clade and were named LHCX1, LHCX2, and LHCX3. Alignments of the three proteins from *N. oceanica* with other LHCX/LHCSR proteins from these organisms showed conserved regions, possible chlorophyll-binding sites, and putative protonatable sites involved in pH sensing (SI Figure 1). A single gene encoding a protein with homology to plant-type VDE (41% identity) and diatom DDE (46% identity to TpDDE; SI Figure 2) is encoded in the *N. oceanica* genome and has previously been

confirmed to function as a VDE by transient expression in *Nicotiana benthamiana* (Leonelli et al., 2016).

Here, we describe the generation of knock-out mutants of VDE and LHCX genes in *N. oceanica*. These mutants have been used previously for spectroscopic analysis (Park et al., 2019) and assessment of biomass productivity (Perin et al., 2023). We used three strategies for mutant generation. For LHCX2 and VDE, we took advantage of the capacity for homologous recombination (HR) in *Nannochloropsis* (Kilian et al., 2011). For LHCX2, a construct containing a zeocin-resistance cassette flanked by 1 kb homology arms, corresponding to the 1 kb upstream and 1 kb downstream of the LHCX2 coding region (Figure 3b), was transformed into WT. For VDE, the construct contained a hygromycin-resistance cassette with 1 kb flanking regions of the first 254 bp

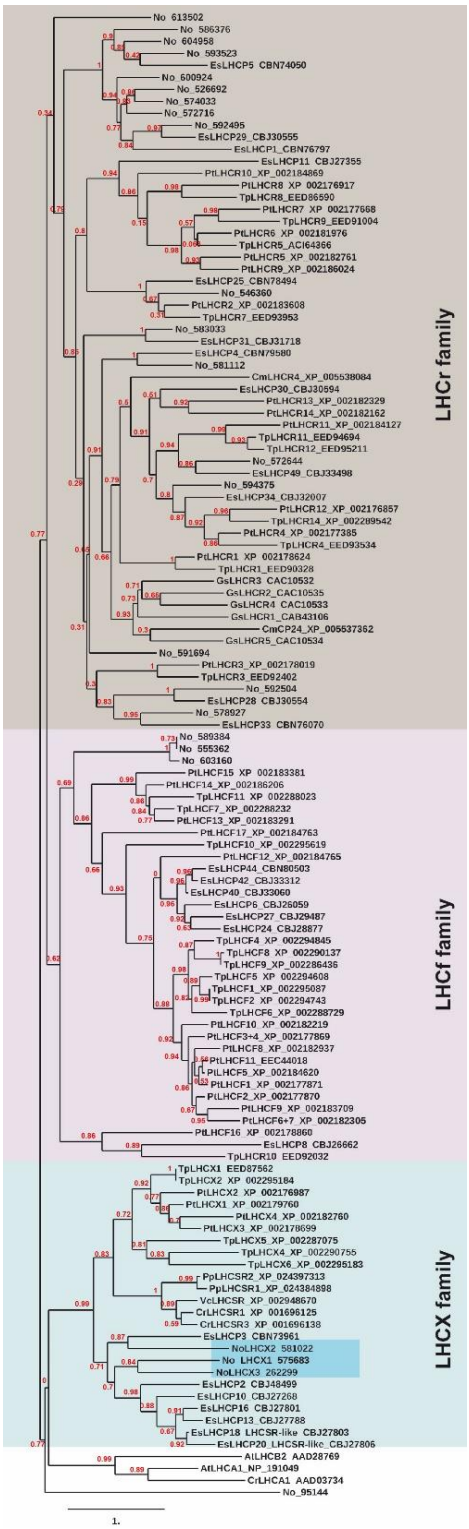



***Figure 2. Phylogenetic tree of selected chlorophyll a/b-binding protein sequences.*** *Protein sequences in the tree include N. oceanica CCMP1779 (No), Phaeodactylum tricornutum CCAP1055 (Pt), Thalassiosira pseudonana CCMP1335 (Tp), Ectocarpus siliculosus (Es), Cyanidioschyzon merolae 10D (Cm), Galdieria sulphuraria 074W (Gs), Chlamydomonas reinhardtii (Cr), Phycomitrium patens (Pp), and Arabidopsis thaliana (At) as an outgroup. LHCX1, 2, and 3 in N. oceanica are highlighted by the blue box and clustered together. These proteins were selected for subsequent knockout mutant generation.*

of the coding region (Figure 3c). These constructs were successfully integrated into the LHCX2 and VDE gene loci by HR with a frequency of about 1% and 3%, respectively. Mutants were identified by genotyping transformants using primers located upstream of the 5’ homology arm and within the 3’ homolog army (Figure 3b, c). Successful HR and

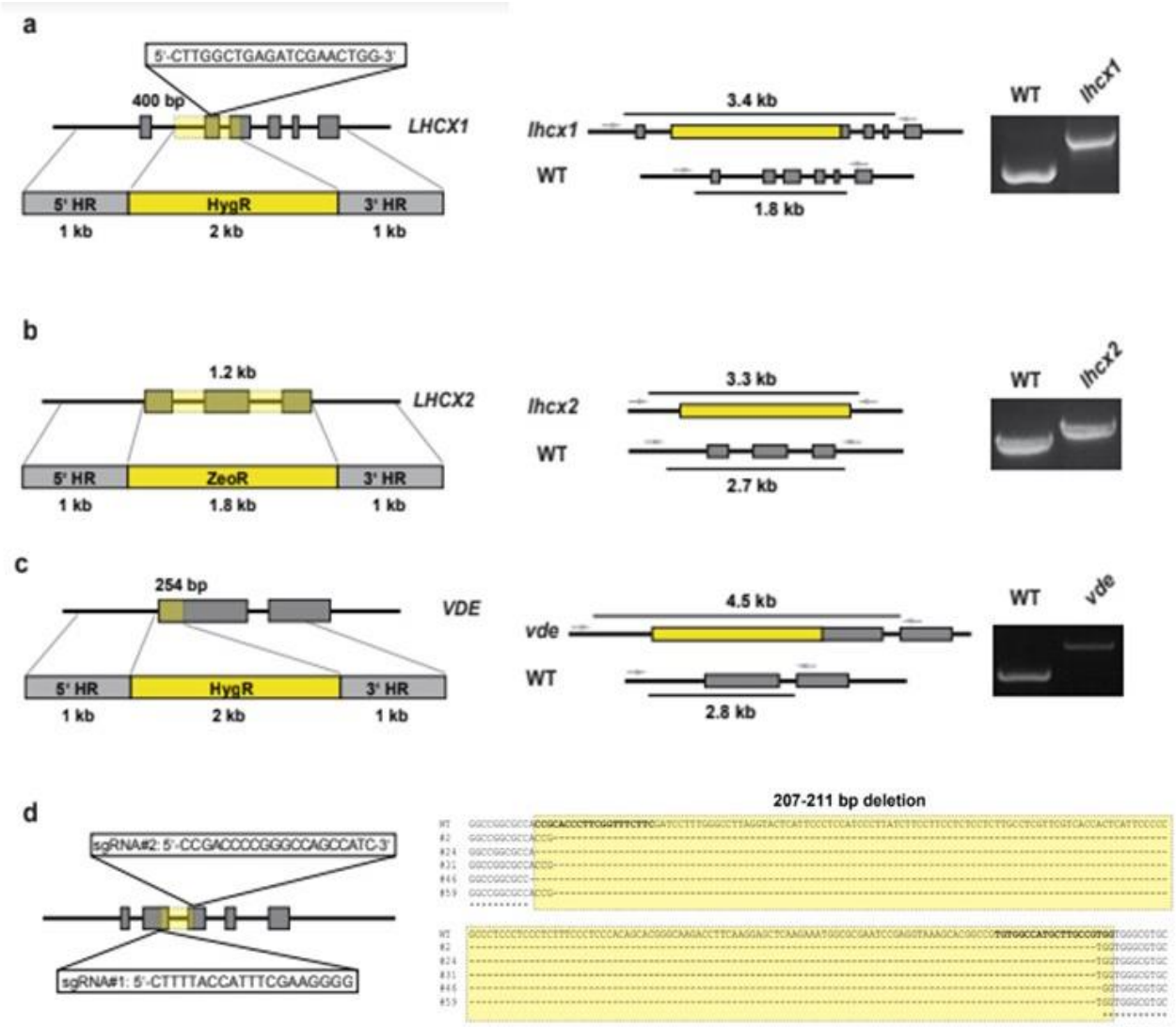


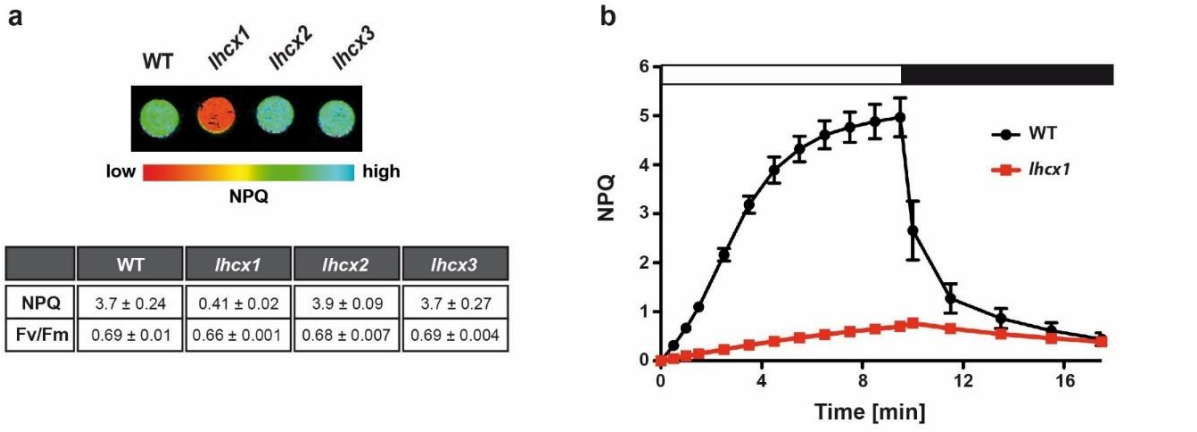

***Figure 3. Generation of lhcx and vde knockout mutants. (****a) Knockout of LHCX1 by Cas9 RNP-mediated HR to introduce a repair template containing a hygromycin-resistance cassette (HygR). A gRNA located in the second exon was used to target the Cas9 enzyme. (b) Knockout of LHCX2 by HR with a zeocin-resistance cassette (ZeoR). (c) Knockout of VDE by HR with a HygR cassette. (d) Knockout of LHCX3 by CRISPR-Cas9 with two gRNAs. Gene models with the deleted/replaced region highlighted in yellow are shown on the left, and examples for gene integration and PCR genotyping of mutants are shown on the right. Locations of primers used for genotyping are indicated as arrows.*

replacement of the target sequence by a larger resistance cassette led to a size shift of the PCR product (Figure 3b, c).

For *LHCX1*, we used CRISPR/Cas9 to generate a double-strand break to stimulate HR efficiency with a repair template containing a hygromycin-resistance cassette flanked by 1 kb homology arms (Figure 2a). The Cas9 enzyme and a synthetic guide RNA (gRNA) targeting the second exon of *LHCX1* were assembled in vitro, and the ribonucleotide protein (RNP) complex was co-transformed into cells with the repair template (Figure 2a; see Materials and Methods for details). The repair template integrated into the *LHCX1* locus with an 85% efficiency, 30-80-fold higher than by HR

alone (Figure 2a). In the case of *LHCX3*, we were not able to amplify the 5' flanking region of its coding sequence and therefore were not able target the gene by HR. Instead, we decided to induce mutations with the CRISPR-Cas9 system only. A construct for stable expression of CRISPR-Cas9 and two gRNAs targeting the second and third exon (Figure 2d) were transformed into the wild type. The transformants were genotyped using primers flanking the targeted region (Figure 2d; see Materials and Methods for details) to identify deletions occurring between the two gRNA sites.

LHCX1 is the main stress-related LHC protein required for qE




| | WT | *lhcx1* | *lhcx2* | *lhcx3* |
|---|---|---|---|---|
| NPQ | 3.7 ± 0.24 | 0.41 ± 0.02 | 3.9 ± 0.09 | 3.7 ± 0.27 |
| Fv/Fm | 0.69 ± 0.01 | 0.66 ± 0.001 | 0.68 ± 0.007 | 0.69 ± 0.004 |

DES:

| | 0 | 5 | 10 |
|---|---|---|---|
| - DTT | 0.06 ± 0.006 | 0.15 ± 0.003 | 0.24 ± 0.018 |
| + DTT | 0.06 ± 0.006 | 0.06 ± 0.004 | 0.06 ± 0.005 |

***Figure 4. NPQ phenotypes of lhcx mutants****. (a) False-color image of NPQ, values for NPQ at the end of the 10-min induction period, and $F_v/F_m$ of dark-acclimated samples grown on agar medium. (b) NPQ induction and relaxation kinetics of lhcx1 compared to wild-type cells measured using a PAM fluorometer. The white bar indicates exposure to 800 µmol photons $m^{-2}$ $s^{-1}$ white actinic light, and the black bar indicates recovery in the dark. (c) Effect of 10 mM DTT on NPQ induction in lhcx1 after co-incubation during 30-min dark-acclimation of samples. (d) Quantification of VAZ cycle pigments by HPLC and table of de-epoxidation states (DES) of cells after 0, 5 and 10 min of exposure to 800 µmol photons $m^{-2}$ $s^{-1}$ white actinic light with and without DTT treatment. V = violaxanthin; A = antheraxanthin; Z = zeaxanthin; DES = de-epoxidation state ((A+Z)/(V+A+Z))*

To understand the relative contributions of each *LHCX* gene to NPQ, we first screened three independent mutant lines per gene by measuring NPQ using an imaging pulse-amplitude modulated (PAM) fluorometer (Figure 4a). In the low-light grown cells (50 µmol photons $m^{-2}$ $s^{-1}$), *lhcx1* exhibited an 89% reduction in NPQ when compared to the wild type (Figure 3a). The $F_v/F_m$ value of *lhcx1*, reflecting the maximum quantum efficiency of PSII, was not significantly different from that of the wild type.

The *lhcx2* and *lhcx3* mutants had wild-type levels of NPQ and $F_v/F_m$ (Figure 4a). To confirm the lack of a significant *lhcx2* or *lhcx3* NPQ phenotype, we used time-correlated single photon counting (TCSPC) to monitor the changes in average Chl *a* fluorescence lifetime. Consistent with the PAM NPQ phenotypes, we observed fluorescence lifetimes nearly identical to those of the wild type during the induction and relaxation of NPQ (SI Figure 3a). This contrasted with the impaired quenching of Chl *a* fluorescence lifetime in *lhcx1* and *vde* mutants (Park et al., 2019). We then performed ultrafast transient absorption (TA) spectroscopy on *lhcx2* and *lhcx3* cells by exciting Chl *a* at 665 nm and probing for Car S1 excited state absorption ($S_1$-$S_n$ transition) at 540 nm, in the dark and after 15 min of exposure to high light (SI Figure 3b, c). For both mutants, we see a clear decay of the signal with a time constant representative of the Car $S_1$ lifetime indicative of excitation energy transfer (EET) quenching. This decay is also present in the wild type but absent in either *lhcx1* or *vde* mutant cells (Park et al., 2019).

To further investigate the NPQ induction and relaxation kinetics of *lhcx1*, we measured Chl *a* fluorescence of cell cultures grown in liquid culture. As shown in Figure 4b, the rapidly inducible NPQ corresponding to qE is completely absent in the *lhcx1* mutant. Only a slowly and linearly inducible NPQ component (possibly qZ) was still present that relaxed in a similar linear fashion after the light was turned off. NPQ in the *lhcx1* mutant (~20% of the wild-type level) was decreased further by 50% after treatment with DTT (Figure 4c), consistent with the possibility that the residual NPQ in the *lhcx1* mutant is qZ. We then examined levels of VAZ cycle pigments using HPLC with and without the addition of DTT in dark-acclimated *lhcx1* cells. We compared cells exposed to 0, 5, and 10 min of high actinic light (800 µmol photons $m^{-2}$ $s^{-1}$) and found that in cells not treated with DTT, the DES increased by three-fold due to an increase in Z xand Ax levels and a concomitant decrease in Vx (Figure 4d). In DTT-treated cells, the DES remained constant, and the amount of Zx and Ax did not change upon illumination (Figure 4d). These results are similar to our findings in WT cells grown in the same conditions and treated with and without DTT (Figure 1b).

To investigate whether increased expression of LHCX1 functionally compensates for the absence of LHCX2 or LHCX3 in their respective mutant backgrounds, we conducted immunoblot analysis on *lhcx2* and *lhcx3* cells grown in low-light conditions. Our results indicate that LHCX1 is not upregulated to compensate for the lack of either of these two proteins (Figure 5, SI Figure 4).

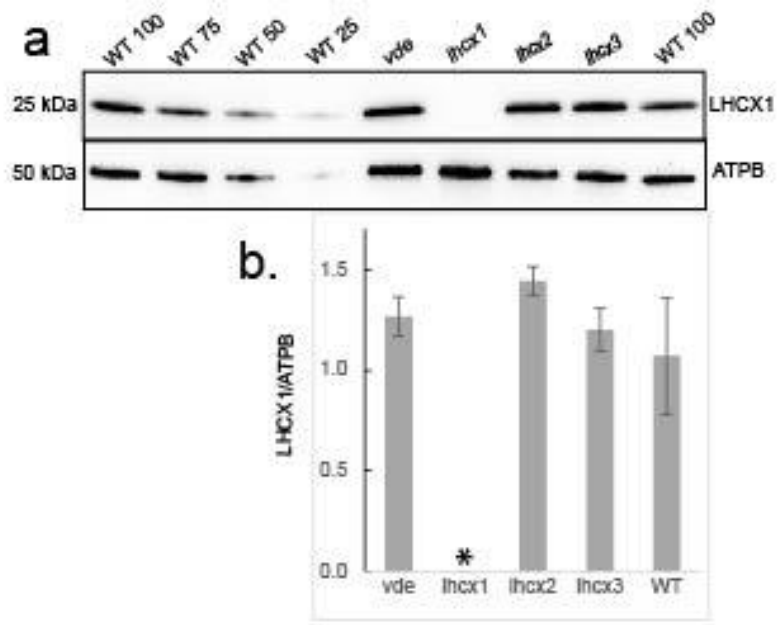



***Figure 5. Immunoblot analysis of LHCX1 accumulation in wild type (WT) and knockout mutants of lhcx1, lhcx2, lhcx3, and vde.*** *(a) Representative immunoblot showing LHCX1 protein accumulation (top) and ATP-β as a loading control (bottom). Wild-type protein dilution series loaded at 100%, 75%, 50%, and 25% and protein accumulation in the four knockout mutants are shown. (b) Quantification of the amount of LHCX1 relative to ATPβ. Asterisk indicates statistically significant differences from the wild type in LHCX1 protein accumulation (student's t-test, p-value <0.05). Data shown are mean values ± SD of three biological replicates. Cells were grown in low light at 50 µmol photons m-2 s-1.*

The xanthophyll cycle is essential for qE and qZ in *N. oceanica.*

Our results with DTT treatment indicate that NPQ in *N. oceanica* depends on the VAZ cycle in the wild type and *lhcx1* mutant (Figure 1, 4c, 4d). This was confirmed by analysis of *vde* mutants, which exhibited a 95% decrease in NPQ when compared to the wild type (Figure 6a). However, the $F_v/F_m$ value was also compromised even in low-light-grown *vde* cells by 26% when compared to the wild type with values of 0.51 and 0.69, respectively. We then measured chlorophyll *a* fluorescence of cells grown in liquid medium. As shown in Figure 6b, NPQ in the *vde* mutant reached around 0.2, even lower than in the *lhcx1* mutant (Figure 4b). Both the rapidly inducible qE and slowly and linearly inducible qZ were absent in the *vde* mutant.

We then examined accumulation of VAZ pigments using HPLC in the WT and *vde* cells exposed to 0, 5, and 10 min of high actinic light (800 µmol photons $m^{-2}$ $s^{-1}$). De-epoxidation of Vx occurred as expected in WT cells, and the DES increased by four-fold upon exposure to high light (an increase in DES from 0.06 to 0.24 over 10 min, Figure 6c). In contrast, the de-epoxidation of Vx was blocked in the *vde* mutant, and the DES remained constant, even after a 10 min exposure to high light (Figure 6c). Our results indicate that the absence of Zx correlates with the loss of qE and qZ and that the presence of LHCX1 without Z is not sufficient for NPQ.

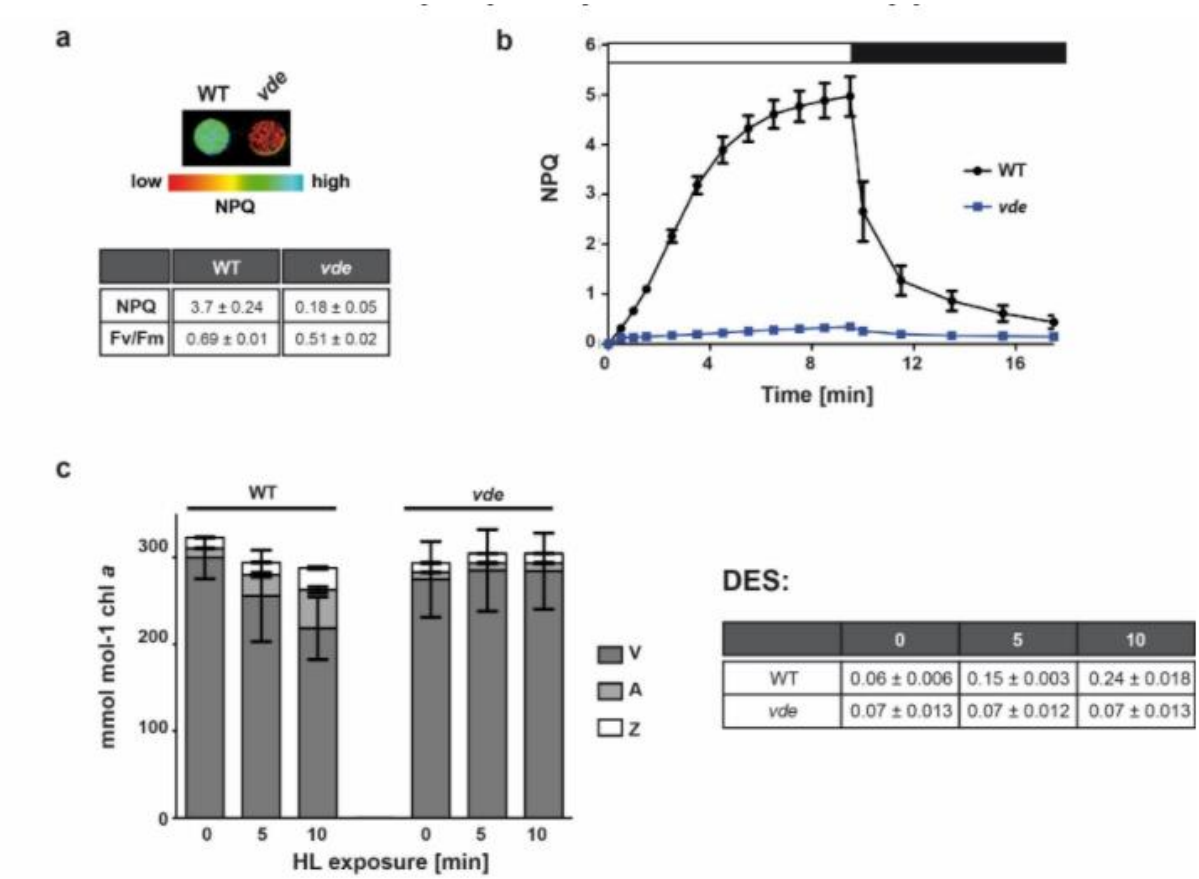



***Figure 6. NPQ and DES phenotypes of the vde mutant.*** *(a) False-color image of NPQ at the end of the 10-min induction period, values for NPQ, and Fv/Fm of dark-acclimated cells grown on agar medium. (b) NPQ induction and relaxation kinetics of vde. The white bar indicates exposure to 800 µmol photons m-2 s-1 white actinic light, and the black bar indicates recovery in the dark. (c) Quantification of VAZ cycle pigments by HPLC analysis in wild type and vde mutant and table of de-epoxidation states (DES) of cells after 0, 5 and 10 min of exposure to 800 µmol photons m-2 s-1 white actinic light. V = violaxanthin; A = antheraxanthin; Z = zeaxanthin; DES = de-epoxidation state ((A+Z)/(V+A+Z))*

## Discussion

Wild-type *N. oceanica* CCMP1779 has a relatively high NPQ capacity of ~5 (Figure 1a). Based on inhibitor treatments and mutant analysis, this NPQ consists mostly of qE along with some qZ that relaxes more slowly. We found that LHCX1 is completely required for qE in *N. oceanica*, whereas LHCX2 and LHCX3 do not appear to be involved in NPQ under our experimental conditions (Figure 4, SI Figure 3). DTT inhibition of VDE indicated that the residual NPQ in the *lhcx1* mutant is qZ (Figure 4c, d), and this was confirmed by analysis of the *vde* mutant, which is unable to de-epoxidize Vx to Zx and has even less NPQ than the *lhcx1* mutant (Figure 6). Thus, the *vde* mutant seemed to lack both qE and qZ. The complete lack of qE in the *vde* mutant, which accumulates wild-type levels of LHCX1 (Figure 5), suggests that LHCX1-dependent qE is highly dependent on Zx, which is consistent with our recent modeling that shows Zx is ~9 times more effective than Vx in inducing qE in *N. oceanica* (Short et al., 2023). As suggested previously (Park et al., 2019; Perin et al., 2023; Short et al.,

2022; Short et al., 2023), we hypothesize that LHCX1 and Zx interact to induce qE. These components may interact directly in a pigment-protein complex, or activation of LHCX1 by ΔpH may cause conformational changes that enable Zx quenching when it is bound to other proteins.

The LHCX1-dependent qE appears to be similar to that in diatoms. Unlike LHCSR3 in *C. reinhardtii*, diatom LHCX1 is constitutively expressed in non-stressful conditions and is required for efficient light responses and growth (Bailleul et al., 2010; Buck et al., 2019, 2021; Giovagnetti et al., 2022; Taddei et al., 2018). Our immunoblots analysis of *N. oceanica* showed that LHCX1 is present in wild-type cells grown in low light (Figure 5, SI Figure 4). On the structural level, LHCX1 in *N. oceanica* contains three-transmembrane α-helices like LHCSR3 in *C. reinhardtii*, and pigment-binding sites or protonatable sites may be conserved. Thus, it is possible that LHCX1 may be involved in pH sensing due to the presence of acidic residues that are predicted to be exposed to the lumen (SI Fig 1, Ballottari et al., 2016). However, when compared to LHCSR, mutagenesis of individual, conserved putative protonatable sites in *P. tricornutum* LHCX1 does not alter qE capacity, suggesting that PtLHCX1 is not involved in sensing lumen pH (Buck et al., 2021; Giovagnetti et al., 2022). This may indicate a functional divergence between PtLHCX1 and CrLHCSR3 in modulating NPQ. However, it is not yet clear whether qE in *N. oceanica* is induced by the protonation of acidic residues in the same way. Future experiments involving incubating wild-type cells with DCCD, an inhibitor of protonatable residues (Walters et al., 1996), or making mutations in conserved residues in NoLHCX1 could address this. Notably, point mutations in amino acid W133 in PtLHCX1 reduced qE capacity, indicating that this site could be a Ddx/Dtx binding pocket and important in quenching absorbed energy (Buck et al., 2021). This residue is conserved in NoLHCX1, which might allow it to directly bind Zx and form a pigment-protein complex that is directly involved in qE.

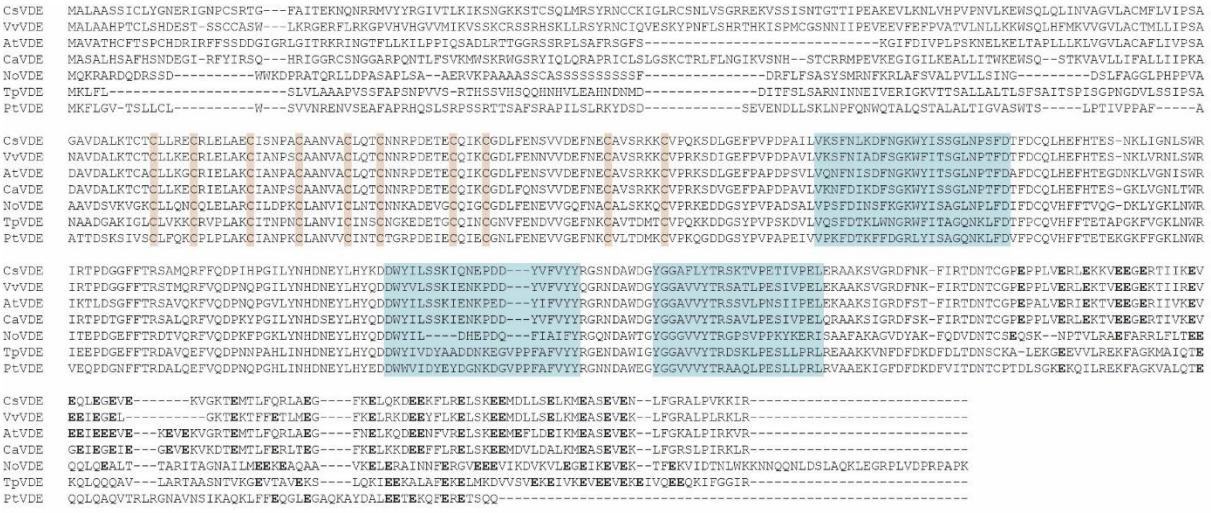
Our results show that LHCX2 and LHXC3 do not contribute to qE in low-light grown cells (Figure 3, SI Figure 3), although again there may be parallels between *N. oceanica* and diatoms. We know that LHCX1 is the main contributor to qE in both *N. oceanica* and *P. tricorutum*, whereas LHCX2 and LHCX3 have been shown to have roles in prolonged HL in diatoms, providing flexibility to various light environments (Buck et al., 2021; Lepetit et al., 2017; Taddei et al., 2018). Additionally, PtLHCX2 has been suggested to aid in acclimation to low iron due to its upregulation in iron-deplete conditions when compared to the wild type (Buck et al., 2022). It is possible that LHCX2 and LHCX3 have similar roles in *N. oceanica*, for instance an LHCX3 homolog in *N. gaditana* was upregulated in high light (Chukhutsina et al., 2017). Further analysis of these proteins is necessary.

We have identified LHCX1 as the main stress-related LHC and investigated the role of Zx in NPQ in *N. oceanica*. We can use this knowledge to improve light-energy utilization by modifying NPQ and improving biomass productivity by optimizing VAZ cycle dynamics in *N. oceanica,* which can lead to faster relaxation of these photoprotective mechanisms as shown previously in tobacco (Kromdijk et al., 2016), soybean (De Souza et al., 2022), and more recently in *N. gaditana* (Perin et al., 2023).

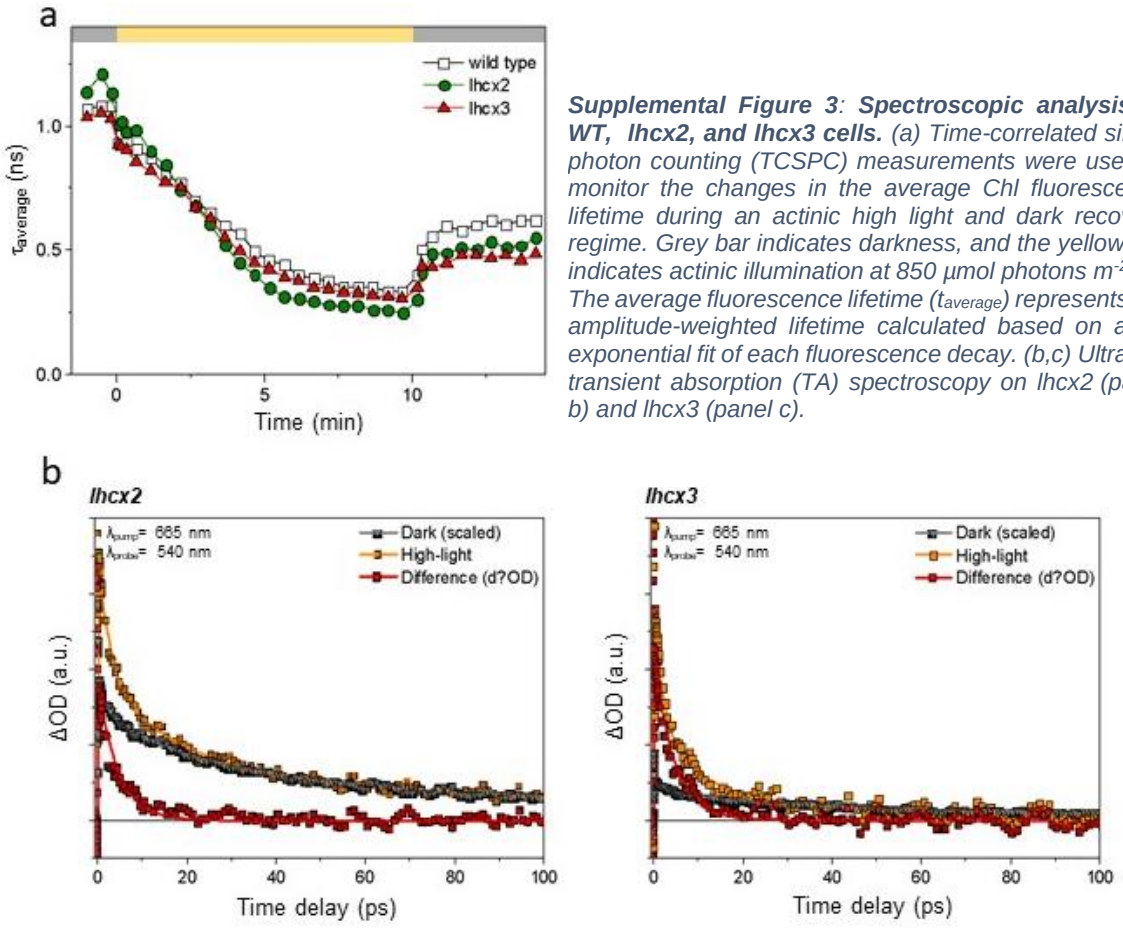
Supplemental Materials



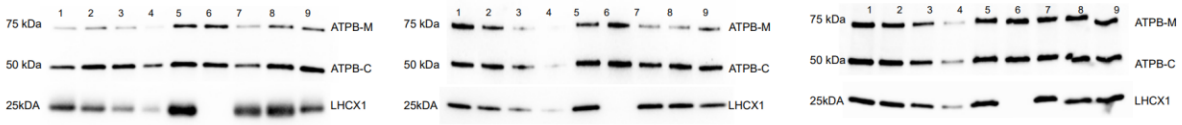
***SI Figure 1****. Sequence alignment of LHCX/LHCSR homologs showing conserved regions (black box), putative Chl a-binding sites (green), and lumen-exposed protonatable residues (blue and red). No = Nannochloropsis oceanica, Es = Ectocarpus siliculosus, Tp= Thalassiosira pseudonana, Pt= Phaeodactylum tricornutum, Cr= Chlamydomonas reinhardtii, Vc= Volvox carterii.*




***SI Figure 2.*** *Sequence alignment of VDE proteins from selected plants (top four sequences), Nannochloropsis oceanica, (No), Thalassiosira pseudonana (Tp), and Phaeodactylum tricornutum (Pt). Blue boxes indicate conserved lipocalin domains, beige indicates conserved cysteine residues, and bolded letters indicate glutamate residues in the C-terminal domain.*




***Supplemental Figure 3***: ***Spectroscopic analysis of WT, lhcx2, and lhcx3 cells.*** *(a) Time-correlated single photon counting (TCSPC) measurements were used to monitor the changes in the average Chl fluorescence lifetime during an actinic high light and dark recovery regime. Grey bar indicates darkness, and the yellow bar indicates actinic illumination at 850 µmol photons $m^{-2}$ $s^{-1}$. The average fluorescence lifetime ($t_{average}$) represents the amplitude-weighted lifetime calculated based on a bi-exponential fit of each fluorescence decay. (b,c) Ultrafast transient absorption (TA) spectroscopy on lhcx2 (panel b) and lhcx3 (panel c).*




Blot 1 Densitometry

| Lane | Sample | LHCX1 | ATPB-M | ATPB-C |
|---|---|---|---|---|
| 1 | WT 100 | 29935 | 9205 | 22035 |
| 2 | WT 75 | 27402 | 12847 | 32705 |
| 3 | WT 50 | 18324 | 10419 | 32031 |
| 4 | WT 25 | 13200 | 3940 | 20744 |
| 5 | vde | 41703 | 23621 | 36064 |
| 6 | lhcx1 | 0 | 23502 | 29979 |
| 7 | lhcx2 | 29308 | 10085 | 19767 |
| 8 | lhcx3 | 31997 | 18004 | 28738 |
| 9 | WT | 26661 | 15725 | 31422 |

Blot 2 Densitometry

| Lane | Sample | LHCX1 | ATPB-M | ATPB-C |
|---|---|---|---|---|
| 1 | WT 100 | 23401 | 26643 | 25461 |
| 2 | WT 75 | 20044 | 15778 | 23195 |
| 3 | WT 50 | 13036 | 7008 | 15571 |
| 4 | WT 25 | 4679 | 1424 | 4540 |
| 5 | vde | 35462 | 15810 | 26,931 |
| 6 | lhcx1 | 0 | 23615 | 31501 |
| 7 | lhcx2 | 31587 | 10746 | 21254 |
| 8 | lhcx3 | 30177 | 10386 | 25867 |
| 9 | WT | 27533 | 14199 | 28636 |

Blot 3 Densitometry

| Lane | Sample | LHCX1 | ATPB-M | ATPB-C |
|---|---|---|---|---|
| 1 | WT 100 | 39765 | 38360 | 41701 |
| 2 | WT 75 | 34284 | 25608 | 33361 |
| 3 | WT 50 | 26141 | 17082 | 22175 |
| 4 | WT 25 | 15994 | 4706 | 10169 |
| 5 | vde | 35696 | 22622 | 26,916 |
| 6 | lhcx1 | 0 | 29815 | 28894 |
| 7 | lhcx2 | 35865 | 26307 | 26397 |
| 8 | lhcx3 | 32274 | 24600 | 24418 |
| 9 | WT | 35279 | 24080 | 25254 |

***Supplemental Figure 4. Immunoblot replicates showing the accumulation of LHCX1 in wild type (WT) and mutant lines.*** *Wild-type protein dilution series loaded at 100%, 75%, 50%, and 25% are shown in lanes 1-4, and protein accumulation in the four knockout mutants are shown in lanes 5-9. ATPB-C indicates the chloroplast ATPB loading control probed using the ATB-Beta subunit antibody, whereas ATPB-M indicates the mitochondrial ATP-Beta subunit in the solubilized whole-cell sample. Each replicate has a densitometry table below with density values for each band.*

| Name | Sequence 5'-3' | Experiment |
|---|---|---|
| VDE_3'_HR_F | GGGGACAACTTTGTATAATAAAGTTGTACCTCTCTATCAATGGGGACA | VDE HR |
| VDE_3'_HR_R | GGGGACCACTTTGTACAAGAAAGCTGGGTTTATTGTCCACGTCCTGAAAT | VDE HR |
| VDE_5'_HR_F | GGGGACAAGTTTGTACAAAAAAGCAGGCTTAAAGGGGCGGCACCGGGCTG G | VDE HR |
| VDE_5'_HR_R | GGGGACAACTTTGTATAGAAAAGTTGGGTGATTTGTTGTGGAAGAAGTGG | VDE HR |
| Fus1_F | TAGAAATGCGGCCGCTAA | sgRNA vector assembly |
| Fus1_R | CTCTTGATCGGATCAAAGTCTCAGAGAAGAGCGTCCCATGC | sgRNA vector assembly |
| Fus2_F | GCATGGGACGCTCTTCTCTGAGACTTTGATCCGATCAAGAG | sgRNA vector assembly |
| Fus2_R | TCTATGAGACGTCCGGAAAGTTGCGAGAGTAAAC | sgRNA vector assembly |
| HygR_F | AAGATGGAGTGGATGGAGGAG | HygR cassette amplification |
| HygR_R | TCTAGTGGATCAGCTTGCATGC | HygR cassette amplification |
| LHCX1_genoF | TTCCGCACCTTCGCACCTGG | Genotyping LHCX1 |
| LHCX1_genoR | GGGAAGGCGTGACGTACCGTG | Genotyping LHCX1 |
| LHCX1_gRNA | CUUGGCUGAGAUCGAACUGG | CRISPR-Cas9 gRNA |
| LHCX1_HR_F | AAAGCATGGCTTGGAGGACAA | Amplification LHCX1 repair template |
| LHCX1_HR_R | CAACGCACACACGACACAAATC | Amplification LHCX1 repair template |
| LHCX2_genoF | GCAATGCACGCGTCGTTGTTC | Genotyping LHCX2 |
| LHCX3_genoF | CGTGCCTTTGAGAAGCTTGGC | Genotyping LHCX3 |
| LHCX3_genoR | GCCTTATCTTCGAGGAGGGCG | Genotyping LHCX3 |
| LHCX3_gRNA_F | CUUUUACCAUUUCGAAGGGG | CRISPR-Cas9 guideRNA oligo |
| LHCX3_gRNA_R | CCGACCCCGGGCCAGCCAUC | CRISPR-Cas9 guideRNA oligo |
| U6-F | GCTTGGGTTGAGCATCG | U6 promoter amplification |
| U6-R | TTCTCGATTTGTGCGTGTCA | U6 promoter amplification |
| VDE_genoF | GATGGCAGGCATCCATCCCC | Genotyping VDE |
| VDE_genoR | GTGCTGGACTGAATCGTATG | Genotping VDE |
| VDE_HR_F | AAGGGGCGGCACCGGGCTGG | Cloning VDE repair template |
| VDE_HR_R | TATTGTCCACGTCCTGAAAT | Cloning LHCX1 repair template |
| VDE_HygR_HR_F | GGGGACAACTTTTCTATACAAAGTTGTAAAGATGGAGTGGATGGAGGAG | HygR assembly |
| VDE_HygR_HR_R | GGGGACAACTTTATTATACAAAGTTGTTCTAGTGGATCAGCTTGCATGC | HygR assembly |

***Table 1.*** *List of primers used in materials and methods.*

## References

Ahumada-Fierro, N. V., García-Mendoza, E., Sandoval-Gil, J. M., & Band-Schmidt, C. J. (2021). Photosynthesis and photoprotection characteristics related to ROS

production in three Chattonella (Raphidophyceae) species. *Journal of Phycology*, *57*(3), 941–954. https://doi.org/10.1111/JPY.13138

Ajjawi, I., Verruto, J., Aqui, M., Soriaga, L. B., Coppersmith, J., Kwok, K., Peach, L., Orchard, E., Kalb, R., Xu, W., Carlson, T. J., Francis, K., Konigsfeld, K., Bartalis, J., Schultz, A., Lambert, W., Schwartz, A. S., Brown, R., & Moellering, E. R. (2017). Lipid production in Nannochloropsis gaditana is doubled by decreasing expression of a single transcriptional regulator. *Nature Biotechnology*, *35*(7), 647–652. https://doi.org/10.1038/NBT.3865

Alboresi, A., Le Quiniou, C., Yadav, S. K. N., Scholz, M., Meneghesso, A., Gerotto, C., Simionato, D., Hippler, M., Boekema, E. J., Croce, R., & Morosinotto, T. (2017). Conservation of core complex subunits shaped the structure and function of photosystem I in the secondary endosymbiont alga Nannochloropsis gaditana. *New Phytologist*, *213*, 714–726. https://doi.org/10.1111/nph.14156

Alboresi, A., Perin, G., Vitulo, N., Diretto, G., Block, M., Jouhet, J., Meneghesso, A., Valle, G., Giuliano, G., Maréchal, E., & Morosinotto, T. (2016). Light Remodels Lipid Biosynthesis in Nannochloropsis gaditana by Modulating Carbon Partitioning between Organelles. *Plant Physiology*, *171*(4), 2468–2482. https://doi.org/10.1104/PP.16.00599

Alboresia, A., Gerottob, C., Giacomettib, G. M., Bassia, R., & Morosinotto, T. (2010). Physcomitrella patens mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(24), 11128–11133. https://doi.org/10.1073/PNAS.1002873107

Amaral, R., Sérgio Seixas de Melo, J., & Maria Antunes dos Santos, L. (2021). Pigments from Eustigmatophyceae: an interesting class of microalgae for carotenoid production. *Journal of Applied Phycology*, *33*, 371–384. https://doi.org/10.1007/s10811-020-02312-z/Published

Antoshvili, M., Caspy, I., Hippler, · Michael, & Nelson, · Nathan. (2019). Structure and function of photosystem I in Cyanidioschyzon merolae. *Photosynthesis Research*, *139*, 499–508. https://doi.org/10.1007/s11120-018-0501-4

Armbrust, E. V., Berges, J. A., Bowler, C., Green, B. R., Martinez, D., Putnam, N. H., Zhou, S., Allen, A. E., Apt, K. E., Bechner, M., Brzezinski, M. A., Chaal, B. K., Chiovitti, A., Davis, A. K., Demarest, M. S., Detter, J. C., Glavina, T., Goodstein, D., Hadi, M. Z., … Rokhsar, D. S. (2004). The genome of the diatom Thalassiosira Pseudonana: Ecology, evolution, and metabolism. *Science*, *306*(5693), 79–86. https://doi.org/10.1126/science.1101156

Arsalane, W., Rousseau, B., & Duval, J. -C. (1994). INFLUENCE OF THE POOL SIZE OF THE XANTHOPHYLL CYCLE ON THE EFFECTS OF LIGHT STRESS IN A DIATOM: COMPETITION BETWEEN PHOTOPROTECI'ION AND

PHOTOINHIBITION. *Photochemistry and Photobiology*, *60*(3), 237–243. https://doi.org/10.1111/J.1751-1097.1994.TB05097.X

Arshad, R., Calvaruso, C., Boekema, E. J., Buchel, C., & Kouril, R. (2021). Revealing the architecture of the photosynthetic apparatus in the diatom Thalassiosira pseudonana. *Plant Physiology*, *186*(4), 2124–2136. https://doi.org/10.1093/PLPHYS/KIAB208

Bai, Y., Cao, T., Dautermann, O., Buschbeck, P., Cantrell, M. B., Chen, Y., Lein, C. D., Shi, X., Ware, M. A., Yang, F., Zhang, H., Zhang, L., Peers, G., Li, X., & Lohr, M. (2022). Green diatom mutants reveal an intricate biosynthetic pathway of fucoxanthin. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(38). https://doi.org/10.1073/PNAS.2203708119/-/DCSUPPLEMENTAL

Bailey, J. C., Bidigare, R. R., Christensen, S. J., & Andersen, R. A. (1998). Phaeothamniophyceae Classis Nova: A New Lineage of Chromophytes Based upon Photosynthetic Pigments, rbcL Sequence Analysis and Ultrastructure. *Protist*, *149*(3), 245–263. https://doi.org/10.1016/S1434-4610(98)70032-X

Bailleul, B., Rogato, A., De Martino, A., Coesel, S., Cardol, P., Bowler, C., Falciatore, A., & Finazzi, G. (2010). An atypical member of the light-harvesting complex stress-related protein family modulates diatom responses to light. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(42), 18214–18219. https://doi.org/10.1073/pnas.1007703107

Ballottari, M., Truong, T. B., De Re, E., Erickson, E., Stella, G. R., Fleming, G. R., Bassi, R., & Niyogi, K. K. (2016). Identification of pH-sensing Sites in the Light Harvesting Complex Stress-related 3 Protein Essential for Triggering Non-photochemical Quenching in Chlamydomonas reinhardtii. *The Journal of Biological Chemistry*, *291*(14), 7334–7346. https://doi.org/10.1074/jbc.M115.704601

Baroli, I., Do, A. D., Yamane, T., & Niyogi, K. K. (2003). Zeaxanthin Accumulation in the Absence of a Functional Xanthophyll Cycle Protects Chlamydomonas reinhardtii from Photooxidative Stress. *The Plant Cell*, *15*(4), 992–1008. https://doi.org/10.1105/TPC.010405

Bassi, R., & Dall'osto, L. (2021). *Dissipation of Light Energy Absorbed in Excess: The Molecular Mechanisms*. https://doi.org/10.1146/annurev-arplant-071720

Basso, S., Simionato, D., Gerotto, C., Segalla, A., Giacometti, G. M., & Morosinotto, T. (2014). Characterization of the photosynthetic apparatus of the Eustigmatophycean Nannochloropsis gaditana: Evidence of convergent evolution in the supramolecular organization of photosystem I. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(2), 306–314. https://doi.org/10.1016/J.BBABIO.2013.11.019

Benedetti, M., Vecchi, V., Barera, S., & Dall'Osto, L. (2018). Biomass from microalgae: The potential of domestication towards sustainable biofactories. *Microbial Cell Factories*, *17*(1). https://doi.org/10.1186/S12934-018-1019-3

Berne, N., Fabryova, T., Istaz, B., Cardol, P., & Bailleul, B. (2018). The peculiar NPQ regulation in the stramenopile Phaeomonas sp. challenges the xanthophyll cycle dogma. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1859*(7), 491–500. https://doi.org/10.1016/J.BBABIO.2018.03.013

Betancor, S., Domínguez, B., Tuya, F., Figueroa, F. L., & Haroun, R. (2015). Photosynthetic performance and photoprotection of Cystoseira humilis (Phaeophyceae) and Digenea simplex (Rhodophyceae) in an intertidal rock pool. *Aquatic Botany*, *121*, 16–25. https://doi.org/10.1016/J.AQUABOT.2014.10.008

Bína, D., Bouda, K., & Litvín, R. (2017). A two-component nonphotochemical fluorescence quenching in eustigmatophyte algae. *Photosynthesis Research*, *131*(1), 65–77. https://doi.org/10.1007/S11120-016-0299-X/FIGURES/9

Bína, D., Durchan, M., Kuznetsova, V., Vácha, F., Litvín, R., & Polívka, T. (2018). *Energy transfer dynamics in a red-shifted violaxanthin-chlorophyll a light-harvesting complex*. https://doi.org/10.1016/j.bbabio.2018.11.006

Bína, D., Gardian, Z., Herbstová, M., & Litvín, R. (2017). Modular antenna of photosystem I in secondary plastids of red algal origin: a Nannochloropsis oceanica case study. *Photosynthesis Research*, *131*(3), 255–266. https://doi.org/10.1007/s11120-016-0315-1

Bird, D. F., & Kalff, J. (1987). Algal phagotrophy: Regulating factors and importance relative to photosynthesis in Dinobryon (Chrysophyceae). *Limnology and Oceanography*, *32*(2), 277–284. https://doi.org/10.4319/LO.1987.32.2.0277

Bonente, G., Ballottari, M., Truong, T. B., Morosinotto, T., Ahn, T. K., Fleming, G. R., Niyogi, K. K., & Bassi, R. (2011). Analysis of LHcSR3, a protein essential for feedback de-excitation in the green alga Chlamydomonas reinhardtii. *PLoS Biology*, *9*(1). https://doi.org/10.1371/JOURNAL.PBIO.1000577

Borowitzka, M. A. (2013). High-value products from microalgae—their development and commercialisation. *Journal of Applied Phycology 2013 25:3*, *25*(3), 743–756. https://doi.org/10.1007/S10811-013-9983-9

Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., Otillar, R. P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde, M., Katinka, M., Mock, T., Valentin, K., … Grigoriev, I. V. (2008a). The Phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature 2008 456:7219*, *456*(7219), 239–244. https://doi.org/10.1038/nature07410

Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., Otillar, R. P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde, M., Katinka, M., Mock, T., Valentin, K., … Grigoriev, I. V. (2008b). The Phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature 2008 456:7219*, *456*(7219), 239–244. https://doi.org/10.1038/nature07410

Brenner, S., & Horne, R. W. (1959). A NEGATIVE STAINING METHOD FOR HIGH RESOLUTION ELECTRON MICROSCOPY OF VIRUSES. *Biochimica Et Biophysica Acta* , *34*, 103–110.

Büchel, C. (2015a). Evolution and function of light harvesting proteins. *Journal of Plant Physiology*, *172*, 62–75. https://doi.org/10.1016/J.JPLPH.2014.04.018

Büchel, C. (2015b). Evolution and function of light harvesting proteins. *Journal of Plant Physiology*, *172*, 62–75. https://doi.org/10.1016/J.JPLPH.2014.04.018

Büchel, C. (2020a). Light harvesting complexes in chlorophyll c-containing algae. *Biochimica et Biophysica Acta - Bioenergetics*, *1861*(4). https://doi.org/10.1016/j.bbabio.2019.05.003

Büchel, C. (2020b). Light harvesting complexes in chlorophyll c-containing algae. *Biochimica et Biophysica Acta - Bioenergetics*, *1861*(4). https://doi.org/10.1016/J.BBABIO.2019.05.003

Büchel, C. (2020c). *Light-Harvesting Complexes of Diatoms: Fucoxanthin-Chlorophyll Proteins*. 441–457. https://doi.org/10.1007/978-3-030-33397-3_16

Büchel, C., & Garab, G. (1995). Electrochromic absorbance changes in the chlorophyll-c-containing alga Pleurochloris meiringensis (Xanthophyceae). *Photosynthesis Research*, *43*, 49–56.

Büchel, C., Wilhelm, C., & Lenartz-Weiler, I. (1988). The Molecular Analysis of the Light Adaptation Reactions in the Yellow-green Alga Pleurochloris meiringensis (Xanthophyceae). *Botanica Acta*, *101*(4), 306–310. https://doi.org/10.1111/J.1438-8677.1988.TB00049.X

Buck, J. M., Kroth, P. G., & Lepetit, B. (2021). Identification of sequence motifs in Lhcx proteins that confer qE-based photoprotection in the diatom Phaeodactylum tricornutum. *The Plant Journal*, *108*(6), 1721–1734. https://doi.org/10.1111/TPJ.15539

Buck, J. M., Sherman, J., Bártulos, C. R., Serif, M., Halder, M., Henkel, J., Falciatore, A., Lavaud, J., Gorbunov, M. Y., Kroth, P. G., Falkowski, P. G., & Lepetit, B. (2019). Lhcx proteins provide photoprotection via thermal dissipation of absorbed light in the diatom Phaeodactylum tricornutum. *Nature Communications 2019 10:1*, *10*(1), 1–12. https://doi.org/10.1038/s41467-019-12043-6

Buck, J. M., Wünsch, M., Schober, A. F., Kroth, P. G., & Lepetit, B. (2022). Impact of Lhcx2 on Acclimation to Low Iron Conditions in the Diatom Phaeodactylum tricornutum. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/FPLS.2022.841058

Buck-Wiese, H., Andskog, M. A., Nguyen, N. P., Bligh, M., Asmala, E., Vidal-Melgosa, S., Liebeke, M., Gustafsson, C., & Hehemann, J. H. (2023). Fucoid brown algae inject fucoidan carbon into the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(1), e2210561119.

https://doi.org/10.1073/PNAS.2210561119/SUPPL_FILE/PNAS.2210561119.SAPP.PDF

Burton-Smith, R. N., Akimasa Watanabe, X., Ryutaro Tokutsu, X., Song, C., Kazuyoshi Murata, X., & Jun Minagawa, X. (2019). Structural determination of the large photosystem IIâ€“light-harvesting complex II supercomplex of Chlamydomonas reinhardtii using nonionic amphipol. *J. Biol. Chem*, *294*(41), 15003. https://doi.org/10.1074/jbc.RA119.009341

Cancela, A., Pérez, L., Febrero, A., Sánchez, A., Salgueiro, J. L., & Ortiz, L. (2019). Exploitation of Nannochloropsis gaditana biomass for biodiesel and pellet production. *Renewable Energy*, *133*, 725–730. https://doi.org/10.1016/j.renene.2018.10.075

Cao, S., Zhang, X., Fan, X., Qiao, H., Liang, C., Xu, D., Mou, S., Wang, W., & Ye, N. (2013). Phylogeny and characterisation of Nannochloropsis oceanica var. sinensis var. nov. (Eustigmatophyceae), a new oleaginous alga from China. *Phycologia*, *52*(6), 573–577. https://doi.org/10.2216/13-164.1

Cao, S., Zhang, X., Xu, D., Fan, X., Mou, S., Wang, Y., Ye, N., & Wang, W. (2013). A transthylakoid proton gradient and inhibitors induce a non-photochemical fluorescence quenching in unicellular algae Nannochloropsis sp. *FEBS Letters*, *587*(9), 1310–1315. https://doi.org/10.1016/J.FEBSLET.2012.12.031

Carbonera, D., Agostini, A., Di Valentin, M., Gerotto, C., Basso, S., Giacometti, G. M., & Morosinotto, T. (2014). Photoprotective sites in the violaxanthin-chlorophyll a binding Protein (VCP) from Nannochloropsis gaditana. *Biochimica et Biophysica Acta - Bioenergetics*, *1837*(8), 1235–1246. https://doi.org/10.1016/J.BBABIO.2014.03.014

Castillo, T., Ramos, D., García-Beltrán, T., Brito-Bazan, M., & Galindo, E. (2021). Mixotrophic cultivation of microalgae: An alternative to produce high-value metabolites. *Biochemical Engineering Journal*, *176*, 108183. https://doi.org/10.1016/j.bej.2021.108183

Christa, G., Pütz, L., Sickinger, C., Clavijo, J. M., Laetz, E. M. J., Greve, C., & Serôdio, J. (2018). Photoprotective Non-photochemical Quenching Does Not Prevent Kleptoplasts From Net Photoinactivation. *Frontiers in Ecology and Evolution | Www.Frontiersin.Org*, *1*, 121. https://doi.org/10.3389/fevo.2018.00121

Chukhutsina, V. U., Fristedt, R., Morosinotto, T., & Croce, R. (2017). Photoprotection strategies of the alga Nannochloropsis gaditana. *Biochimica et Biophysica Acta - Bioenergetics*, *1858*(7), 544–552. https://doi.org/10.1016/J.BBABIO.2017.05.003

Cock, J. M., Sterck, L., Rouzé, P., Scornet, D., Allen, A. E., Amoutzias, G., Anthouard, V., Artiguenave, F., Aury, J.-M., Badger, J. H., Beszteri, B., Billiau, K., Bonnet, E., Bothwell, J. H., Bowler, C., Boyen, C., Brownlee, C., Carrano, C. J., Charrier, B., … Wincker, P. (2010). *The Ectocarpus genome and the independent evolution of multicellularity in brown algae*. https://doi.org/10.1038/nature09016

Correa-Galvis, V., Redekop, P., Guan, K., Griess, A., Truong, T. B., Wakao, S., Niyogi, K. K., & Jahns, P. (2016). Photosystem II Subunit PsbS Is Involved in the Induction of LHCSR Protein-dependent Energy Dissipation in Chlamydomonas reinhardtii*. *Journal of Biological Chemistry*, *291*(33), 17478–17487. https://doi.org/10.1074/JBC.M116.737312

Cruz, S., Cartaxana, P., Newcomer, R., Dionísio, G., Calado, R., Serôdio, J., Pelletreau, K. N., Rumpho, M. E., & Biological, M. (2015). *Photoprotection in sequestered plastids of sea slugs and respective algal sources*. https://doi.org/10.1038/srep07904

D'Amici, G. M., Huber, C. G., & Zolla, L. (2009). Separation of thylakoid membrane proteins by sucrose gradient ultracentrifugation or blue native-SDS-PAGE two-dimensional electrophoresis. *Methods in Molecular Biology (Clifton, N.J.)*, *528*, 61–70. https://doi.org/10.1007/978-1-60327-310-7_4/COVER

Daskalakis, V., Papadatos, S., & Kleinekathöfer, U. (2019). Fine tuning of the photosystem II major antenna mobility within the thylakoid membrane of higher plants. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1861*(12), 183059. https://doi.org/10.1016/J.BBAMEM.2019.183059

De Meyer, F., Maddalena Venturoli, & Berend Smit. (2008). Hydrophobic Mismatch Can Induce Specific and Selective Membrane Protein Interactions. *The 2008 Spring National Meeting*.

De Souza, A. P., Burgess, S. J., Doran, L., Hansen, J., Manukyan, L., Maryn, N., Gotarkar, D., Leonelli, L., Niyogi, K. K., & Long, S. P. (2022). Soybean photosynthesis and crop yield are improved by accelerating recovery from photoprotection. *Science*, *377*(6608), 851–854. https://doi.org/10.1126/SCIENCE.ADC9831/SUPPL_FILE/SCIENCE.ADC9831_MDAR_REPRODUCIBILITY_CHECKLIST.PDF

Demmig-Adams, B. (1990). Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1020*(1), 1–24. https://doi.org/10.1016/0005-2728(90)90088-L

Demmig-Adams, B., & Adams, W. W. (1996). The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant Science*, *1*(1), 21–26. https://doi.org/10.1016/S1360-1385(96)80019-7

Demmig-Adams, B., & Adams, W. W. (2006a). Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New Phytol.*, *172*(1), 11–21. https://doi.org/10.1111/j.1469-8137.2006.01835.x

Demmig-Adams, B., & Adams, W. W. (2006b). Photoprotection in an ecological context: The remarkable complexity of thermal energy dissipation. *New Phytologist*, *172*(1), 11–21. https://doi.org/10.1111/J.1469-8137.2006.01835.X

Demmig-Adams, B., Koh, S.-C., Cohu, C. M., Muller, O., Stewart, J. J., & Iii, W. W. A. (2014). Non-Photochemical Fluorescence Quenching in Contrasting Plant Species

and Environments. *Advances in Photosynthesis and Respiration*, *40*, 531–552. https://doi.org/10.1007/978-94-017-9032-1_24

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., & Gascuel, O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, *36*(Web Server issue). https://doi.org/10.1093/NAR/GKN180

Derelle, R., López-García, P., Timpano, H., & Moreira, D. (2016). A Phylogenomic Framework to Study the Diversity and Evolution of Stramenopiles (=Heterokonts). *Molecular Biology and Evolution*, *33*(11), 2890–2898. https://doi.org/10.1093/MOLBEV/MSW168

Dimier, C., Brunet, C., Geider, R., & Raven, J. (2009). Growth and photoregulation dynamics of the picoeukaryote Pelagomonas calceolata in fluctuating light. *Limnology and Oceanography*, *54*(3), 823–836. https://doi.org/10.4319/LO.2009.54.3.0823

Dittami, S. M., Michel, G., Collén, J., Boyen, C., & Tonon, T. (2010a). Chlorophyll-binding proteins revisited - A multigenic family of light-harvesting and stress proteins from a brown algal perspective. *BMC Evolutionary Biology*, *10*(1), 1–14. https://doi.org/10.1186/1471-2148-10-365/FIGURES/5

Dittami, S. M., Michel, G., Collén, J., Boyen, C., & Tonon, T. (2010b). Chlorophyll-binding proteins revisited - A multigenic family of light-harvesting and stress proteins from a brown algal perspective. *BMC Evolutionary Biology*, *10*(1), 1–14. https://doi.org/10.1186/1471-2148-10-365/FIGURES/5

Dorrell, R. G., & Bowler, C. (2017). Secondary Plastids of Stramenopiles. *Advances in Botanical Research*, *84*, 57–103. https://doi.org/10.1016/BS.ABR.2017.06.003

Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, *346*(6213). https://doi.org/10.1126/SCIENCE.1258096/ASSET/2313E70A-5C58-4755-A0E6-2E64EE240A09/ASSETS/GRAPHIC/346_1258096_F6.JPEG

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797. https://doi.org/10.1093/NAR/GKH340

Eilers, U., Dietzel, L., Breitenbach, J., Büchel, C., & Sandmann, G. (2016). Identification of genes coding for functional zeaxanthin epoxidases in the diatom Phaeodactylum tricornutum. *Journal of Plant Physiology*, *192*, 64–70. https://doi.org/10.1016/j.jplph.2016.01.006

Eliáš, M. (2021). Protist diversity: Novel groups enrich the algal tree of life. *Current Biology*, *31*(11), R733–R735. https://doi.org/10.1016/J.CUB.2021.04.025

Eppard, M., Krumbein, W. E., Von Haeseler, A., & Rhiel, E. (2000). Characterization of fcp4 and fcp12, two additional genes encoding light harvesting proteins of Cyclotella cryptica (Bacillariophyceae) and phylogenetic analysis of this complex

gene family. *Plant Biology*, *2*(3), 283–289. https://doi.org/10.1055/S-2000-3702/ID/36/BIB

Fan, M., Li, M., Liu, Z., Cao, P., Pan, X., Zhang, H., Zhao, X., Zhang, J., & Chang, W. (2015). Crystal structures of the PsbS protein essential for photoprotection in plants. *Nature Structural & Molecular Biology*, *22*. https://doi.org/10.1038/nsmb.3068

Fattore, N., Bellan, A., Pedroletti, L., Vitulo, N., & Morosinotto, T. (2021). Acclimation of photosynthesis and lipids biosynthesis to prolonged nitrogen and phosphorus limitation in Nannochloropsis gaditana. *Algal Research*, *58*, 102368. https://doi.org/10.1016/J.ALGAL.2021.102368

Feng, Y., Li, Z., Li, X., Shen, L., Liu, X., Zhou, C., Zhang, J., Sang, M., Han, G., Yang, W., Kuang, T., Wang, W., & Shen, J.-R. (2023). Structure of a diatom photosystem II supercomplex containing a member of Lhcx family and dimeric FCPII. *Science Advances*.

Fernández-Marín, B., Míguez, F., Becerril, J. M., & García-Plazaola, J. I. (2011). Activation of violaxanthin cycle in darkness is a common response to different abiotic stresses: A case study in Pelvetia canaliculata. *BMC Plant Biology*, *11*(1), 1–11. https://doi.org/10.1186/1471-2229-11-181/FIGURES/8

Figueroa, F. L., Celis-Plá, P. S. M., Martínez, B., Korbee, N., Trilla, A., & Arenas, F. (2019). Yield losses and electron transport rate as indicators of thermal stress in Fucus serratus (Ochrophyta). *Algal Research*, *41*, 101560. https://doi.org/10.1016/J.ALGAL.2019.101560

Fu, F. X., Zhang, Y., Warner, M. E., Feng, Y., Sun, J., & Hutchins, D. A. (2008). A comparison of future increased CO2 and temperature effects on sympatric Heterosigma akashiwo and Prorocentrum minimum. *Harmful Algae*, *7*(1), 76–90. https://doi.org/10.1016/J.HAL.2007.05.006

García-Mendoza, E., & Colombo-Pallotta, M. F. (2007). The giant kelp Macrocystis pyrifera presents a different nonphotochemical quenching control than higher plants. *New Phytologist*, *173*(3), 526–536. https://doi.org/10.1111/J.1469-8137.2006.01951.X

Garcia-Mendoza, E., Ocampo-Alvarez, H., & Govindjee. (2011). Photoprotection in the brown alga Macrocystis pyrifera: Evolutionary implications. *Journal of Photochemistry and Photobiology B: Biology*, *104*(1–2), 377–385. https://doi.org/10.1016/j.jphotobiol.2011.04.004

Gardian, Z., Litvín, R., Bína, D., & Vácha, F. (2014). Supramolecular organization of fucoxanthin-chlorophyll proteins in centric and pennate diatoms. *Photosynthesis Research*, *121*, 79–86. https://doi.org/10.1007/s11120-014-9998-3

Gardian, Z., Tichy, J., & Vácha, F. (2011). Structure of PSI, PSII and antennae complexes from yellow-green alga Xanthonema debile. *Photosynthesis Research*, *108*, 25–32. https://doi.org/10.1007/s11120-011-9647-z

Ghazaryan, A., Akhtar, P., Garab, G., Lambrev, P. H., & Büchel, C. (2016a). *Involvement of the Lhcx protein Fcp6 of the diatom Cyclotella meneghiniana in the macro-organisation and structural flexibility of thylakoid membranes*. https://doi.org/10.1016/j.bbabio.2016.04.288

Ghazaryan, A., Akhtar, P., Garab, G., Lambrev, P. H., & Büchel, C. (2016b). Involvement of the Lhcx protein Fcp6 of the diatom Cyclotella meneghiniana in the macro-organisation and structural flexibility of thylakoid membranes. *Biochimica et Biophysica Acta - Bioenergetics*, *1857*(9), 1373–1379. https://doi.org/10.1016/j.bbabio.2016.04.288

Gibbs, P. B., & Biggins, J. (1989). Regulation of the distribution of excitation energy in Ochromonas danica, an organism containing a chlorophyll-A/C/carotenoid light harvesting antenna. *Photosynthesis Research*, *21*, 81–91.

Giovagnetti, V., Cataldo, M. L., Conversano, F., & Brunet, C. (2010). Functional relation between growth, photosynthetic rate and regulation in the coastal picoeukaryote Phaeomonas sp. RCC 503 (Pinguiophyceae, Stramenopiles). *Journal of Plankton Research*, *32*(11), 1501–1511. https://doi.org/10.1093/plankt/fbq074

Giovagnetti, V., Jaubert, M., Shukla, M. K., Ungerer, P., Bouly, J. P., Falciatore, A., & Ruban, A. V. (2022). Biochemical and molecular properties of LHCX1, the essential regulator of dynamic photoprotection in diatoms. *Plant Physiology*, *188*(1), 509–525. https://doi.org/10.1093/PLPHYS/KIAB425

Giovagnetti, V., & Ruban, A. V. (2018). The evolution of the photoprotective antenna proteins in oxygenic photosynthetic eukaryotes. *Biochemical Society Transactions*, *46*(5), 1263–1277. https://doi.org/10.1042/BST20170304

Goss, R., Ann Pinto, E., Wilhelm, C., & Richter, M. (2006). The importance of a highly active and ΔpH-regulated diatoxanthin epoxidase for the regulation of the PS II antenna function in diadinoxanthin cycle containing algae. *Journal of Plant Physiology*, *163*(10), 1008–1021. https://doi.org/10.1016/J.JPLPH.2005.09.008

Goss, R., & Jakob, T. (2010a). Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynthesis Research 2010 106:1*, *106*(1), 103–122. https://doi.org/10.1007/S11120-010-9536-X

Goss, R., & Jakob, T. (2010b). Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynthesis Research 2010 106:1*, *106*(1), 103–122. https://doi.org/10.1007/S11120-010-9536-X

Goss, R., & Lepetit, B. (2015). Biodiversity of NPQ. *Journal of Plant Physiology*, *172*, 13–32. https://doi.org/10.1016/j.jplph.2014.03.004

Graf, L., Yang, E. C., Han, K. Y., Küpper, F. C., Benes, K. M., Oyadomari, J. K., Herbert, R. J. H., Verbruggen, H., Wetherbee, R., Andersen, R. A., & Yoon, H. S. (2020). Multigene Phylogeny, Morphological Observation and Re-examination of the Literature Lead to the Description of the Phaeosacciophyceae Classis Nova

and Four New Species of the Heterokontophyta SI Clade. *Protist*, *171*(6), 125781. https://doi.org/10.1016/J.PROTIS.2020.125781

Graiff, A., Bartsch, I., Glaser, K., & Karsten, U. (2021). Seasonal Photophysiological Performance of Adult Western Baltic Fucus vesiculosus (Phaeophyceae) Under Ocean Warming and Acidification. *Frontiers in Marine Science*, *8*, 666493. https://doi.org/10.3389/FMARS.2021.666493/BIBTEX

Grossman, A. R., Bhaya, D., Apt, K. E., & Kehoe, D. M. (1995). LIGHT-HARVESTING COMPLEXES IN OXYGENIC PHOTOSYNTHESIS: Diversity, Control, and Evolution. *Https://Doi.Org/10.1146/Annurev.Ge.29.120195.001311*, *29*, 231–288. https://doi.org/10.1146/ANNUREV.GE.29.120195.001311

Grouneva, I., Rokka, A., & Aro, E. M. (2011). The thylakoid membrane proteome of two marine diatoms outlines both diatom-specific and species-specific features of the photosynthetic machinery. *Journal of Proteome Research*, *10*(12), 5338–5353. https://doi.org/10.1021/PR200600F/SUPPL_FILE/PR200600F_SI_015.PDF

Guillou, L., Chrétiennot-Dinet, M. J., Medlin, L. K., Claustre, H., Loiseaux-de Goër, S., & Vaulot, D. (1999). BOLIDOMONAS: A NEW GENUS WITH TWO SPECIES BELONGING TO A NEW ALGAL CLASS, THE BOLIDOPHYCEAE (HETEROKONTA). *Journal of Phycology*, *35*(2), 368–381. https://doi.org/10.1046/J.1529-8817.1999.3520368.X

Gundermann, K., Wagner, V., Mittag, M., & Büchel, C. (2019). Fucoxanthin-Chlorophyll Protein Complexes of the Centric Diatom Cyclotella Meneghiniana Differ in Lhcx1 and Lhcx6_1 Content. *Plant Physiology*, *179*(4), 1779–1795. https://doi.org/10.1104/PP.18.01363

Hao, T. Bin, Jiang, T., Dong, H. P., Ou, L. jian, He, X., & Yang, Y. F. (2018). Light-harvesting protein Lhcx3 is essential for high light acclimation of Phaeodactylum tricornutum. *AMB Express*, *8*(1), 1–11. https://doi.org/10.1186/S13568-018-0703-3/TABLES/1

Havaux, M., & Niyogi, K. K. (1999). The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proc. Natl Acad. Sci. USA*, *96*(15), 8762–8767. https://doi.org/10.1073/pnas.96.15.8762

Hennige, S. J., Coyne, K. J., Macintyre, H., Liefer, J., & Warner, M. E. (2013). *THE PHOTOBIOLOGY OF HETEROSIGMA AKASHIWO. PHOTOACCLIMATION, DIURNAL PERIODICITY, AND ITS ABILITY TO RAPIDLY EXPLOIT EXPOSURE TO HIGH LIGHT 1*. https://doi.org/10.1111/jpy.12043

Horn, S., Ehlers, K., Fritzsch, G., Gil-Rodríguez, C., Wilhelm, C., & Schnetter, R. (2007). Synchroma grande spec. nov. (Synchromophyceae class. nov., Heterokontophyta): An Amoeboid Marine Alga with Unique Plastid Complexes. *Protist*, *158*, 277–293. https://doi.org/10.1016/j.protis.2007.02.004

Hupel, M., Lecointre, C., Meudec, A., Poupart, N., & Gall, E. A. (2011). Comparison of photoprotective responses to UV radiation in the brown seaweed Pelvetia

canaliculata and the marine angiosperm Salicornia ramosissima. *Journal of Experimental Marine Biology and Ecology*, *401*(1–2), 36–47. https://doi.org/10.1016/J.JEMBE.2011.03.004

Ichinomiya, M., Dos Santos, A. L., Gourvil, P., Yoshikawa, S., Kamiya, M., Ohki, K., Audic, S., De Vargas, C., Noël, M. H., Vaulot, D., & Kuwata, A. (2016). Diversity and oceanic distribution of the Parmales (Bolidophyceae), a picoplanktonic group closely related to diatoms. *The ISME Journal 2016 10:10*, *10*(10), 2419–2434. https://doi.org/10.1038/ismej.2016.38

Iwai, M., Grob, P., Iavarone, A. T., Nogales, E., & Niyogi, K. K. (2018). A unique supramolecular organization of photosystem I in the moss Physcomitrella patens. *Nature Plants*, *4*(11), 904–909. https://doi.org/10.1038/s41477-018-0271-1

Jakob, T., Goss, R., & Wilhelm, C. (1999). Activation of Diadinoxanthin De-Epoxidase Due to a Chiororespiratory Proton Gradient in the Dark in the Diatom Phaeodactylum tricornutum. *Plant Biology*, *1*(1), 76–82. https://doi.org/10.1111/J.1438-8677.1999.TB00711.X

Jesus, B., Ventura, P., & Calado, G. (2010). Behaviour and a functional xanthophyll cycle enhance photo-regulation mechanisms in the solar-powered sea slug Elysia timida (Risso, 1818). *Journal of Experimental Marine Biology and Ecology*, *395*(1–2), 98–105. https://doi.org/10.1016/J.JEMBE.2010.08.021

Jimel, M. S., Kv Iderov, J., & Elster, J. (2020). *Annual Cycle of Mat-FOrming Filamentous Alga Tribonema CF. Minus (Stramenopiles, Xanthophyceae) in Hydro-terrestrial habitats In the High Arctic Revealed By Multiparameter Fluorescence Staining*. https://doi.org/10.1111/jpy.13109-20-203

Johnson, M. P., & Ruban, A. v. (2011). Restoration of Rapidly Reversible Photoprotective Energy Dissipation in the Absence of PsbS Protein by Enhanced ΔpH *. *Journal of Biological Chemistry*, *286*(22), 19973–19981. https://doi.org/10.1074/JBC.M111.237255

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. A. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., … Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature 2021 596:7873*, *596*(7873), 583–589. https://doi.org/10.1038/s41586-021-03819-2

Kai, A., Yoshii, Y., Nakayama, T., & Inouye, I. (2008). Aurearenophyceae classis nova, a new class of Heterokontophyta based on a new marine unicellular alga Aurearena cruciata gen. et sp. nov. inhabiting sandy beaches. *Protist*, *159*(3), 435–457. https://doi.org/10.1016/J.PROTIS.2007.12.003

Kastner, B., Fischer, N., Golas, M. M., Sander, B., Dube, P., Boehringer, D., Hartmuth, K., Deckert, J., Hauer, F., Wolf, E., Uchtenhagen, H., Urlaub, H., Herzog, F., Peters, J. M., Poerschke, D., Lührmann, R., & Stark, H. (2007). GraFix: sample

preparation for single-particle electron cryomicroscopy. *Nature Methods 2008 5:1*, *5*(1), 53–55. https://doi.org/10.1038/nmeth1139

Kawachi, M., Inouye, I., Honda, D., Charles, ‡, O'kelly, J., Bailey, J. C., Robert, §, Bidigare, R., & Andersen, R. A. (2002). The Pinguiophyceae classis nova , a new class of photosynthetic stramenopiles whose members produce large amounts of omega-3 fatty acids. *Phycological Research*, *50*, 31–47. https://doi.org/10.1046/j.1440-1835.2002.00260.x

Keeling, P. J., & Burki, F. (2019). Progress towards the Tree of Eukaryotes. *Current Biology*, *29*, R808–R817. https://doi.org/10.1016/j.cub.2019.07.031

Khoroshyy, P., David Bína, ·, Gardian, Z., Litvín, R., Alster, J., & Pšenčík, J. (2018). Quenching of chlorophyll triplet states by carotenoids in algal light-harvesting complexes related to fucoxanthin-chlorophyll protein. *Photosynthesis Research*, *135*, 213–225. https://doi.org/10.1007/s11120-017-0416-5

Kilian, O., Benemann, C. S. E., Niyogi, K. K., & Vick, B. (2011). High-efficiency homologous recombination in the oil-producing alga Nannochloropsis sp. *Proceedings of the National Academy of Sciences*, *108*(52), 21265–21269. https://doi.org/10.1073/pnas.1105861108

Kirst, H., Formighieri, C., & Melis, A. (2014). Maximizing photosynthetic efficiency and culture productivity in cyanobacteria upon minimizing the phycobilisome light-harvesting antenna size. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(10), 1653–1664. https://doi.org/10.1016/J.BBABIO.2014.07.009

Kirst, H., Gabilly, S. T., Niyogi, K. K., Lemaux, P. G., & Melis, A. (2017). Photosynthetic antenna engineering to improve crop yields. *Planta*, *245*(5), 1009–1020. https://doi.org/10.1007/s00425-017-2659-y

Korbee, N., Navarro, N. P., García-Sánchez, M., Celis-Plá, P. S., Quintano, E., Copertino, M. S., Pedersen, A., Mariath, R., Mangaiyarkarasi, N., Pérez-Ruzafa, Figueroa, F. L., & Martínez, B. (2014). A novel in situ system to evaluate the effect of high CO2 on photosynthesis and biochemistry of seaweeds. *Aquatic Biology*, *22*, 245–259. https://doi.org/10.3354/AB00594

Kouřil, R., Dekker, J. P., & Boekema, E. J. (2012). Supramolecular organization of photosystem II in green plants. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1817*(1), 2–12. https://doi.org/10.1016/J.BBABIO.2011.05.024

Krause, G. H., & Krause, G. H. (1988). Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiologia Plantarum*, *74*(3), 566–574. https://doi.org/10.1111/J.1399-3054.1988.TB02020.X

Krogmann, D. W., Jagendorf, A. T., & Avron, M. (1959). Uncouplers of Spinach Chloroplast Photosynthetic Phosphorylation. *Plant Physiology*, *34*(3), 272. https://doi.org/10.1104/PP.34.3.272

Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S. T., Iwai, M., Niyogi, K. K., & Long, S. P. (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science*, *354*(6314). https://doi.org/10.1126/science.aai8878

Kuczynska, P., Jemiola-Rzeminska, M., Nowicka, B., Jakubowska, A., Strzalka, W., Burda, K., & Strzalka, K. (2020). The xanthophyll cycle in diatom Phaeodactylum tricornutum in response to light stress. *Plant Physiology and Biochemistry*, *152*, 125–137. https://doi.org/10.1016/J.PLAPHY.2020.04.043

Kumazawa, M., Nishide, H., Nagao, | Ryo, Inoue-Kashino, N., Shen, J.-R., Nakano, T., Uchiyama, I., Kashino, Y., & Ifuku, K. (2021). *Molecular phylogeny of fucoxanthin-chlorophyll a/c proteins from Chaetoceros gracilis and Lhcq/Lhcf diversity*. https://doi.org/10.1111/ppl.13598

Kurita, T., Iwai, M., Ohta, H., Sakuma, T., & Yamamoto, T. (2023). Genome editing for biodiesel production in oleaginous microalga, Nannochloropsis species. *Gene and Genome Editing*, *6*, 100027. https://doi.org/10.1016/J.GGEDIT.2023.100027

Kvernvik, A. C., Hoppe, C. J. M., Lawrenz, E., Prášil, O., Greenacre, M., Wiktor, J. M., & Leu, E. (2018). Fast reactivation of photosynthesis in arctic phytoplankton during the polar night. *Journal of Phycology*, *54*(4), 461–470. https://doi.org/10.1111/JPY.12750

Kvíderová, J., Souquieres, C.-E., & Elster, J. (2018). *Ecophysiology of photosynthesis of Vaucheria sp. mats in a Svalbard tidal flat*. https://doi.org/10.1016/j.polar.2018.11.006

Lacour, T., Babin, M., & Lavaud, J. (2020). Diversity in Xanthophyll Cycle Pigments Content and Related Nonphotochemical Quenching (NPQ) Among Microalgae: Implications for Growth Strategy and Ecology. *Journal of Phycology*, *56*(2), 245–263. https://doi.org/10.1111/JPY.12944

Lacour, T., Morin, P. I., Sciandra, T., Donaher, N., Campbell, D. A., Ferland, J., & Babin, M. (2019). Decoupling light harvesting, electron transport and carbon fixation during prolonged darkness supports rapid recovery upon re-illumination in the Arctic diatom Chaetoceros neogracilis. *Polar Biology*, *42*(10), 1787–1799. https://doi.org/10.1007/S00300-019-02507-2/FIGURES/7

Lavaud, J., & Goss, R. (2014). The Peculiar Features of Non-Photochemical Fluorescence Quenching in Diatoms and Brown Algae. *Advances in Photosynthesis and Respiration*, *40*, 421–443. https://doi.org/10.1007/978-94-017-9032-1_20

Lavaud, J., & Kroth, P. G. (2006). In diatoms, the transthylakoid proton gradient regulates the photoprotective non-photochemical fluorescence quenching beyond its control on the xanthophyll cycle. *Plant and Cell Physiology*, *47*(7), 1010–1016. https://doi.org/10.1093/PCP/PCJ058

Lavaud, J., Materna, A. C., Sturm, S., Vugrinec, S., & Kroth, P. G. (2012). Silencing of the Violaxanthin De-Epoxidase Gene in the Diatom Phaeodactylum tricornutum

Reduces Diatoxanthin Synthesis and Non-Photochemical Quenching. *PLOS ONE*, *7*(5), e36806. https://doi.org/10.1371/JOURNAL.PONE.0036806

Lavaud, J., Rousseau, B., & Etienne, A. (2004). General features of photoprotection by energy dissipation in planktonic diatoms (Bacillariophyceae). *J Phycol*, *40*.

Lavaud, J., Rousseau, B., Van Gorkom, H. J., & Etienne, A. L. (2002). Influence of the Diadinoxanthin Pool Size on Photoprotection in the Marine Planktonic Diatom Phaeodactylum tricornutum. *Plant Physiology*, *129*(3), 1398–1406. https://doi.org/10.1104/PP.002014

Leonelli, L., Erickson, E., Lyska, D., & Niyogi, K. K. (2016). Transient expression in Nicotiana benthamiana for rapid functional analysis of genes involved in non-photochemical quenching and carotenoid biosynthesis. *The Plant Journal*, *88*(3), 375–386. https://doi.org/10.1111/TPJ.13268

Lepetit, B., Gélin, G., Lepetit, M., Sturm, S., Vugrinec, S., Rogato, A., Kroth, P. G., Falciatore, A., & Lavaud, J. (2017). The diatom Phaeodactylum tricornutum adjusts nonphotochemical fluorescence quenching capacity in response to dynamic light via fine-tuned Lhcx and xanthophyll cycle pigment synthesis. *New Phytologist*, *214*(1), 205–218. https://doi.org/10.1111/NPH.14337

Li, D. H., Wilson, S., Mastroianni, G., & Ruban, A. V. (2023). Altered lipid acyl chain length controls energy dissipation in light-harvesting complex II proteoliposomes by hydrophobic mismatch. *Journal of Photochemistry and Photobiology B: Biology*, *246*, 112758. https://doi.org/10.1016/J.JPHOTOBIOL.2023.112758

Li, X. M., Zhang, Q. S., Tang, Y. Z., Yu, Y. Q., Liu, H. L., & Li, L. X. (2014). Highly efficient photoprotective responses to high light stress in Sargassum thunbergii germlings, a representative brown macroalga of intertidal zone. *Journal of Sea Research*, *85*, 491–498. https://doi.org/10.1016/J.SEARES.2013.08.004

Li, X. P., Björkman, O., Shih, C., Grossman, A. R., Rosenquist, M., Jansson, S., & Niyogi, K. K. (2000). A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature 2000 403:6768*, *403*(6768), 391–395. https://doi.org/10.1038/35000131

Li, X. P., Gilmore, A. M., Caffarri, S., Bassi, R., Golan, T., Kramer, D., & Niyogi, K. K. (2004). Regulation of Photosynthetic Light Harvesting Involves Intrathylakoid Lumen pH Sensing by the PsbS Protein. *Journal of Biological Chemistry*, *279*(22), 22866–22874. https://doi.org/10.1074/JBC.M402461200

Li, Z., Peers, G., Dent, R. M., Bai, Y., Yang, S. Y., Apel, W., Leonelli, L., & Niyogi, K. K. (2016). Evolution of an atypical de-epoxidase for photoprotection in the green lineage. *Nature Plants 2016 2:10*, *2*(10), 1–6. https://doi.org/10.1038/nplants.2016.140

Lichtlé, C., Arsalane, W., Duval, J. C., & Passaquet, C. (1995). CHARACTERIZATION OF THE LIGHT-HARVESTING COMPLEX OF GIRAUDYOPSIS STELLIFER

(CHRYSOPHYCEAE) AND EFFECTS OF LIGHT STRESS1. *Journal of Phycology*, *31*(3), 380–387. https://doi.org/10.1111/J.0022-3646.1995.00380.X

Lie, A. A. Y., Liu, Z., Terrado, R., Tatters, A. O., Heidelberg, K. B., & Caron, D. A. (2018). *A tale of two mixotrophic chrysophytes: Insights into the metabolisms of two Ochromonas species (Chrysophyceae) through a comparison of gene expression*. https://doi.org/10.1371/journal.pone.0192439

Liesner, D., Fouqueau, L., Valero, M., Roleda, M. Y., Pearson, G. A., Bischof, K., Valentin, K., & Bartsch, I. (2020). Heat stress responses and population genetics of the kelp Laminaria digitata (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations. *Ecology and Evolution*, *10*(17), 9144–9177. https://doi.org/10.1002/ECE3.6569

Litvín, R., Bína, D., Herbstová, M., & Gardian, Z. (2016). Architecture of the light-harvesting apparatus of the eustigmatophyte alga Nannochloropsis oceanica. *Photosynthesis Research*, *130*(1–3), 137–150. https://doi.org/10.1007/S11120-016-0234-1

Litvín, R., David Bína, ·, Miroslava Herbstová, ·, Pazderník, M., Kotabová, E., Gardian, Z., Trtílek, M., Prášil, · Ondřej, & Vácha, F. (2019). *Red-shifted light-harvesting system of freshwater eukaryotic alga Trachydiscus minutus (Eustigmatophyta, Stramenopila)*. *142*, 137–151. https://doi.org/10.1007/s11120-019-00662-5

Liu, J., Song, Y., & Qiu, W. (2017a). Oleaginous microalgae Nannochloropsis as a new model for biofuel production: Review & analysis. *Renewable and Sustainable Energy Reviews*, *72*, 154–162. https://doi.org/10.1016/J.RSER.2016.12.120

Liu, J., Song, Y., & Qiu, W. (2017b). Oleaginous microalgae Nannochloropsis as a new model for biofuel production: Review & analysis. *Renewable and Sustainable Energy Reviews*, *72*, 154–162. https://doi.org/10.1016/J.RSER.2016.12.120

Liu, M., Ding, W., Pan, Y., Hu, H., & Liu, J. (2023). Zeaxanthin epoxidase is involved in the carotenoid biosynthesis and light-dependent growth of the marine alga Nannochloropsis oceanica. *Biotechnology for Biofuels and Bioproducts*, *16*(1), 74. https://doi.org/10.1186/S13068-023-02326-Y

Lohr, M., & Wilhelm, C. (1999a). Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(15), 8784–8789. https://doi.org/10.1073/PNAS.96.15.8784

Lohr, M., & Wilhelm, C. (1999b). Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(15), 8784–8789. https://doi.org/10.1073/PNAS.96.15.8784

Malnoë, A., Schultink, A., Shahrasbi, S., Rumeau, D., Havaux, M., & Niyogi, K. K. (2018). The Plastid Lipocalin LCNP Is Required for Sustained Photoprotective

Energy Dissipation in Arabidopsis. *The Plant Cell*, *30*(1), 196–208. https://doi.org/10.1105/tpc.17.00536

Marshall, J. A., & Newman, S. (2002). Differences in photoprotective pigment production between Japanese and Australian strains of Chattonella marina (Raphidophyceae). *Journal of Experimental Marine Biology and Ecology*, *272*, 13–27. www.elsevier.com/locate/jembe

Mazor, Y., Borovikova, A., Caspy, I., & Nelson, N. (2017). *Structure of the plant photosystem I supercomplex at 2.6 Å resolution*. https://doi.org/10.1038/nplants.2017.14

Meng, E. C., Goddard, T. D., Pettersen, E. F., Couch, G. S., Pearson, Z. J., Morris, J. H., Ferrin, T. E., & Thomas Ferrin, C. E. (2023). UCSF ChimeraX: Tools for structure building and analysis. *Protein Science*, *32*(11), e4792. https://doi.org/10.1002/PRO.4792

Michel, G., Tonon, T., Scornet, D., Cock, J. M., & Kloareg, B. (2010). Central and storage carbon metabolism of the brown alga Ectocarpus siliculosus: Insights into the origin and evolution of storage carbohydrates in Eukaryotes. *New Phytologist*, *188*(1), 67–81. https://doi.org/10.1111/J.1469-8137.2010.03345.X

Mock, T., Otillar, R. P., Strauss, J., McMullan, M., Paajanen, P., Schmutz, J., Salamov, A., Sanges, R., Toseland, A., Ward, B. J., Allen, A. E., Dupont, C. L., Frickenhaus, S., Maumus, F., Veluchamy, A., Wu, T., Barry, K. W., Falciatore, A., Ferrante, M. I., … Grigoriev, I. V. (2017). Evolutionary genomics of the cold-adapted diatom Fragilariopsis cylindrus. *Nature 2017 541:7638*, *541*(7638), 536–540. https://doi.org/10.1038/nature20803

Müller, P., Li, X.-P., & Niyogi, K. K. (2001). *Update on Photosynthesis Non-Photochemical Quenching. A Response to Excess Light Energy*. www.plantphysiol.org

Müller-Moulé, P., Conklin, P. L., & Niyogi, K. K. (2002). Ascorbate Deficiency Can Limit Violaxanthin De-Epoxidase Activity in Vivo. *Plant Physiology*, *128*(3), 970–977. https://doi.org/10.1104/PP.010924

Murakami', S., & Packer, L. (1970). Light-induced Changes in the Conformation and Configuration of the Thylakoid Membrane of Ulva and Porphyra Chloroplasts in Vivo. *Plant Physiol*, *45*, 289–299. https://academic.oup.com/plphys/article/45/3/289/6090237

Murchie, E. H., & Ruban, A. V. (2020). Dynamic non-photochemical quenching in plants: from molecular mechanism to productivity. *The Plant Journal*, *101*(4), 885–896. https://doi.org/10.1111/TPJ.14601

Nagao, R., Kato, K., Ifuku, K., Suzuki, T., Kumazawa, M., Uchiyama, I., Kashino, Y., Dohmae, N., Akimoto, S., Shen, J.-R., Miyazaki, N., & Akita, F. (2020). Structural basis for assembly and function of a diatom photosystem I-light-harvesting

supercomplex. *Nature Communications*. https://doi.org/10.1038/s41467-020-16324-3

Nagao, R., Kato, K., Suzuki, T., Ifuku, K., Uchiyama, I., Kashino, Y., Dohmae, N., Akimoto, S., Shen, J. R., Miyazaki, N., & Akita, F. (2019). Structural basis for energy harvesting and dissipation in a diatom PSII–FCPII supercomplex. *Nature Plants*, *5*(8), 890–901. https://doi.org/10.1038/s41477-019-0477-x

Nan, G. N., Zhou, X. Q., Zhang, X. M., Zhang, Q. S., Hu, Z. M., Huang, R. P., & Zhang, D. (2022). Xanthophyll cycle-related non-photochemical quenching protects Sargassum thunbergii from high light-induced photoinhibition. *Frontiers in Marine Science*, *9*, 1067596. https://doi.org/10.3389/FMARS.2022.1067596/BIBTEX

Nawrocki, W. J., Liu, X., Raber, B., Hu, C., De Vitry, C., Bennett, D. I. G., & Croce, R. (2021). Molecular origins of induction and loss of photoinhibition-related energy dissipation qI. *Science Advances*, *7*(52), 55. https://doi.org/10.1126/SCIADV.ABJ0055/SUPPL_FILE/SCIADV.ABJ0055_SM.PDF

Nepper-Davidsen, J. N., Andersen, D. T., & Foldager, M. F. (2019). Exposure to simulated heatwave scenarios causes long-term reductions in performance in Saccharina latissima. *Marine Ecology Progress Series*, *630*(630), 25–39. https://doi.org/10.3354/meps13133

Neubauer, C. (1993). Multiple Effects of Dithiothreitol on Nonphotochemical Fluorescence Quenching in Intact Chloroplasts (Influence on Violaxanthin De-epoxidase and Ascorbate Peroxidase Activity). *Plant Physiology*, *103*(2), 575–583. https://doi.org/10.1104/PP.103.2.575

Nilkens, M., Kress, E., Lambrev, P., Miloslavina, Y., Müller, M., Holzwarth, A. R., & Jahns, P. (2010). Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in Arabidopsis. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1797*(4), 466–475. https://doi.org/10.1016/J.BBABIO.2010.01.001

Niyogi, K. K., Björkman, O., & Grossman, A. R. (1997a). Chlamydomonas Xanthophyll Cycle Mutants Identified by Video Imaging of Chlorophyll Fluorescence Quenching. *The Plant Cell*, *9*(8), 1369–1380. https://doi.org/10.1105/TPC.9.8.1369

Niyogi, K. K., Björkman, O., & Grossman, A. R. (1997b). The roles of specific xanthophylls in photoprotection. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(25), 14162–14167. https://doi.org/10.1073/PNAS.94.25.14162

Niyogi, K. K., Grossman, A. R., & Björkman, O. (1998). Arabidopsis mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. *The Plant Cell*, *10*(7), 1121–1134. https://doi.org/10.1105/TPC.10.7.1121

Niyogi, K. K., Shih, C., Soon Chow, W., Pogson, B. J., DellaPenna, D., & Björkman, O. (2001). Photoprotection in a zeaxanthin- and lutein-deficient double mutant of Arabidopsis. *Photosynthesis Research 2001 67:1*, *67*(1), 139–145. https://doi.org/10.1023/A:1010661102365

Niyogi, K. K., & Truong, T. B. (2013). Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Current Opinion in Plant Biology*, *16*(3), 307–314. https://doi.org/10.1016/J.PBI.2013.03.011

Nymark, M., Valle, K. C., Brembu, T., Hancke, K., Winge, P., Andresen, K., Johnsen, G., & Bones, A. M. (2009). An Integrated Analysis of Molecular Acclimation to High Light in the Marine Diatom Phaeodactylum tricornutum. *PLOS ONE*, *4*(11), e7743. https://doi.org/10.1371/JOURNAL.PONE.0007743

Nymark, M., Valle, K. C., Hancke, K., Winge, P., Andresen, K., Johnsen, G., Bones, A. M., & Brembu, T. (2013). Molecular and Photosynthetic Responses to Prolonged Darkness and Subsequent Acclimation to Re-Illumination in the Diatom Phaeodactylum tricornutum. *PLOS ONE*, *8*(3), e58722. https://doi.org/10.1371/JOURNAL.PONE.0058722

Ocampo-Alvarez, H., & García-Mendoza, E. (2013). Antagonist effect between violaxanthin and de-epoxidated pigments in nonphotochemical quenching induction in the qE deficient brown alga Macrocystis pyrifera. *Biochimica et Biophysica Acta*, *1827*, 427–437. https://doi.org/10.1016/j.bbabio.2012.12.006

Ohi, M., Li, Y., Cheng, Y., & Walz, T. (2004). Negative Staining and Image Classification - Powerful Tools in Modern Electron Microscopy. *Biological Procedures Online*, *6*(1), 23–34. https://doi.org/10.1251/BPO70

Olaizola, M., La Roche, J., Kolber, Z., & Falkowski, P. G. (1994). Non-photochemical fluorescence quenching and the diadinoxanthin cycle in a marine diatom. *Photosynthesis Research 1994 41:2*, *41*(2), 357–370. https://doi.org/10.1007/BF00019413

Owens, T. G., Gallagher, J. C., & Alberte, R. S. (1987). PHOTOSYNTHETIC LIGHT-HARVESTING FUNCTION OF VIOLAXANTHIN IN NANNOCHLOROPSIS SPP. (EUSTIGMATOPHYCEAE). *Journal of Phycology*, *23*(2), 79–85. https://doi.org/10.1111/J.1529-8817.1987.TB04429.X

Park, S., Steen, C. J., Lyska, D., Fischer, A. L., Endelman, B., Iwai, M., Niyogi, K. K., & Fleming, G. R. (2019). Chlorophyll-carotenoid excitation energy transfer and charge transfer in Nannochloropsis oceanica for the regulation of photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(9), 3385–3390. https://doi.org/10.1073/pnas.1819011116

Peers, G., Truong, T. B., Ostendorf, E., Busch, A., Elrad, D., Grossman, A. R., Hippler, M., & Niyogi, K. K. (2009). An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. *Nature*, *462*(7272), 518–521. https://doi.org/10.1038/NATURE08587

Perin, G., Bellan, A., Lyska, D., Niyogi, K. K., & Morosinotto, T. (2022). *Modulation of xanthophyll cycle impacts biomass productivity in the marine microalga Nannochloropsis 2 3*. https://doi.org/10.1101/2022.08.16.504082

Perin, G., Bellan, A., Michelberger, T., Lyska, D., Wakao, S., Niyogi, K. K., & Morosinotto, T. (2023). Modulation of xanthophyll cycle impacts biomass productivity in the marine microalga Nannochloropsis . *Proceedings of the National Academy of Sciences*, *120*(25), e2214119120. https://doi.org/10.1073/PNAS.2214119120/SUPPL_FILE/PNAS.2214119120.SAPP.PDF

Perin, G., Bellan, A., Segalla, A., Meneghesso, A., Alboresi, A., & Morosinotto, T. (2015). Generation of random mutants to improve light-use efficiency of Nannochloropsis gaditana cultures for biofuel production. *Biotechnology for Biofuels*, *8*(1), 1–13. https://doi.org/10.1186/S13068-015-0337-5/FIGURES/7

Pi, X., Tian, L., Dai, H. E., Qin, X., Cheng, L., Kuang, T., Sui, S. F., & Shen, J. R. (2018). Unique organization of photosystem I-light-harvesting supercomplex revealed by cryo-EM from a red alga. *Proc. Natl Acad. Sci. USA*, *115*(17), 4423–4428. https://doi.org/10.1073/pnas.1722482115

Pi, X., Zhao, S., Wang, W., Liu, D., Xu, C., Han, G., Kuang, T., Sui, S.-F., & Shen, J.-R. (2019). The pigment-protein network of a diatom photosystem II-light-harvesting antenna supercomplex. *Science (New York, N.Y.)*, *365*(6452), eaax4406. https://doi.org/10.1126/science.aax4406

Pinnola, A., Dall'Osto, L., Gerotto, C., Morosinotto, T., Bassi, R., & Alboresi, A. (2013). Zeaxanthin Binds to Light-Harvesting Complex Stress-Related Protein to Enhance Nonphotochemical Quenching in Physcomitrella patens. *The Plant Cell*, *25*(9), 3519–3534. https://doi.org/10.1105/TPC.113.114538

Porra, R. J., Thompson, W. A., & Kriedemann, P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *975*(3), 384–394. https://doi.org/10.1016/S0005-2728(89)80347-0

Qin, X., Pi, X., Wang, W., Han, G., Zhu, L., Liu, M., Cheng, L., Shen, J. R., Kuang, T., & Sui, S. F. (2019). Structure of a green algal photosystem I in complex with a large number of light-harvesting complex I subunits. *Nat. Plants*, *5*(3), 263–272. https://doi.org/10.1038/s41477-019-0379-y

Qin, X., Suga, M., Kuang, T., & Shen, J. R. (2015). Photosynthesis. Structural basis for energy transfer pathways in the plant PSI-LHCI supercomplex. *Science*, *348*(6238), 989–995. https://doi.org/10.1126/science.aab0214

Quaas, T., Berteotti, S., Ballottari, M., Flieger, K., Bassi, R., Wilhelm, C., & Goss, R. (2015). Non-photochemical quenching and xanthophyll cycle activities in six green

algal species suggest mechanistic differences in the process of excess energy dissipation. *Journal of Plant Physiology*, *172*, 92–103. https://doi.org/10.1016/J.JPLPH.2014.07.023

Rico, C., Matsuda, Y., Lin, X., Litvin, R., Blommaert, L., Vancaester, E., J Huysman, M. J., Osuna-Cruz, C. M., Lavaud, J., Lepetit, B., Winge, P., Bones, A. M., Vandepoele, K., Vyverman, W., & Sabbe, K. (2020). *Light Regulation of LHCX Genes in the Benthic Diatom Seminavis robusta*. https://doi.org/10.3389/fmars.2020.00192

Semchonok, D. A., Sathish Yadav, K. N., Xu, P., Drop, B., Croce, R., & Boekema, E. J. (2017). Interaction between the photoprotective protein LHCSR3 and C2S2 Photosystem II supercomplex in Chlamydomonas reinhardtii. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1858*(5), 379–385. https://doi.org/10.1016/J.BBABIO.2017.02.015

Shen, L., Huang, Z., Chang, S., Wang, W., Wang, J., Kuang, T., Han, G., Shen, J. R., & Zhang, X. (2019). Structure of a C2S2M2N2-type PSII–LHCII supercomplex from the green alga Chlamydomonas reinhardtii. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(42), 21246–21255. https://doi.org/10.1073/pnas.1912462116

Shimasaki, Y., Mukai, K., Takai, Y., Qiu, X., & Oshima, Y. (2021). *antioxidants Recent Progress in the Study of Peroxiredoxin in the Harmful Algal Bloom Species Chattonella marina*. https://doi.org/10.3390/antiox10020162

Short, A., Fay, T. P., Crisanto, T., Mangal, R., Niyogi, K. K., Limmer, D. T., & Fleming, G. R. (2023). Kinetics of the xanthophyll cycle and its role in photoprotective memory and response. *Nature Communications 2023 14:1*, *14*(1), 1–10. https://doi.org/10.1038/s41467-023-42281-8

Short, A. H., Fay, T. P., Crisanto, T., Hall, J., Steen, C. J., Niyogi, K. K., Limmer, D. T., & Fleming, G. R. (2022). Xanthophyll-cycle based model of the rapid photoprotection of Nannochloropsis in response to regular and irregular light/dark sequences. *The Journal of Chemical Physics*, *156*(20), 205102. https://doi.org/10.1063/5.0089335

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology*, *7*. https://doi.org/10.1038/MSB.2011.75

Simionato, D., Basso, S., Giacometti, G. M., & Morosinotto, T. (2013). Optimization of light use efficiency for biofuel production in algae. *Biophys Chem*, *182*, 71–78. https://doi.org/10.1016/j.bpc.2013.06.017

Simionato, D., Block, M. A., la Rocca, N., Jouhet, J., Maréchal, E., Finazzi, G., & Morosinotto, T. (2013). The response of Nannochloropsis gaditana to nitrogen

starvation includes de novo biosynthesis of triacylglycerols, a decrease of chloroplast galactolipids, and reorganization of the photosynthetic apparatus. *Eukaryot Cell*, *12*(5), 665–676. https://doi.org/10.1128/ec.00363-12

Simionato, D., Sforza, E., Corteggiani Carpinelli, E., Bertucco, A., Giacometti, G. M., & Morosinotto, T. (2011a). Acclimation of Nannochloropsis gaditana to different illumination regimes: Effects on lipids accumulation. *Bioresource Technology*, *102*(10), 6026–6032. https://doi.org/10.1016/J.BIORTECH.2011.02.100

Simionato, D., Sforza, E., Corteggiani Carpinelli, E., Bertucco, A., Giacometti, G. M., & Morosinotto, T. (2011b). Acclimation of Nannochloropsis gaditana to different illumination regimes: Effects on lipids accumulation. *Bioresource Technology*, *102*(10), 6026–6032. https://doi.org/10.1016/J.BIORTECH.2011.02.100

Six, C., Finkel, Z. V., Rodriguez, F., Marie, D., Partensky, F., & Campbell, D. A. (2008). Contrasting photoacclimation costs in ecotypes of the marine eukaryotic picoplankter Ostreococcus. *Limnology and Oceanography*, *53*(1), 255–265. https://doi.org/10.4319/LO.2008.53.1.0255

Soule, K. M., & Rumpho, M. E. (2012). Light-regulated photosynthetic gene expression and phosphoribulokinase enzyme activity in the heterokont alga vaucheria litorea (xanthophyceae) and its symbiotic molluskan partner elysia chlorotica (gastropoda). *Journal of Phycology*, *48*(2), 373–383. https://doi.org/10.1111/J.1529-8817.2012.01111.X

Streckaite, S., Gardian, Z., Li, F., Pascal, A. A., Litvin, R., Robert, B., & Llansola-Portoles, M. J. (2018). Pigment configuration in the light-harvesting protein of the xanthophyte alga Xanthonema debile. *Photosynthesis Research*, *138*, 139–148. https://doi.org/10.1007/s11120-018-0557-1

Suga, M., Ozawa, S. I., Yoshida-Motomura, K., Akita, F., Miyazaki, N., & Takahashi, Y. (2019). Structure of the green algal photosystem I supercomplex with a decameric light-harvesting complex I. *Nature Plants 2019 5:6*, *5*(6), 626–636. https://doi.org/10.1038/s41477-019-0438-4

Sukenik, A., Livne, A., Apt, K. E., & Grossman, A. R. (2000). CHARACTERIZATION OF A GENE ENCODING THE LIGHT-HARVESTING VIOLAXANTHIN-CHLOROPHYLL PROTEIN OF NANNOCHLOROPSIS SP. (EUSTIGMATOPHYCEAE). *Journal of Phycology*, *36*(3), 563–570. https://doi.org/10.1046/J.1529-8817.2000.99115.X

Sun, K.-M., Gao, C., Zhang, J., Tang, · Xuexi, Wang, Z., Zhang, X., & Li, Y. (2020). Rapid formation of antheraxanthin and zeaxanthin in seconds in microalgae and its relation to non-photochemical quenching. *Photosynthesis Research*, *144*, 317–326. https://doi.org/10.1007/s11120-020-00739-6

Sylak-Glassman, E. J., Malnoe, A., De Re, E., Brooks, M. D., Fischer, A. L., Niyogi, K. K., & Fleming, G. R. (2014). Distinct roles of the photosystem II protein PsbS and zeaxanthin in the regulation of light harvesting in plants revealed by fluorescence

lifetime snapshots. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(49), 17498–17503. https://doi.org/10.1073/PNAS.1418317111

Taddei, L., Chukhutsina, V. U., Lepetit, B., Stella, G. R., Bassi, R., van Amerongen, H., Bouly, J. P., Jaubert, M., Finazzi, G., & Falciatore, A. (2018). Dynamic Changes between Two LHCX-Related Energy Quenching Sites Control Diatom Photoacclimation. *Plant Physiology*, *177*(3), 953–965. https://doi.org/10.1104/PP.18.00448

Taddei, L., Stella, G. R., Rogato, A., Bailleul, B., Fortunato, A. E., Annunziata, R., Sanges, R., Thaler, M., Lepetit, B., Lavaud, J., Jaubert, M., Finazzi, G., Bouly, J. P., & Falciatore, A. (2016). Multisignal control of expression of the LHCX protein family in the marine diatom Phaeodactylum tricornutum. *Journal of Experimental Botany*, *67*(13), 3939–3951. https://doi.org/10.1093/JXB/ERW198

Takahashi, H., Iwai, M., Takahashi, Y., & Minagawa, J. (2006). Identification of the mobile light-harvesting complex II polypeptides for state transitions in Chlamydomonas reinhardtii. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(2), 477–482. https://doi.org/10.1073/PNAS.0509952103

Tanabe, Y., Shitara, T., Kashino, Y., Hara, Y., & Kudoh, S. (2009). Utilizing the Effective Xanthophyll Cycle for Blooming of Ochromonas smithii and O. itoi (Chrysophyceae) on the Snow Surface. *Plos One*. https://doi.org/10.1371/journal.pone.0014690

Teo, C. L., Atta, M., Bukhari, A., Taisir, M., Yusuf, A. M., & Idris, A. (2014). Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths. *Bioresource Technology*, *162*, 38–44. https://doi.org/10.1016/J.BIORTECH.2014.03.113

Tian, L., Nawrocki, W. J., Liu, X., Polukhina, I., Van Stokkum, I. H. M., & Croce, R. (2019). PH dependence, kinetics and light-harvesting regulation of nonphotochemical quenching in Chlamydomonas. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(17), 8320–8325. https://doi.org/10.1073/PNAS.1817796116/SUPPL_FILE/PNAS.1817796116.SAPP.PDF

Tibiletti, T., Auroy, P., Peltier, G., & Caffarri, S. (2016). Chlamydomonas reinhardtii PsbS Protein Is Functional and Accumulates Rapidly and Transiently under High Light. *Plant Physiology*, *171*(4), 2717–2730. https://doi.org/10.1104/PP.16.00572

Uhrmacher, S., Hanelt, D., & Nultsch, W. (1995). Zeaxanthin content and the degree of photoinhibition are linearly correlated in the brown alga Dictyota dichotoma. *Marine Biology*, *123*, 159–165.

Umetani, I., Kunugi, M., Yokono, M., Takabayashi, A., & Tanaka, A. (2018). Evidence of the supercomplex organization of photosystem II and light-harvesting complexes in

Nannochloropsis granulata. *Photosynthesis Research*, *136*(1), 49–61. https://doi.org/10.1007/s11120-017-0438-z

Vieler, A., Wu, G., Tsai, C. H., Bullard, B., Cornish, A. J., Harvey, C., Reca, I. B., Thornburg, C., Achawanantakun, R., Buehl, C. J., Campbell, M. S., Cavalier, D., Childs, K. L., Clark, T. J., Deshpande, R., Erickson, E., Armenia Ferguson, A., Handee, W., Kong, Q., … Benning, C. (2012a). Genome, Functional Gene Annotation, and Nuclear Transformation of the Heterokont Oleaginous Alga Nannochloropsis oceanica CCMP1779. *PLoS Genetics*, *8*(11). https://doi.org/10.1371/journal.pgen.1003064

Vieler, A., Wu, G., Tsai, C.-H., Bullard, B., Cornish, A. J., Harvey, C., Reca, I.-B., Thornburg, C., Achawanantakun, R., Buehl, C. J., Campbell, M. S., Cavalier, D., Childs, K. L., Clark, T. J., Deshpande, R., Erickson, E., Armenia Ferguson, A., Handee, W., Kong, Q., … Benning, C. (2012b). Genome, Functional Gene Annotation, and Nuclear Transformation of the Heterokont Oleaginous Alga Nannochloropsis oceanica CCMP1779. *PLoS Genetics*, *8*(11), e1003064. https://doi.org/10.1371/journal.pgen.1003064

Walter, J., & Kromdijk, J. (2022). Here comes the sun: How optimization of photosynthetic light reactions can boost crop yields. *Journal of Integrative Plant Biology*, *64*(2), 564–591. https://doi.org/10.1111/JIPB.13206

Walters, R. G., Ruban, A. v., & Horton, P. (1996). Identification of proton-active residues in a higher plant light-harvesting complex. *Proceedings of the National Academy of Sciences*, *93*(24), 14204–14209. https://doi.org/10.1073/PNAS.93.24.14204

Wang, H., Ji, B., Wang, J., Guo, F., Zhou, W., Gao, L., & Liu, T. Z. (2014). Growth and biochemical composition of filamentous microalgae Tribonema sp. as potential biofuel feedstock. *Bioprocess and Biosystems Engineering*, *37*(12), 2607–2613. https://doi.org/10.1007/S00449-014-1238-X/TABLES/2

Wang, W., Yu, L. J., Xu, C., Tomizaki, T., Zhao, S., Umena, Y., Chen, X., Qin, X., Xin, Y., Suga, M., Han, G., Kuang, T., & Shen, J. R. (2019). Structural basis for blue-green light harvesting and energy dissipation in diatoms. *Science*, *363*(6427). https://doi.org/10.1126/SCIENCE.AAV0365/SUPPL_FILE/AAV0365-WANG-SM.PDF

Wang, W., Zhao, S., Pi, X., Kuang, T., Sui, S. F., & Shen, J. R. (2020). Structural features of the diatom photosystem II–light-harvesting antenna complex. *The FEBS Journal*, *287*(11), 2191–2200. https://doi.org/10.1111/FEBS.15183

Wang, W., Zhao, S., Shen, L., Li, X., Tao, Q., Li, Z., Xu, C., Zhou, C., Yang, Y., Sang, M., & Han, G. (2023). Structural insights into photosystem II supercomplex and trimeric FCP antennae of a centric diatom Cyclotella meneghiniana. *Preprint*. https://doi.org/10.21203/rs.3.rs-3094926/v1

Wetherbee, R., Jackson, C. J., Repetti, S. I., Clementson, L. A., Costa, J. F., van de Meene, A., Crawford, S., & Verbruggen, H. (2019). The golden paradox - a new

heterokont lineage with chloroplasts surrounded by two membranes. *Journal of Phycology*, *55*(2), 257–278. https://doi.org/10.1111/JPY.12822

Wilken, S., Choi, C. J., & Worden, A. Z. (2020). Contrasting Mixotrophic Lifestyles Reveal Different Ecological Niches in Two Closely Related Marine Protists. *Journal of Phycology*, *56*(1), 52–67. https://doi.org/10.1111/JPY.12920

Wilken, S., Huisman, J., Naus-Wiezer, S., & Van Donk, E. (2013). Mixotrophic organisms become more heterotrophic with rising temperature. *Ecology Letters*, *16*(2), 225–233. https://doi.org/10.1111/ELE.12033

Xu, C., Pi, X., Huang, Y., Han, G., Chen, X., Qin, X., Huang, G., Zhao, S., Yang, Y., Kuang, T., Wang, W., Sui, S. F., & Shen, J. R. (2020). Structural basis for energy transfer in a huge diatom PSI-FCPI supercomplex. *Nature Communications 2020 11:1*, *11*(1), 1–12. https://doi.org/10.1038/s41467-020-18867-x

Yang, R., Wei, D., & Xie, J. (2020). Diatoms as cell factories for high-value products: chrysolaminarin, eicosapentaenoic acid, and fucoxanthin. *Critical Reviews in Biotechnology*, *40*(7), 993–1009. https://doi.org/10.1080/07388551.2020.1805402

Yoon, H. S., Andersen, R. A., Boo, S. M., & Bhattacharya, D. (2009). Stramenopiles. *Encyclopedia of Microbiology, Third Edition*, 721–731. https://doi.org/10.1016/B978-012373944-5.00253-4

You, X., Zhang, X., Cheng, J., Xiao, Y., Ma, J., Sun, S., Zhang, X., Wang, H.-W., & Sui, S.-F. (2023). In situ structure of the red algal phycobilisome-PSII-PSI-LHC megacomplex. *Nature*, *616*. https://doi.org/10.1038/s41586-023-05831-0

Yuasa, K., Shikata, T., Kitatsuji, S., Yamasaki, Y., & Nishiyama, Y. (2020). *Extracellular secretion of superoxide is regulated by photosynthetic electron transport in the noxious red-tide-forming raphidophyte Chattonella antiqua*. https://doi.org/10.1016/j.jphotobiol.2020.111839

Zhang, X., Xu, D., Guan, Z., Wang, S., Zhang, Y., Wang, W., Zhang, X., Fan, X., Li, F., & Ye, N. (2020). Elevated CO 2 concentrations promote growth and photosynthesis of the brown alga Saccharina japonica. *Journal of Applied Phycology*, *32*, 1949–1959. https://doi.org/10.1007/s10811-020-02108-1

Zhao, L.-S., Wang, P., Li, K., Zhang, Q.-B., He, F.-Y., Li, C.-Y., Su, H.-N., Chen, X.-L., Liu, L.-N., & Zhang, Y.-Z. (2023). *Structural basis and evolution of the photosystem I-light-harvesting supercomplex of cryptophyte algae*. https://doi.org/10.1093/plcell/koad087

Zhu, S. H., & Green, B. R. (2010). Photoprotection in the diatom Thalassiosira pseudonana: Role of LI818-like proteins in response to high light stress. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1797*(8), 1449–1457. https://doi.org/10.1016/J.BBABIO.2010.04.003

# Chapter 3 : Investigating the Role of LHCX1 in Non-Photochemical Quenching Induction in *Nannochloropsis oceanica*


## Abstract

This chapter explores the intricacies of non-photochemical quenching (NPQ) induction with a focus on the stress-related LHCX1 protein in *Nannochloropsis oceanica* that is required for qE. To test the hypothesis that LHCX1 is involved in sensing a decrease in thylakoid lumen pH in excess light, knock-in mutations targeting putative pH-sensing sites were generated in the native NoLHCX1 gene. Mutants affecting a conserved acidic residue (E215Q) or all lumen-facing acidic residues in LHCX1 revealed no discernible impact on qE capacity. This observation aligns with prior diatom studies and raises questions about how, or if, LHCX1 is involved in sensing the ΔpH to induce qE. Generation and analysis of the W143M mutant in NoLHCX1 also revealed no impact on a putative pigment binding site that may affect qE capacity. However, generation and analysis of the W143M mutant revealed that a mutation in this amino acid doesn't impact a putative pigment binding site involved in qE. This contrasts with previous results in diatoms, suggesting species-specific divergence in *N. oceanica*. This contrasts with previous results with diatoms and provides intriguing insights into the species-specific mechanisms that govern NPQ induction. This work contributes to the evolving understanding of NPQ induction in *Nannochloropsis* but also prompts a reconsideration of ΔpH-sensing mechanisms in photosynthetic organisms.


## Introduction

Peripheral antenna proteins and pigments make up the diverse light-harvesting complex (LHC) associated with photosystems I and II. The proteins in the LHC predominantly facilitate interactions between chlorophyll and pigment molecules for light-energy transfer to the reaction centers. Because photosynthetic organisms experience variations in light conditions, they have evolved stress-related LHC proteins and mechanisms for photoprotection. Well-studied stress-related LHCs in green algae and plants include LHCSR3 from *Chlamydomonas reinhardtiii* (CrLHCSR3) and PsbS from *Arabidopsis thaliana* (AtPsbS; Li et al., 2000; Peers et al., 2009). Both proteins are involved in a crucial photoprotective mechanism called non-photochemical quenching (NPQ), which dissipates excess light energy as heat (Demmig-Adams & Adams, 2006a; Müller et al., 2001). NPQ encompasses components with kinetics ranging from fast-acting responses within seconds, to slower responses that take hours to relax (Müller et al., 2001). This chapter focuses mainly on the qE component and the use of LHC proteins for NPQ induction.

qE is known for its flexibility and rapid activation/relaxation within seconds to minutes. Inducing qE involves the formation of a high ΔpH by thylakoid lumen acidification, the presence stress-related LHCs, and the synthesis of zeaxanthin (Zx) or diatoxanthin (Dtx) controlled by a xanthophyll cycle (Goss & Lepetit, 2015; Müller et al., 2001; Niyogi & Truong, 2013). Both CrLHCSR3 and AtPsbS have been shown to be important for qE induction, with the protonation of specific lumen-facing glutamate and aspartate residues playing a role. In AtPsbS, mutagenesis of two glutamate residues, E122 and E226, to glutamine resulted in loss of qE (X. P. Li et al., 2004). The same

approach in CrLHCSR3 revealed that A117, E221, and E224 were essential regulators of qE (Ballottari et al., 2016). These works have revealed that AtPsbS and CrLHCSR3 function in sensing changes in the thylakoid lumen pH to trigger qE. However, this is not the case in studies done on stress-related LHC proteins found in photosynthetic stramenopiles (stramenochromes).

Stramenochromes are descendants of a secondary endosymbiotic event of a red alga and are comprised of very diverse groups of algae ranging from microscopic phytoplankton to macroscopic giant kelp. Stramenochromes contain stress-related LHCs, called LHCX proteins, to help them cope with dynamic light environments. The most well studied class of stramenochromes are the diatoms (Bacillariophyceae). Interestingly, diatoms contain many genes encoding for LHCX proteins ranging from 4 in *Phaeodactylum tricornutum* to 17 in *Seminavis robusta* (Bowler et al., 2008b; Rico et al., 2020). Among the different LHCX proteins, LHCX1 is the most important for qE in many diatoms, although other LHCX proteins are involved in high light and stress responses (Bailleul et al., 2010; Hao et al., 2018; Lepetit et al., 2017; Nymark et al., 2009; Taddei et al., 2018; Zhu & Green, 2010). Unlike *CrLHCSR3*, which is upregulated in high light (Peers et al., 2009), *PtLHCX1* is constitutively expressed and upregulated under intermittent light (Bailleul et al., 2010; Buck et al., 2019; Giovagnetti et al., 2022). How PtLHCX1 induces NPQ has been explored, and there appear to be functional differences between stramenochromes and the green lineage. Targeted mutagenesis of protonatable residues conserved between CrLHCSR3 and PtLHCX1 showed that D94 and E205 did not play a role in sensing ΔpH, and qE was unaffected in cells in which these residues were mutated (Buck et al., 2021; Giovagnetti et al., 2022). However, the potential involvement of additional protonatable amino acid residues facing the lumen was not examined in these studies and needs further investigation. Recent studies on transmembrane motifs by Buck et al. (2021) have identified two peptide motifs in PtLHCX1 that are crucial for qE induction. Notably, one of these motifs, harboring a tryptophan residue (W133), exerts significant influence on qE induction and is predicted to be in close proximity to a Dtx-binding site.

Eustigmatophytes, a different class within the stramenochromes, harbor stress-related LHCX proteins resembling those found in diatoms (Litvín et al., 2016; Vieler et al., 2012b). Numerous investigations have shown the significance of NPQ in *Nannochloropsis sp*., attributing it to the buildup of ΔpH, de-epoxidized xanthophylls, and the contribution of LHCX proteins to both qE and qZ ; (Chapter 2; Cao et al., 2013; Chukhutsina et al., 2017). As elucidated in Chapter 2, NoLHCX1 plays a pivotal role in qE induction, and both qE and qZ depend strongly on the presence of Zx (Bína, Bouda, et al., 2017; Park et al., 2019; Perin et al., 2023). However, the mechanisms underlying NoLHCX1's involvement in qE remain ambiguous, raising questions about the function of lumen-facing amino acid residues capable of sensing ΔpH. Additionally, the similarity of NoLHCX1 and PtLHCX1 suggests that stramenochromes may have evolved mechanisms for inducing qE that are distinct from those in the green lineage. This chapter delves into the functional role of NoLHCX1 in pH sensing and qE induction, unraveling potential distinctions in NPQ mechanisms across diverse photosynthetic organisms.

## Materials and Methods

### Protein Alignments and Prediction Modelling

Protein sequence alignments were generated using Clustal Omega (Sievers et al., 2011). The predicted 3-D protein structure of LHCX1 was computed using AlphaFold (Jumper et al., 2021). Molecular graphics were performed and visualized using UCSF Chimera X (Resource for Biocomputing, Visualization, and Informatics at UCSF, USA) following previously described methods (Meng et al., 2023).

### Algal strain and cultivation

*Nannochloropsis oceanica* CCMP1779 was obtained from The Provasoli-Guillard National Center for Culture of Marine Phytoplankton (https://ncma.bigelow.org/) and cultivated in F2N medium (Kilian et al., 2011). Culturing methods for each experiment are described below.

### Algal Transformation

Liquid cultures were grown to 1 x $10^6$ cells/mL in F2N media under continuous light at 50 µmol photons $m^{-2}$ $s^{-1}$ at 23°C. The *LHCX1* gene was accessed through the *N. oceanica* genome (JGI, https://genome.jgi.doe.gov/Nanoce1779_2, transcript ID 575769; protein ID 575683). The *LHCX1* KI Control (KC line) was generated as described in Chapter 2, with the HygR cassette now following the *LHCX1* gene instead of disrupting it. The plasmid map is shown in the Supplemental Materials.

The DNA fragment containing the All-Site (AS) domain mutant version of *LHCX1* was synthesized by Genescript (USA) in plasmid PCC1. The AS fragment was amplified from the plasmid using PCR and primers TC105/106. All primers are listed in Table 1 in Supplemental Materials. Both the AS fragment and *LHCX1* KI Cassette were double digested using restriction enzymes, MsCI and KasI, with 1xCutSmart Buffer (New England BioLabs, USA). The digested DNA was run on a 1% agarose gel before being assembled using a HiFI DNA Assembly kit (New England BioLabs, USA). The AS fragment sequence is shown in Supplemental Materials.

W143M and E215Q mutants were generated using the *LHCX1* KI Cassette and Q5 Site-Directed Mutagenesis Kit (New England Bio-Rad, USA). E215Q was mutated using primers TC168/169, and W143M was mutated using primers TC166/167.

All plasmids were transformed into *E. coli* and screened using PCR with primers TC 107/108 for the correct mutated sequence. Plasmids were linearized using TC116/117 prior to transformation into *N. oceanica*.

Transformation of *N. oceanica* cells was performed as described previously (Kilian et al., 2011), with the modification that cells were transformed using Cas9 ribonucleotide protein (RNP)-mediated homologous recombination (HR; see Chapter 2 for detailed methods). Two increase the cleavage efficiency of Cas9, two gRNAs (TC002/085) were used along with tracrRNA to form two gRNA:tracrRNA duplexes (Doudna & Charpentier, 2014). This was incubated with the Cas9 enzyme to form the Cas9 RNP and added to *N. oceanica* cells with template DNA prior to transformation. gRNA sequences and primers used for genotyping are listed in Table 1. Transformants were selected using 0.9% Bacto Agar and F2N plates with 50 µg/ml hygromycin. Selection plates were grown in medium light at 100 µmol photons $m^{-2}$ $s^{-1}$ at 23 °C.

Genotypic analysis by PCR and Sanger sequencing

Transformants were screened using PCR and Sanger sequencing facilitated by the UC Berkeley Barker Sequencing Facility. PCR genotyping amplified from the 35S terminator region inside the *LHCX1* KI cassette to a region outside of the 3' UTR using primers TC119/122 for a primary reaction and TC158/159 for a secondary reaction. This method would only generate bands for mutants, and no bands for the *lhcx1 KO* or WT, which lack the sequence for the region inside the *LHCX1* KI cassette. PCR reactions were done using Plant Phire Direct PCR Master Mix (Thermo Fischer Scientific, USA). To ensure that the *N. oceanica* colonies contained the correct mutations, the *LHCX1* gene was amplified using primers TC170/171 for the primary reaction and TC172/173 for the secondary reaction. This reaction also excluded WT and *lhcx1 KO* targets in that it amplified from outside the 5' UTR to inside the Arf terminator of the *LHCX1* KI cassette. The resulting fragments were sent for Sanger sequencing and analyzed on Benchling (https://benchling.com).

Phenotypic analysis by PAM fluorometry

Cells were spotted on plates and grown in a diurnal cycle of 12 hours at 50 µmol photons $m^{-2}$ $s^{-1}$ and 12 hours in darkness (12:12) for 6 days. Respective cells were then transferred to continuous low light (LL) at 50 µmol photons $m^{-2}$ $s^{-1}$, continuous medium light (ML) at 100 µmol photons $m^{-2}$ $s^{-1}$, or continuous high light (HL) at 350 µmol photons $m^{-2}$ $s^{-1}$ for an additional 4 days until they reached mid-log phase (~1-5x$10^7$ cells). All cells were grown at 23°C.

$F_v/F_m$ and NPQ were measured using pulse-amplitude modulated (PAM) chlorophyll fluorometry through an Imaging-PAM (Walz, Germany). Cells were dark acclimated for 30 min prior to measurements. Cells were exposed to 800 µmol photons $m^{-2}$ $sec^{-1}$ of actinic light and 1800 µmol photons $m^{-2}$ $sec^{-1}$ of saturating light pulses.

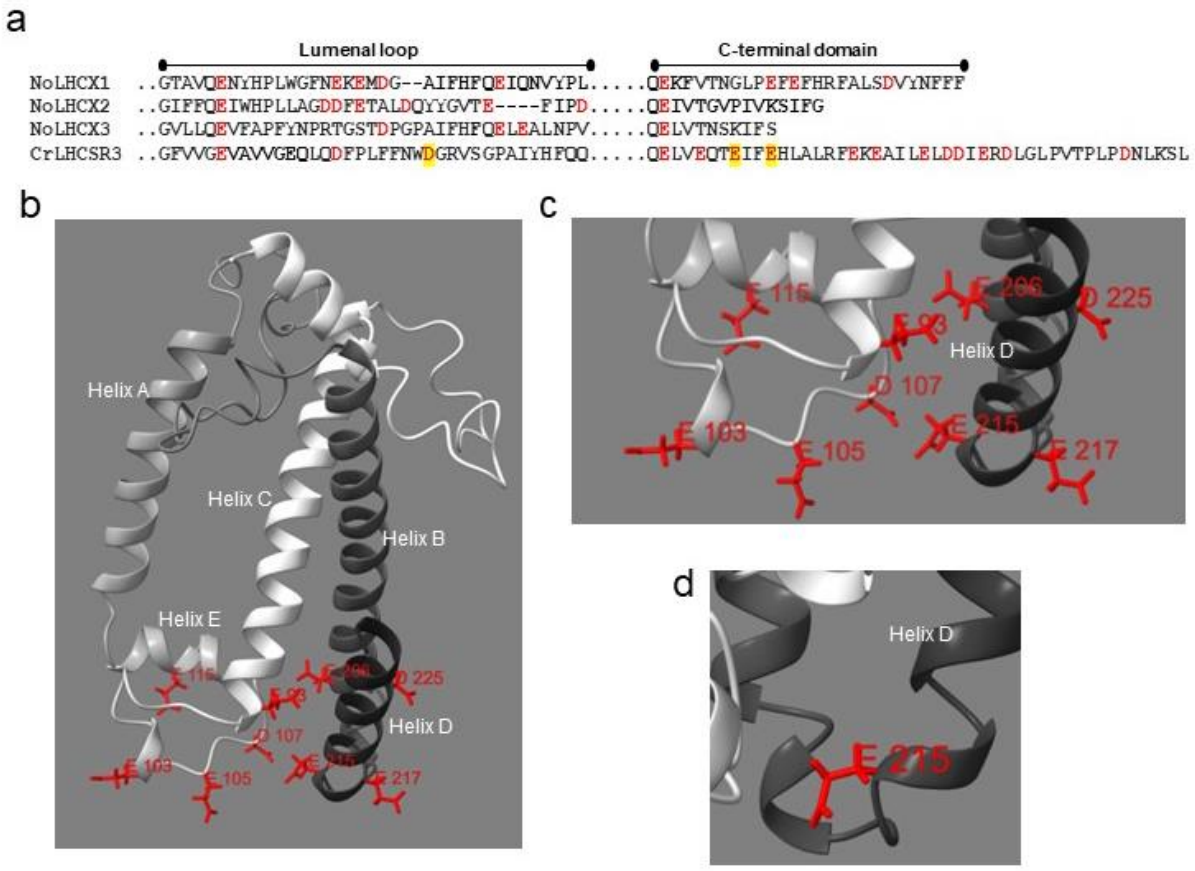



***Figure 1. Putative protonatable residues in LHCX1.*** *(a) An alignment of the three LHCX proteins in N. oceanica with LHCSR3 from C. reinhardtii. The predicted lumen-facing acidic amino acidic residues in the lumenal loop and C-terminal domain are highlighted in red. The three residues highlighted in yellow have been characterized as important for qE induction in CrLHCSR3 (Ballottari et al., 2016). (b) Predicted structure of LHCX1 from AlphaFold, visualized using UCSF ChimeraX. The nine putative protonatable residues predicted to be located on the lumen side of the protein are shown in red. (c) Close up of the nine putative protonatable residues in the All-Site (AS) mutant. (d) Close up of E215 in NoLHCX1.*

## Testing the ability to knock-in DNA into the endogenous *LHCX1* locus using Cas9-mediated RNP

In a preceding investigation detailed in Chapter 2, we demonstrated the efficacy of Cas9-mediated ribonucleotide protein (RNP) technology for knocking out genes in *N. oceanica*, and the significance of LHCX1 in qE induction was established through the

generation of knock-out mutants. Notably, the protein features a conserved acidic amino acid shared with CrLHCSR3, suggesting its potential importance in pH-sensing and qE

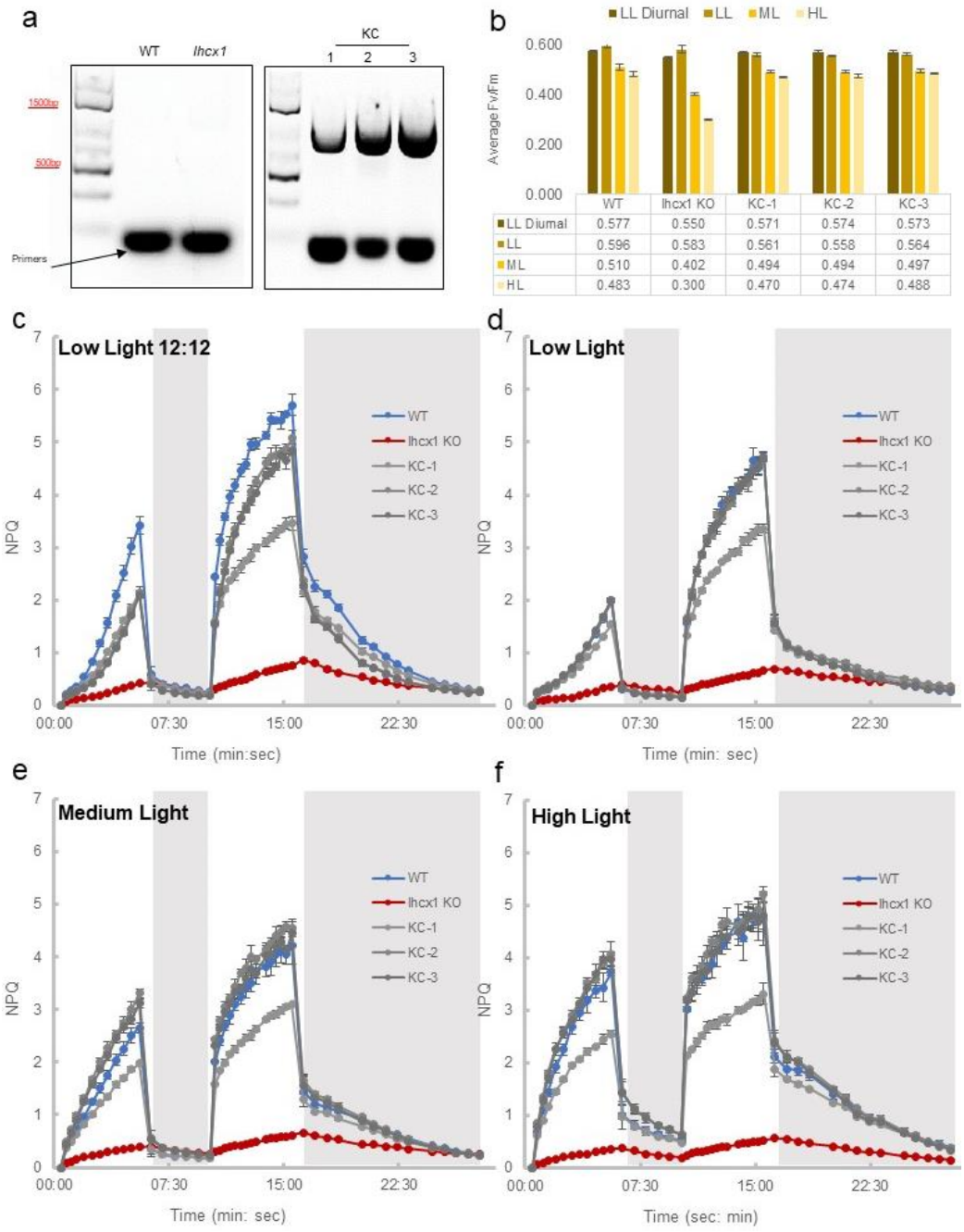



***Figure 2. Characterization of three LHCX1 Knock-In Control (KC) lines in N. oceanica.*** *(a) Genetic characterization using PCR. Fragments were successfully amplified only in the mutant lines (~1200 bp in size) and not in WT or the lhcx1 KO mutant. Residual primers can be seen at the bottom of each lane. (b) $F_v/F_m$ of WT (blue), lhcx1 KO (red), and three KC mutant lines (grey) under four different light conditions. (c-f) NPQ measurements of WT, lhcx1 KO, and KC lines grown in (c) low light for 12 hours of light and 12 hours of darkness, (d) continuous low light, (e) continuous medium light, and (f) continuous high light. White and grey bars in NPQ graphs indicate that cells were exposed to 5 min of high light, 5 min of darkness, 5 min of high light, then 10 min of darkness.*

induction (Figure 1a). This observation prompted an inquiry into whether other acidic residues, beyond the conserved one, might contribute to qE induction in Eustigmatophytes. To address this hypothesis, we devised a strategy to generate mutants that are incapable of sensing lumen acidification. Importantly, we developed a novel method to accomplish this without the need for complementation into the knockout line, which inherently possesses low NPQ. This involved the generation of knock-in mutants, exemplified by the *LHCX1* KI-Control (KC) line (Figure 2). The KC line, encompassing the native promoter, *LHCX1* gene, a HygR cassette, and the *LHCX1* 3' UTR, was transformed into the endogenous *LHCX1* locus using Cas9 RNP-mediated homologous recombination (Figure 3). Characterization of these knock-in mutants involved the utilization of PCR to verify the integration of the constructs into the *LHCX1* locus and confirm the transformation of the correct strain into wild-type (WT) (Figure 2a).

Subsequently, the cultures were cultivated under varying light conditions to assess the potential impact of light variations on downstream pH-sensing mutants. Comparative analysis of the maximum quantum yield of photosystem II ($F_v/F_m$) between the three KC lines and wild type (WT) demonstrated similar photosynthetic performances (Figure 2b). Conversely, the *lhcx1* knockout (KO) exhibited a decline in $F_v/F_m$ with increasing light intensities, suggesting photoinhibitory effects and

photodamage attributable to the absence of the photoprotective protein. NPQ traces revealed that two KC lines maintained wild-type level NPQ phenotypes, while KC-1 consistently displayed lower NPQ overall (Figures 2c-f). This discrepancy may be attributed to the potentially diminished expression of the construct in the KC-1 line. To further elucidate this, Western blot analysis will be conducted to investigate how LHCX1 protein expression correlates with qE capacity. The observed elevated NPQ values under the low-light 12:12 regime, consistently observed across subsequent lines, could potentially stem from the upregulation of LHCX1, mirroring observations with diatom LHCX1 (Figure 2C; Bailleul et al., 2010). In summary, the successful generation of KC lines with wild-type NPQ induction and relaxation demonstrates that knock-in of the HygR selectable marker cassette at the *LHCX1* locus does not affect expression or function of LHCX1.

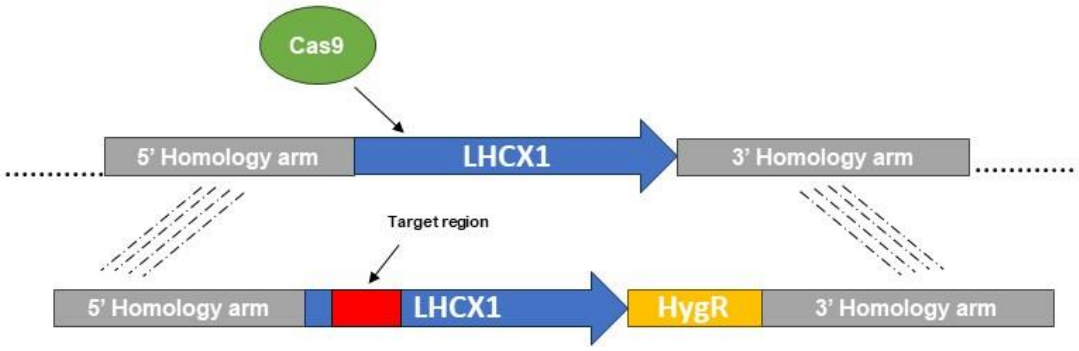



***Figure 3. Diagram showing knock-in (KI) method.*** *Cas9 ribonucleotide protein (RNP) is used to target the endogenous LHCX1 locus. Through homologous recombination the LHCX1 KI cassette is incorporated into the N. oceanica genome, and the target mutation is introduced.*

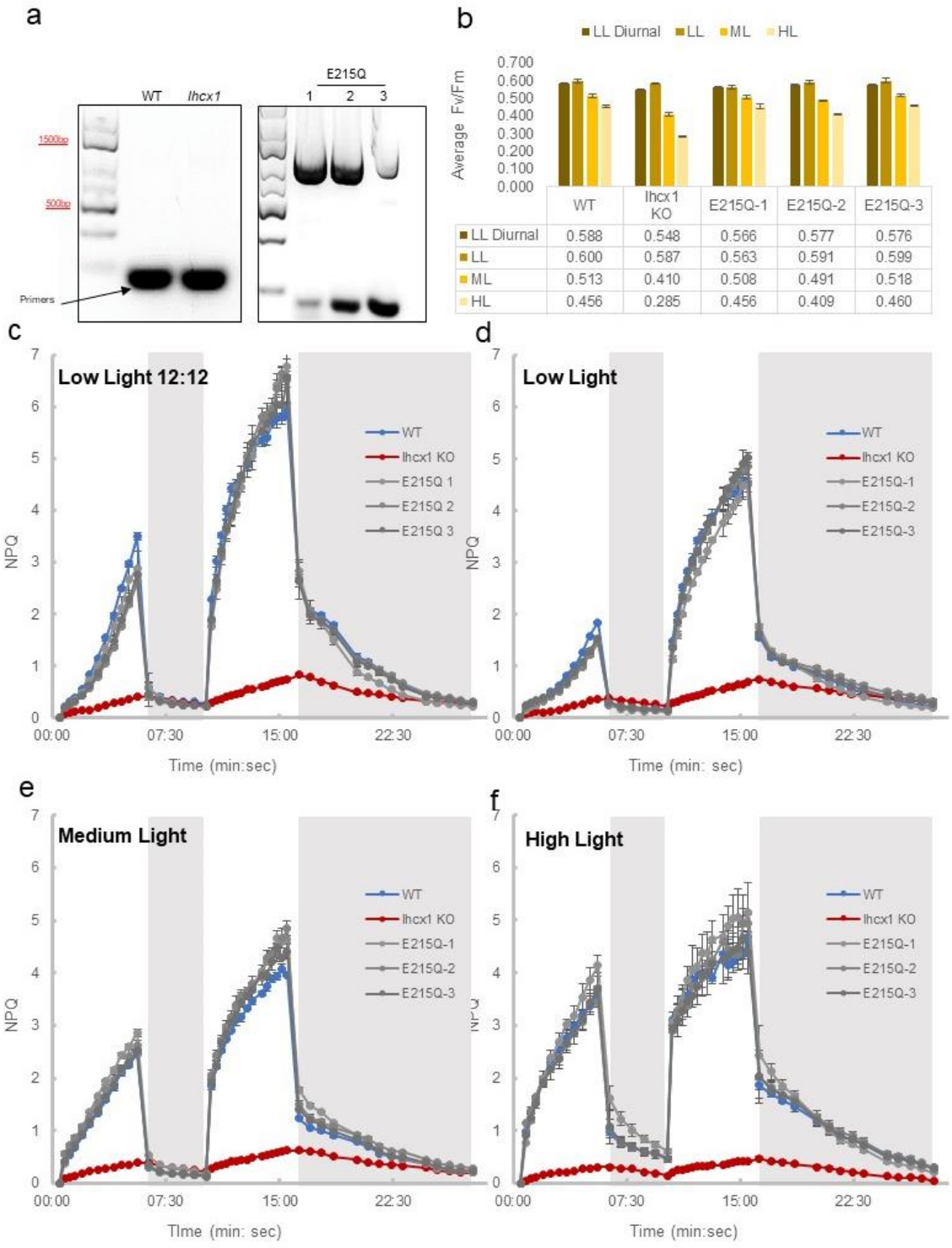



***Figure 4. Characterization of three E215Q lines in N. oceanica.*** *(a) Predicted location of E215 residue using AlphaFold (left). Genetic characterization using PCR (right). For details, see Figure 2 legend. (b) $F_v/F_m$ of WT (blue), Ihcx1 KO (red), and three E215Q mutant lines (grey) under four different light conditions. (c-f) NPQ measurements of WT, Ihcx1 KO, and E215Q lines grown in (c) low light 12:12, and continuous (d) low light, (e) medium light, and (f) high light.*

Testing the importance of Lhcx protonation for qE

Earlier investigations demonstrated the significance of three protonatable sites for pH-sensing in *C. reinhardtii,* and a tryptophan residue in *P. tricornutum,* for qE induction (Ballottari et al., 2016, Buck et al., 2021). When aligning CrLHCSR3 with the three LHCX proteins in *N. oceanica*, only one site was conserved in LHCX1 (E215), and none of these sites was conserved in LHCX2 or LHCX3 (Figure 1a). This could indicate that pH-sensing residues have evolved independently in NoLHCX1 and CrLHCSR3. Indeed, LHCX1 structural predictions using AlphaFold and regions of protein sequence conservation identified nine putative acidic amino acid residues that might be oriented toward the thylakoid lumen and potentially involved in pH sensing (Figure 1b, c). To ascertain the involvement of LHCX1 in pH sensing for qE induction, mutants lacking these putative pH-sensing sites were engineered (Figures 4 and 5).

We first investigated the significance of E215, the sole conserved site shared between NoLHCX1 and CrLHCSR3, in qE induction. We used site-directed mutagenesis to generate mutants with glutamine substituted for glutamate at position 215 of LHCX1. Three independent E215Q knock-in lines were successfully generated, but they exhibited no alterations in NPQ or other deviations from WT phenotypes (Figure 4). It is noteworthy that mutants affecting two conserved acidic residues in LHCX1 in the diatom *P. tricornutum* also had no impact on qE (Buck et al., 2021; Giovagnetti et al., 2022).

Next, we decided to examine the possible involvement of other, non-conserved acidic residues in LHCX1 in sensing lumen pH, by mutating eight glutamate residues to glutamine and one aspartate residue to asparagine. Knock-in mutants lacking all nine putative lumen-facing acidic residues were successfully generated, termed All-Site (AS) mutants (Figure 5a). Three independent AS mutant lines exhibited comparable $F_v/F_m$ to WT (Figure 5b). Surprisingly, despite the presumed absence of the ability to sense changes in lumen pH, all three AS lines displayed NPQ phenotypes very similar to those of the WT (Figures 4c-f). In the low-light 12:12 condition, where LHCX1 might be upregulated, there appears to be a modest impact on NPQ in the AS-1 line (Figure 4c). However, this effect is absent in medium light or high light growth conditions, where any influence on NPQ phenotypes would be more conspicuous if LHCX1 were significantly impaired. These results strongly suggest that protonation of LHCX1 is not pivotal for qE and that LHCX1 does not participate in sensing lumen acidification. Moreover, NoLHCX1 may function more similarly to other LHCX proteins in stramenochromes rather than to LHCSR proteins in the green lineage.

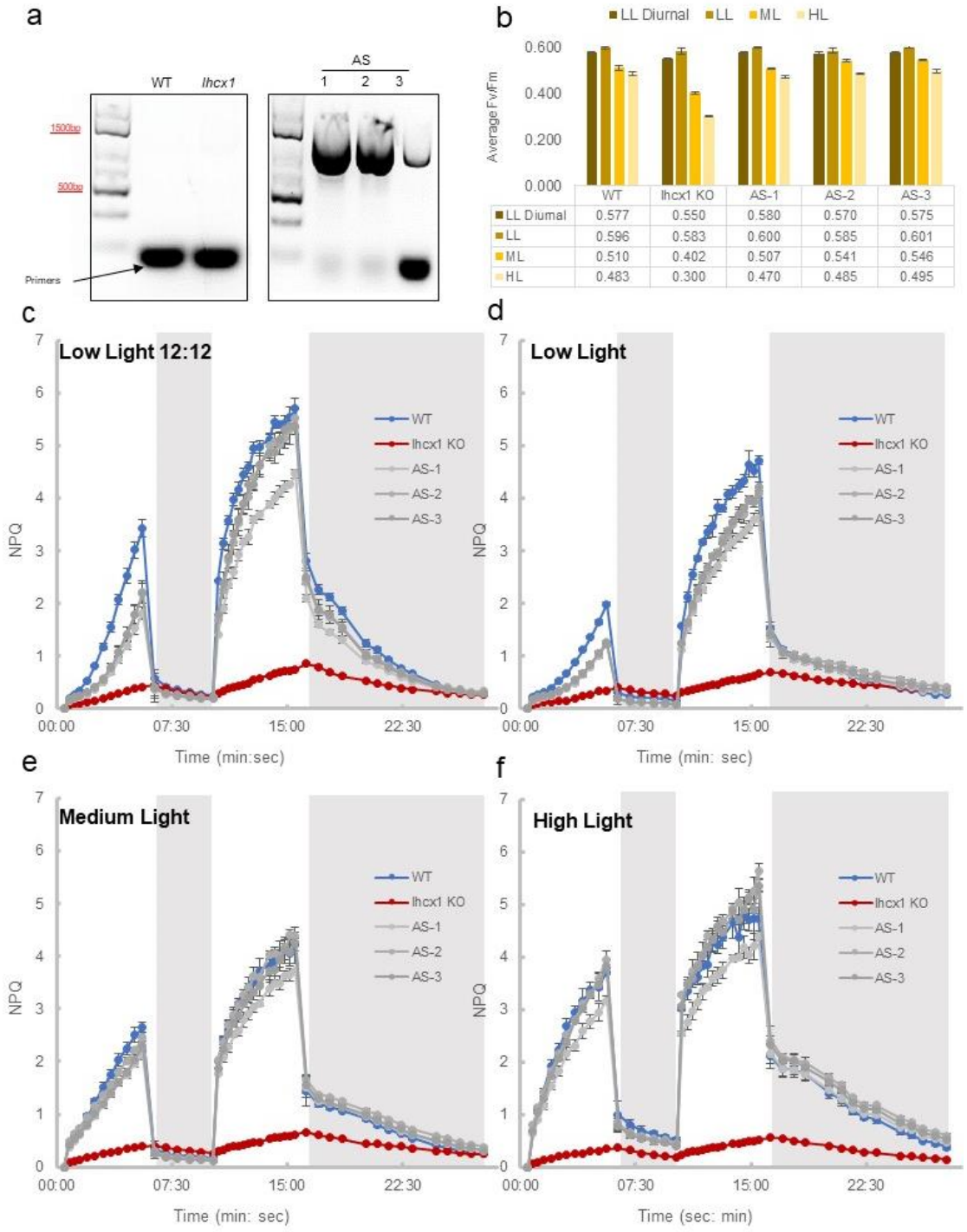



***Figure 5. Characterization of three All-Site (AS) lines in N. oceanica.*** *(a) Genetic characterization using PCR. For details, see Figure 2 legend. (b) Fv/Fm of WT (blue), lhcx1 KO (red), and three AS mutant lines (grey) under four different light conditions. (c-f) NPQ measurements of WT, lhcx1 KO, and AS lines grown in (c) low light 12:12, and continuous (d) low light, (e) medium light, and (f) high light.*

Investigating the role of an evolutionarily conserved tryptophan for qE

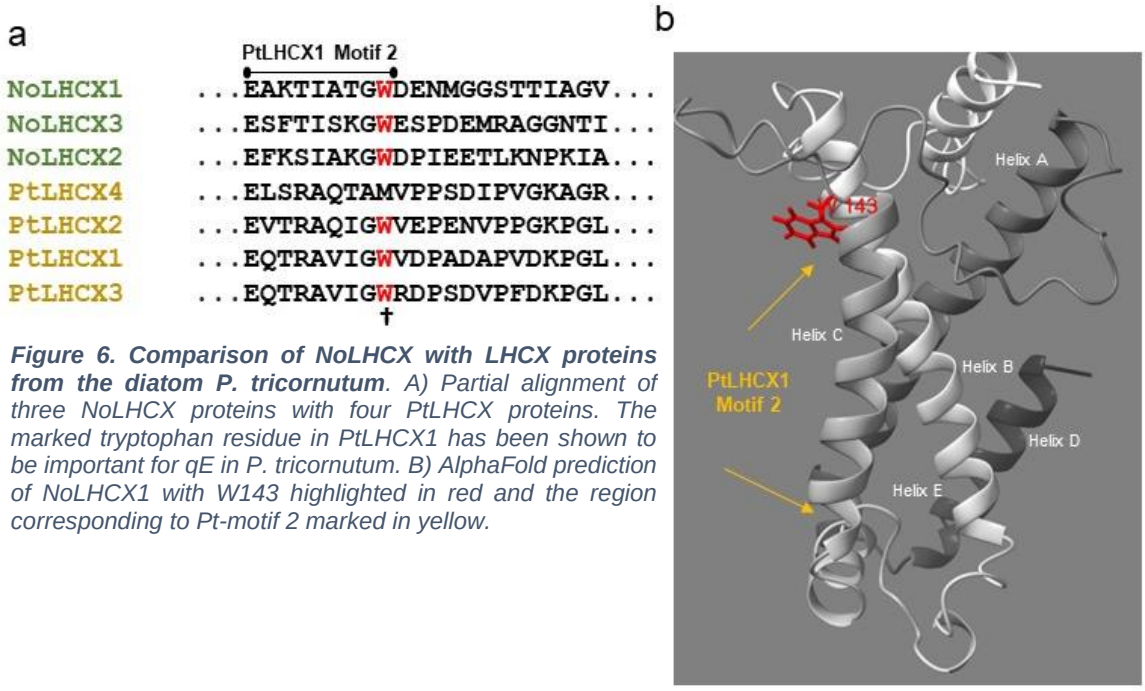



***Figure 6. Comparison of NoLHCX with LHCX proteins from the diatom P. tricornutum****. A) Partial alignment of three NoLHCX proteins with four PtLHCX proteins. The marked tryptophan residue in PtLHCX1 has been shown to be important for qE in P. tricornutum. B) AlphaFold prediction of NoLHCX1 with W143 highlighted in red and the region corresponding to Pt-motif 2 marked in yellow.*

Like NoLHCX1, the LHCX1 protein in the diatom *P. tricornutum* (PtLHCX1) has been investigated by site-directed mutagenesis (Buck et al., 2021). A specific region denoted as motif 2 within helix C was shown to be important for establishing qE. Within this motif, a tryptophan (W) residue was identified as critical, potentially serving as a pigment-binding site involved in NPQ. Alignment of NoLHCX proteins with PtLHCX proteins highlighted the conservation of the crucial W residue in motif 2 (Figure 6a), reinforcing its potential significance across LHCX proteins, excluding PtLHCX4, which may not be functional in qE. The AlphaFold prediction for the structure of NoLHCX1 confirmed that W143's position is homologous to the critical W site in PtLHCX1, with motif 2 spanning the transmembrane domain (Figure 6b; Buck et al., 2021).

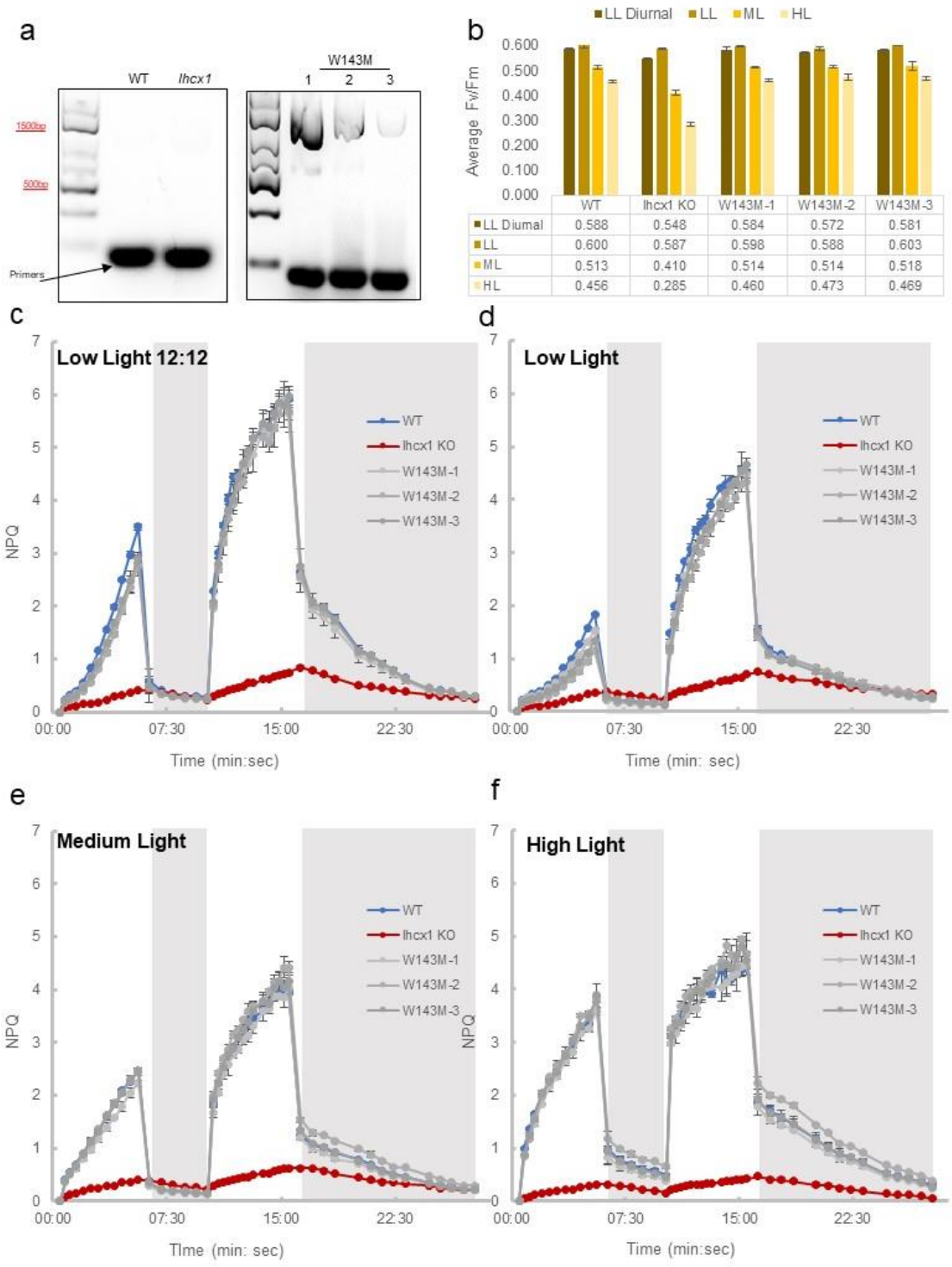



***Figure 7. Characterization of three W143M lines in N. oceanica.*** *(a) Genetic characterization using PCR. For details, see Figure 2 legend. (b) $F_v/F_m$ of WT (blue), lhcx1 KO (red), and three W143M mutant lines (grey) under four different light conditions. (c-f) NPQ measurements of WT, lhcx1 KO, and AS lines grown in (c) low light 12:12, and continuous (d) low light, (e) medium light, and (f) high light.*

To assess the importance of W143 in NoLHCX1, mutant lines were generated through site-directed mutagenesis, substituting the tryptophan residue with methionine. Genotyping and sequencing confirmed the presence of the mutant sequence (Figure 7a). $F_v/F_m$ in the W143M mutant was unaltered compared to the WT, indicating unaffected PSII efficiency (Figure 7b). Surprisingly, this mutation did not cause any changes in NPQ, with all three W143M mutants exhibiting NPQ phenotypes identical to WT in all growth conditions tested (Figure 7c-f).

## Discussion

In this study, we applied a Cas9-mediated ribonucleotide protein (RNP) approach to knock-in DNA into the endogenous *LHCX1* locus in *N. oceanica* (Figure 1). This method avoids the problem of position effects on expression of randomly integrated DNA and eliminates the need to screen hundreds of transformants per construct to find a few with WT expression of the protein of interest (Ballottari et al., 2016). We first validated the approach by knocking in a WT copy of the *LHCX1* gene along with the HygR selectable marker gene at the native *LHCX1* locus, which yielded lines with NPQ very similar to that of the WT (Figure 2).

We then proceeded to structure-function analysis of LHCX1 using knock-in of site-directed mutants. Mutants lacking E215, a conserved residue possibly involved in sensing lumen pH to induce qE, exhibited no changes in NPQ (Figure 4), aligning with previous observations in the diatom *P. tricornutum* (Buck et al., 2021; Giovagnetti et al., 2022). Surprisingly, AS mutants lacking all nine putative lumen-facing acidic residues also exhibited NPQ phenotypes very similar to the WT (Figure 5), suggesting that the function of LHCX1 does not involve direct sensing of the lumen pH. Notably, this differs from the well-established paradigm in the green lineage, where CrLHCSR3 and AtPsbS proteins are pivotal for pH- sensing and qE induction (Liguori et al., 2013; Ballottari et al., 2016; Peers et al., 2009; Quaas et al., 2015). The unexpected results with NoLHCX1 and PtLHCX1 prompt a reevaluation of the current understanding of NPQ induction mechanisms in stramenochromes and raise questions about how LHCX proteins function in qE.

Is LHCX1 involved in sensing the ΔpH in excess light, and if so, how? One possibility is that it senses the relatively small increase in stromal pH instead of the decrease in lumenal pH. A precedent for this exists in the atypical CVDE enzyme of *C. reinhardtii*, which is located in the stroma instead of the lumen (Z. Li et al., 2016). CVDE exhibits nigericin-sensitive violaxanthin de-epoxidase activity, indicating that it is responding to a change in stromal pH in excess light. Another possibility is that LHCX1 might respond to a putative ΔpH-dependent decrease in the thickness of the thylakoid membrane in excess light (Murakami' & Packer, 1970), which could cause a conformational change in LHCX1 via hydrophobic mismatch (Daskalakis et al., 2019; De Meyer et al., 2008; D. H. Li et al., 2023). Alternatively, it is conceivable that another protein is involved in ΔpH sensing and that LHCX1 has a different essential role in qE. For example, it could serve as a pigment-binding site, facilitating energy transfer by binding Vx/Zx, akin to TpLHCX6_1 and PtLHCF4 with the binding of Ddx/Dtx (Feng et al., 2023; W. Wang et al., 2019).

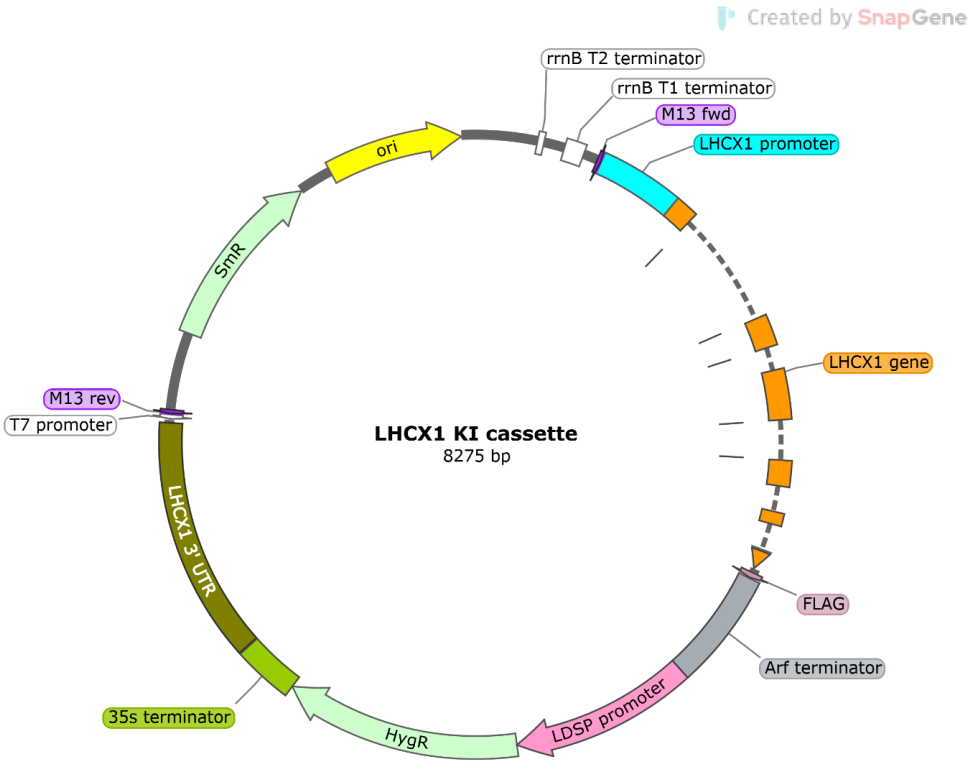
We also explored the role of an evolutionarily conserved tryptophan residue in LHCXI that is necessary for qE in the diatom *P. tricornutum* (Buck et al., 2021). However, the W143M mutation in NoLHCX1 did not affect PSII efficiency or NPQ (Figure 7), indicating species-specific variations in the functional significance of this amino acid and motif. These results underscore the complexity of qE mechanisms in nature, highlight the importance of species-specific investigations in understanding the evolution of NPQ, and set the stage for the next phase in unraveling the complex interplay between structure and function in NoLHCX1.

Supplemental Materials

| Experiment | Primer Name | Description | Oligo Sequence (5' -> 3') |
|---|---|---|---|
| Cas9-RNP HR | TC002f | gRNA | CGCCGCCGGCATCACGATCC |
| | TC085r | gRNA | aagaaggaatggaaaaaagg |
| HIFI Assembly | TC105f | All-site mutated version of Lhcx1 w/ 5'overhang | tctcctcaaccgacagGCCGAGTGGCCATGCTTG |
| | TC106r | All-site mutated version of Lhcx1 w/ 3' overhang | gtcgtcgtcgtccttgtaatcggcgcctttGAGACAAGG |
| *E. coli* screening | TC107f | For all mutated plasmids | GAAGCCGAGCTCAAACACGg |
| | TC108r | For all mutated plasmids | ccggtgaatgacctggcc |
| *Nannochloropsis* transformation | TC116f | Plasmid linearization | AAAGCATGGCTTGGAGGACAA |
| | TC117r | Plasmid linearization | CAACGCACACACGACACAAATC |
| Site-Directed Mutagenesis | TC168f | E215Q Q5 Kit SDM with E Coli | CGGCCTCCCCcagTTCGAATTTC |
| | TC169r | E215Q Q5 Kit SDM with E Coli | TTGGTCACAAACTTCTCCTGTAG |
| | TC166 | W143M Q5 Kit SDM with E Coli | CGCCACAGGGatGGACGAGAAC |
| | TC167 | W143M Q5 Kit SDM with E Coli | ATGGTCTTGGCCTCGATG |
| LHCX1 KI Genotyping | TC119f | Primary PCR, Cassette Insertion genotyping | cggagtccgcaaaaatcacc |
| | TC122r | Primary PCR, Cassette Insertion genotyping | taccactaccaccacgtcca |
| | TC158f | Secondary PCR, Cassette Insertion genotyping | gctcatgtgttgagcatataagaaaccctagtatg |
| | TC159r | Secondary PCR, Cassette Insertion genotyping | CGAAACAAACTCTCCTGACAAAACAATG |
| | TC170f | Primary PCR, Lhcx1 gene sequencing | GGTTCTAGCAGTGCCAGTGCG |
| | TC171r | Primary PCR, Lhcx1 gene sequencing | ccgtacggtgaaataaggtacaatcga |
| | TC172f | Secondary PCR, Lhcx1 gene sequencing | GACGAGTATCCCACACAGTTCGC |
| | TC173r | Secondary PCR, Lhcx1 gene sequencing | cgacgaccccacccatccatc |

***Table 1. Primers used in materials and methods.***

*LHCX1* KI Cassette Sequence




CTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAG
CTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGG
AAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTC
ATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAA
CGCAATTAATACGCGTACCGCTAGCCAGGAAGAGTTTGTAGAAACGCAAAAAGGC
CATCCGTCAGGATGGCCTTCTGCTTAGTTTGATGCCTGGCAGTTTATGGCGGGCG
TCCTGCCCGCCACCCTCCGGGCCGTTGCTTCACAACGTTCAAATCCGCTCCCGGC
GGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGG
CCCAGTCTTCCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCAGTTCCCTACTCTC
GCGTTAACGCTAGCATGGATGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCA
GTCAAAGCATGGCTTGGAGGACAATCGCGTGCCTGTCGATTTATAAGACGCATAAA
CAAGAATAGCTGGGAGATGCGGCAACAGATGTGTCGCATTTGGACCATGTGGATA
CTGTATTTCCAAGTTGGCGACGAGAAGGATATGAACTTCCCTTCCCCGACTTCCTG
CAAAGTTCTGTTGTATGTTTTGCTCGTGGTGACGACGCACACTAGTGTTGGGCATT
TGCTCACCCTACCGCGGTCCGCTCATTCTGCCAACGCTTCTTTCATCTCGCTTCTC
GCACTGGCTCCCTGCCTCGTTTGCCCTGTTCCCGGCAACAAGCTCACCTCTTCATC
CTTTTCTCTTCCCCACCCAGAAGAACCATGCGTGTCCTCTCTTTCCTCGCCATGCT
CGGCACTGCCGCCGCTTTTGTCAAGCCCACCCTCCCTTCTGCGGGGTCCCGCACC
CGTGCCGGTGCCCTCCGgtaagtgagtgctatgtgtgttcaggtccctgcgcttgactgcattacccgtgtggttg

aacccaaaagacgcgacgggaaaaacgaatatcaggcctgtacgtgtgtttgtgtacaagttgcgggcctcttcccccgc
atcgcctgccttcccccctgtacaccctaatcaaccttcttacagagaagttcggtcgcgccgtacctttgatttcccctggcat
tgaaaccaagttccccaaaagatcgtcctcttccttttcctcccgtggcagattcctatacccacattcctcgctcacaacacc
aacaccaacacgaacacgacaccaccatccccatcgtaccttgagtcatgactggaccgtgcgtttctatcacctcaccgt
ccctcctttcacccctctcccccccggccccatcttttctccttctcgaacgaggcatctgttttaccttcgctcaccctctccgctt
ctcccctcttttcccctctccctcatccatcagCATGAACTTGGCTGAGATCGAACTGGAGGCCGGC
AAGACCTCCCCCTTCCCTGAGGGCTTTGACCCCCTTGGCCTGTCCAAAGACAAGT
CCTTCAAGGAGCTCAAGAAATGGCGCGAAGCCGAGCTCAAACACGgtacgtcacgccttc
ccccccccccccttttttccattccttcttcattggtctcaccacatgctcttcttcccgtcccttcccttctcctcaaccgacagGC
CGAGTGGCCATGCTTGCCGTGCTGGGCACCGCGGTGCAGGAGAACTACCACCCC
CTGTGGGGCTTTAACGAGAAGGAGATGGACGGCGCTATCTTCCACTTCCAGGAAA
TCCAAAACGTTTACCCCCTTTTCTGGACTGCCCTCCTCTTCATCATCGGCGTCATC
GAGGCCAAGACCATCGCCACAGGGTGGGACGAGAACATGGGCGGCTCCgtgagtacc
ttccattccttcctcccttcctctccttcccctccctccctctctgtcctccaaacagtcaattgttgttttcttcagtgtatgcttttccc
ctttcctcccaactcactccaataaccatctcgccctccttctctccctcctttccctccctattcaccagACCACCATCG
CCGGCGTGAAGGAGGATTACATCtgcGGCAACCTGGGCCTTGACCCCCTCAAGATC
ATCGAGAACGAGGACGAGGAGGCCTTCCTCTCCTACCGCAACAAGgtgtgctttccctccc
acctcctctcccgccgtctatgggcctacagcttgtctagatcccattctcatcctgccccctcccccctttcctccctctctctttc
atgctacagGAGCTCAACAACGGCCGACTTGCCATGATCGCCGCCGCCGGCATCACG
ATCCAGgttcgtccgcccttcccttccctccctccctccctccctcccccttcctcgcttttcccaaccatccctgccccccg
ctcacccccttcctcctctacgatttcccccccctccttgctcctacagGAGAAGTTTGTGACCAACGGCCTCC
CCGAGTTCGAATTTCACCGATTCGCCCTCTCTGATGTCTACAACTTCTTCTTCTAAG
CCTTGTCTCaaaggcgccgattacaaggacgacgacgacaagTAGaggatggcattagtgaggaggaaggg
aagtgatgaatgagggccaggtcattcaccggttttaatcacacctgttgggggttaggtgggttgatggatgggtggggtc
gtcgatttttcgattgtaccttatttcaccgtacggccctccatgttctccttgcctttcctttcttcattcacacaacatataccgtgc
aacaacaaaaaagaaacggaggcaattggcgctactttcaaaaaatcaaaaaacaaacattcgttttatttttatttttagttct
cccctcccgtgagtccaacaaattggaggcgacgcctgcgcatatatcgaaacacaaagaaaggtggagaagtggga
cggaggagaggagtggtgtacggaagccgaaagtctagcgagaaaagagcgaagggaagaaacgacgaagaag
aatgggtccctttatttttgtggatgatgaaaagaaaaaaagacgaaggagaattaaagaaggaagaagatggagtggat
ggaggaggagGCGAGCGTAGCAGCAAGCGTGAGTTATACAGCCAGGCACATGTCGCA
ATCCTTCGGTCTCGGGCTTAAAATCCACGCACTAATCACGCTGGGCCATGCAAAGA
GCAATGCCGAGGCCCACCACACAAAACGCTGTGTCGCGCGTTGCGGCCTGAAGC
TTCATACTTCTTAGTCGCCGCCAAAAGGGCTCGAGAGACGAGACCCGTTGGCATG
ACCGATGTTGTTCGACGCGGTTTGCTTCGTCACAGTCGACGTGATTCAGGAATCTG
GAGCCTGCAGATCATTTTTTTCAGCCTGATATCGTTCTTTTCCACTGAGAACCATCA
GACCACCTTTTCTTCCATTGTGTGAAGGAGTAGGAGTTGCCGTGCTGCTTTGTGGG
AGACATCTGCGATGGTGACCAGCCTCCCGTCGTCTGGTCGACGTGACGAGCCTCT
TCACTGTTCTTCGACGGAGAGACGCAAGCGAGACGGCTCTAGACCTTTTGGACAC
GCATTCTGTGTGTGAACTAGTGGACAGTGATACCACGTCTGAAAGCTCACCACTGC
CCATGGTGCAGCTACTTGTCACAAAGTTTTGACTCCGTCGGTATCACCATTCGCGC

TCGTGTGCCTGGTTGTTCCGCCACGCCGGCCTGCCCCGGGGCGGGGCAATATTC
TAAAATCTCACGCAAAACACCGCACTTACCCCTCACACATATTCGTGATAGACCAC
CACCAATCTCAGCCCGCATCAACACAGgagggccccgggggcaataagatatgaaaaagcctga
actcaccgcgacgtctgtcgagaagtttctgatcgaaaagttcgacagcgtctccgacctgatgcagctctcggagggcg
aagaatctcgtgctttcagcttcgatgtaggagggcgtggatatgtcctgcgggtaaatagctgcgccgatggtttctacaaa
gatcgttatgtttatcggcactttgcatcggccgcgctcccgattccggaagtgcttgacattggggaattcagcgagagcct
gacctattgcatctcccgccgtgcacagggtgtcacgttgcaagacctgcctgaaaccgaactgcccgctgttctgcagcc
ggtcgcggaggccatggatgcgatcgctgcggccgatcttagccagacgagcgggttcggcccattcggaccgcaagg
aatcggtcaatacactacatggcgtgatttcatatgcgcgattgctgatccccatgtgtatcactggcaaactgtgatggacg
acaccgtcagtgcgtccgtcgcgcaggctctcgatgagctgatgctttgggccgaggactgccccgaagtccggcacctc
gtgcacgcggatttcggctccaacaatgtcctgacggacaatggccgcataacagcggtcattgactggagcgaggcga
tgttcggggattcccaatacgaggtcgccaacatcttcttctggaggccgtggttggcttgtatggagcagcagacgcgcta
cttcgagcggaggcatccggagcttgcaggatcgccgcggctccgggcgtatatgctccgcattggtcttgaccaactctat
cagagcttggttgacggcaatttcgatgatgcagcttgggcgcagggtcgatgcgacgcaatcgtccgatccggagccgg
gactgtcgggcgtacacaaatcgcccgcagaagcgcggccgtctggaccgatggctgtgtagaagtactcgccgatagt
ggaaaccgacgccccagcactcgtccgagggcaaaggaatagagtagatgccgaccggagtccgcaaaaatcacc
agtctctctctacaaatctatctctctctatttttctccagaataatgtgtgagtagttcccagataagggaattagggttcttatag
ggtttcgctcatgtgttgagcatataagaaaccccttagtatgtatttgtatttgtaaaatacttctatcaataaaatttctaattccta
aaaccaaaatccagtgacctgcaggcatgcaagctgatccactagaggtacCCGCATCAAGAAAAAAGAA
GGGGCCGAGAGCGATGGAGAGAAGAGGAGAGGGTAGCTTTTTACGCGCGAAAGG
GGATTTAGTAAGGAGTGGTCGGTCTGGGTTTAGCCAGTGTCAAAGTTAAAGTGAGT
GAGAGTGCGTTGTTAGGCCTGGGAAGGTGGTGTGATGGGGGGCGTCTGTAAGGA
GGCCCTGTCTTGTCCCATCATCTTCTGTAATTGTATGTGTGTGTGCGGCGGTCA
GAGGACCGCCGCACATGAAAGGGGGGGAGGGAGGGAGGGAGAGAGGAGGAAGG
GAGAGGGATCCAGTGGTTTCGGCCTCTGCTTTTAAACCATTTTCTCCCCTTCCCCC
TTAAAGGCTTCCTTCTTTTGTTGTCTCCCACCGTACGGGCACACACACTTGATTTGA
GATGCCGTATTAATGTGCCTGACGAATAGTGATGGAGGTGGCGGAGGAGAGAGG
GGTTTGAGTATCAGAAGAATGAAATCAATCTCCAGGGAAATAGGAAGGTTCTCACC
GACAGAGTTGCGTTTCATTTGTTTACTTTTCGTGTTGGTGAGTGTTGTTCTGTCGGG
GACAAGAGACGAGGTCTATTGAAGGAGGAGGAGGCAGAGGAGGAGGAGGAGGC
CTTAGAAAGGACTTTGTCGGTATCAATTGTCGTGGTTGACGGAGGGGAGGAAGGC
AAAGAATGTCATTTGACATACGCATGGCGTTTACCAGAAGCTCTCTGTGGATGTCC
GATGCATCTCTTGGGTATGCCCTTCCTTGTTTCCCTTTATTTCCTTTTTTATCTTCAC
TTCCTTCTATCGTCTTCTTTTTAATTCCTCTTCTCTTCTTTCTCCTCCTTTGCCAGTTA
CTTTCCTGCTTTGACCCTCCTTATTATTCCGCCCTCTTTTTTCCCCTCCCTTCTCTTC
TCTTTTCTTTGCCATCATATTCGATGTCCTCCTCGTGGGTGTTTATTCATTGTTTGAC
AAAAGAGAATTCGTTTCGTGTGTGCGTTGCTTGTTTTGCTCTACGAGAAGGATTTGT
GTCGTGTGTGCGTTGgtacccagctgatatcccCTATAGTGAGTCGTATTACATGGTCATAG
CTGTTTCCTGGCAGCTCTGGCCCGTGTCTCAAAATCTCTGATGTTACATTGCACAA
GATAAAATAATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAA

GGGGTGTTagatccATGCGCTCACGCAACTGGTCCAGAACCTTGACCGAACGCAGC
GGTGGTAACGGCGCAGTGGCGGTTTTCATGGCTTGTTATGACTGTTTTTTTGGGGT
ACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGTCGATGT
TTGATGTTATGGAGCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCTAAAACAA
AGTTAAACATTATGAGGGAAGCGGTGATCGCCGAAGTATCGACTCAACTATCAGAG
GTAGTTGGCGTCATCGAGCGCCATCTCGAACCGACGTTGCTGGCCGTACATTTGT
ACGGCTCCGCAGTGGATGGCGGCCTGAAGCCACACAGTGATATTGATTTGCTGGT
TACGGTGACCGTAAGGCTTGATGAAACAACGCGGCGAGCTTTGATCAACGACCTT
TTGGAAACTTCGGCTTCCCCTGGAGAGAGCGAGATTCTCCGCGCTGTAGAAGTCA
CCATTGTTGTGCACGACGACATCATTCCGTGGCGTTATCCAGCTAAGCGCGAACTG
CAATTTGGAGAATGGCAGCGCAATGACATTCTTGCgGGTATCTTCGAGCCAGCCAC
GATCGACATTGATCTGGCTATCTTGCTGACAAAAGCAAGAGAACATAGCGTTGCCT
TGGTAGGTCCAGCGGCGGAGGAACTCTTTGATCCGGTTCCTGAACAGGATCTATTT
GAGGCGCTAAATGAAACCTTAACGCTATGGAACTCGCCGCCCGACTGGGCTGGCG
ATGAGCGAAATGTAGTGCTTACGTTGTCCCGCATTTGGTACAGCGCAGTAACCGG
CAAAATCGCGCCGAAGGATGTCGCTGCCGACTGGGCAATGGAGCGCCTGCCGGC
CCAGTATCAGCCCGTCATACTTGAAGCTAGACAGGCTTATCTTGGACAAGAAGAAG
ATCGCTTGGCCTCGCGCGCAGATCAGTTGGAAGAATTTGTCCACTACGTGAAAGG
CGAGATCACCAAGGTAGTCGGCAAATAAgaatgcAGAATTGGTTAATTGGTTGTAACA
CTGGCAGAGCATTACGCTGACTTGACGGGACGGCGCAAGCTCATGACCAAAATCC
CTTAACGTGAGTTACGCGTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAA
AGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAA
ACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTC
CGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAG
CCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCT
GCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGG
TTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGG
GGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACC
TACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACA
GGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAG
GGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAG
CGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCA
ACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTT

All-Site fragment sequence of *LHCX1* starting from primer TC105f and ending in TC106r.

tctcctcaaccgacagGCCGAGTGGCCATGCTTGCCGTGCTGGGCACCGCGGTGCAGCA
GAACTACCACCCCCTGTGGGGCTTTAACCAGAAGCAGATGAACGGCGCTATCTTC
CACTTCCAGCAAATCCAAAACGTTTACCCCCTTTTCTGGACTGCCCTCCTCTTCATC
ATCGGCGTCATCGAGGCCAAGACCATCGCCACAGGGTGGGACGAGAACATGGGC
GGCTCCgtgagtaccttccattccttcctcccttcctctccttcccctccctcctctctgtcctccaaacagtcaattgttgttt
tcttcagtgtatgcttttccccctttcctcccaactcactccaataaccatctcgccctccttctctccctcctttccctccctattcacc
agACCACCATCGCCGGCGTGAAGGAGGATTACATCtgcGGCAACCTGGGCCTTGAC
CCCCTCAAGATCATCGAGAACGAGGACGAGGAGGCCTTCCTCTCCTACCGCAACA
AGgtgtgctttccctcccacctcctctcccgccgtctatgggcctacagcttgtctagatcccattctcatcctgccccctcccc
ccttcctccctctctctttcatgctacagGAGCTCAACAACGGCCGACTTGCCATGATCGCCGCCG
CCGGCATCACGATCCAGgttcgtccgcccttcccttccctccctccctccctccctccccttcctcgcttttcccaa
ccatccctgccccccgctcacccccttcctcctctacgatttcccccccctccttgctcctacagCAGAAGTTTGTGAC
CAACGGCCTCCCCCAGTTCCAATTTCACCGATTCGCCCTCTCTAATGTCTACAACT
TCTTCTTCTAAGCCTTGTCTCaaaggcgccgattacaaggacgacgacgac

References


Ahumada-Fierro, N. V., García-Mendoza, E., Sandoval-Gil, J. M., & Band-Schmidt, C. J. (2021). Photosynthesis and photoprotection characteristics related to ROS production in three Chattonella (Raphidophyceae) species. *Journal of Phycology*, *57*(3), 941–954. https://doi.org/10.1111/JPY.13138

Ajjawi, I., Verruto, J., Aqui, M., Soriaga, L. B., Coppersmith, J., Kwok, K., Peach, L., Orchard, E., Kalb, R., Xu, W., Carlson, T. J., Francis, K., Konigsfeld, K., Bartalis, J., Schultz, A., Lambert, W., Schwartz, A. S., Brown, R., & Moellering, E. R. (2017). Lipid production in Nannochloropsis gaditana is doubled by decreasing expression of a single transcriptional regulator. *Nature Biotechnology*, *35*(7), 647–652. https://doi.org/10.1038/NBT.3865

Alboresi, A., Le Quiniou, C., Yadav, S. K. N., Scholz, M., Meneghesso, A., Gerotto, C., Simionato, D., Hippler, M., Boekema, E. J., Croce, R., & Morosinotto, T. (2017). Conservation of core complex subunits shaped the structure and function of photosystem I in the secondary endosymbiont alga Nannochloropsis gaditana. *New Phytologist*, *213*, 714–726. https://doi.org/10.1111/nph.14156

Alboresi, A., Perin, G., Vitulo, N., Diretto, G., Block, M., Jouhet, J., Meneghesso, A., Valle, G., Giuliano, G., Maréchal, E., & Morosinotto, T. (2016). Light Remodels Lipid Biosynthesis in Nannochloropsis gaditana by Modulating Carbon Partitioning between Organelles. *Plant Physiology*, *171*(4), 2468–2482. https://doi.org/10.1104/PP.16.00599

Alboresia, A., Gerottob, C., Giacomettib, G. M., Bassia, R., & Morosinotto, T. (2010). Physcomitrella patens mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(24), 11128–11133. https://doi.org/10.1073/PNAS.1002873107

Amaral, R., Sérgio Seixas de Melo, J., & Maria Antunes dos Santos, L. (2021). Pigments from Eustigmatophyceae: an interesting class of microalgae for carotenoid production. *Journal of Applied Phycology*, *33*, 371–384. https://doi.org/10.1007/s10811-020-02312-z/Published

Antoshvili, M., Caspy, I., Hippler, · Michael, & Nelson, · Nathan. (2019). Structure and function of photosystem I in Cyanidioschyzon merolae. *Photosynthesis Research*, *139*, 499–508. https://doi.org/10.1007/s11120-018-0501-4

Armbrust, E. V., Berges, J. A., Bowler, C., Green, B. R., Martinez, D., Putnam, N. H., Zhou, S., Allen, A. E., Apt, K. E., Bechner, M., Brzezinski, M. A., Chaal, B. K., Chiovitti, A., Davis, A. K., Demarest, M. S., Detter, J. C., Glavina, T., Goodstein, D., Hadi, M. Z., … Rokhsar, D. S. (2004). The genome of the diatom Thalassiosira Pseudonana: Ecology, evolution, and metabolism. *Science*, *306*(5693), 79–86. https://doi.org/10.1126/science.1101156

Arsalane, W., Rousseau, B., & Duval, J. -C. (1994). INFLUENCE OF THE POOL SIZE OF THE XANTHOPHYLL CYCLE ON THE EFFECTS OF LIGHT STRESS IN A DIATOM: COMPETITION BETWEEN PHOTOPROTECI'ION AND PHOTOINHIBITION. *Photochemistry and Photobiology*, *60*(3), 237–243. https://doi.org/10.1111/J.1751-1097.1994.TB05097.X

Arshad, R., Calvaruso, C., Boekema, E. J., Buchel, C., & Kouril, R. (2021). Revealing the architecture of the photosynthetic apparatus in the diatom Thalassiosira pseudonana. *Plant Physiology*, *186*(4), 2124–2136. https://doi.org/10.1093/PLPHYS/KIAB208

Bai, Y., Cao, T., Dautermann, O., Buschbeck, P., Cantrell, M. B., Chen, Y., Lein, C. D., Shi, X., Ware, M. A., Yang, F., Zhang, H., Zhang, L., Peers, G., Li, X., & Lohr, M. (2022). Green diatom mutants reveal an intricate biosynthetic pathway of fucoxanthin. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(38). https://doi.org/10.1073/PNAS.2203708119/-/DCSUPPLEMENTAL

Bailey, J. C., Bidigare, R. R., Christensen, S. J., & Andersen, R. A. (1998). Phaeothamniophyceae Classis Nova: A New Lineage of Chromophytes Based upon Photosynthetic Pigments, rbcL Sequence Analysis and Ultrastructure. *Protist*, *149*(3), 245–263. https://doi.org/10.1016/S1434-4610(98)70032-X

Bailleul, B., Rogato, A., De Martino, A., Coesel, S., Cardol, P., Bowler, C., Falciatore, A., & Finazzi, G. (2010). An atypical member of the light-harvesting complex stress-related protein family modulates diatom responses to light. *Proceedings of the*

*National Academy of Sciences of the United States of America*, *107*(42), 18214–18219. https://doi.org/10.1073/pnas.1007703107

Ballottari, M., Truong, T. B., De Re, E., Erickson, E., Stella, G. R., Fleming, G. R., Bassi, R., & Niyogi, K. K. (2016). Identification of pH-sensing Sites in the Light Harvesting Complex Stress-related 3 Protein Essential for Triggering Non-photochemical Quenching in Chlamydomonas reinhardtii. *The Journal of Biological Chemistry*, *291*(14), 7334–7346. https://doi.org/10.1074/jbc.M115.704601

Baroli, I., Do, A. D., Yamane, T., & Niyogi, K. K. (2003). Zeaxanthin Accumulation in the Absence of a Functional Xanthophyll Cycle Protects Chlamydomonas reinhardtii from Photooxidative Stress. *The Plant Cell*, *15*(4), 992–1008. https://doi.org/10.1105/TPC.010405

Bassi, R., & Dall'osto, L. (2021). *Dissipation of Light Energy Absorbed in Excess: The Molecular Mechanisms*. https://doi.org/10.1146/annurev-arplant-071720

Basso, S., Simionato, D., Gerotto, C., Segalla, A., Giacometti, G. M., & Morosinotto, T. (2014). Characterization of the photosynthetic apparatus of the Eustigmatophycean Nannochloropsis gaditana: Evidence of convergent evolution in the supramolecular organization of photosystem I. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(2), 306–314. https://doi.org/10.1016/J.BBABIO.2013.11.019

Benedetti, M., Vecchi, V., Barera, S., & Dall'Osto, L. (2018). Biomass from microalgae: The potential of domestication towards sustainable biofactories. *Microbial Cell Factories*, *17*(1). https://doi.org/10.1186/S12934-018-1019-3

Berne, N., Fabryova, T., Istaz, B., Cardol, P., & Bailleul, B. (2018). The peculiar NPQ regulation in the stramenopile Phaeomonas sp. challenges the xanthophyll cycle dogma. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1859*(7), 491–500. https://doi.org/10.1016/J.BBABIO.2018.03.013

Betancor, S., Domínguez, B., Tuya, F., Figueroa, F. L., & Haroun, R. (2015). Photosynthetic performance and photoprotection of Cystoseira humilis (Phaeophyceae) and Digenea simplex (Rhodophyceae) in an intertidal rock pool. *Aquatic Botany*, *121*, 16–25. https://doi.org/10.1016/J.AQUABOT.2014.10.008

Bína, D., Bouda, K., & Litvín, R. (2017). A two-component nonphotochemical fluorescence quenching in eustigmatophyte algae. *Photosynthesis Research*, *131*(1), 65–77. https://doi.org/10.1007/S11120-016-0299-X/FIGURES/9

Bína, D., Durchan, M., Kuznetsova, V., Vácha, F., Litvín, R., & Polívka, T. (2018). *Energy transfer dynamics in a red-shifted violaxanthin-chlorophyll a light-harvesting complex*. https://doi.org/10.1016/j.bbabio.2018.11.006

Bína, D., Gardian, Z., Herbstová, M., & Litvín, R. (2017). Modular antenna of photosystem I in secondary plastids of red algal origin: a Nannochloropsis oceanica case study. *Photosynthesis Research*, *131*(3), 255–266. https://doi.org/10.1007/s11120-016-0315-1

Bird, D. F., & Kalff, J. (1987). Algal phagotrophy: Regulating factors and importance relative to photosynthesis in Dinobryon (Chrysophyceae). *Limnology and Oceanography*, *32*(2), 277–284. https://doi.org/10.4319/LO.1987.32.2.0277

Bonente, G., Ballottari, M., Truong, T. B., Morosinotto, T., Ahn, T. K., Fleming, G. R., Niyogi, K. K., & Bassi, R. (2011). Analysis of LHcSR3, a protein essential for feedback de-excitation in the green alga Chlamydomonas reinhardtii. *PLoS Biology*, *9*(1). https://doi.org/10.1371/JOURNAL.PBIO.1000577

Borowitzka, M. A. (2013). High-value products from microalgae—their development and commercialisation. *Journal of Applied Phycology 2013 25:3*, *25*(3), 743–756. https://doi.org/10.1007/S10811-013-9983-9

Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., Otillar, R. P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde, M., Katinka, M., Mock, T., Valentin, K., … Grigoriev, I. V. (2008a). The Phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature 2008 456:7219*, *456*(7219), 239–244. https://doi.org/10.1038/nature07410

Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., Otillar, R. P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde, M., Katinka, M., Mock, T., Valentin, K., … Grigoriev, I. V. (2008b). The Phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature 2008 456:7219*, *456*(7219), 239–244. https://doi.org/10.1038/nature07410

Brenner, S., & Horne, R. W. (1959). A NEGATIVE STAINING METHOD FOR HIGH RESOLUTION ELECTRON MICROSCOPY OF VIRUSES. *Biochimica Et Biophysica Acta* , *34*, 103–110.

Büchel, C. (2015a). Evolution and function of light harvesting proteins. *Journal of Plant Physiology*, *172*, 62–75. https://doi.org/10.1016/J.JPLPH.2014.04.018

Büchel, C. (2015b). Evolution and function of light harvesting proteins. *Journal of Plant Physiology*, *172*, 62–75. https://doi.org/10.1016/J.JPLPH.2014.04.018

Büchel, C. (2020a). Light harvesting complexes in chlorophyll c-containing algae. *Biochimica et Biophysica Acta - Bioenergetics*, *1861*(4). https://doi.org/10.1016/j.bbabio.2019.05.003

Büchel, C. (2020b). Light harvesting complexes in chlorophyll c-containing algae. *Biochimica et Biophysica Acta - Bioenergetics*, *1861*(4). https://doi.org/10.1016/J.BBABIO.2019.05.003

Büchel, C. (2020c). *Light-Harvesting Complexes of Diatoms: Fucoxanthin-Chlorophyll Proteins*. 441–457. https://doi.org/10.1007/978-3-030-33397-3_16

Büchel, C., & Garab, G. (1995). Electrochromic absorbance changes in the chlorophyll-c-containing alga Pleurochloris meiringensis (Xanthophyceae). *Photosynthesis Research*, *43*, 49–56.

Büchel, C., Wilhelm, C., & Lenartz-Weiler, I. (1988). The Molecular Analysis of the Light Adaptation Reactions in the Yellow-green Alga Pleurochloris meiringensis (Xanthophyceae). *Botanica Acta*, *101*(4), 306–310. https://doi.org/10.1111/J.1438-8677.1988.TB00049.X

Buck, J. M., Kroth, P. G., & Lepetit, B. (2021). Identification of sequence motifs in Lhcx proteins that confer qE-based photoprotection in the diatom Phaeodactylum tricornutum. *The Plant Journal*, *108*(6), 1721–1734. https://doi.org/10.1111/TPJ.15539

Buck, J. M., Sherman, J., Bártulos, C. R., Serif, M., Halder, M., Henkel, J., Falciatore, A., Lavaud, J., Gorbunov, M. Y., Kroth, P. G., Falkowski, P. G., & Lepetit, B. (2019). Lhcx proteins provide photoprotection via thermal dissipation of absorbed light in the diatom Phaeodactylum tricornutum. *Nature Communications 2019 10:1*, *10*(1), 1–12. https://doi.org/10.1038/s41467-019-12043-6

Buck, J. M., Wünsch, M., Schober, A. F., Kroth, P. G., & Lepetit, B. (2022). Impact of Lhcx2 on Acclimation to Low Iron Conditions in the Diatom Phaeodactylum tricornutum. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/FPLS.2022.841058

Buck-Wiese, H., Andskog, M. A., Nguyen, N. P., Bligh, M., Asmala, E., Vidal-Melgosa, S., Liebeke, M., Gustafsson, C., & Hehemann, J. H. (2023). Fucoid brown algae inject fucoidan carbon into the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(1), e2210561119. https://doi.org/10.1073/PNAS.2210561119/SUPPL_FILE/PNAS.2210561119.SAPP.PDF

Burton-Smith, R. N., Akimasa Watanabe, X., Ryutaro Tokutsu, X., Song, C., Kazuyoshi Murata, X., & Jun Minagawa, X. (2019). Structural determination of the large photosystem IIâ€“light-harvesting complex II supercomplex of Chlamydomonas reinhardtii using nonionic amphipol. *J. Biol. Chem*, *294*(41), 15003. https://doi.org/10.1074/jbc.RA119.009341

Cancela, A., Pérez, L., Febrero, A., Sánchez, A., Salgueiro, J. L., & Ortiz, L. (2019). Exploitation of Nannochloropsis gaditana biomass for biodiesel and pellet production. *Renewable Energy*, *133*, 725–730. https://doi.org/10.1016/j.renene.2018.10.075

Cao, S., Zhang, X., Fan, X., Qiao, H., Liang, C., Xu, D., Mou, S., Wang, W., & Ye, N. (2013). Phylogeny and characterisation of Nannochloropsis oceanica var. sinensis var. nov. (Eustigmatophyceae), a new oleaginous alga from China. *Phycologia*, *52*(6), 573–577. https://doi.org/10.2216/13-164.1

Cao, S., Zhang, X., Xu, D., Fan, X., Mou, S., Wang, Y., Ye, N., & Wang, W. (2013). A transthylakoid proton gradient and inhibitors induce a non-photochemical fluorescence quenching in unicellular algae Nannochloropsis sp. *FEBS Letters*, *587*(9), 1310–1315. https://doi.org/10.1016/J.FEBSLET.2012.12.031

Carbonera, D., Agostini, A., Di Valentin, M., Gerotto, C., Basso, S., Giacometti, G. M., & Morosinotto, T. (2014). Photoprotective sites in the violaxanthin-chlorophyll a binding Protein (VCP) from Nannochloropsis gaditana. *Biochimica et Biophysica Acta - Bioenergetics*, *1837*(8), 1235–1246. https://doi.org/10.1016/J.BBABIO.2014.03.014

Castillo, T., Ramos, D., García-Beltrán, T., Brito-Bazan, M., & Galindo, E. (2021). Mixotrophic cultivation of microalgae: An alternative to produce high-value metabolites. *Biochemical Engineering Journal*, *176*, 108183. https://doi.org/10.1016/j.bej.2021.108183

Christa, G., Pütz, L., Sickinger, C., Clavijo, J. M., Laetz, E. M. J., Greve, C., & Serôdio, J. (2018). Photoprotective Non-photochemical Quenching Does Not Prevent Kleptoplasts From Net Photoinactivation. *Frontiers in Ecology and Evolution | Www.Frontiersin.Org*, *1*, 121. https://doi.org/10.3389/fevo.2018.00121

Chukhutsina, V. U., Fristedt, R., Morosinotto, T., & Croce, R. (2017). Photoprotection strategies of the alga Nannochloropsis gaditana. *Biochimica et Biophysica Acta - Bioenergetics*, *1858*(7), 544–552. https://doi.org/10.1016/J.BBABIO.2017.05.003

Cock, J. M., Sterck, L., Rouzé, P., Scornet, D., Allen, A. E., Amoutzias, G., Anthouard, V., Artiguenave, F., Aury, J.-M., Badger, J. H., Beszteri, B., Billiau, K., Bonnet, E., Bothwell, J. H., Bowler, C., Boyen, C., Brownlee, C., Carrano, C. J., Charrier, B., … Wincker, P. (2010). *The Ectocarpus genome and the independent evolution of multicellularity in brown algae*. https://doi.org/10.1038/nature09016

Correa-Galvis, V., Redekop, P., Guan, K., Griess, A., Truong, T. B., Wakao, S., Niyogi, K. K., & Jahns, P. (2016). Photosystem II Subunit PsbS Is Involved in the Induction of LHCSR Protein-dependent Energy Dissipation in Chlamydomonas reinhardtii*. *Journal of Biological Chemistry*, *291*(33), 17478–17487. https://doi.org/10.1074/JBC.M116.737312

Cruz, S., Cartaxana, P., Newcomer, R., Dionísio, G., Calado, R., Serôdio, J., Pelletreau, K. N., Rumpho, M. E., & Biological, M. (2015). *Photoprotection in sequestered plastids of sea slugs and respective algal sources*. https://doi.org/10.1038/srep07904

D'Amici, G. M., Huber, C. G., & Zolla, L. (2009). Separation of thylakoid membrane proteins by sucrose gradient ultracentrifugation or blue native-SDS-PAGE two-dimensional electrophoresis. *Methods in Molecular Biology (Clifton, N.J.)*, *528*, 61–70. https://doi.org/10.1007/978-1-60327-310-7_4/COVER

Daskalakis, V., Papadatos, S., & Kleinekathöfer, U. (2019). Fine tuning of the photosystem II major antenna mobility within the thylakoid membrane of higher plants. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1861*(12), 183059. https://doi.org/10.1016/J.BBAMEM.2019.183059

De Meyer, F., Maddalena Venturoli, & Berend Smit. (2008). Hydrophobic Mismatch Can Induce Specific and Selective Membrane Protein Interactions. *The 2008 Spring National Meeting*.

De Souza, A. P., Burgess, S. J., Doran, L., Hansen, J., Manukyan, L., Maryn, N., Gotarkar, D., Leonelli, L., Niyogi, K. K., & Long, S. P. (2022). Soybean photosynthesis and crop yield are improved by accelerating recovery from photoprotection. *Science*, *377*(6608), 851–854. https://doi.org/10.1126/SCIENCE.ADC9831/SUPPL_FILE/SCIENCE.ADC9831_MDAR_REPRODUCIBILITY_CHECKLIST.PDF

Demmig-Adams, B. (1990). Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1020*(1), 1–24. https://doi.org/10.1016/0005-2728(90)90088-L

Demmig-Adams, B., & Adams, W. W. (1996). The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant Science*, *1*(1), 21–26. https://doi.org/10.1016/S1360-1385(96)80019-7

Demmig-Adams, B., & Adams, W. W. (2006a). Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New Phytol.*, *172*(1), 11–21. https://doi.org/10.1111/j.1469-8137.2006.01835.x

Demmig-Adams, B., & Adams, W. W. (2006b). Photoprotection in an ecological context: The remarkable complexity of thermal energy dissipation. *New Phytologist*, *172*(1), 11–21. https://doi.org/10.1111/J.1469-8137.2006.01835.X

Demmig-Adams, B., Koh, S.-C., Cohu, C. M., Muller, O., Stewart, J. J., & Iii, W. W. A. (2014). Non-Photochemical Fluorescence Quenching in Contrasting Plant Species and Environments. *Advances in Photosynthesis and Respiration*, *40*, 531–552. https://doi.org/10.1007/978-94-017-9032-1_24

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., & Gascuel, O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, *36*(Web Server issue). https://doi.org/10.1093/NAR/GKN180

Derelle, R., López-García, P., Timpano, H., & Moreira, D. (2016). A Phylogenomic Framework to Study the Diversity and Evolution of Stramenopiles (=Heterokonts). *Molecular Biology and Evolution*, *33*(11), 2890–2898. https://doi.org/10.1093/MOLBEV/MSW168

Dimier, C., Brunet, C., Geider, R., & Raven, J. (2009). Growth and photoregulation dynamics of the picoeukaryote Pelagomonas calceolata in fluctuating light. *Limnology and Oceanography*, *54*(3), 823–836. https://doi.org/10.4319/LO.2009.54.3.0823

Dittami, S. M., Michel, G., Collén, J., Boyen, C., & Tonon, T. (2010a). Chlorophyll-binding proteins revisited - A multigenic family of light-harvesting and stress

proteins from a brown algal perspective. *BMC Evolutionary Biology*, *10*(1), 1–14. https://doi.org/10.1186/1471-2148-10-365/FIGURES/5

Dittami, S. M., Michel, G., Collén, J., Boyen, C., & Tonon, T. (2010b). Chlorophyll-binding proteins revisited - A multigenic family of light-harvesting and stress proteins from a brown algal perspective. *BMC Evolutionary Biology*, *10*(1), 1–14. https://doi.org/10.1186/1471-2148-10-365/FIGURES/5

Dorrell, R. G., & Bowler, C. (2017). Secondary Plastids of Stramenopiles. *Advances in Botanical Research*, *84*, 57–103. https://doi.org/10.1016/BS.ABR.2017.06.003

Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, *346*(6213). https://doi.org/10.1126/SCIENCE.1258096/ASSET/2313E70A-5C58-4755-A0E6-2E64EE240A09/ASSETS/GRAPHIC/346_1258096_F6.JPEG

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797. https://doi.org/10.1093/NAR/GKH340

Eilers, U., Dietzel, L., Breitenbach, J., Büchel, C., & Sandmann, G. (2016). Identification of genes coding for functional zeaxanthin epoxidases in the diatom Phaeodactylum tricornutum. *Journal of Plant Physiology*, *192*, 64–70. https://doi.org/10.1016/j.jplph.2016.01.006

Eliáš, M. (2021). Protist diversity: Novel groups enrich the algal tree of life. *Current Biology*, *31*(11), R733–R735. https://doi.org/10.1016/J.CUB.2021.04.025

Eppard, M., Krumbein, W. E., Von Haeseler, A., & Rhiel, E. (2000). Characterization of fcp4 and fcp12, two additional genes encoding light harvesting proteins of Cyclotella cryptica (Bacillariophyceae) and phylogenetic analysis of this complex gene family. *Plant Biology*, *2*(3), 283–289. https://doi.org/10.1055/S-2000-3702/ID/36/BIB

Fan, M., Li, M., Liu, Z., Cao, P., Pan, X., Zhang, H., Zhao, X., Zhang, J., & Chang, W. (2015). Crystal structures of the PsbS protein essential for photoprotection in plants. *Nature Structural & Molecular Biology*, *22*. https://doi.org/10.1038/nsmb.3068

Fattore, N., Bellan, A., Pedroletti, L., Vitulo, N., & Morosinotto, T. (2021). Acclimation of photosynthesis and lipids biosynthesis to prolonged nitrogen and phosphorus limitation in Nannochloropsis gaditana. *Algal Research*, *58*, 102368. https://doi.org/10.1016/J.ALGAL.2021.102368

Feng, Y., Li, Z., Li, X., Shen, L., Liu, X., Zhou, C., Zhang, J., Sang, M., Han, G., Yang, W., Kuang, T., Wang, W., & Shen, J.-R. (2023). Structure of a diatom photosystem II supercomplex containing a member of Lhcx family and dimeric FCPII. *Science Advances*.

Fernández-Marín, B., Míguez, F., Becerril, J. M., & García-Plazaola, J. I. (2011). Activation of violaxanthin cycle in darkness is a common response to different

abiotic stresses: A case study in Pelvetia canaliculata. *BMC Plant Biology*, *11*(1), 1–11. https://doi.org/10.1186/1471-2229-11-181/FIGURES/8

Figueroa, F. L., Celis-Plá, P. S. M., Martínez, B., Korbee, N., Trilla, A., & Arenas, F. (2019). Yield losses and electron transport rate as indicators of thermal stress in Fucus serratus (Ochrophyta). *Algal Research*, *41*, 101560. https://doi.org/10.1016/J.ALGAL.2019.101560

Fu, F. X., Zhang, Y., Warner, M. E., Feng, Y., Sun, J., & Hutchins, D. A. (2008). A comparison of future increased CO2 and temperature effects on sympatric Heterosigma akashiwo and Prorocentrum minimum. *Harmful Algae*, *7*(1), 76–90. https://doi.org/10.1016/J.HAL.2007.05.006

García-Mendoza, E., & Colombo-Pallotta, M. F. (2007). The giant kelp Macrocystis pyrifera presents a different nonphotochemical quenching control than higher plants. *New Phytologist*, *173*(3), 526–536. https://doi.org/10.1111/J.1469-8137.2006.01951.X

Garcia-Mendoza, E., Ocampo-Alvarez, H., & Govindjee. (2011). Photoprotection in the brown alga Macrocystis pyrifera: Evolutionary implications. *Journal of Photochemistry and Photobiology B: Biology*, *104*(1–2), 377–385. https://doi.org/10.1016/j.jphotobiol.2011.04.004

Gardian, Z., Litvín, R., Bína, D., & Vácha, F. (2014). Supramolecular organization of fucoxanthin-chlorophyll proteins in centric and pennate diatoms. *Photosynthesis Research*, *121*, 79–86. https://doi.org/10.1007/s11120-014-9998-3

Gardian, Z., Tichy, J., & Vácha, F. (2011). Structure of PSI, PSII and antennae complexes from yellow-green alga Xanthonema debile. *Photosynthesis Research*, *108*, 25–32. https://doi.org/10.1007/s11120-011-9647-z

Ghazaryan, A., Akhtar, P., Garab, G., Lambrev, P. H., & Büchel, C. (2016a). *Involvement of the Lhcx protein Fcp6 of the diatom Cyclotella meneghiniana in the macro-organisation and structural flexibility of thylakoid membranes*. https://doi.org/10.1016/j.bbabio.2016.04.288

Ghazaryan, A., Akhtar, P., Garab, G., Lambrev, P. H., & Büchel, C. (2016b). Involvement of the Lhcx protein Fcp6 of the diatom Cyclotella meneghiniana in the macro-organisation and structural flexibility of thylakoid membranes. *Biochimica et Biophysica Acta - Bioenergetics*, *1857*(9), 1373–1379. https://doi.org/10.1016/j.bbabio.2016.04.288

Gibbs, P. B., & Biggins, J. (1989). Regulation of the distribution of excitation energy in Ochromonas danica, an organism containing a chlorophyll-A/C/carotenoid light harvesting antenna. *Photosynthesis Research*, *21*, 81–91.

Giovagnetti, V., Cataldo, M. L., Conversano, F., & Brunet, C. (2010). Functional relation between growth, photosynthetic rate and regulation in the coastal picoeukaryote Phaeomonas sp. RCC 503 (Pinguiophyceae, Stramenopiles). *Journal of Plankton Research*, *32*(11), 1501–1511. https://doi.org/10.1093/plankt/fbq074

Giovagnetti, V., Jaubert, M., Shukla, M. K., Ungerer, P., Bouly, J. P., Falciatore, A., & Ruban, A. V. (2022). Biochemical and molecular properties of LHCX1, the essential regulator of dynamic photoprotection in diatoms. *Plant Physiology*, *188*(1), 509–525. https://doi.org/10.1093/PLPHYS/KIAB425

Giovagnetti, V., & Ruban, A. V. (2018). The evolution of the photoprotective antenna proteins in oxygenic photosynthetic eukaryotes. *Biochemical Society Transactions*, *46*(5), 1263–1277. https://doi.org/10.1042/BST20170304

Goss, R., Ann Pinto, E., Wilhelm, C., & Richter, M. (2006). The importance of a highly active and ΔpH-regulated diatoxanthin epoxidase for the regulation of the PS II antenna function in diadinoxanthin cycle containing algae. *Journal of Plant Physiology*, *163*(10), 1008–1021. https://doi.org/10.1016/J.JPLPH.2005.09.008

Goss, R., & Jakob, T. (2010a). Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynthesis Research 2010 106:1*, *106*(1), 103–122. https://doi.org/10.1007/S11120-010-9536-X

Goss, R., & Jakob, T. (2010b). Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynthesis Research 2010 106:1*, *106*(1), 103–122. https://doi.org/10.1007/S11120-010-9536-X

Goss, R., & Lepetit, B. (2015). Biodiversity of NPQ. *Journal of Plant Physiology*, *172*, 13–32. https://doi.org/10.1016/j.jplph.2014.03.004

Graf, L., Yang, E. C., Han, K. Y., Küpper, F. C., Benes, K. M., Oyadomari, J. K., Herbert, R. J. H., Verbruggen, H., Wetherbee, R., Andersen, R. A., & Yoon, H. S. (2020). Multigene Phylogeny, Morphological Observation and Re-examination of the Literature Lead to the Description of the Phaeosacciophyceae Classis Nova and Four New Species of the Heterokontophyta SI Clade. *Protist*, *171*(6), 125781. https://doi.org/10.1016/J.PROTIS.2020.125781

Graiff, A., Bartsch, I., Glaser, K., & Karsten, U. (2021). Seasonal Photophysiological Performance of Adult Western Baltic Fucus vesiculosus (Phaeophyceae) Under Ocean Warming and Acidification. *Frontiers in Marine Science*, *8*, 666493. https://doi.org/10.3389/FMARS.2021.666493/BIBTEX

Grossman, A. R., Bhaya, D., Apt, K. E., & Kehoe, D. M. (1995). LIGHT-HARVESTING COMPLEXES IN OXYGENIC PHOTOSYNTHESIS: Diversity, Control, and Evolution. *Https://Doi.Org/10.1146/Annurev.Ge.29.120195.001311*, *29*, 231–288. https://doi.org/10.1146/ANNUREV.GE.29.120195.001311

Grouneva, I., Rokka, A., & Aro, E. M. (2011). The thylakoid membrane proteome of two marine diatoms outlines both diatom-specific and species-specific features of the photosynthetic machinery. *Journal of Proteome Research*, *10*(12), 5338–5353. https://doi.org/10.1021/PR200600F/SUPPL_FILE/PR200600F_SI_015.PDF

Guillou, L., Chrétiennot-Dinet, M. J., Medlin, L. K., Claustre, H., Loiseaux-de Goër, S., & Vaulot, D. (1999). BOLIDOMONAS: A NEW GENUS WITH TWO SPECIES BELONGING TO A NEW ALGAL CLASS, THE BOLIDOPHYCEAE

(HETEROKONTA). *Journal of Phycology*, *35*(2), 368–381. https://doi.org/10.1046/J.1529-8817.1999.3520368.X

Gundermann, K., Wagner, V., Mittag, M., & Büchel, C. (2019). Fucoxanthin-Chlorophyll Protein Complexes of the Centric Diatom Cyclotella Meneghiniana Differ in Lhcx1 and Lhcx6_1 Content. *Plant Physiology*, *179*(4), 1779–1795. https://doi.org/10.1104/PP.18.01363

Hao, T. Bin, Jiang, T., Dong, H. P., Ou, L. jian, He, X., & Yang, Y. F. (2018). Light-harvesting protein Lhcx3 is essential for high light acclimation of Phaeodactylum tricornutum. *AMB Express*, *8*(1), 1–11. https://doi.org/10.1186/S13568-018-0703-3/TABLES/1

Havaux, M., & Niyogi, K. K. (1999). The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proc. Natl Acad. Sci. USA*, *96*(15), 8762–8767. https://doi.org/10.1073/pnas.96.15.8762

Hennige, S. J., Coyne, K. J., Macintyre, H., Liefer, J., & Warner, M. E. (2013). *THE PHOTOBIOLOGY OF HETEROSIGMA AKASHIWO. PHOTOACCLIMATION, DIURNAL PERIODICITY, AND ITS ABILITY TO RAPIDLY EXPLOIT EXPOSURE TO HIGH LIGHT 1*. https://doi.org/10.1111/jpy.12043

Horn, S., Ehlers, K., Fritzsch, G., Gil-Rodríguez, C., Wilhelm, C., & Schnetter, R. (2007). Synchroma grande spec. nov. (Synchromophyceae class. nov., Heterokontophyta): An Amoeboid Marine Alga with Unique Plastid Complexes. *Protist*, *158*, 277–293. https://doi.org/10.1016/j.protis.2007.02.004

Hupel, M., Lecointre, C., Meudec, A., Poupart, N., & Gall, E. A. (2011). Comparison of photoprotective responses to UV radiation in the brown seaweed Pelvetia canaliculata and the marine angiosperm Salicornia ramosissima. *Journal of Experimental Marine Biology and Ecology*, *401*(1–2), 36–47. https://doi.org/10.1016/J.JEMBE.2011.03.004

Ichinomiya, M., Dos Santos, A. L., Gourvil, P., Yoshikawa, S., Kamiya, M., Ohki, K., Audic, S., De Vargas, C., Noël, M. H., Vaulot, D., & Kuwata, A. (2016). Diversity and oceanic distribution of the Parmales (Bolidophyceae), a picoplanktonic group closely related to diatoms. *The ISME Journal 2016 10:10*, *10*(10), 2419–2434. https://doi.org/10.1038/ismej.2016.38

Iwai, M., Grob, P., Iavarone, A. T., Nogales, E., & Niyogi, K. K. (2018). A unique supramolecular organization of photosystem I in the moss Physcomitrella patens. *Nature Plants*, *4*(11), 904–909. https://doi.org/10.1038/s41477-018-0271-1

Jakob, T., Goss, R., & Wilhelm, C. (1999). Activation of Diadinoxanthin De-Epoxidase Due to a Chiororespiratory Proton Gradient in the Dark in the Diatom Phaeodactylum tricornutum. *Plant Biology*, *1*(1), 76–82. https://doi.org/10.1111/J.1438-8677.1999.TB00711.X

Jesus, B., Ventura, P., & Calado, G. (2010). Behaviour and a functional xanthophyll cycle enhance photo-regulation mechanisms in the solar-powered sea slug Elysia

timida (Risso, 1818). *Journal of Experimental Marine Biology and Ecology*, *395*(1–2), 98–105. https://doi.org/10.1016/J.JEMBE.2010.08.021

Jimel, M. S., Kv Iderov, J., & Elster, J. (2020). *Annual Cycle of Mat-FOrming Filamentous Alga Tribonema CF. Minus (Stramenopiles, Xanthophyceae) in Hydro-terrestrial habitats In the High Arctic Revealed By Multiparameter Fluorescence Staining*. https://doi.org/10.1111/jpy.13109-20-203

Johnson, M. P., & Ruban, A. v. (2011). Restoration of Rapidly Reversible Photoprotective Energy Dissipation in the Absence of PsbS Protein by Enhanced ΔpH *. *Journal of Biological Chemistry*, *286*(22), 19973–19981. https://doi.org/10.1074/JBC.M111.237255

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. A. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., … Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature 2021 596:7873*, *596*(7873), 583–589. https://doi.org/10.1038/s41586-021-03819-2

Kai, A., Yoshii, Y., Nakayama, T., & Inouye, I. (2008). Aurearenophyceae classis nova, a new class of Heterokontophyta based on a new marine unicellular alga Aurearena cruciata gen. et sp. nov. inhabiting sandy beaches. *Protist*, *159*(3), 435–457. https://doi.org/10.1016/J.PROTIS.2007.12.003

Kastner, B., Fischer, N., Golas, M. M., Sander, B., Dube, P., Boehringer, D., Hartmuth, K., Deckert, J., Hauer, F., Wolf, E., Uchtenhagen, H., Urlaub, H., Herzog, F., Peters, J. M., Poerschke, D., Lührmann, R., & Stark, H. (2007). GraFix: sample preparation for single-particle electron cryomicroscopy. *Nature Methods 2008 5:1*, *5*(1), 53–55. https://doi.org/10.1038/nmeth1139

Kawachi, M., Inouye, I., Honda, D., Charles, ‡, O'kelly, J., Bailey, J. C., Robert, §, Bidigare, R., & Andersen, R. A. (2002). The Pinguiophyceae classis nova , a new class of photosynthetic stramenopiles whose members produce large amounts of omega-3 fatty acids. *Phycological Research*, *50*, 31–47. https://doi.org/10.1046/j.1440-1835.2002.00260.x

Keeling, P. J., & Burki, F. (2019). Progress towards the Tree of Eukaryotes. *Current Biology*, *29*, R808–R817. https://doi.org/10.1016/j.cub.2019.07.031

Khoroshyy, P., David Bína, ·, Gardian, Z., Litvín, R., Alster, J., & Pšenčík, J. (2018). Quenching of chlorophyll triplet states by carotenoids in algal light-harvesting complexes related to fucoxanthin-chlorophyll protein. *Photosynthesis Research*, *135*, 213–225. https://doi.org/10.1007/s11120-017-0416-5

Kilian, O., Benemann, C. S. E., Niyogi, K. K., & Vick, B. (2011). High-efficiency homologous recombination in the oil-producing alga Nannochloropsis sp. *Proceedings of the National Academy of Sciences*, *108*(52), 21265–21269. https://doi.org/10.1073/pnas.1105861108

Kirst, H., Formighieri, C., & Melis, A. (2014). Maximizing photosynthetic efficiency and culture productivity in cyanobacteria upon minimizing the phycobilisome light-harvesting antenna size. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(10), 1653–1664. https://doi.org/10.1016/J.BBABIO.2014.07.009

Kirst, H., Gabilly, S. T., Niyogi, K. K., Lemaux, P. G., & Melis, A. (2017). Photosynthetic antenna engineering to improve crop yields. *Planta*, *245*(5), 1009–1020. https://doi.org/10.1007/s00425-017-2659-y

Korbee, N., Navarro, N. P., García-Sánchez, M., Celis-Plá, P. S., Quintano, E., Copertino, M. S., Pedersen, A., Mariath, R., Mangaiyarkarasi, N., Pérez-Ruzafa, Figueroa, F. L., & Martínez, B. (2014). A novel in situ system to evaluate the effect of high CO2 on photosynthesis and biochemistry of seaweeds. *Aquatic Biology*, *22*, 245–259. https://doi.org/10.3354/AB00594

Kouřil, R., Dekker, J. P., & Boekema, E. J. (2012). Supramolecular organization of photosystem II in green plants. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1817*(1), 2–12. https://doi.org/10.1016/J.BBABIO.2011.05.024

Krause, G. H., & Krause, G. H. (1988). Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiologia Plantarum*, *74*(3), 566–574. https://doi.org/10.1111/J.1399-3054.1988.TB02020.X

Krogmann, D. W., Jagendorf, A. T., & Avron, M. (1959). Uncouplers of Spinach Chloroplast Photosynthetic Phosphorylation. *Plant Physiology*, *34*(3), 272. https://doi.org/10.1104/PP.34.3.272

Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S. T., Iwai, M., Niyogi, K. K., & Long, S. P. (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science*, *354*(6314). https://doi.org/10.1126/science.aai8878

Kuczynska, P., Jemiola-Rzeminska, M., Nowicka, B., Jakubowska, A., Strzalka, W., Burda, K., & Strzalka, K. (2020). The xanthophyll cycle in diatom Phaeodactylum tricornutum in response to light stress. *Plant Physiology and Biochemistry*, *152*, 125–137. https://doi.org/10.1016/J.PLAPHY.2020.04.043

Kumazawa, M., Nishide, H., Nagao, | Ryo, Inoue-Kashino, N., Shen, J.-R., Nakano, T., Uchiyama, I., Kashino, Y., & Ifuku, K. (2021). *Molecular phylogeny of fucoxanthin-chlorophyll a/c proteins from Chaetoceros gracilis and Lhcq/Lhcf diversity*. https://doi.org/10.1111/ppl.13598

Kurita, T., Iwai, M., Ohta, H., Sakuma, T., & Yamamoto, T. (2023). Genome editing for biodiesel production in oleaginous microalga, Nannochloropsis species. *Gene and Genome Editing*, *6*, 100027. https://doi.org/10.1016/J.GGEDIT.2023.100027

Kvernvik, A. C., Hoppe, C. J. M., Lawrenz, E., Prášil, O., Greenacre, M., Wiktor, J. M., & Leu, E. (2018). Fast reactivation of photosynthesis in arctic phytoplankton during the polar night. *Journal of Phycology*, *54*(4), 461–470. https://doi.org/10.1111/JPY.12750

Kvíderová, J., Souquieres, C.-E., & Elster, J. (2018). *Ecophysiology of photosynthesis of Vaucheria sp. mats in a Svalbard tidal flat*. https://doi.org/10.1016/j.polar.2018.11.006

Lacour, T., Babin, M., & Lavaud, J. (2020). Diversity in Xanthophyll Cycle Pigments Content and Related Nonphotochemical Quenching (NPQ) Among Microalgae: Implications for Growth Strategy and Ecology. *Journal of Phycology*, *56*(2), 245–263. https://doi.org/10.1111/JPY.12944

Lacour, T., Morin, P. I., Sciandra, T., Donaher, N., Campbell, D. A., Ferland, J., & Babin, M. (2019). Decoupling light harvesting, electron transport and carbon fixation during prolonged darkness supports rapid recovery upon re-illumination in the Arctic diatom Chaetoceros neogracilis. *Polar Biology*, *42*(10), 1787–1799. https://doi.org/10.1007/S00300-019-02507-2/FIGURES/7

Lavaud, J., & Goss, R. (2014). The Peculiar Features of Non-Photochemical Fluorescence Quenching in Diatoms and Brown Algae. *Advances in Photosynthesis and Respiration*, *40*, 421–443. https://doi.org/10.1007/978-94-017-9032-1_20

Lavaud, J., & Kroth, P. G. (2006). In diatoms, the transthylakoid proton gradient regulates the photoprotective non-photochemical fluorescence quenching beyond its control on the xanthophyll cycle. *Plant and Cell Physiology*, *47*(7), 1010–1016. https://doi.org/10.1093/PCP/PCJ058

Lavaud, J., Materna, A. C., Sturm, S., Vugrinec, S., & Kroth, P. G. (2012). Silencing of the Violaxanthin De-Epoxidase Gene in the Diatom Phaeodactylum tricornutum Reduces Diatoxanthin Synthesis and Non-Photochemical Quenching. *PLOS ONE*, *7*(5), e36806. https://doi.org/10.1371/JOURNAL.PONE.0036806

Lavaud, J., Rousseau, B., & Etienne, A. (2004). General features of photoprotection by energy dissipation in planktonic diatoms (Bacillariophyceae). *J Phycol*, *40*.

Lavaud, J., Rousseau, B., Van Gorkom, H. J., & Etienne, A. L. (2002). Influence of the Diadinoxanthin Pool Size on Photoprotection in the Marine Planktonic Diatom Phaeodactylum tricornutum. *Plant Physiology*, *129*(3), 1398–1406. https://doi.org/10.1104/PP.002014

Leonelli, L., Erickson, E., Lyska, D., & Niyogi, K. K. (2016). Transient expression in Nicotiana benthamiana for rapid functional analysis of genes involved in non-photochemical quenching and carotenoid biosynthesis. *The Plant Journal*, *88*(3), 375–386. https://doi.org/10.1111/TPJ.13268

Lepetit, B., Gélin, G., Lepetit, M., Sturm, S., Vugrinec, S., Rogato, A., Kroth, P. G., Falciatore, A., & Lavaud, J. (2017). The diatom Phaeodactylum tricornutum adjusts nonphotochemical fluorescence quenching capacity in response to dynamic light via fine-tuned Lhcx and xanthophyll cycle pigment synthesis. *New Phytologist*, *214*(1), 205–218. https://doi.org/10.1111/NPH.14337

Li, D. H., Wilson, S., Mastroianni, G., & Ruban, A. V. (2023). Altered lipid acyl chain length controls energy dissipation in light-harvesting complex II proteoliposomes by hydrophobic mismatch. *Journal of Photochemistry and Photobiology B: Biology*, *246*, 112758. https://doi.org/10.1016/J.JPHOTOBIOL.2023.112758

Li, X. M., Zhang, Q. S., Tang, Y. Z., Yu, Y. Q., Liu, H. L., & Li, L. X. (2014). Highly efficient photoprotective responses to high light stress in Sargassum thunbergii germlings, a representative brown macroalga of intertidal zone. *Journal of Sea Research*, *85*, 491–498. https://doi.org/10.1016/J.SEARES.2013.08.004

Li, X. P., Björkman, O., Shih, C., Grossman, A. R., Rosenquist, M., Jansson, S., & Niyogi, K. K. (2000). A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature 2000 403:6768*, *403*(6768), 391–395. https://doi.org/10.1038/35000131

Li, X. P., Gilmore, A. M., Caffarri, S., Bassi, R., Golan, T., Kramer, D., & Niyogi, K. K. (2004). Regulation of Photosynthetic Light Harvesting Involves Intrathylakoid Lumen pH Sensing by the PsbS Protein. *Journal of Biological Chemistry*, *279*(22), 22866–22874. https://doi.org/10.1074/JBC.M402461200

Li, Z., Peers, G., Dent, R. M., Bai, Y., Yang, S. Y., Apel, W., Leonelli, L., & Niyogi, K. K. (2016). Evolution of an atypical de-epoxidase for photoprotection in the green lineage. *Nature Plants 2016 2:10*, *2*(10), 1–6. https://doi.org/10.1038/nplants.2016.140

Lichtlé, C., Arsalane, W., Duval, J. C., & Passaquet, C. (1995). CHARACTERIZATION OF THE LIGHT-HARVESTING COMPLEX OF GIRAUDYOPSIS STELLIFER (CHRYSOPHYCEAE) AND EFFECTS OF LIGHT STRESS1. *Journal of Phycology*, *31*(3), 380–387. https://doi.org/10.1111/J.0022-3646.1995.00380.X

Lie, A. A. Y., Liu, Z., Terrado, R., Tatters, A. O., Heidelberg, K. B., & Caron, D. A. (2018). *A tale of two mixotrophic chrysophytes: Insights into the metabolisms of two Ochromonas species (Chrysophyceae) through a comparison of gene expression*. https://doi.org/10.1371/journal.pone.0192439

Liesner, D., Fouqueau, L., Valero, M., Roleda, M. Y., Pearson, G. A., Bischof, K., Valentin, K., & Bartsch, I. (2020). Heat stress responses and population genetics of the kelp Laminaria digitata (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations. *Ecology and Evolution*, *10*(17), 9144–9177. https://doi.org/10.1002/ECE3.6569

Litvín, R., Bína, D., Herbstová, M., & Gardian, Z. (2016). Architecture of the light-harvesting apparatus of the eustigmatophyte alga Nannochloropsis oceanica. *Photosynthesis Research*, *130*(1–3), 137–150. https://doi.org/10.1007/S11120-016-0234-1

Litvín, R., David Bína, ·, Miroslava Herbstová, ·, Pazderník, M., Kotabová, E., Gardian, Z., Trtílek, M., Prášil, · Ondřej, & Vácha, F. (2019). *Red-shifted light-harvesting*

*system of freshwater eukaryotic alga Trachydiscus minutus (Eustigmatophyta, Stramenopila)*. *142*, 137–151. https://doi.org/10.1007/s11120-019-00662-5

Liu, J., Song, Y., & Qiu, W. (2017a). Oleaginous microalgae Nannochloropsis as a new model for biofuel production: Review & analysis. *Renewable and Sustainable Energy Reviews*, *72*, 154–162. https://doi.org/10.1016/J.RSER.2016.12.120

Liu, J., Song, Y., & Qiu, W. (2017b). Oleaginous microalgae Nannochloropsis as a new model for biofuel production: Review & analysis. *Renewable and Sustainable Energy Reviews*, *72*, 154–162. https://doi.org/10.1016/J.RSER.2016.12.120

Liu, M., Ding, W., Pan, Y., Hu, H., & Liu, J. (2023). Zeaxanthin epoxidase is involved in the carotenoid biosynthesis and light-dependent growth of the marine alga Nannochloropsis oceanica. *Biotechnology for Biofuels and Bioproducts*, *16*(1), 74. https://doi.org/10.1186/S13068-023-02326-Y

Lohr, M., & Wilhelm, C. (1999a). Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(15), 8784–8789. https://doi.org/10.1073/PNAS.96.15.8784

Lohr, M., & Wilhelm, C. (1999b). Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(15), 8784–8789. https://doi.org/10.1073/PNAS.96.15.8784

Malnoë, A., Schultink, A., Shahrasbi, S., Rumeau, D., Havaux, M., & Niyogi, K. K. (2018). The Plastid Lipocalin LCNP Is Required for Sustained Photoprotective Energy Dissipation in Arabidopsis. *The Plant Cell*, *30*(1), 196–208. https://doi.org/10.1105/tpc.17.00536

Marshall, J. A., & Newman, S. (2002). Differences in photoprotective pigment production between Japanese and Australian strains of Chattonella marina (Raphidophyceae). *Journal of Experimental Marine Biology and Ecology*, *272*, 13–27. www.elsevier.com/locate/jembe

Mazor, Y., Borovikova, A., Caspy, I., & Nelson, N. (2017). *Structure of the plant photosystem I supercomplex at 2.6 Å resolution*. https://doi.org/10.1038/nplants.2017.14

Meng, E. C., Goddard, T. D., Pettersen, E. F., Couch, G. S., Pearson, Z. J., Morris, J. H., Ferrin, T. E., & Thomas Ferrin, C. E. (2023). UCSF ChimeraX: Tools for structure building and analysis. *Protein Science*, *32*(11), e4792. https://doi.org/10.1002/PRO.4792

Michel, G., Tonon, T., Scornet, D., Cock, J. M., & Kloareg, B. (2010). Central and storage carbon metabolism of the brown alga Ectocarpus siliculosus: Insights into the origin and evolution of storage carbohydrates in Eukaryotes. *New Phytologist*, *188*(1), 67–81. https://doi.org/10.1111/J.1469-8137.2010.03345.X

Mock, T., Otillar, R. P., Strauss, J., McMullan, M., Paajanen, P., Schmutz, J., Salamov, A., Sanges, R., Toseland, A., Ward, B. J., Allen, A. E., Dupont, C. L., Frickenhaus, S., Maumus, F., Veluchamy, A., Wu, T., Barry, K. W., Falciatore, A., Ferrante, M. I., … Grigoriev, I. V. (2017). Evolutionary genomics of the cold-adapted diatom Fragilariopsis cylindrus. *Nature 2017 541:7638*, *541*(7638), 536–540. https://doi.org/10.1038/nature20803

Müller, P., Li, X.-P., & Niyogi, K. K. (2001). *Update on Photosynthesis Non-Photochemical Quenching. A Response to Excess Light Energy*. www.plantphysiol.org

Müller-Moulé, P., Conklin, P. L., & Niyogi, K. K. (2002). Ascorbate Deficiency Can Limit Violaxanthin De-Epoxidase Activity in Vivo. *Plant Physiology*, *128*(3), 970–977. https://doi.org/10.1104/PP.010924

Murakami', S., & Packer, L. (1970). Light-induced Changes in the Conformation and Configuration of the Thylakoid Membrane of Ulva and Porphyra Chloroplasts in Vivo. *Plant Physiol*, *45*, 289–299. https://academic.oup.com/plphys/article/45/3/289/6090237

Murchie, E. H., & Ruban, A. V. (2020). Dynamic non-photochemical quenching in plants: from molecular mechanism to productivity. *The Plant Journal*, *101*(4), 885–896. https://doi.org/10.1111/TPJ.14601

Nagao, R., Kato, K., Ifuku, K., Suzuki, T., Kumazawa, M., Uchiyama, I., Kashino, Y., Dohmae, N., Akimoto, S., Shen, J.-R., Miyazaki, N., & Akita, F. (2020). Structural basis for assembly and function of a diatom photosystem I-light-harvesting supercomplex. *Nature Communications*. https://doi.org/10.1038/s41467-020-16324-3

Nagao, R., Kato, K., Suzuki, T., Ifuku, K., Uchiyama, I., Kashino, Y., Dohmae, N., Akimoto, S., Shen, J. R., Miyazaki, N., & Akita, F. (2019). Structural basis for energy harvesting and dissipation in a diatom PSII–FCPII supercomplex. *Nature Plants*, *5*(8), 890–901. https://doi.org/10.1038/s41477-019-0477-x

Nan, G. N., Zhou, X. Q., Zhang, X. M., Zhang, Q. S., Hu, Z. M., Huang, R. P., & Zhang, D. (2022). Xanthophyll cycle-related non-photochemical quenching protects Sargassum thunbergii from high light-induced photoinhibition. *Frontiers in Marine Science*, *9*, 1067596. https://doi.org/10.3389/FMARS.2022.1067596/BIBTEX

Nawrocki, W. J., Liu, X., Raber, B., Hu, C., De Vitry, C., Bennett, D. I. G., & Croce, R. (2021). Molecular origins of induction and loss of photoinhibition-related energy dissipation qI. *Science Advances*, *7*(52), 55. https://doi.org/10.1126/SCIADV.ABJ0055/SUPPL_FILE/SCIADV.ABJ0055_SM.PDF

Nepper-Davidsen, J. N., Andersen, D. T., & Foldager, M. F. (2019). Exposure to simulated heatwave scenarios causes long-term reductions in performance in

Saccharina latissima. *Marine Ecology Progress Series*, *630*(630), 25–39. https://doi.org/10.3354/meps13133

Neubauer, C. (1993). Multiple Effects of Dithiothreitol on Nonphotochemical Fluorescence Quenching in Intact Chloroplasts (Influence on Violaxanthin De-epoxidase and Ascorbate Peroxidase Activity). *Plant Physiology*, *103*(2), 575–583. https://doi.org/10.1104/PP.103.2.575

Nilkens, M., Kress, E., Lambrev, P., Miloslavina, Y., Müller, M., Holzwarth, A. R., & Jahns, P. (2010). Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in Arabidopsis. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1797*(4), 466–475. https://doi.org/10.1016/J.BBABIO.2010.01.001

Niyogi, K. K., Björkman, O., & Grossman, A. R. (1997a). Chlamydomonas Xanthophyll Cycle Mutants Identified by Video Imaging of Chlorophyll Fluorescence Quenching. *The Plant Cell*, *9*(8), 1369–1380. https://doi.org/10.1105/TPC.9.8.1369

Niyogi, K. K., Björkman, O., & Grossman, A. R. (1997b). The roles of specific xanthophylls in photoprotection. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(25), 14162–14167. https://doi.org/10.1073/PNAS.94.25.14162

Niyogi, K. K., Grossman, A. R., & Björkman, O. (1998). Arabidopsis mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. *The Plant Cell*, *10*(7), 1121–1134. https://doi.org/10.1105/TPC.10.7.1121

Niyogi, K. K., Shih, C., Soon Chow, W., Pogson, B. J., DellaPenna, D., & Björkman, O. (2001). Photoprotection in a zeaxanthin- and lutein-deficient double mutant of Arabidopsis. *Photosynthesis Research 2001 67:1*, *67*(1), 139–145. https://doi.org/10.1023/A:1010661102365

Niyogi, K. K., & Truong, T. B. (2013). Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Current Opinion in Plant Biology*, *16*(3), 307–314. https://doi.org/10.1016/J.PBI.2013.03.011

Nymark, M., Valle, K. C., Brembu, T., Hancke, K., Winge, P., Andresen, K., Johnsen, G., & Bones, A. M. (2009). An Integrated Analysis of Molecular Acclimation to High Light in the Marine Diatom Phaeodactylum tricornutum. *PLOS ONE*, *4*(11), e7743. https://doi.org/10.1371/JOURNAL.PONE.0007743

Nymark, M., Valle, K. C., Hancke, K., Winge, P., Andresen, K., Johnsen, G., Bones, A. M., & Brembu, T. (2013). Molecular and Photosynthetic Responses to Prolonged Darkness and Subsequent Acclimation to Re-Illumination in the Diatom Phaeodactylum tricornutum. *PLOS ONE*, *8*(3), e58722. https://doi.org/10.1371/JOURNAL.PONE.0058722

Ocampo-Alvarez, H., & García-Mendoza, E. (2013). Antagonist effect between violaxanthin and de-epoxidated pigments in nonphotochemical quenching induction

in the qE deficient brown alga Macrocystis pyrifera. *Biochimica et Biophysica Acta*, *1827*, 427–437. https://doi.org/10.1016/j.bbabio.2012.12.006

Ohi, M., Li, Y., Cheng, Y., & Walz, T. (2004). Negative Staining and Image Classification - Powerful Tools in Modern Electron Microscopy. *Biological Procedures Online*, *6*(1), 23–34. https://doi.org/10.1251/BPO70

Olaizola, M., La Roche, J., Kolber, Z., & Falkowski, P. G. (1994). Non-photochemical fluorescence quenching and the diadinoxanthin cycle in a marine diatom. *Photosynthesis Research 1994 41:2*, *41*(2), 357–370. https://doi.org/10.1007/BF00019413

Owens, T. G., Gallagher, J. C., & Alberte, R. S. (1987). PHOTOSYNTHETIC LIGHT-HARVESTING FUNCTION OF VIOLAXANTHIN IN NANNOCHLOROPSIS SPP. (EUSTIGMATOPHYCEAE). *Journal of Phycology*, *23*(2), 79–85. https://doi.org/10.1111/J.1529-8817.1987.TB04429.X

Park, S., Steen, C. J., Lyska, D., Fischer, A. L., Endelman, B., Iwai, M., Niyogi, K. K., & Fleming, G. R. (2019). Chlorophyll-carotenoid excitation energy transfer and charge transfer in Nannochloropsis oceanica for the regulation of photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(9), 3385–3390. https://doi.org/10.1073/pnas.1819011116

Peers, G., Truong, T. B., Ostendorf, E., Busch, A., Elrad, D., Grossman, A. R., Hippler, M., & Niyogi, K. K. (2009). An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. *Nature*, *462*(7272), 518–521. https://doi.org/10.1038/NATURE08587

Perin, G., Bellan, A., Lyska, D., Niyogi, K. K., & Morosinotto, T. (2022). *Modulation of xanthophyll cycle impacts biomass productivity in the marine microalga Nannochloropsis 2 3*. https://doi.org/10.1101/2022.08.16.504082

Perin, G., Bellan, A., Michelberger, T., Lyska, D., Wakao, S., Niyogi, K. K., & Morosinotto, T. (2023). Modulation of xanthophyll cycle impacts biomass productivity in the marine microalga Nannochloropsis . *Proceedings of the National Academy of Sciences*, *120*(25), e2214119120. https://doi.org/10.1073/PNAS.2214119120/SUPPL_FILE/PNAS.2214119120.SAPP.PDF

Perin, G., Bellan, A., Segalla, A., Meneghesso, A., Alboresi, A., & Morosinotto, T. (2015). Generation of random mutants to improve light-use efficiency of Nannochloropsis gaditana cultures for biofuel production. *Biotechnology for Biofuels*, *8*(1), 1–13. https://doi.org/10.1186/S13068-015-0337-5/FIGURES/7

Pi, X., Tian, L., Dai, H. E., Qin, X., Cheng, L., Kuang, T., Sui, S. F., & Shen, J. R. (2018). Unique organization of photosystem I-light-harvesting supercomplex revealed by cryo-EM from a red alga. *Proc. Natl Acad. Sci. USA*, *115*(17), 4423–4428. https://doi.org/10.1073/pnas.1722482115

Pi, X., Zhao, S., Wang, W., Liu, D., Xu, C., Han, G., Kuang, T., Sui, S.-F., & Shen, J.-R. (2019). The pigment-protein network of a diatom photosystem II-light-harvesting antenna supercomplex. *Science (New York, N.Y.)*, *365*(6452), eaax4406. https://doi.org/10.1126/science.aax4406

Pinnola, A., Dall'Osto, L., Gerotto, C., Morosinotto, T., Bassi, R., & Alboresi, A. (2013). Zeaxanthin Binds to Light-Harvesting Complex Stress-Related Protein to Enhance Nonphotochemical Quenching in Physcomitrella patens. *The Plant Cell*, *25*(9), 3519–3534. https://doi.org/10.1105/TPC.113.114538

Porra, R. J., Thompson, W. A., & Kriedemann, P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *975*(3), 384–394. https://doi.org/10.1016/S0005-2728(89)80347-0

Qin, X., Pi, X., Wang, W., Han, G., Zhu, L., Liu, M., Cheng, L., Shen, J. R., Kuang, T., & Sui, S. F. (2019). Structure of a green algal photosystem I in complex with a large number of light-harvesting complex I subunits. *Nat. Plants*, *5*(3), 263–272. https://doi.org/10.1038/s41477-019-0379-y

Qin, X., Suga, M., Kuang, T., & Shen, J. R. (2015). Photosynthesis. Structural basis for energy transfer pathways in the plant PSI-LHCI supercomplex. *Science*, *348*(6238), 989–995. https://doi.org/10.1126/science.aab0214

Quaas, T., Berteotti, S., Ballottari, M., Flieger, K., Bassi, R., Wilhelm, C., & Goss, R. (2015). Non-photochemical quenching and xanthophyll cycle activities in six green algal species suggest mechanistic differences in the process of excess energy dissipation. *Journal of Plant Physiology*, *172*, 92–103. https://doi.org/10.1016/J.JPLPH.2014.07.023

Rico, C., Matsuda, Y., Lin, X., Litvin, R., Blommaert, L., Vancaester, E., J Huysman, M. J., Osuna-Cruz, C. M., Lavaud, J., Lepetit, B., Winge, P., Bones, A. M., Vandepoele, K., Vyverman, W., & Sabbe, K. (2020). *Light Regulation of LHCX Genes in the Benthic Diatom Seminavis robusta*. https://doi.org/10.3389/fmars.2020.00192

Semchonok, D. A., Sathish Yadav, K. N., Xu, P., Drop, B., Croce, R., & Boekema, E. J. (2017). Interaction between the photoprotective protein LHCSR3 and C2S2 Photosystem II supercomplex in Chlamydomonas reinhardtii. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1858*(5), 379–385. https://doi.org/10.1016/J.BBABIO.2017.02.015

Shen, L., Huang, Z., Chang, S., Wang, W., Wang, J., Kuang, T., Han, G., Shen, J. R., & Zhang, X. (2019). Structure of a C2S2M2N2-type PSII–LHCII supercomplex from the green alga Chlamydomonas reinhardtii. *Proceedings of the National Academy*

*of Sciences of the United States of America*, *116*(42), 21246–21255. https://doi.org/10.1073/pnas.1912462116

Shimasaki, Y., Mukai, K., Takai, Y., Qiu, X., & Oshima, Y. (2021). *antioxidants Recent Progress in the Study of Peroxiredoxin in the Harmful Algal Bloom Species Chattonella marina*. https://doi.org/10.3390/antiox10020162

Short, A., Fay, T. P., Crisanto, T., Mangal, R., Niyogi, K. K., Limmer, D. T., & Fleming, G. R. (2023). Kinetics of the xanthophyll cycle and its role in photoprotective memory and response. *Nature Communications 2023 14:1*, *14*(1), 1–10. https://doi.org/10.1038/s41467-023-42281-8

Short, A. H., Fay, T. P., Crisanto, T., Hall, J., Steen, C. J., Niyogi, K. K., Limmer, D. T., & Fleming, G. R. (2022). Xanthophyll-cycle based model of the rapid photoprotection of Nannochloropsis in response to regular and irregular light/dark sequences. *The Journal of Chemical Physics*, *156*(20), 205102. https://doi.org/10.1063/5.0089335

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology*, *7*. https://doi.org/10.1038/MSB.2011.75

Simionato, D., Basso, S., Giacometti, G. M., & Morosinotto, T. (2013). Optimization of light use efficiency for biofuel production in algae. *Biophys Chem*, *182*, 71–78. https://doi.org/10.1016/j.bpc.2013.06.017

Simionato, D., Block, M. A., la Rocca, N., Jouhet, J., Maréchal, E., Finazzi, G., & Morosinotto, T. (2013). The response of Nannochloropsis gaditana to nitrogen starvation includes de novo biosynthesis of triacylglycerols, a decrease of chloroplast galactolipids, and reorganization of the photosynthetic apparatus. *Eukaryot Cell*, *12*(5), 665–676. https://doi.org/10.1128/ec.00363-12

Simionato, D., Sforza, E., Corteggiani Carpinelli, E., Bertucco, A., Giacometti, G. M., & Morosinotto, T. (2011a). Acclimation of Nannochloropsis gaditana to different illumination regimes: Effects on lipids accumulation. *Bioresource Technology*, *102*(10), 6026–6032. https://doi.org/10.1016/J.BIORTECH.2011.02.100

Simionato, D., Sforza, E., Corteggiani Carpinelli, E., Bertucco, A., Giacometti, G. M., & Morosinotto, T. (2011b). Acclimation of Nannochloropsis gaditana to different illumination regimes: Effects on lipids accumulation. *Bioresource Technology*, *102*(10), 6026–6032. https://doi.org/10.1016/J.BIORTECH.2011.02.100

Six, C., Finkel, Z. V., Rodriguez, F., Marie, D., Partensky, F., & Campbell, D. A. (2008). Contrasting photoacclimation costs in ecotypes of the marine eukaryotic picoplankter Ostreococcus. *Limnology and Oceanography*, *53*(1), 255–265. https://doi.org/10.4319/LO.2008.53.1.0255

Soule, K. M., & Rumpho, M. E. (2012). Light-regulated photosynthetic gene expression and phosphoribulokinase enzyme activity in the heterokont alga vaucheria litorea (xanthophyceae) and its symbiotic molluskan partner elysia chlorotica (gastropoda). *Journal of Phycology*, *48*(2), 373–383. https://doi.org/10.1111/J.1529-8817.2012.01111.X

Streckaite, S., Gardian, Z., Li, F., Pascal, A. A., Litvin, R., Robert, B., & Llansola-Portoles, M. J. (2018). Pigment configuration in the light-harvesting protein of the xanthophyte alga Xanthonema debile. *Photosynthesis Research*, *138*, 139–148. https://doi.org/10.1007/s11120-018-0557-1

Suga, M., Ozawa, S. I., Yoshida-Motomura, K., Akita, F., Miyazaki, N., & Takahashi, Y. (2019). Structure of the green algal photosystem I supercomplex with a decameric light-harvesting complex I. *Nature Plants 2019 5:6*, *5*(6), 626–636. https://doi.org/10.1038/s41477-019-0438-4

Sukenik, A., Livne, A., Apt, K. E., & Grossman, A. R. (2000). CHARACTERIZATION OF A GENE ENCODING THE LIGHT-HARVESTING VIOLAXANTHIN-CHLOROPHYLL PROTEIN OF NANNOCHLOROPSIS SP. (EUSTIGMATOPHYCEAE). *Journal of Phycology*, *36*(3), 563–570. https://doi.org/10.1046/J.1529-8817.2000.99115.X

Sun, K.-M., Gao, C., Zhang, J., Tang, · Xuexi, Wang, Z., Zhang, X., & Li, Y. (2020). Rapid formation of antheraxanthin and zeaxanthin in seconds in microalgae and its relation to non-photochemical quenching. *Photosynthesis Research*, *144*, 317–326. https://doi.org/10.1007/s11120-020-00739-6

Sylak-Glassman, E. J., Malnoe, A., De Re, E., Brooks, M. D., Fischer, A. L., Niyogi, K. K., & Fleming, G. R. (2014). Distinct roles of the photosystem II protein PsbS and zeaxanthin in the regulation of light harvesting in plants revealed by fluorescence lifetime snapshots. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(49), 17498–17503. https://doi.org/10.1073/PNAS.1418317111

Taddei, L., Chukhutsina, V. U., Lepetit, B., Stella, G. R., Bassi, R., van Amerongen, H., Bouly, J. P., Jaubert, M., Finazzi, G., & Falciatore, A. (2018). Dynamic Changes between Two LHCX-Related Energy Quenching Sites Control Diatom Photoacclimation. *Plant Physiology*, *177*(3), 953–965. https://doi.org/10.1104/PP.18.00448

Taddei, L., Stella, G. R., Rogato, A., Bailleul, B., Fortunato, A. E., Annunziata, R., Sanges, R., Thaler, M., Lepetit, B., Lavaud, J., Jaubert, M., Finazzi, G., Bouly, J. P., & Falciatore, A. (2016). Multisignal control of expression of the LHCX protein family in the marine diatom Phaeodactylum tricornutum. *Journal of Experimental Botany*, *67*(13), 3939–3951. https://doi.org/10.1093/JXB/ERW198

Takahashi, H., Iwai, M., Takahashi, Y., & Minagawa, J. (2006). Identification of the mobile light-harvesting complex II polypeptides for state transitions in

Chlamydomonas reinhardtii. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(2), 477–482. https://doi.org/10.1073/PNAS.0509952103

Tanabe, Y., Shitara, T., Kashino, Y., Hara, Y., & Kudoh, S. (2009). Utilizing the Effective Xanthophyll Cycle for Blooming of Ochromonas smithii and O. itoi (Chrysophyceae) on the Snow Surface. *Plos One*. https://doi.org/10.1371/journal.pone.0014690

Teo, C. L., Atta, M., Bukhari, A., Taisir, M., Yusuf, A. M., & Idris, A. (2014). Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths. *Bioresource Technology*, *162*, 38–44. https://doi.org/10.1016/J.BIORTECH.2014.03.113

Tian, L., Nawrocki, W. J., Liu, X., Polukhina, I., Van Stokkum, I. H. M., & Croce, R. (2019). PH dependence, kinetics and light-harvesting regulation of nonphotochemical quenching in Chlamydomonas. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(17), 8320–8325. https://doi.org/10.1073/PNAS.1817796116/SUPPL_FILE/PNAS.1817796116.SAPP.PDF

Tibiletti, T., Auroy, P., Peltier, G., & Caffarri, S. (2016). Chlamydomonas reinhardtii PsbS Protein Is Functional and Accumulates Rapidly and Transiently under High Light. *Plant Physiology*, *171*(4), 2717–2730. https://doi.org/10.1104/PP.16.00572

Uhrmacher, S., Hanelt, D., & Nultsch, W. (1995). Zeaxanthin content and the degree of photoinhibition are linearly correlated in the brown alga Dictyota dichotoma. *Marine Biology*, *123*, 159–165.

Umetani, I., Kunugi, M., Yokono, M., Takabayashi, A., & Tanaka, A. (2018). Evidence of the supercomplex organization of photosystem II and light-harvesting complexes in Nannochloropsis granulata. *Photosynthesis Research*, *136*(1), 49–61. https://doi.org/10.1007/s11120-017-0438-z

Vieler, A., Wu, G., Tsai, C. H., Bullard, B., Cornish, A. J., Harvey, C., Reca, I. B., Thornburg, C., Achawanantakun, R., Buehl, C. J., Campbell, M. S., Cavalier, D., Childs, K. L., Clark, T. J., Deshpande, R., Erickson, E., Armenia Ferguson, A., Handee, W., Kong, Q., … Benning, C. (2012a). Genome, Functional Gene Annotation, and Nuclear Transformation of the Heterokont Oleaginous Alga Nannochloropsis oceanica CCMP1779. *PLoS Genetics*, *8*(11). https://doi.org/10.1371/journal.pgen.1003064

Vieler, A., Wu, G., Tsai, C.-H., Bullard, B., Cornish, A. J., Harvey, C., Reca, I.-B., Thornburg, C., Achawanantakun, R., Buehl, C. J., Campbell, M. S., Cavalier, D., Childs, K. L., Clark, T. J., Deshpande, R., Erickson, E., Armenia Ferguson, A., Handee, W., Kong, Q., … Benning, C. (2012b). Genome, Functional Gene Annotation, and Nuclear Transformation of the Heterokont Oleaginous Alga Nannochloropsis oceanica CCMP1779. *PLoS Genetics*, *8*(11), e1003064. https://doi.org/10.1371/journal.pgen.1003064

Walter, J., & Kromdijk, J. (2022). Here comes the sun: How optimization of photosynthetic light reactions can boost crop yields. *Journal of Integrative Plant Biology*, *64*(2), 564–591. https://doi.org/10.1111/JIPB.13206

Walters, R. G., Ruban, A. v., & Horton, P. (1996). Identification of proton-active residues in a higher plant light-harvesting complex. *Proceedings of the National Academy of Sciences*, *93*(24), 14204–14209. https://doi.org/10.1073/PNAS.93.24.14204

Wang, H., Ji, B., Wang, J., Guo, F., Zhou, W., Gao, L., & Liu, T. Z. (2014). Growth and biochemical composition of filamentous microalgae Tribonema sp. as potential biofuel feedstock. *Bioprocess and Biosystems Engineering*, *37*(12), 2607–2613. https://doi.org/10.1007/S00449-014-1238-X/TABLES/2

Wang, W., Yu, L. J., Xu, C., Tomizaki, T., Zhao, S., Umena, Y., Chen, X., Qin, X., Xin, Y., Suga, M., Han, G., Kuang, T., & Shen, J. R. (2019). Structural basis for blue-green light harvesting and energy dissipation in diatoms. *Science*, *363*(6427). https://doi.org/10.1126/SCIENCE.AAV0365/SUPPL_FILE/AAV0365-WANG-SM.PDF

Wang, W., Zhao, S., Pi, X., Kuang, T., Sui, S. F., & Shen, J. R. (2020). Structural features of the diatom photosystem II–light-harvesting antenna complex. *The FEBS Journal*, *287*(11), 2191–2200. https://doi.org/10.1111/FEBS.15183

Wang, W., Zhao, S., Shen, L., Li, X., Tao, Q., Li, Z., Xu, C., Zhou, C., Yang, Y., Sang, M., & Han, G. (2023). Structural insights into photosystem II supercomplex and trimeric FCP antennae of a centric diatom Cyclotella meneghiniana. *Preprint*. https://doi.org/10.21203/rs.3.rs-3094926/v1

Wetherbee, R., Jackson, C. J., Repetti, S. I., Clementson, L. A., Costa, J. F., van de Meene, A., Crawford, S., & Verbruggen, H. (2019). The golden paradox - a new heterokont lineage with chloroplasts surrounded by two membranes. *Journal of Phycology*, *55*(2), 257–278. https://doi.org/10.1111/JPY.12822

Wilken, S., Choi, C. J., & Worden, A. Z. (2020). Contrasting Mixotrophic Lifestyles Reveal Different Ecological Niches in Two Closely Related Marine Protists. *Journal of Phycology*, *56*(1), 52–67. https://doi.org/10.1111/JPY.12920

Wilken, S., Huisman, J., Naus-Wiezer, S., & Van Donk, E. (2013). Mixotrophic organisms become more heterotrophic with rising temperature. *Ecology Letters*, *16*(2), 225–233. https://doi.org/10.1111/ELE.12033

Xu, C., Pi, X., Huang, Y., Han, G., Chen, X., Qin, X., Huang, G., Zhao, S., Yang, Y., Kuang, T., Wang, W., Sui, S. F., & Shen, J. R. (2020). Structural basis for energy transfer in a huge diatom PSI-FCPI supercomplex. *Nature Communications 2020 11:1*, *11*(1), 1–12. https://doi.org/10.1038/s41467-020-18867-x

Yang, R., Wei, D., & Xie, J. (2020). Diatoms as cell factories for high-value products: chrysolaminarin, eicosapentaenoic acid, and fucoxanthin. *Critical Reviews in Biotechnology*, *40*(7), 993–1009. https://doi.org/10.1080/07388551.2020.1805402

Yoon, H. S., Andersen, R. A., Boo, S. M., & Bhattacharya, D. (2009). Stramenopiles. *Encyclopedia of Microbiology, Third Edition*, 721–731. https://doi.org/10.1016/B978-012373944-5.00253-4

You, X., Zhang, X., Cheng, J., Xiao, Y., Ma, J., Sun, S., Zhang, X., Wang, H.-W., & Sui, S.-F. (2023). In situ structure of the red algal phycobilisome-PSII-PSI-LHC megacomplex. *Nature*, *616*. https://doi.org/10.1038/s41586-023-05831-0

Yuasa, K., Shikata, T., Kitatsuji, S., Yamasaki, Y., & Nishiyama, Y. (2020). *Extracellular secretion of superoxide is regulated by photosynthetic electron transport in the noxious red-tide-forming raphidophyte Chattonella antiqua*. https://doi.org/10.1016/j.jphotobiol.2020.111839

Zhang, X., Xu, D., Guan, Z., Wang, S., Zhang, Y., Wang, W., Zhang, X., Fan, X., Li, F., & Ye, N. (2020). Elevated CO 2 concentrations promote growth and photosynthesis of the brown alga Saccharina japonica. *Journal of Applied Phycology*, *32*, 1949–1959. https://doi.org/10.1007/s10811-020-02108-1

Zhao, L.-S., Wang, P., Li, K., Zhang, Q.-B., He, F.-Y., Li, C.-Y., Su, H.-N., Chen, X.-L., Liu, L.-N., & Zhang, Y.-Z. (2023). *Structural basis and evolution of the photosystem I-light-harvesting supercomplex of cryptophyte algae*. https://doi.org/10.1093/plcell/koad087

Zhu, S. H., & Green, B. R. (2010). Photoprotection in the diatom Thalassiosira pseudonana: Role of LI818-like proteins in response to high light stress. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1797*(8), 1449–1457. https://doi.org/10.1016/J.BBABIO.2010.04.003

# Chapter 4: Towards the Structures of PSI-VCPI and PSII-VCPII Supercomplexes Containing LHCX1 in *Nannochloropsis oceanica*


## Abstract

Recent advancements in single particle cryo-EM have allowed for the determination of high-resolution structures of photosynthetic supercomplexes. Our study focuses on developing methods towards unraveling the architecture and protein makeup of PSI-VCPI and PSII-VCPII in the Eustigmatophyte alga *Nannochloropsis oceanica*. While PSI and PSII reaction centers exhibit evolutionary conservation across eukaryotes, our investigation delves into variations in associated light-harvesting proteins and pigments, crucial for modulating light absorption and facilitating non-photochemical quenching (NPQ). Our methods encompass optimizing isolation of PSI-VCPI and PSII-VCPII supercomplexes that contain LHCX1 and assessing sample quality for single-particle analysis. Negative stain TEM and maltose density gradients with gradient fixation (GraFix) improved our sample quality and supercomplex isolation. Our refined methods set the stage for the first high-resolution structural analyses of Eustigmatophyte supercomplexes through single-particle cryo-EM. This in-depth structural analysis will reveal the overall architecture, protein-protein interactions, and pigment locations in the photosynthetic supercomplexes, leading to inferences on energy-transfer and energy-dissipation pathways in *N. oceanica* and other diverse algae groups.


## Preface

The transmission electron microscopy (TEM) in Figure 1d was done by Reena Zakour. The negative stain TEM was done by Radhika Khera. All other work was done by Thien Crisanto.

## Introduction

Photosystem supercomplexes consist of a reaction center (RC) and accessory light-harvesting complexes (LHCs). The two photosystems, PSI and PSII, are involved in light-energy conversion and contain RCs that use photon energy to drive photochemical electron transport. PSI/II RCs are highly conserved in photosynthetic organisms, but the evolutionary diversity among the supercomplexes arises from variations in the associated LHCs and bound pigments. These LHC and pigment interactions are responsible for modulating the amount of light energy that is harvested and transferring that energy to the RC. They can also be involved in dissipating excess light energy through a photoprotective process called non-photochemical quenching (NPQ). The structures of the photosynthetic supercomplexes have been solved using high-resolution methods like single particle cryogenic electron microscopy (cryo-EM). Cryo-EM allows for biological samples to be imaged in their native state using rapid freezing preservation techniques. With this method, protein-protein interactions and protein-pigment interactions can be resolved at angstrom resolution, allowing for in-depth structural analysis on the overall architecture of photosynthetic supercomplexes.

These high-resolution structural advancements have led to inferences on energy-transfer pathways and broadened our knowledge on evolution amongst photosynthetic organisms.

The PSI RC contains nine evolutionarily conserved subunits called PsaA/B/C/D/E/F/I/J/L, and additional subunits can be found in various groups (Pi et al., 2018; Qin et al., 2015).The PSII RC contains four large subunits, several small subunits, and subunits that make up the lumen-facing oxygen-evolving complex. The four large subunits of PSII are PsbA (D1), PsbD (D2), PsbC (CP43), and PsbB (CP47). Advances have also been made resolving the PS-LHC supercomplexes in Archaeplastida, descendants of primary endosymbiosis. In land plants, such as *Pisum sativum*, the LHCI antenna was found to form a crescent-shaped belt around the PSI core (Mazor et al., 2017). Typically, LHCI forms one belt around the PSI RC, although some species of green algae can contain two LHCI belts (Qin et al., 2019; Suga et al., 2019). The LHCI antenna primarily consists of Lhca proteins that bind carotenoids and chlorophyll (Chl) molecules, with both Chl *a* and *b* located in various locations. For example, green alga *Chlamydomonas reinhardtii* LHCI contains Chl *a*, Chl *b*, and accessory pigments like lutein (Lut), violaxanthin (Vx), and β-carotene (βcar; Suga et al., 2019). The green algal PSII supercomplex is similar in structure to that of plants, with LHCII trimers linked to the PSII core via monomeric LHCII antenna proteins (Burton-Smith et al., 2019; Kouřil et al., 2012). LHCII is composed of Lhcb proteins and Chl *a*/*b*, neoxanthin (Nx), Vx, Lut, βcar, and some green algae contain loroxanthin (Shen et al., 2019, Caffarri et al., 2004). Both green algae and land plants also have stress-related LHCs associated mainly with LHCII called LHCSR and PsbS (X. P. Li et al., 2000; Peers et al., 2009). These proteins are important for NPQ induction, although their cryo-EM structures in association with photosystems have remained elusive (Fan et al., 2015; Semchonok et al., 2017).

The architecture and protein makeup of red algal supercomplexes differ from those in the “green lineage”, accounting for evolutionary diversification after primary endosymbiosis. In the red alga *Cyanidioschyzon merolae*, Lhcr and RedCAP proteins are red-lineage LHCs (Büchel, 2020a) that form a crescent shape around PSI (Antoshvili et al., 2019). *C. merolae* contains the 9 conserved PSI subunits with additions of PsaO/M/K. Differing from the green lineage, red algal PSII does not utilize LHCs and instead utilizes phycobilisomes (PB). PBs are ancient light-harvesting antennas found in cyanobacteria, glaucophytes, and red algae (Grossman et al., 1995). However, the red algal antenna does contain Chl *a* (You et al., 2023). Red algae gave rise to many groups of algae through secondary and higher order endosymbiosis, one of which is the stramenopiles.

Photosynthetic stramenopiles (stramenochromes) include diatoms (Bacillariophyceae), Eustigmatophytes, and brown algae (Phaeophyceae). The photosynthetic apparatus in stramenochromes reveals key diversification and retention of pigments and proteins across evolutionary steps (Büchel, 2015a). For instance, stramenochromes utilize fucoxanthin-chlorophyll *a/c* proteins (FCP) that make up their antenna, which mainly binds Chl *a/c,* fucoxanthin (Fx), diadinoxanthin (Ddx) and/or Vx, vaucheriaxanthin (Vau), and βcar (Büchel, 2020b). These antennas lack Chl *b,* Lut, and Lor pigments. The antenna in stramenochromes more closely resembles LHCs from the green lineage, and they contain LHCSR proteins (called LHCX proteins in this group) involved in photoprotection. However, some components from red algae were retained

like Lhcr proteins, which make up the major light-harvesting proteins in FCPI (Xu et al., 2020), whereas Lhcf proteins make up FCPII (Feng et al., 2023; Nagao et al., 2019; Pi et al., 2019). Some stramenochromes also contain Lhcy and Lhcz proteins that can be also found in other secondary and tertiary endosymbiotic groups. These Lhcs mainly function in light-harvesting, although other roles are still being investigated, and their structures have not been resolved (Dittami et al., 2010a; Kumazawa et al., 2021; Nymark et al., 2013; Umetani et al., 2018).

Diatoms are the most well studied stramenochromes in terms of photosynthesis and photoprotection. Recent work using cryo-EM has revealed the PSI-FCPI and PSII-FCPII structures in diatoms, although overall structure can differ between centric and pennate forms and within species (Wang et al., 2020, Arshad et al., 2021; Wang et al., 2023). Two independent studies on PSI-FCPI revealed that the centric diatom *Chaetoceros gracilis* (Cg) has a very large supercomplex (Nagao et al., 2020; Xu et al., 2020). The analysis showed that 16-24 different FPCI subunits form an inner, middle, and outer layer around the PSI core. This large FCPI has similarities to red algal LHCI and a 4-belted antenna found in a Cryptophyte, *Chroomonas placoidea* (Zhao et al., 2023, Antoshvili et al., 2019). Recent cryo-EM studies on PSII-FCPII in centric diatoms shows structural variability among species. For example, Cg has tetrameric FCPII associations with the PSII core (Nagao et al., 2019; Pi et al., 2019), whereas *Thalassiosira pseudonana* contains homodimeric and heterodimeric forms of FCPII (Feng et al., 2023). This variability among FCPII structures may allow each organism to take advantage of different ecological niches and light environments.

Recently, a member of the LHCX/LHCSR family has been resolved using cryo-EM. Previously, *T. pseudonana* and *Cyclotella meneghiniana* LHCX6_1 were shown to be constitutively expressed and may be involved in NPQ (Grouneva et al., 2011; Gundermann et al., 2019). The structure of monomeric TpLHCX6_1 was shown in complex with PSII-FCPII, binding Chl *a/c*, Fx, and Ddx molecules (Feng et al., 2023). TpLHCX6_1 also directly links homodimer LHCF7 to the PSII core. These new insights suggest that TpLHCX6_1 may be involved in quenching excess energy at the Ddx/Dtx site close to the core and may mediate aggregation of quenching from more flexible peripheral FCPs like LHCF7.

While structural analysis on diatoms provides valuable insights for photosynthesis and photoprotection in stramenochromes, there is still much to learn in other groups. Eustigmatophytes are distinct from other stramenochromes in that they lack both Chl *c* and Fx and mainly utilize Chl *a* and Vx for light harvesting (Amaral et al., 2021). As such, Eustigmatophytes utilize Lhcv proteins to make up their violaxanthin chlorophyll *a*-binding protein (VCP) antenna (Litvín et al., 2016). Eustigmatophytes in the genus *Nannochloropsis* have been touted as high-performing feedstocks for biofuel production due to their high lipid content and ability to generate rapid biomass rapidly (J. Liu et al., 2017b). There has been evidence for the supercomplex organization in *Nannochloropsis* through pigment and protein characterization and TEM, although no studies have resolved these structures using single particle cryo-EM (Alboresi et al., 2017; Basso et al., 2014; Bína, Gardian, et al., 2017; Carbonera et al., 2014; Litvín et al., 2016). Resolving the structures of the PS-VCP supercomplexes in this alga will broaden the scope of work that can be done towards increasing photosynthetic efficiency for lipid production. We also know that LHCX1 is an LHCX protein that is

important for NPQ in *N. oceanica* (Chapter 2, Park et al., 2019; Perin et al., 2023) and seek to resolve its structure to make inferences about energy-dissipation pathways and NPQ in this organism. In this study we describe progress towards resolving the PSI-VCPI and PSII-VCPII supercomplexes with LHCX1 through single particle cryo-EM.

## Methods

### Strain and Growth Conditions

*Nannochloropsis oceanica* CCMP1779 was obtained from The National Center for Marine Algae and Microbiota (https://ncma.bigelow.org/) and cultivated in F2N medium (Kilian et al., 2011). Algal cells were cultured in 100 mL of F2N to mid-log phase (1 x $10^7$ cells/mL) before being transferred to 13 L of F2N and grown with ambient $CO_2$ bubbling and stirring, until they reached ~1x $10^{12}$ cells total (about 3 weeks after transfer). All cultures were grown under continuous 30 µmol photons $m^{-2}$ $s^{-1}$ in 23 °C.

### Sucrose Cushion Centrifugation and Chloroplast Isolation

Cells from liquid culture were centrifuged at 4000 RPM for 10 min. Cells were resuspended in residual F2N and brought up to 45 mL with 0.33 MS buffer (25 mM MES-NaOH pH 6.5, 0.33 M sucrose, 1.5 mM NaCl, 0.2 mM benzamidine, 1 mM aminocaproic acid) before being split into 6 tubes. About 8 mL of cells were broken open at a time via a Multi Shot Cell Disruptor (Pressure BioSciences Inc., South Easton, MA, USA) at 0, 10, 20, and 30 kPSI with 1 or 2 passages through the cell disruptor. 20 kPSI x 1 was used for the remaining experiments. Disrupted cells were then pipetted onto 6 sucrose cushions. The sucrose cushion is as follows from bottom to top: 10 mL of 1.8 MS buffer (25mM MES-NaOH pH 6.5, 1.8 M sucrose, 1.5mM NaCl), 10 mL of 1.3 MS buffer (25 mM MES-NaOH pH 6.5, 1.3M sucrose, 1.5mM NaCl), and ~4mL of 0.33 MS buffer + 8mL of disrupted cells on top. Sucrose cushion tubes were ultracentrifuged at 27,000 RPM (SW 32 Ti rotor, Beckman Coulter) at 4°C for 30 min, and the separated chloroplast layer was collected by pipetting off the top band. This layer was then pelleted by centrifugation at 15,000 RPM (JA-20 rotor, Beckman Coulter) for 15 min, and the resulting pellets were resuspended in less than 400 µL of 0.33 MS buffer. Chlorophyll (Chl) *a* concentration was determined by diluting a small sample of chloroplasts (1:200) with 80% acetone and following methods in Porra et al., 1989. Total Chl *a* measured about 2000-2500 µg Chl *a*. Chloroplasts were divided into aliquots of 200 µg Chl *a* then flash frozen in liquid nitrogen for storage at -80°C.

### Bright-Field and Transmission Electron Microscopy (TEM)

Bright-field (BF) microscopy was conducted using an Axio Imager 2 (Zeiss, Germany) using 10 µL of liquid samples from each sucrose cushion band. For transmission electron microscopy (TEM), sample preparation and sectioning were performed by the University of California Berkeley Electron Microscope Laboratory. Cell pellets were fixed for 30 min at room temperature in 2.5% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4. Fixed cells were stabilized in 1% very low melting-point

agarose and then cut into small cubes. The cubed sample was then rinsed three times at room temperature for 10 min in 0.05 M sodium cacodylate buffer, pH 7.4 and then immersed in 1% osmium tetroxide with 1.6% potassium ferricyanide in 0.05 M cacodylate buffer for an hour in the dark on a rocker. Samples were later rinsed three times with cacodylate buffer and then subjected to an ascending series of acetone for 10 min each (35%, 50%, 75%, 80%, 90%, 100%, 100%). Samples were progressively infiltrated with Epon resin (EMS, Hatfield, PA, USA) while rocking and later polymerized at 60°C for 24 h. 70 nm thin sections were cut using an Ultracut E (Leica, Wetzlar, Germany) and collected on 100µm mesh formvar-coated copper grids. The grids were further stained for 5 min with 2% aqueous uranyl acetate and 4 min with Reynold lead citrate. The sections were imaged using a Tecnai 12 TEM at 120 KV (FEI, Hillsboro, OR, USA), and images were collected using UltraScan 1000 digital micrograph software (Gatan Inc., Pleasanton, CA, USA).

## Protein Complex Solubilization, Gradients, and Ultracentrifugation

### Protein Complex Solubilization

Chloroplast aliquots were quickly thawed using warm water and placed on ice. The chloroplasts were then washed using a 25 mM MES buffer (added up to 5 mL) to remove residual sucrose and centrifuged at 14,000 RPM for 7 min at 4°C. The liquid was decanted before resuspending the chloroplasts in ~50 uL of 25 mM MES buffer (pH 6.5). Chloroplasts were transferred to bead-beating tubes with 0.32 g of lysing matrix D beads with detergent added, before bead beating using a FastPrep-24 (MP Biomedicals LLC) for 10 s at 4 m/s. Solubilization conditions were ~200 µg Chl at 0.5 mg Chl/mL, or 1 mg Chl/mL, with 1% (w/v) of α- or β-DM. The resulting chloroplast lysate was removed from the beads and incubated for 30 min on ice with gentle agitation every 5 min. The incubated lysate was centrifuged at 14,000 RPM for 5 min, and the supernatant was loaded onto a sucrose or maltose gradient.

### Sucrose and Maltose Step Gradients

Sucrose and maltose step gradients and ultracentrifugation were carried out as described (Iwai et al., 2018), with few modifications. Density gradient centrifuge tubes were prepared by depositing 6 discrete layers ranging from 0.1–1.4 M maltose, or 0.1–1.8 M sucrose, with 25 mM MES-NaOH (pH 6.5) and 0.03% (w/v) α-DM or β-DM. Lysed and solubilized chloroplasts were loaded onto the prepared step gradient centrifugation tubes and ultracentrifuged at 30,000 RPM (SW 41 Ti rotor, Beckman Coulter) for 24 h at 4°C. The gradient was collected dropwise from the bottom of the tube into a 96-well plate. These samples were immediately concentrated using centrifugal filters (Amicon Ultra, 100 kDa MWCO) or solubilized and processed for SDS-PAGE analysis.

## Maltose Density Linear Gradients with GraFix

Supercomplex fractionation via linear gradient with gradient fixation (GraFix) was performed according to Kastner et al., 2007 with some modifications. The maltose density gradient was provided by a continuous 0.1M to 1.4 M maltose with 25 mM MES-NaOH (pH 6.5) and 0.03% (w/v) α-DM or β-DM, and 0.15% glutaraldehyde fixative was added to the 1.4 M maltose solution. Additionally, a cushion of detergent-containing 0.05 M maltose, 25 mM MES pH 6.5, 0.03% α- or β-DM detergent was added to the top of the gradient to cushion the samples before being loaded. The tubes were ultracentrifuged and processed as indicated above.

## SDS-PAGE, Silver Staining, and Western blotting

Fractionated protein samples were solubilized using 5X sample buffer (20% lithium dodecyl sulfate (LDS), 1 M Tris-HCl (pH 9.0), 1 M Tris-HCl (pH 8.0), 1 M dithiothreitol (DTT), 60% (w/v) sucrose) and dye solution (1% (w/v) bromophenol blue (BPB), 100% (w/v) glycerol). Samples were incubated at room temperature for 30 min with continuous shaking or regular vortexing and centrifuged at 14,000 RPM for 5 min to solubilize proteins and remove insoluble material. Solubilized protein samples were loaded onto a Criterion TGX Precast Midi Protein Gels (Bio-Rad, USA) for SDS-PAGE analysis. For silver staining of protein gels, the protocol was followed via the Pierce Silver Stain Kit (Thermo Fisher Scientific, USA). Silver-stained gels were imaged using a Bio-Rad ChemiDoc Imaging System (Bio-Rad USA).

For Western blotting, the protein gels were blotted onto a PVDF membrane by way of a conventional wet transfer (Bio-Rad, USA). The resulting blotted membranes were blocked with blocking buffer (5% (w/v) powdered milk solution in 1x TBS-T) and washed three times with 1x TBS-T for 15 min with shaking. Membranes were probed with 1:5000 anti-LHCX1, PsaA, PsbC, or VCP primary antibody solutions in TBS-T and allowed to incubate at room temperature for 1 h or overnight at 4°C, then washed three times more with 1x TBS-T. Following the primary antibody reaction, membranes were then incubated with 1:10,000 donkey anti-rabbit HRP secondary antibody solution in 1x TBS-T for 1 h at room temperature. Following secondary antibody reaction, the membranes were washed three times more with TBS-T as described, and protein bands were visualized via ECL reaction using the SuperSignal West Femto Maximum Sensitivity Substrate kit as directed (Thermo Fisher Scientific, USA). Chemiluminescence imaging was provided via the Bio-Rad ChemiDoc Imaging System (Bio-Rad USA).

## HPLC and pigment analysis

Cells were grown to a cell density of 1-2 x $10^7$ cells/ml, and a volume equal to 2 x $10^8$ cells was centrifuged for 3 min at 14,000 RPM. The supernatant was removed, and the pellet was frozen in liquid nitrogen. The cells were thawed at room temperature and frozen in liquid nitrogen a second time. The frozen material was powdered (6.5 m/sec for 60 s) using a Fastprep-24 (MP Bio), resuspended in 100 µl of 80% acetone, and vortexed for 15 s. After centrifugation at 14,000 RPM for 3 min, the supernatant was

collected, and the pellet was extracted a second time as described above. The supernatants were pooled, filtered through a 0.45 µm nylon filter (Thermo Fisher Scientific, Waltham, MA, USA), and separated on Spherisorb S5 column (Waters, Milford, MA) as described by (Müller-Moulé et al., 2002).

## Negative Staining Electron Microscopy

Negative-staining protocols were generally followed using methods described previously (Brenner & Horne, 1959; Ohi et al., 2004). The carbon-layered TEM grids (400µm mesh copper grids) were glow-discharged before applying the protein sample onto the grid. 4 µL of the sample was applied to the grid 1 min to enable the adsorption of protein molecules. The grid was then washed with three 50 µL drops of 1% uranyl formate by inverting the grid and gently rotating it over the drops of the stain placed on parafilm. After the wash steps, the excess stain was blotted away using filter paper. Once the grid was dried, it was imaged with a 120 kV Tecnai 12 TEM (FEI, Hillsboro, OR, USA).

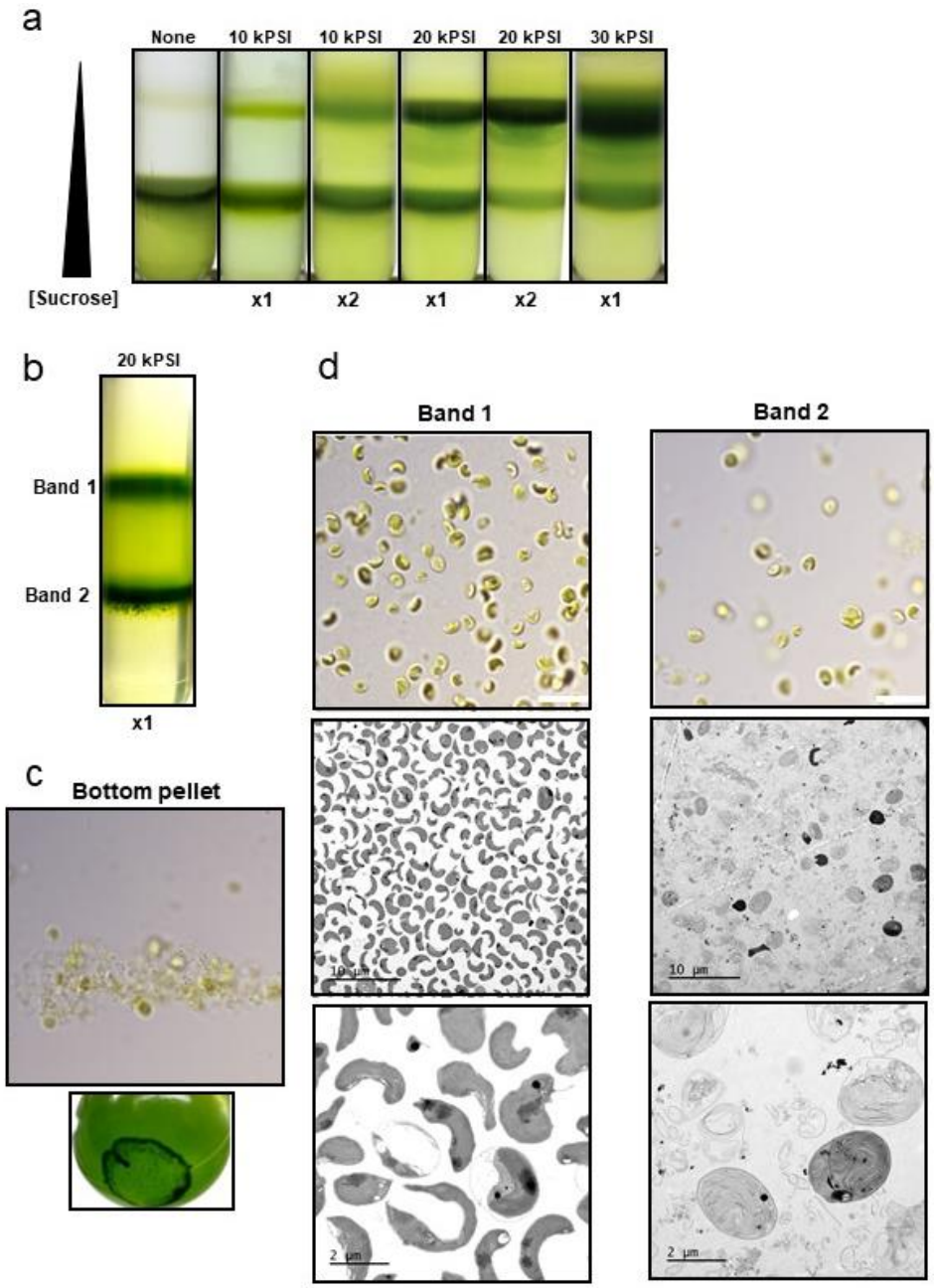



***Figure 1. Sucrose cushion centrifugation and microscopic image analysis****. (a) Sucrose cushion optimization on disrupted N. oceanica cells subjected to various pressures from 0, 10, 20, and 30 kPSI, with one or two passages through the cell disruptor. (b) 20 kPSI x 1 sucrose cushion was used for microscopic image analysis. (c) Bright-field microscopy (BFM) revealed that the the bottom pellet contained mostly broken cell debris. (d) Band 1 from the sucrose cushion contains isolated chloroplasts as shown by BFM in the top panel and transmission electron microscopy (TEM) in the middle and bottom panels. Band 2 of the sucrose cushion contains unbroken cells and debris as revealed by BFM in the top panel (1:10 dilution), and TEM in the middle and bottom panels. White scale bars in BFM images are ~10 µm (top panel). Scale bars in TEM images are 10 µm (middle panel) and 2 µm (bottom panel).*

## Isolation of *Nannochloropsis oceanica* chloroplasts by fine tuning cell disruption methods

Following growth and collection of large cultures of *N. oceanica* cells, high concentrations of isolated chloroplasts were needed before supercomplex isolation and high-resolution microscopy could be conducted. Cell disruption methods were tested and optimized using various pressures to break open cells while maintaining intact chloroplasts (Figure 1a). Cells were subjected to no breaking or pressures of 10 kPSI, 20 kPSI, and 30 kPSI with passages once and twice through the cell disruptor. The resulting sucrose cushions provided a range of band sizes, with 30 kPSI x 1 having the largest top band (Band 1). Bright-field microscopy (BFM) on Band 1 revealed that 20 kPSI x 1 contained what looked like intact chloroplasts (Figure 1d top panel). BFM on other conditions resulted in broken cell debris that resembled the bottom pellet in Figure 1c. TEM imaging on Band 1 revealed that the circular and crescent-shaped objects identified from BFM were indeed intact chloroplasts, while Band 2 contained a mixture of whole cells, dead cells, and cell debris (Figure 1d). Following these results, subsequent experiments for isolating intact chloroplast disrupted cells at 20 kPSI with one passage through the cell disruptor. Band 1 was collected and used for further analysis.

## Sucrose-density step gradients (SDSG) reveal co-migration of PSII-VCPII with LHCX1

Sucrose-density gradients were conducted via the “step” method using α-DM and β-DM to solubilize isolated chloroplasts (Figure 2a; (D’Amici et al., 2009). A side-by-side comparison of both detergents revealed that α-DM offered greater band resolution than β-DM and possibly acts as a better detergent for solubilizing proteins. However, this may not be favorable for isolation of protein supercomplexes, as the use of β-DM yielded heavier bands, indicative of higher molecular weight protein associations. Silver staining and western blotting were used to investigate the composition of each protein complex. In the silver-stained gel, bands around 75 kDa are indicative of the PSI core proteins PsaA and PsaB, bands from 37-47 kDa are indicative of PSII RC subunits like CP43, CP47, D1, and D2, and bands under 25 kD are indicative of VCP proteins. Bands around 50 kD are typically indicative of Atp-β and RbcL, common protein contaminants in thylakoid protein density gradients. The silver-stained gel in Figure 2B revealed that the top bands in each gradient (A1-A2, B1-3) contain an abundance of VCP antenna proteins. Analysis of bands A1-A3 and B2-B5 revealed the presence of PSII core proteins, and lower bands A1-A4 and B2-B2 showed the presence of PSI core proteins. Western blot analysis in Figure 2C led to a more concrete conclusion of where proteins and complexes migrate within the gradients. PsaA, indicative of PSI RC, accumulates mostly in A4-A6 and B5, towards the bottom of the gradients. PsbC (CP43) accumulates in a range of bands from A2-A5 in α-DM and B1-B5 in β-DM, with the greatest accumulation in B3-B5. LHCX1 is present mostly in bands A1-A3, and B1-B3 with the greatest accumulation at the top of the gradient. There is overlap between PsbC and LHCX1 in both gradients, as seen in bands A2 and B1-B2 in the western blots (Figure 2C). There is also overlap between PSI and PSII accumulation in bands A4-A5 and B4-B5. Taken together, these data revealed that VCP antenna proteins are migrating close to the top of the gradient, PSII core in the middle, and PSI core closer to

the bottom. The accumulation of VCP at the top of the gradients suggests that the proteins are not in complex with PSI or PSII, and that they may exist as monomers or oligomers. LHCX1 migrates mostly with other VCP antenna proteins, and further optimization is needed for stronger supercomplex formation.

The isolation of supercomplexes in heavier fractions can be achieved by adjusting the ratio of proteins/Chl to detergent. This was done by increasing the quantity of Chl from 0.5 to 1 mg Chl/mL, thus increasing the amount of protein in the sample. This was tested using α-DM and β-DM as shown in Figure 2D. With an increase in the ratio of Chl to detergent, band migrations changed within the gradient. Both gradients with 1 mg Chl/mL showed migration of the VCP antenna band to a lower position in the

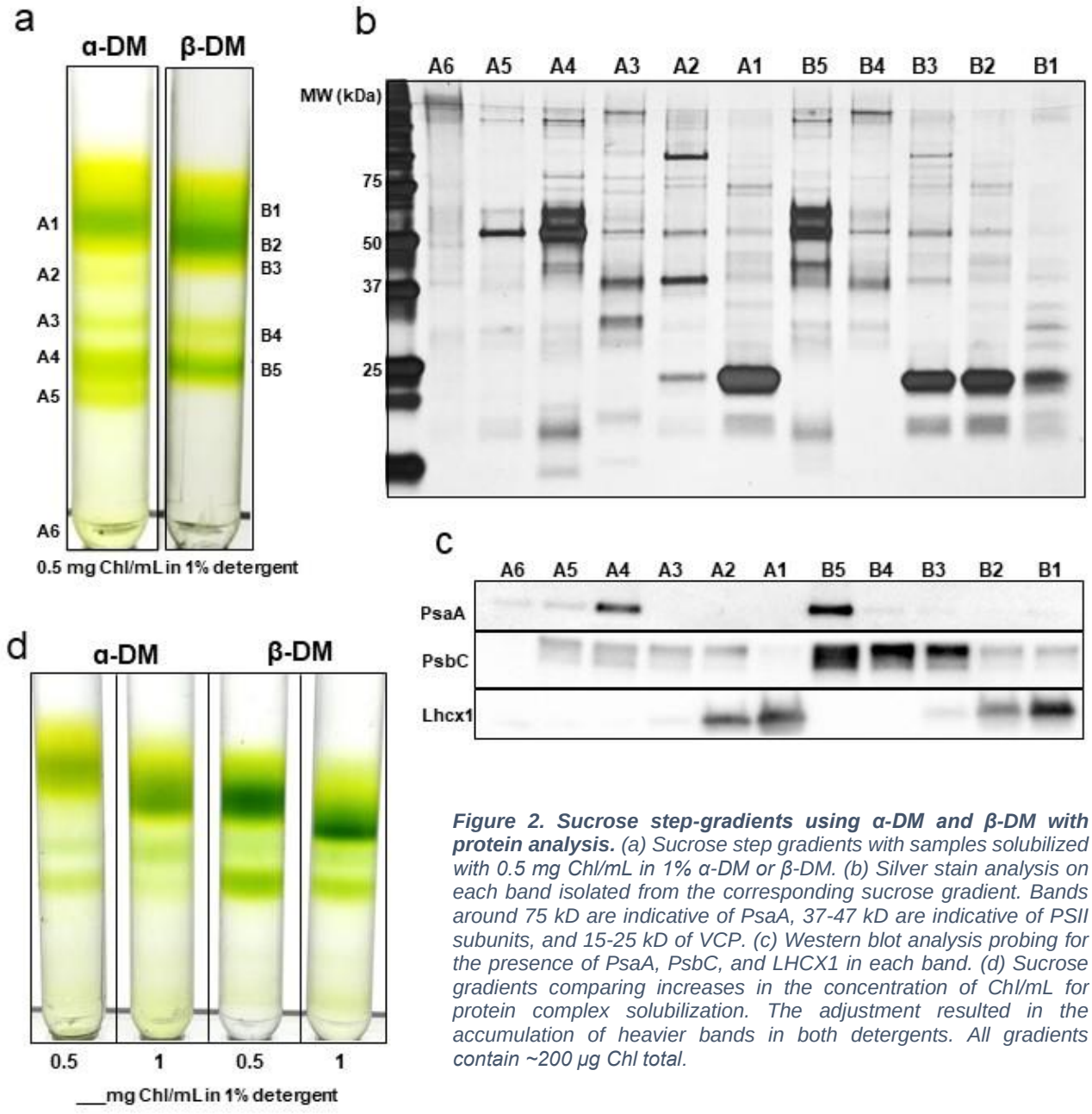



***Figure 2. Sucrose step-gradients using α-DM and β-DM with protein analysis.*** *(a) Sucrose step gradients with samples solubilized with 0.5 mg Chl/mL in 1% α-DM or β-DM. (b) Silver stain analysis on each band isolated from the corresponding sucrose gradient. Bands around 75 kD are indicative of PsaA, 37-47 kD are indicative of PSII subunits, and 15-25 kD of VCP. (c) Western blot analysis probing for the presence of PsaA, PsbC, and LHCX1 in each band. (d) Sucrose gradients comparing increases in the concentration of Chl/mL for protein complex solubilization. The adjustment resulted in the accumulation of heavier bands in both detergents. All gradients contain ~200 µg Chl total.*

gradient, indicating that a higher molecular weight protein association was present. In both conditions, β-DM resulted in lower migrating bands than α-DM. These results indicated that 1 mg Chl/mL in 1% β-DM should be used moving forward.

Following Figure 2, further steps were taken to prepare the sample for negative staining TEM. Negative staining TEM is a straightforward tool to rapidly check sample quality prior to further structural investigation via single-particle cryo-EM. Negative staining primarily works by casting heavy atoms (typically uranium) around the protein molecules absorbed onto the TEM grid. This enhances the signal-noise ratio (SNR) or contrast of the protein molecules against the background. While this method provides high-contrast images and overall shapes, the signal mainly originates from the heavy atoms surrounding the protein, thus causing a lack of high-resolution information.

To move forward towards negative staining TEM, we tested both α-DM and β-DM SDSG once again with one minor change. Here we added the detergent to the sample prior to bead beating; prior to this the detergent was added after bead beating. This resulted in better formation and solubilization of heavier bands within the gradient (Figure 3a compared to Figure 2d). β-DM continued to show heavier bands in gradients, indicating that there may be proteins bound together in higher molecular weight states, and we moved forward with this sample. Silver-stained gel analysis revealed that VCP antenna proteins greatly accumulate towards the middle of the gradient, corresponding to the thick green band (B1) in Figure 3a. PSII core proteins can also be seen co-migrating with VCP in B1, while PSI core proteins accumulate more towards the bottom of the gradient. This accumulation and migration pattern is consistent with our findings in Figure 2, where VCP/LHCX1 accumulates more at the top, PSII in the middle, and PSI at the bottom. Western blot analysis, now including α-VCP, shows that PsbC, LHCX1, and VCP accumulate together in band B1, though VCP can be seen accumulating in low amounts throughout the gradient, even with PsaA (Figure 3C). Taken together this indicates that PSII, LHCX1, and VCPII are co-migrating in the gradient, although the abundance of LHCX1 is higher at the top of the gradient. The top of the gradient likely contains monomeric PSII, and monomeric or oligomeric LHCX1 and VCP subunits. It is likely that the heavier bands in the gradient are PSII-VCPII and PSI-VCPI.

We then used negative stain TEM to further assess the contents of band B1 and check the quality of our sample (Figure 3d). Undiluted band B1 resulted in overcrowding and heterogeneity among particles. Diluting the sample by 1:10 resulted in less overcrowding, although the sample was still heterogenous, and the image was not acceptable for further analysis. We further worked to optimize the homogeneity, overall quality, and reduce the noise level of our sample. An acceptable sample for single particle cryo-EM should be homogenous and dilute enough for single particles to be sorted into classes, a process called 2D and 3D classification. 2D classification is a crucial first step towards resolving high-resolution structures and involves sorting a large collection of particles (~100K) into classes based on their orientation. This can be done using negative staining TEM images to check the quality of the particles, or with cryo-

EM. When using cryo-EM the 2D classes are further sorted into 3D classes, which are then refined and offer high-resolution 3D structures.

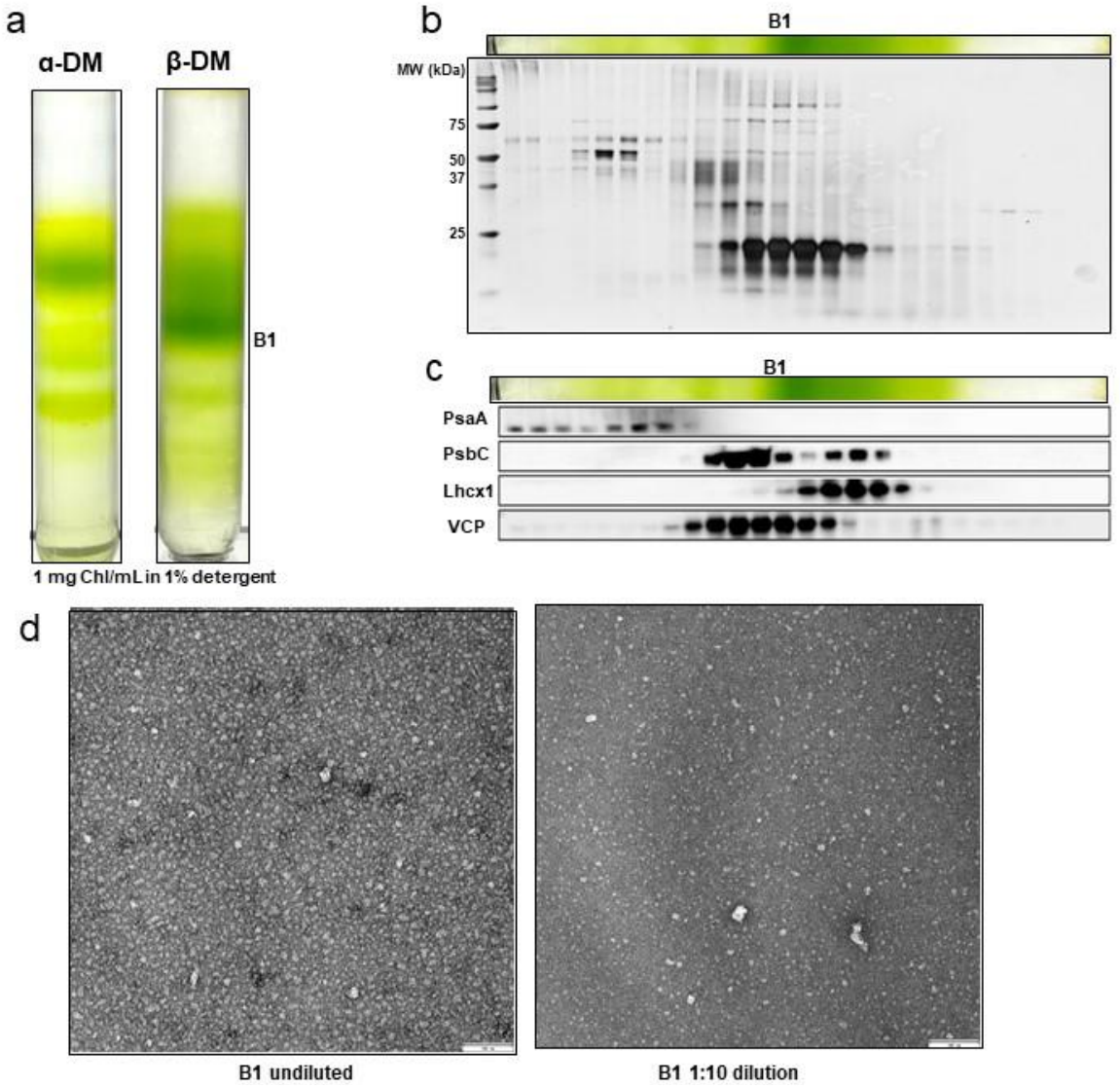



***Figure 3. Protein and negative stain TEM analysis on sucrose and β-DM sucrose-density step gradients (SDSG).*** *(a) Side-by-side comparison of SDSG using 1 mg Chl/mL in 1% α-DM or β-DM. Each gradient contains ~200 µg Chl. (b) Silver stain analysis of the proteins fractionated from the β-DM gradient. Bands around 75 kD are indicative of PsaA, 37-47 kD are indicative of PSII subunits, and 15-25 kD of VCP. (c) Western blot analysis probing for the presence of PsaA, PsbC, LHCX1, and the VCP antenna in the β-DM gradient. (d) Undiluted and 1:10 diluted negative stain TEM images from band B1 β-DM. Scale bars are 100 nm.*

Maltose-density linear gradients and GraFix improve the quality of samples for single particle analysis.

To increase the contrast of the images using TEM, we switched from using sucrose to maltose in the density gradients (Figure 4a left panel). The band corresponding to a possible PSII-VCPII supercomplex (Figure 3a-c) was isolated and

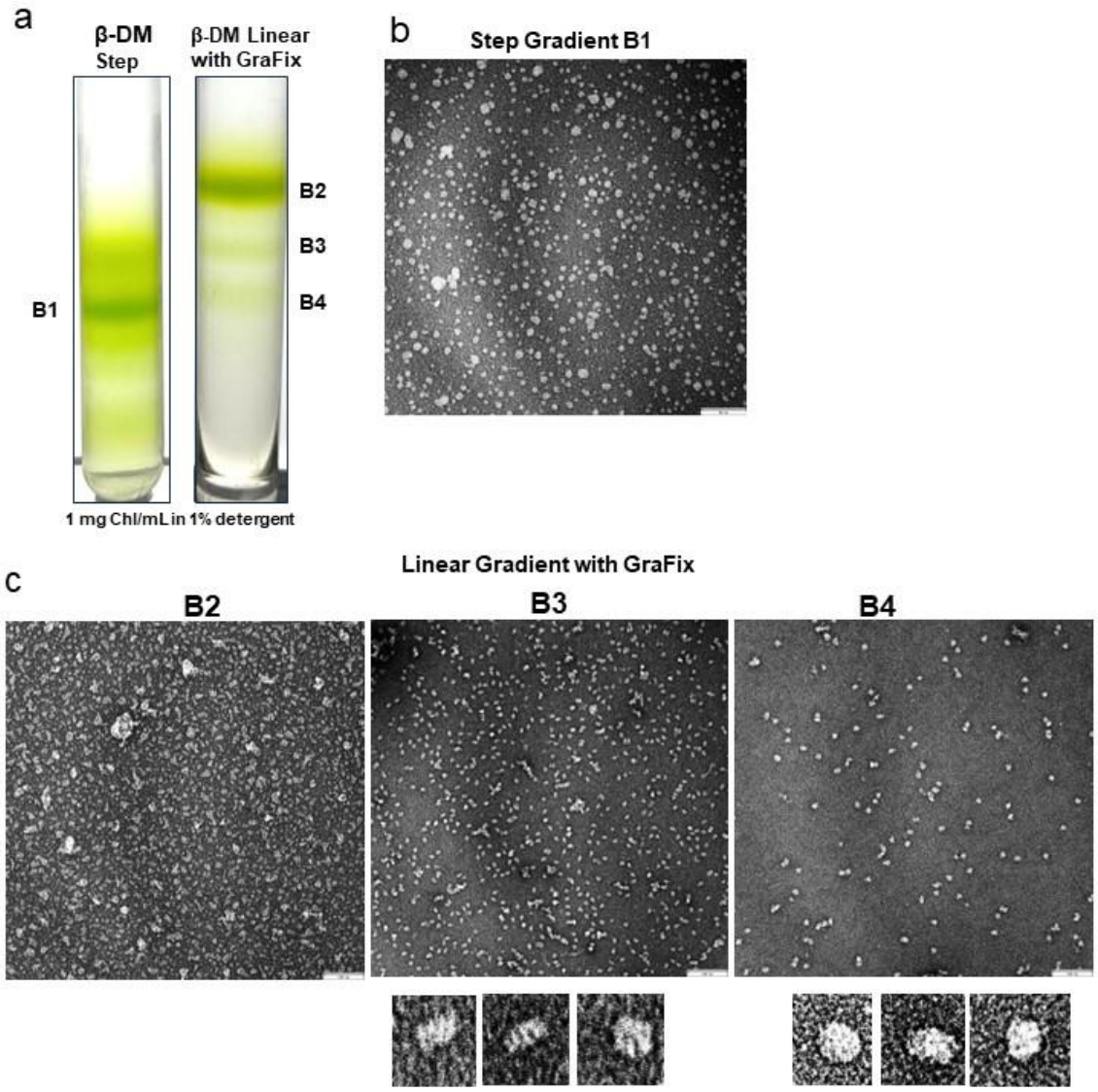



***Figure 4. Maltose-density linear-gradients (MDLG) with GraFix imaged by negative staining TEM****. (a) Comparison of a maltose-density step gradient (MDSG, left) and a MDLG with GraFix (right). 0.75% of glutaraldehyde fixative was added to the MDLG. Each gradient contained ~100 µg Chl solubilized at a concentration of 1 mg Chl/mL in 1% β-DM. Bands B1, B2, B3, and B4 were selected for TEM. (b) Band B1 from MDSG imaged using negative staining TEM. C) Bands B2, B3, and B4 from MDLG imaged using negative staining TEM. B3 and B4 contain selected particles indicative of circular PSI-VCPI and rectangular PSII-VPCII. Scale bars are 100 nm.*

imaged by negative staining EM (Figure 4b). Band 1 in this sample was better in overall contrast and quality than the corresponding band in the SDSG, however single particles were still unable to be distinguished due to low SNR, heterogeneity, and crowding. We then adjusted our protocol to introduce a maltose-density linear-gradient (MDLG) with the addition of glutaraldehyde as a fixative. This Gradient Fixation (GraFix) method cross-links the protein complexes together and stabilizes macromolecules (Kastner et al., 2007). This method resulted in sharper bands in the gradient, though the bands

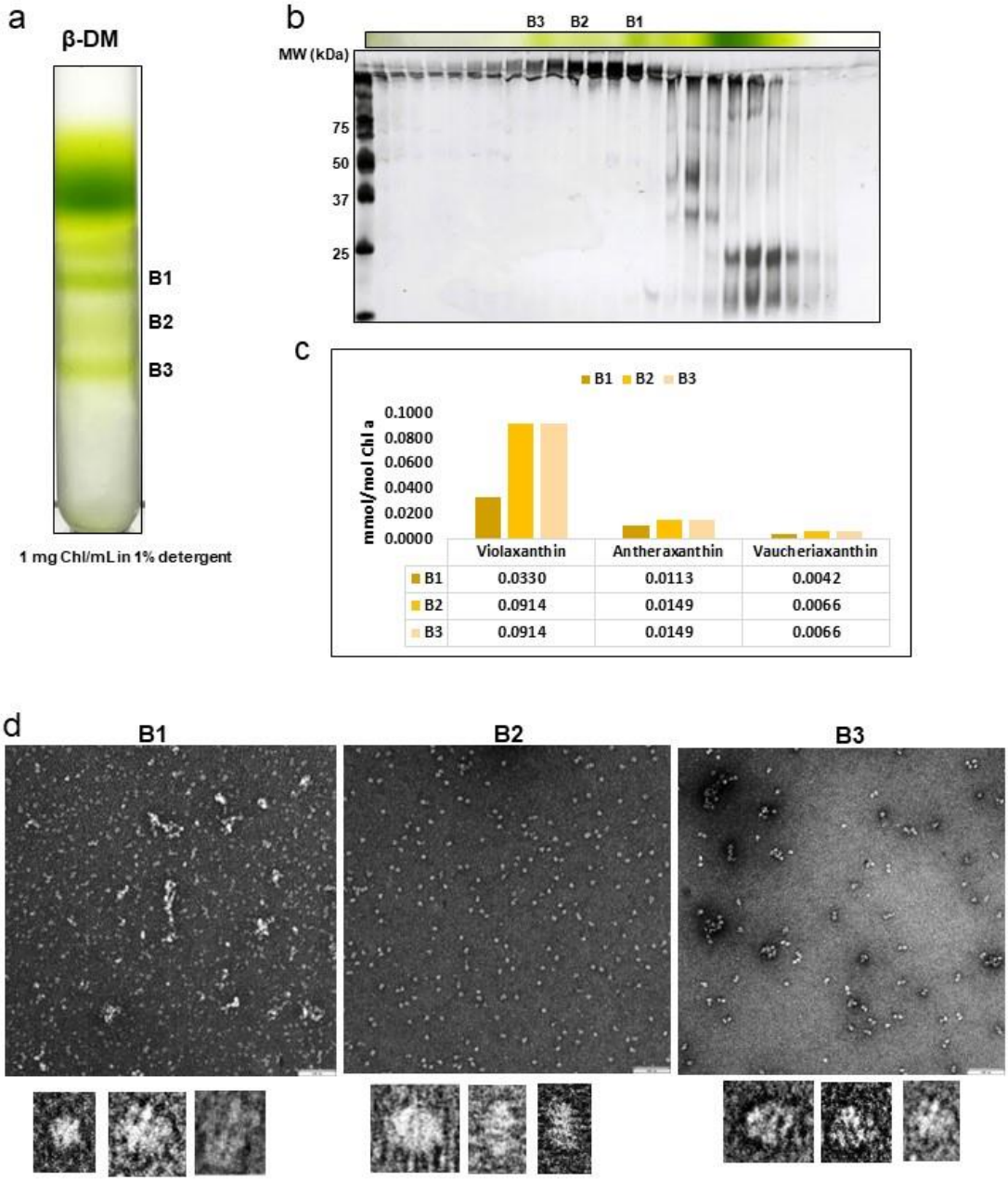



***Figure 5. Protein, pigment, and TEM analysis on bands from maltose-density linear-gradient (MDLG).*** *A) MDLG with GraFix (0.15% fixative added). Maltose gradient contains ~200µg Chl solubilized in a concentration of 1mg Chl/mL in 1% β-DM. Bands B1, B2, and B3 were isolated for further analysis. B) Silver-stained gel shows the migration of proteins in the gradient, though cross-linked proteins were unable to move through the gel. C) HPLC analysis on accessory pigments accumulating in each band, indicative of the presence of VCP. The pigments are normalized by total Chl a in the sample D) Negative staining TEM images of bands B1, B2, and B3 including selected particles indicative of circular PSI-VCPI and rectangular PSII-VPCII. Scale bars are 100nm.*

were now located closer to the top of the gradient (Figure 4a, right panel). Negative staining TEM images on the three MDLG bands revealed that B2 was like B1 in the MDSG (Figure 4c left panel, Figure 4b). However, images of bands B3 and B4 were of promising quality. Band B3 resulted in particles resembling circular PSI-VCPI and

rectangular PSII-VCPII (Figure 4c middle panel), while B4 resulted in fewer particles, although they resembled mostly PSI-VCPI. This is consistent with Western blot results from Figure 3c even though the methods for generating gradients changed. This promising result facilitated further optimization and characterization of protein contents in each band.

MDLG using GraFix was repeated with an increase of fixative from 0.075% to 0.15% glutaraldehyde (Figure 5a). The increase in fixative resulted in the formation of four to five bands, compared to three in Figure 4a (right panel). Silver-staining gel analysis was done to reveal where proteins accumulated in the gradient, although the cross-linked samples could not migrate into the gel due to their large size. Proteins from the top of the gradient were able to run through the gel possibly because of the lower concentration of fixative at the top or the higher concentration of proteins unable to be fixed. The proteins here migrated on the gel at around 25 kD, indicative of VCP antenna proteins and 37-47 kD indicative of PSII RC subunits. Western blot analysis was attempted, although the lack of protein migration made it difficult to reach conclusions with the proteins trapped in the wells. From previous data (Figures 2 and 3), we know that the topmost band in the gradient contains mainly VCP proteins and is too light to be in a complex with a reaction center. Heavier bands from previous data were indicative of supercomplex formation (Figures 2b, c and 3b, c). Thus, the bottom three bands were isolated and analyzed using HPLC and negative stain TEM.

Due to the inability to confirm the presence of VCP through silver-stained gel or Western blot analysis, HPLC was conducted to identify the presence of accessory pigments associated with VCP. HPLC was conducted on bands B1, B2, and B3 from the MDLG with GraFix (Figure 5c). The main accessory pigment for light harvesting in *N. oceanica* is violaxanthin (Vx), although antheraxanthin (Ax) and vaucheriaxanthin (Vau) are also present (Basso et al., 2014). Because cells were grown in low light, no zeaxanthin (Zx) was detected. All three bands contained Vx, Ax, and Vau, indicating that the VCP antenna is present. B2 and B3 contained the highest amounts of Vx when compared to B1 (Figure 5c). Subsequent negative staining TEM showed quality isolation of particles that resemble supercomplexes (Figure 5d). While B1 is heterogenous and noisy, a few supercomplex particles were able to be identified by eye. B2 contained a mixture of both supercomplexes but mostly PSII-VCPII, while B3 contained mostly PSI-VCPI. This is consistent with previous TEM images (Figure 4c) and protein analysis (Figures 2b, c and 3b, c). The quality of both B3 and B4 allows for further 2D classification of single particles, however B3 is of highest interest, because both supercomplexes migrate here. Moving forward, we will use our optimized methods for isolating chloroplasts and MDLG to prepare samples for single particle cryo-EM.

## Discussion

In our pursuit of resolving the high-resolution structures of the photosynthetic supercomplexes from *N. oceanica,* we refined methods for isolating PSI-VCPI and PSII-VCPII in the Eustigmatophyte alga *Nannochloropsis oceanica*. We optimized our methods for obtaining intact chloroplasts at a high concentration of isolates (Figure 1), employed sucrose-density step-gradients (SDSG) to investigate the co-migration of supercomplexes with VCP and LHCX1 (Figure 2), and used negative staining TEM to

make improvements in the quality of our sample (Figure 3). Further improvements were made by switching to maltose-density linear-gradients (MDLG) and employing the GraFix method (Kastner et al., 2007). This approach enhanced protein stabilization, with bands of interest containing particles that support evidence for supercomplex formation (Figures 4 and 5). The quality of the obtained particles, especially in Band 3, supports further 2D and 3D classification for high-resolution structural analysis through single-particle cryo-EM (Figure 5d). In the future we can test the use of 2D gels and mass spectroscopy to confirm the presence of key proteins like LHCX1 in our samples.

Our carefully refined methods represent a significant step forward in the study of photosynthetic supercomplexes in *N. oceanica*. In prior efforts to elucidate the structures of these complexes, negative-staining TEM successfully captured images of PSI-VCPI (Basso et al., 2014; Litvín et al., 2016). These investigations unveiled the modular nature of VCPI, forming both small and large subunits in a crescent-shaped band around PSI (Litvín et al., 2016). Further study indicated that the VCPI antenna exists in both monomeric and dimeric forms, featuring Chl *a*, Vx, and Vau as the most prevalent pigments (Basso et al., 2014). Despite these advancements, VCP proteins exhibited a stronger association with PSI and a looser connection with PSII, posing difficulties in isolating and imaging the PSII-VCPII supercomplex. Although evidence supports the existence of supercomplexes, the detailed structures of PSI-VCPI and PSII-VCPII remain unresolved through high-resolution single-particle cryo-EM.

By presenting methods for isolating these supercomplexes, our research addresses critical gaps in understanding the structural organization of these unique structures. Further exploration of the location of the LHCX1 protein, a pivotal player in NPQ and a member of the LHCX/LHCSR family (Park et al., 2019; Perin et al., 2023), will provide insights into energy-dissipation pathways and NPQ mechanisms in *N. oceanica.* Recent strides in cryo-EM have led to the resolution of the structure of LHCX6_1 in the centric diatom *Thalassiosira pseudonana*, revealing a Ddx/Dtx binding site and its impact on linking LHCF7 to the PSII core (Feng et al., 2023).

Drawing parallels with the localization of TpLHCX6_1 within the PSII supercomplex and its proposed role as an energy-quenching site, our study anticipates a deeper understanding of NoLHCX1's associations and functions. Future experiments aim to shed light on the structure, functional roles, and interactions of NoLHCX1 within the antenna subunits and the photosystem core. Future experiments, such as single-particle cryo-EM on high-light-grown cells, could unveil the locations and structures of upregulated LHCX proteins like LHCX3 (Chapter 2).

Our research significantly contributes to the growing body of knowledge on photosynthetic supercomplexes, propelling advancements in understanding these structures and their implications for optimizing photosynthetic processes across diverse organisms.

## References

Ahumada-Fierro, N. V., García-Mendoza, E., Sandoval-Gil, J. M., & Band-Schmidt, C. J. (2021). Photosynthesis and photoprotection characteristics related to ROS production in three Chattonella (Raphidophyceae) species. *Journal of Phycology*, *57*(3), 941–954. https://doi.org/10.1111/JPY.13138

Ajjawi, I., Verruto, J., Aqui, M., Soriaga, L. B., Coppersmith, J., Kwok, K., Peach, L., Orchard, E., Kalb, R., Xu, W., Carlson, T. J., Francis, K., Konigsfeld, K., Bartalis, J., Schultz, A., Lambert, W., Schwartz, A. S., Brown, R., & Moellering, E. R. (2017). Lipid production in Nannochloropsis gaditana is doubled by decreasing expression of a single transcriptional regulator. *Nature Biotechnology*, *35*(7), 647–652. https://doi.org/10.1038/NBT.3865

Alboresi, A., Le Quiniou, C., Yadav, S. K. N., Scholz, M., Meneghesso, A., Gerotto, C., Simionato, D., Hippler, M., Boekema, E. J., Croce, R., & Morosinotto, T. (2017). Conservation of core complex subunits shaped the structure and function of photosystem I in the secondary endosymbiont alga Nannochloropsis gaditana. *New Phytologist*, *213*, 714–726. https://doi.org/10.1111/nph.14156

Alboresi, A., Perin, G., Vitulo, N., Diretto, G., Block, M., Jouhet, J., Meneghesso, A., Valle, G., Giuliano, G., Maréchal, E., & Morosinotto, T. (2016). Light Remodels Lipid Biosynthesis in Nannochloropsis gaditana by Modulating Carbon Partitioning between Organelles. *Plant Physiology*, *171*(4), 2468–2482. https://doi.org/10.1104/PP.16.00599

Alboresia, A., Gerottob, C., Giacomettib, G. M., Bassia, R., & Morosinotto, T. (2010). Physcomitrella patens mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(24), 11128–11133. https://doi.org/10.1073/PNAS.1002873107

Amaral, R., Sérgio Seixas de Melo, J., & Maria Antunes dos Santos, L. (2021). Pigments from Eustigmatophyceae: an interesting class of microalgae for carotenoid production. *Journal of Applied Phycology*, *33*, 371–384. https://doi.org/10.1007/s10811-020-02312-z/Published

Antoshvili, M., Caspy, I., Hippler, · Michael, & Nelson, · Nathan. (2019). Structure and function of photosystem I in Cyanidioschyzon merolae. *Photosynthesis Research*, *139*, 499–508. https://doi.org/10.1007/s11120-018-0501-4

Armbrust, E. V., Berges, J. A., Bowler, C., Green, B. R., Martinez, D., Putnam, N. H., Zhou, S., Allen, A. E., Apt, K. E., Bechner, M., Brzezinski, M. A., Chaal, B. K., Chiovitti, A., Davis, A. K., Demarest, M. S., Detter, J. C., Glavina, T., Goodstein, D., Hadi, M. Z., … Rokhsar, D. S. (2004). The genome of the diatom Thalassiosira Pseudonana: Ecology, evolution, and metabolism. *Science*, *306*(5693), 79–86. https://doi.org/10.1126/science.1101156

Arsalane, W., Rousseau, B., & Duval, J. -C. (1994). INFLUENCE OF THE POOL SIZE OF THE XANTHOPHYLL CYCLE ON THE EFFECTS OF LIGHT STRESS IN A DIATOM: COMPETITION BETWEEN PHOTOPROTECI'ION AND PHOTOINHIBITION. *Photochemistry and Photobiology*, *60*(3), 237–243. https://doi.org/10.1111/J.1751-1097.1994.TB05097.X

Arshad, R., Calvaruso, C., Boekema, E. J., Buchel, C., & Kouril, R. (2021). Revealing the architecture of the photosynthetic apparatus in the diatom Thalassiosira pseudonana. *Plant Physiology*, *186*(4), 2124–2136. https://doi.org/10.1093/PLPHYS/KIAB208

Bai, Y., Cao, T., Dautermann, O., Buschbeck, P., Cantrell, M. B., Chen, Y., Lein, C. D., Shi, X., Ware, M. A., Yang, F., Zhang, H., Zhang, L., Peers, G., Li, X., & Lohr, M. (2022). Green diatom mutants reveal an intricate biosynthetic pathway of fucoxanthin. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(38). https://doi.org/10.1073/PNAS.2203708119/-/DCSUPPLEMENTAL

Bailey, J. C., Bidigare, R. R., Christensen, S. J., & Andersen, R. A. (1998). Phaeothamniophyceae Classis Nova: A New Lineage of Chromophytes Based upon Photosynthetic Pigments, rbcL Sequence Analysis and Ultrastructure. *Protist*, *149*(3), 245–263. https://doi.org/10.1016/S1434-4610(98)70032-X

Bailleul, B., Rogato, A., De Martino, A., Coesel, S., Cardol, P., Bowler, C., Falciatore, A., & Finazzi, G. (2010). An atypical member of the light-harvesting complex stress-related protein family modulates diatom responses to light. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(42), 18214–18219. https://doi.org/10.1073/pnas.1007703107

Ballottari, M., Truong, T. B., De Re, E., Erickson, E., Stella, G. R., Fleming, G. R., Bassi, R., & Niyogi, K. K. (2016). Identification of pH-sensing Sites in the Light Harvesting Complex Stress-related 3 Protein Essential for Triggering Non-photochemical Quenching in Chlamydomonas reinhardtii. *The Journal of Biological Chemistry*, *291*(14), 7334–7346. https://doi.org/10.1074/jbc.M115.704601

Baroli, I., Do, A. D., Yamane, T., & Niyogi, K. K. (2003). Zeaxanthin Accumulation in the Absence of a Functional Xanthophyll Cycle Protects Chlamydomonas reinhardtii from Photooxidative Stress. *The Plant Cell*, *15*(4), 992–1008. https://doi.org/10.1105/TPC.010405

Bassi, R., & Dall'osto, L. (2021). *Dissipation of Light Energy Absorbed in Excess: The Molecular Mechanisms*. https://doi.org/10.1146/annurev-arplant-071720

Basso, S., Simionato, D., Gerotto, C., Segalla, A., Giacometti, G. M., & Morosinotto, T. (2014). Characterization of the photosynthetic apparatus of the Eustigmatophycean Nannochloropsis gaditana: Evidence of convergent evolution in the supramolecular organization of photosystem I. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(2), 306–314. https://doi.org/10.1016/J.BBABIO.2013.11.019

Benedetti, M., Vecchi, V., Barera, S., & Dall'Osto, L. (2018). Biomass from microalgae: The potential of domestication towards sustainable biofactories. *Microbial Cell Factories*, *17*(1). https://doi.org/10.1186/S12934-018-1019-3

Berne, N., Fabryova, T., Istaz, B., Cardol, P., & Bailleul, B. (2018). The peculiar NPQ regulation in the stramenopile Phaeomonas sp. challenges the xanthophyll cycle

dogma. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1859*(7), 491–500. https://doi.org/10.1016/J.BBABIO.2018.03.013

Betancor, S., Domínguez, B., Tuya, F., Figueroa, F. L., & Haroun, R. (2015). Photosynthetic performance and photoprotection of Cystoseira humilis (Phaeophyceae) and Digenea simplex (Rhodophyceae) in an intertidal rock pool. *Aquatic Botany*, *121*, 16–25. https://doi.org/10.1016/J.AQUABOT.2014.10.008

Bína, D., Bouda, K., & Litvín, R. (2017). A two-component nonphotochemical fluorescence quenching in eustigmatophyte algae. *Photosynthesis Research*, *131*(1), 65–77. https://doi.org/10.1007/S11120-016-0299-X/FIGURES/9

Bína, D., Durchan, M., Kuznetsova, V., Vácha, F., Litvín, R., & Polívka, T. (2018). *Energy transfer dynamics in a red-shifted violaxanthin-chlorophyll a light-harvesting complex*. https://doi.org/10.1016/j.bbabio.2018.11.006

Bína, D., Gardian, Z., Herbstová, M., & Litvín, R. (2017). Modular antenna of photosystem I in secondary plastids of red algal origin: a Nannochloropsis oceanica case study. *Photosynthesis Research*, *131*(3), 255–266. https://doi.org/10.1007/s11120-016-0315-1

Bird, D. F., & Kalff, J. (1987). Algal phagotrophy: Regulating factors and importance relative to photosynthesis in Dinobryon (Chrysophyceae). *Limnology and Oceanography*, *32*(2), 277–284. https://doi.org/10.4319/LO.1987.32.2.0277

Bonente, G., Ballottari, M., Truong, T. B., Morosinotto, T., Ahn, T. K., Fleming, G. R., Niyogi, K. K., & Bassi, R. (2011). Analysis of LHcSR3, a protein essential for feedback de-excitation in the green alga Chlamydomonas reinhardtii. *PLoS Biology*, *9*(1). https://doi.org/10.1371/JOURNAL.PBIO.1000577

Borowitzka, M. A. (2013). High-value products from microalgae—their development and commercialisation. *Journal of Applied Phycology 2013 25:3*, *25*(3), 743–756. https://doi.org/10.1007/S10811-013-9983-9

Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., Otillar, R. P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde, M., Katinka, M., Mock, T., Valentin, K., … Grigoriev, I. V. (2008a). The Phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature 2008 456:7219*, *456*(7219), 239–244. https://doi.org/10.1038/nature07410

Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., Otillar, R. P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde, M., Katinka, M., Mock, T., Valentin, K., … Grigoriev, I. V. (2008b). The Phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature 2008 456:7219*, *456*(7219), 239–244. https://doi.org/10.1038/nature07410

Brenner, S., & Horne, R. W. (1959). A NEGATIVE STAINING METHOD FOR HIGH RESOLUTION ELECTRON MICROSCOPY OF VIRUSES. *Biochimica Et Biophysica Acta* , *34*, 103–110.

Büchel, C. (2015a). Evolution and function of light harvesting proteins. *Journal of Plant Physiology*, *172*, 62–75. https://doi.org/10.1016/J.JPLPH.2014.04.018

Büchel, C. (2015b). Evolution and function of light harvesting proteins. *Journal of Plant Physiology*, *172*, 62–75. https://doi.org/10.1016/J.JPLPH.2014.04.018

Büchel, C. (2020a). Light harvesting complexes in chlorophyll c-containing algae. *Biochimica et Biophysica Acta - Bioenergetics*, *1861*(4). https://doi.org/10.1016/j.bbabio.2019.05.003

Büchel, C. (2020b). Light harvesting complexes in chlorophyll c-containing algae. *Biochimica et Biophysica Acta - Bioenergetics*, *1861*(4). https://doi.org/10.1016/J.BBABIO.2019.05.003

Büchel, C. (2020c). *Light-Harvesting Complexes of Diatoms: Fucoxanthin-Chlorophyll Proteins*. 441–457. https://doi.org/10.1007/978-3-030-33397-3_16

Büchel, C., & Garab, G. (1995). Electrochromic absorbance changes in the chlorophyll-c-containing alga Pleurochloris meiringensis (Xanthophyceae). *Photosynthesis Research*, *43*, 49–56.

Büchel, C., Wilhelm, C., & Lenartz-Weiler, I. (1988). The Molecular Analysis of the Light Adaptation Reactions in the Yellow-green Alga Pleurochloris meiringensis (Xanthophyceae). *Botanica Acta*, *101*(4), 306–310. https://doi.org/10.1111/J.1438-8677.1988.TB00049.X

Buck, J. M., Kroth, P. G., & Lepetit, B. (2021). Identification of sequence motifs in Lhcx proteins that confer qE-based photoprotection in the diatom Phaeodactylum tricornutum. *The Plant Journal*, *108*(6), 1721–1734. https://doi.org/10.1111/TPJ.15539

Buck, J. M., Sherman, J., Bártulos, C. R., Serif, M., Halder, M., Henkel, J., Falciatore, A., Lavaud, J., Gorbunov, M. Y., Kroth, P. G., Falkowski, P. G., & Lepetit, B. (2019). Lhcx proteins provide photoprotection via thermal dissipation of absorbed light in the diatom Phaeodactylum tricornutum. *Nature Communications 2019 10:1*, *10*(1), 1–12. https://doi.org/10.1038/s41467-019-12043-6

Buck, J. M., Wünsch, M., Schober, A. F., Kroth, P. G., & Lepetit, B. (2022). Impact of Lhcx2 on Acclimation to Low Iron Conditions in the Diatom Phaeodactylum tricornutum. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/FPLS.2022.841058

Buck-Wiese, H., Andskog, M. A., Nguyen, N. P., Bligh, M., Asmala, E., Vidal-Melgosa, S., Liebeke, M., Gustafsson, C., & Hehemann, J. H. (2023). Fucoid brown algae inject fucoidan carbon into the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(1), e2210561119.

https://doi.org/10.1073/PNAS.2210561119/SUPPL_FILE/PNAS.2210561119.SAPP.PDF

Burton-Smith, R. N., Akimasa Watanabe, X., Ryutaro Tokutsu, X., Song, C., Kazuyoshi Murata, X., & Jun Minagawa, X. (2019). Structural determination of the large photosystem IIâ€“light-harvesting complex II supercomplex of Chlamydomonas reinhardtii using nonionic amphipol. *J. Biol. Chem*, *294*(41), 15003. https://doi.org/10.1074/jbc.RA119.009341

Cancela, A., Pérez, L., Febrero, A., Sánchez, A., Salgueiro, J. L., & Ortiz, L. (2019). Exploitation of Nannochloropsis gaditana biomass for biodiesel and pellet production. *Renewable Energy*, *133*, 725–730. https://doi.org/10.1016/j.renene.2018.10.075

Cao, S., Zhang, X., Fan, X., Qiao, H., Liang, C., Xu, D., Mou, S., Wang, W., & Ye, N. (2013). Phylogeny and characterisation of Nannochloropsis oceanica var. sinensis var. nov. (Eustigmatophyceae), a new oleaginous alga from China. *Phycologia*, *52*(6), 573–577. https://doi.org/10.2216/13-164.1

Cao, S., Zhang, X., Xu, D., Fan, X., Mou, S., Wang, Y., Ye, N., & Wang, W. (2013). A transthylakoid proton gradient and inhibitors induce a non-photochemical fluorescence quenching in unicellular algae Nannochloropsis sp. *FEBS Letters*, *587*(9), 1310–1315. https://doi.org/10.1016/J.FEBSLET.2012.12.031

Carbonera, D., Agostini, A., Di Valentin, M., Gerotto, C., Basso, S., Giacometti, G. M., & Morosinotto, T. (2014). Photoprotective sites in the violaxanthin-chlorophyll a binding Protein (VCP) from Nannochloropsis gaditana. *Biochimica et Biophysica Acta - Bioenergetics*, *1837*(8), 1235–1246. https://doi.org/10.1016/J.BBABIO.2014.03.014

Castillo, T., Ramos, D., García-Beltrán, T., Brito-Bazan, M., & Galindo, E. (2021). Mixotrophic cultivation of microalgae: An alternative to produce high-value metabolites. *Biochemical Engineering Journal*, *176*, 108183. https://doi.org/10.1016/j.bej.2021.108183

Christa, G., Pütz, L., Sickinger, C., Clavijo, J. M., Laetz, E. M. J., Greve, C., & Serôdio, J. (2018). Photoprotective Non-photochemical Quenching Does Not Prevent Kleptoplasts From Net Photoinactivation. *Frontiers in Ecology and Evolution | Www.Frontiersin.Org*, *1*, 121. https://doi.org/10.3389/fevo.2018.00121

Chukhutsina, V. U., Fristedt, R., Morosinotto, T., & Croce, R. (2017). Photoprotection strategies of the alga Nannochloropsis gaditana. *Biochimica et Biophysica Acta - Bioenergetics*, *1858*(7), 544–552. https://doi.org/10.1016/J.BBABIO.2017.05.003

Cock, J. M., Sterck, L., Rouzé, P., Scornet, D., Allen, A. E., Amoutzias, G., Anthouard, V., Artiguenave, F., Aury, J.-M., Badger, J. H., Beszteri, B., Billiau, K., Bonnet, E., Bothwell, J. H., Bowler, C., Boyen, C., Brownlee, C., Carrano, C. J., Charrier, B., … Wincker, P. (2010). *The Ectocarpus genome and the independent evolution of multicellularity in brown algae*. https://doi.org/10.1038/nature09016

Correa-Galvis, V., Redekop, P., Guan, K., Griess, A., Truong, T. B., Wakao, S., Niyogi, K. K., & Jahns, P. (2016). Photosystem II Subunit PsbS Is Involved in the Induction of LHCSR Protein-dependent Energy Dissipation in Chlamydomonas reinhardtii*. *Journal of Biological Chemistry*, *291*(33), 17478–17487. https://doi.org/10.1074/JBC.M116.737312

Cruz, S., Cartaxana, P., Newcomer, R., Dionísio, G., Calado, R., Serôdio, J., Pelletreau, K. N., Rumpho, M. E., & Biological, M. (2015). *Photoprotection in sequestered plastids of sea slugs and respective algal sources*. https://doi.org/10.1038/srep07904

D'Amici, G. M., Huber, C. G., & Zolla, L. (2009). Separation of thylakoid membrane proteins by sucrose gradient ultracentrifugation or blue native-SDS-PAGE two-dimensional electrophoresis. *Methods in Molecular Biology (Clifton, N.J.)*, *528*, 61–70. https://doi.org/10.1007/978-1-60327-310-7_4/COVER

Daskalakis, V., Papadatos, S., & Kleinekathöfer, U. (2019). Fine tuning of the photosystem II major antenna mobility within the thylakoid membrane of higher plants. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1861*(12), 183059. https://doi.org/10.1016/J.BBAMEM.2019.183059

De Meyer, F., Maddalena Venturoli, & Berend Smit. (2008). Hydrophobic Mismatch Can Induce Specific and Selective Membrane Protein Interactions. *The 2008 Spring National Meeting*.

De Souza, A. P., Burgess, S. J., Doran, L., Hansen, J., Manukyan, L., Maryn, N., Gotarkar, D., Leonelli, L., Niyogi, K. K., & Long, S. P. (2022). Soybean photosynthesis and crop yield are improved by accelerating recovery from photoprotection. *Science*, *377*(6608), 851–854. https://doi.org/10.1126/SCIENCE.ADC9831/SUPPL_FILE/SCIENCE.ADC9831_MDAR_REPRODUCIBILITY_CHECKLIST.PDF

Demmig-Adams, B. (1990). Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1020*(1), 1–24. https://doi.org/10.1016/0005-2728(90)90088-L

Demmig-Adams, B., & Adams, W. W. (1996). The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant Science*, *1*(1), 21–26. https://doi.org/10.1016/S1360-1385(96)80019-7

Demmig-Adams, B., & Adams, W. W. (2006a). Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New Phytol.*, *172*(1), 11–21. https://doi.org/10.1111/j.1469-8137.2006.01835.x

Demmig-Adams, B., & Adams, W. W. (2006b). Photoprotection in an ecological context: The remarkable complexity of thermal energy dissipation. *New Phytologist*, *172*(1), 11–21. https://doi.org/10.1111/J.1469-8137.2006.01835.X

Demmig-Adams, B., Koh, S.-C., Cohu, C. M., Muller, O., Stewart, J. J., & Iii, W. W. A. (2014). Non-Photochemical Fluorescence Quenching in Contrasting Plant Species

and Environments. *Advances in Photosynthesis and Respiration*, *40*, 531–552. https://doi.org/10.1007/978-94-017-9032-1_24

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., & Gascuel, O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, *36*(Web Server issue). https://doi.org/10.1093/NAR/GKN180

Derelle, R., López-García, P., Timpano, H., & Moreira, D. (2016). A Phylogenomic Framework to Study the Diversity and Evolution of Stramenopiles (=Heterokonts). *Molecular Biology and Evolution*, *33*(11), 2890–2898. https://doi.org/10.1093/MOLBEV/MSW168

Dimier, C., Brunet, C., Geider, R., & Raven, J. (2009). Growth and photoregulation dynamics of the picoeukaryote Pelagomonas calceolata in fluctuating light. *Limnology and Oceanography*, *54*(3), 823–836. https://doi.org/10.4319/LO.2009.54.3.0823

Dittami, S. M., Michel, G., Collén, J., Boyen, C., & Tonon, T. (2010a). Chlorophyll-binding proteins revisited - A multigenic family of light-harvesting and stress proteins from a brown algal perspective. *BMC Evolutionary Biology*, *10*(1), 1–14. https://doi.org/10.1186/1471-2148-10-365/FIGURES/5

Dittami, S. M., Michel, G., Collén, J., Boyen, C., & Tonon, T. (2010b). Chlorophyll-binding proteins revisited - A multigenic family of light-harvesting and stress proteins from a brown algal perspective. *BMC Evolutionary Biology*, *10*(1), 1–14. https://doi.org/10.1186/1471-2148-10-365/FIGURES/5

Dorrell, R. G., & Bowler, C. (2017). Secondary Plastids of Stramenopiles. *Advances in Botanical Research*, *84*, 57–103. https://doi.org/10.1016/BS.ABR.2017.06.003

Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, *346*(6213). https://doi.org/10.1126/SCIENCE.1258096/ASSET/2313E70A-5C58-4755-A0E6-2E64EE240A09/ASSETS/GRAPHIC/346_1258096_F6.JPEG

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797. https://doi.org/10.1093/NAR/GKH340

Eilers, U., Dietzel, L., Breitenbach, J., Büchel, C., & Sandmann, G. (2016). Identification of genes coding for functional zeaxanthin epoxidases in the diatom Phaeodactylum tricornutum. *Journal of Plant Physiology*, *192*, 64–70. https://doi.org/10.1016/j.jplph.2016.01.006

Eliáš, M. (2021). Protist diversity: Novel groups enrich the algal tree of life. *Current Biology*, *31*(11), R733–R735. https://doi.org/10.1016/J.CUB.2021.04.025

Eppard, M., Krumbein, W. E., Von Haeseler, A., & Rhiel, E. (2000). Characterization of fcp4 and fcp12, two additional genes encoding light harvesting proteins of Cyclotella cryptica (Bacillariophyceae) and phylogenetic analysis of this complex

gene family. *Plant Biology*, *2*(3), 283–289. https://doi.org/10.1055/S-2000-3702/ID/36/BIB

Fan, M., Li, M., Liu, Z., Cao, P., Pan, X., Zhang, H., Zhao, X., Zhang, J., & Chang, W. (2015). Crystal structures of the PsbS protein essential for photoprotection in plants. *Nature Structural & Molecular Biology*, *22*. https://doi.org/10.1038/nsmb.3068

Fattore, N., Bellan, A., Pedroletti, L., Vitulo, N., & Morosinotto, T. (2021). Acclimation of photosynthesis and lipids biosynthesis to prolonged nitrogen and phosphorus limitation in Nannochloropsis gaditana. *Algal Research*, *58*, 102368. https://doi.org/10.1016/J.ALGAL.2021.102368

Feng, Y., Li, Z., Li, X., Shen, L., Liu, X., Zhou, C., Zhang, J., Sang, M., Han, G., Yang, W., Kuang, T., Wang, W., & Shen, J.-R. (2023). Structure of a diatom photosystem II supercomplex containing a member of Lhcx family and dimeric FCPII. *Science Advances*.

Fernández-Marín, B., Míguez, F., Becerril, J. M., & García-Plazaola, J. I. (2011). Activation of violaxanthin cycle in darkness is a common response to different abiotic stresses: A case study in Pelvetia canaliculata. *BMC Plant Biology*, *11*(1), 1–11. https://doi.org/10.1186/1471-2229-11-181/FIGURES/8

Figueroa, F. L., Celis-Plá, P. S. M., Martínez, B., Korbee, N., Trilla, A., & Arenas, F. (2019). Yield losses and electron transport rate as indicators of thermal stress in Fucus serratus (Ochrophyta). *Algal Research*, *41*, 101560. https://doi.org/10.1016/J.ALGAL.2019.101560

Fu, F. X., Zhang, Y., Warner, M. E., Feng, Y., Sun, J., & Hutchins, D. A. (2008). A comparison of future increased CO2 and temperature effects on sympatric Heterosigma akashiwo and Prorocentrum minimum. *Harmful Algae*, *7*(1), 76–90. https://doi.org/10.1016/J.HAL.2007.05.006

García-Mendoza, E., & Colombo-Pallotta, M. F. (2007). The giant kelp Macrocystis pyrifera presents a different nonphotochemical quenching control than higher plants. *New Phytologist*, *173*(3), 526–536. https://doi.org/10.1111/J.1469-8137.2006.01951.X

Garcia-Mendoza, E., Ocampo-Alvarez, H., & Govindjee. (2011). Photoprotection in the brown alga Macrocystis pyrifera: Evolutionary implications. *Journal of Photochemistry and Photobiology B: Biology*, *104*(1–2), 377–385. https://doi.org/10.1016/j.jphotobiol.2011.04.004

Gardian, Z., Litvín, R., Bína, D., & Vácha, F. (2014). Supramolecular organization of fucoxanthin-chlorophyll proteins in centric and pennate diatoms. *Photosynthesis Research*, *121*, 79–86. https://doi.org/10.1007/s11120-014-9998-3

Gardian, Z., Tichy, J., & Vácha, F. (2011). Structure of PSI, PSII and antennae complexes from yellow-green alga Xanthonema debile. *Photosynthesis Research*, *108*, 25–32. https://doi.org/10.1007/s11120-011-9647-z

Ghazaryan, A., Akhtar, P., Garab, G., Lambrev, P. H., & Büchel, C. (2016a). *Involvement of the Lhcx protein Fcp6 of the diatom Cyclotella meneghiniana in the macro-organisation and structural flexibility of thylakoid membranes*. https://doi.org/10.1016/j.bbabio.2016.04.288

Ghazaryan, A., Akhtar, P., Garab, G., Lambrev, P. H., & Büchel, C. (2016b). Involvement of the Lhcx protein Fcp6 of the diatom Cyclotella meneghiniana in the macro-organisation and structural flexibility of thylakoid membranes. *Biochimica et Biophysica Acta - Bioenergetics*, *1857*(9), 1373–1379. https://doi.org/10.1016/j.bbabio.2016.04.288

Gibbs, P. B., & Biggins, J. (1989). Regulation of the distribution of excitation energy in Ochromonas danica, an organism containing a chlorophyll-A/C/carotenoid light harvesting antenna. *Photosynthesis Research*, *21*, 81–91.

Giovagnetti, V., Cataldo, M. L., Conversano, F., & Brunet, C. (2010). Functional relation between growth, photosynthetic rate and regulation in the coastal picoeukaryote Phaeomonas sp. RCC 503 (Pinguiophyceae, Stramenopiles). *Journal of Plankton Research*, *32*(11), 1501–1511. https://doi.org/10.1093/plankt/fbq074

Giovagnetti, V., Jaubert, M., Shukla, M. K., Ungerer, P., Bouly, J. P., Falciatore, A., & Ruban, A. V. (2022). Biochemical and molecular properties of LHCX1, the essential regulator of dynamic photoprotection in diatoms. *Plant Physiology*, *188*(1), 509–525. https://doi.org/10.1093/PLPHYS/KIAB425

Giovagnetti, V., & Ruban, A. V. (2018). The evolution of the photoprotective antenna proteins in oxygenic photosynthetic eukaryotes. *Biochemical Society Transactions*, *46*(5), 1263–1277. https://doi.org/10.1042/BST20170304

Goss, R., Ann Pinto, E., Wilhelm, C., & Richter, M. (2006). The importance of a highly active and ΔpH-regulated diatoxanthin epoxidase for the regulation of the PS II antenna function in diadinoxanthin cycle containing algae. *Journal of Plant Physiology*, *163*(10), 1008–1021. https://doi.org/10.1016/J.JPLPH.2005.09.008

Goss, R., & Jakob, T. (2010a). Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynthesis Research 2010 106:1*, *106*(1), 103–122. https://doi.org/10.1007/S11120-010-9536-X

Goss, R., & Jakob, T. (2010b). Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynthesis Research 2010 106:1*, *106*(1), 103–122. https://doi.org/10.1007/S11120-010-9536-X

Goss, R., & Lepetit, B. (2015). Biodiversity of NPQ. *Journal of Plant Physiology*, *172*, 13–32. https://doi.org/10.1016/j.jplph.2014.03.004

Graf, L., Yang, E. C., Han, K. Y., Küpper, F. C., Benes, K. M., Oyadomari, J. K., Herbert, R. J. H., Verbruggen, H., Wetherbee, R., Andersen, R. A., & Yoon, H. S. (2020). Multigene Phylogeny, Morphological Observation and Re-examination of the Literature Lead to the Description of the Phaeosacciophyceae Classis Nova

and Four New Species of the Heterokontophyta SI Clade. *Protist*, *171*(6), 125781. https://doi.org/10.1016/J.PROTIS.2020.125781

Graiff, A., Bartsch, I., Glaser, K., & Karsten, U. (2021). Seasonal Photophysiological Performance of Adult Western Baltic Fucus vesiculosus (Phaeophyceae) Under Ocean Warming and Acidification. *Frontiers in Marine Science*, *8*, 666493. https://doi.org/10.3389/FMARS.2021.666493/BIBTEX

Grossman, A. R., Bhaya, D., Apt, K. E., & Kehoe, D. M. (1995). LIGHT-HARVESTING COMPLEXES IN OXYGENIC PHOTOSYNTHESIS: Diversity, Control, and Evolution. *Https://Doi.Org/10.1146/Annurev.Ge.29.120195.001311*, *29*, 231–288. https://doi.org/10.1146/ANNUREV.GE.29.120195.001311

Grouneva, I., Rokka, A., & Aro, E. M. (2011). The thylakoid membrane proteome of two marine diatoms outlines both diatom-specific and species-specific features of the photosynthetic machinery. *Journal of Proteome Research*, *10*(12), 5338–5353. https://doi.org/10.1021/PR200600F/SUPPL_FILE/PR200600F_SI_015.PDF

Guillou, L., Chrétiennot-Dinet, M. J., Medlin, L. K., Claustre, H., Loiseaux-de Goër, S., & Vaulot, D. (1999). BOLIDOMONAS: A NEW GENUS WITH TWO SPECIES BELONGING TO A NEW ALGAL CLASS, THE BOLIDOPHYCEAE (HETEROKONTA). *Journal of Phycology*, *35*(2), 368–381. https://doi.org/10.1046/J.1529-8817.1999.3520368.X

Gundermann, K., Wagner, V., Mittag, M., & Büchel, C. (2019). Fucoxanthin-Chlorophyll Protein Complexes of the Centric Diatom Cyclotella Meneghiniana Differ in Lhcx1 and Lhcx6_1 Content. *Plant Physiology*, *179*(4), 1779–1795. https://doi.org/10.1104/PP.18.01363

Hao, T. Bin, Jiang, T., Dong, H. P., Ou, L. jian, He, X., & Yang, Y. F. (2018). Light-harvesting protein Lhcx3 is essential for high light acclimation of Phaeodactylum tricornutum. *AMB Express*, *8*(1), 1–11. https://doi.org/10.1186/S13568-018-0703-3/TABLES/1

Havaux, M., & Niyogi, K. K. (1999). The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proc. Natl Acad. Sci. USA*, *96*(15), 8762–8767. https://doi.org/10.1073/pnas.96.15.8762

Hennige, S. J., Coyne, K. J., Macintyre, H., Liefer, J., & Warner, M. E. (2013). *THE PHOTOBIOLOGY OF HETEROSIGMA AKASHIWO. PHOTOACCLIMATION, DIURNAL PERIODICITY, AND ITS ABILITY TO RAPIDLY EXPLOIT EXPOSURE TO HIGH LIGHT 1*. https://doi.org/10.1111/jpy.12043

Horn, S., Ehlers, K., Fritzsch, G., Gil-Rodríguez, C., Wilhelm, C., & Schnetter, R. (2007). Synchroma grande spec. nov. (Synchromophyceae class. nov., Heterokontophyta): An Amoeboid Marine Alga with Unique Plastid Complexes. *Protist*, *158*, 277–293. https://doi.org/10.1016/j.protis.2007.02.004

Hupel, M., Lecointre, C., Meudec, A., Poupart, N., & Gall, E. A. (2011). Comparison of photoprotective responses to UV radiation in the brown seaweed Pelvetia

canaliculata and the marine angiosperm Salicornia ramosissima. *Journal of Experimental Marine Biology and Ecology*, *401*(1–2), 36–47. https://doi.org/10.1016/J.JEMBE.2011.03.004

Ichinomiya, M., Dos Santos, A. L., Gourvil, P., Yoshikawa, S., Kamiya, M., Ohki, K., Audic, S., De Vargas, C., Noël, M. H., Vaulot, D., & Kuwata, A. (2016). Diversity and oceanic distribution of the Parmales (Bolidophyceae), a picoplanktonic group closely related to diatoms. *The ISME Journal 2016 10:10*, *10*(10), 2419–2434. https://doi.org/10.1038/ismej.2016.38

Iwai, M., Grob, P., Iavarone, A. T., Nogales, E., & Niyogi, K. K. (2018). A unique supramolecular organization of photosystem I in the moss Physcomitrella patens. *Nature Plants*, *4*(11), 904–909. https://doi.org/10.1038/s41477-018-0271-1

Jakob, T., Goss, R., & Wilhelm, C. (1999). Activation of Diadinoxanthin De-Epoxidase Due to a Chiororespiratory Proton Gradient in the Dark in the Diatom Phaeodactylum tricornutum. *Plant Biology*, *1*(1), 76–82. https://doi.org/10.1111/J.1438-8677.1999.TB00711.X

Jesus, B., Ventura, P., & Calado, G. (2010). Behaviour and a functional xanthophyll cycle enhance photo-regulation mechanisms in the solar-powered sea slug Elysia timida (Risso, 1818). *Journal of Experimental Marine Biology and Ecology*, *395*(1–2), 98–105. https://doi.org/10.1016/J.JEMBE.2010.08.021

Jimel, M. S., Kv Iderov, J., & Elster, J. (2020). *Annual Cycle of Mat-FOrming Filamentous Alga Tribonema CF. Minus (Stramenopiles, Xanthophyceae) in Hydro-terrestrial habitats In the High Arctic Revealed By Multiparameter Fluorescence Staining*. https://doi.org/10.1111/jpy.13109-20-203

Johnson, M. P., & Ruban, A. v. (2011). Restoration of Rapidly Reversible Photoprotective Energy Dissipation in the Absence of PsbS Protein by Enhanced ΔpH *. *Journal of Biological Chemistry*, *286*(22), 19973–19981. https://doi.org/10.1074/JBC.M111.237255

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. A. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., … Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature 2021 596:7873*, *596*(7873), 583–589. https://doi.org/10.1038/s41586-021-03819-2

Kai, A., Yoshii, Y., Nakayama, T., & Inouye, I. (2008). Aurearenophyceae classis nova, a new class of Heterokontophyta based on a new marine unicellular alga Aurearena cruciata gen. et sp. nov. inhabiting sandy beaches. *Protist*, *159*(3), 435–457. https://doi.org/10.1016/J.PROTIS.2007.12.003

Kastner, B., Fischer, N., Golas, M. M., Sander, B., Dube, P., Boehringer, D., Hartmuth, K., Deckert, J., Hauer, F., Wolf, E., Uchtenhagen, H., Urlaub, H., Herzog, F., Peters, J. M., Poerschke, D., Lührmann, R., & Stark, H. (2007). GraFix: sample

preparation for single-particle electron cryomicroscopy. *Nature Methods 2008 5:1*, *5*(1), 53–55. https://doi.org/10.1038/nmeth1139

Kawachi, M., Inouye, I., Honda, D., Charles, ‡, O'kelly, J., Bailey, J. C., Robert, §, Bidigare, R., & Andersen, R. A. (2002). The Pinguiophyceae classis nova , a new class of photosynthetic stramenopiles whose members produce large amounts of omega-3 fatty acids. *Phycological Research*, *50*, 31–47. https://doi.org/10.1046/j.1440-1835.2002.00260.x

Keeling, P. J., & Burki, F. (2019). Progress towards the Tree of Eukaryotes. *Current Biology*, *29*, R808–R817. https://doi.org/10.1016/j.cub.2019.07.031

Khoroshyy, P., David Bína, ·, Gardian, Z., Litvín, R., Alster, J., & Pšenčík, J. (2018). Quenching of chlorophyll triplet states by carotenoids in algal light-harvesting complexes related to fucoxanthin-chlorophyll protein. *Photosynthesis Research*, *135*, 213–225. https://doi.org/10.1007/s11120-017-0416-5

Kilian, O., Benemann, C. S. E., Niyogi, K. K., & Vick, B. (2011). High-efficiency homologous recombination in the oil-producing alga Nannochloropsis sp. *Proceedings of the National Academy of Sciences*, *108*(52), 21265–21269. https://doi.org/10.1073/pnas.1105861108

Kirst, H., Formighieri, C., & Melis, A. (2014). Maximizing photosynthetic efficiency and culture productivity in cyanobacteria upon minimizing the phycobilisome light-harvesting antenna size. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(10), 1653–1664. https://doi.org/10.1016/J.BBABIO.2014.07.009

Kirst, H., Gabilly, S. T., Niyogi, K. K., Lemaux, P. G., & Melis, A. (2017). Photosynthetic antenna engineering to improve crop yields. *Planta*, *245*(5), 1009–1020. https://doi.org/10.1007/s00425-017-2659-y

Korbee, N., Navarro, N. P., García-Sánchez, M., Celis-Plá, P. S., Quintano, E., Copertino, M. S., Pedersen, A., Mariath, R., Mangaiyarkarasi, N., Pérez-Ruzafa, Figueroa, F. L., & Martínez, B. (2014). A novel in situ system to evaluate the effect of high CO2 on photosynthesis and biochemistry of seaweeds. *Aquatic Biology*, *22*, 245–259. https://doi.org/10.3354/AB00594

Kouřil, R., Dekker, J. P., & Boekema, E. J. (2012). Supramolecular organization of photosystem II in green plants. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1817*(1), 2–12. https://doi.org/10.1016/J.BBABIO.2011.05.024

Krause, G. H., & Krause, G. H. (1988). Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiologia Plantarum*, *74*(3), 566–574. https://doi.org/10.1111/J.1399-3054.1988.TB02020.X

Krogmann, D. W., Jagendorf, A. T., & Avron, M. (1959). Uncouplers of Spinach Chloroplast Photosynthetic Phosphorylation. *Plant Physiology*, *34*(3), 272. https://doi.org/10.1104/PP.34.3.272

Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S. T., Iwai, M., Niyogi, K. K., & Long, S. P. (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science*, *354*(6314). https://doi.org/10.1126/science.aai8878

Kuczynska, P., Jemiola-Rzeminska, M., Nowicka, B., Jakubowska, A., Strzalka, W., Burda, K., & Strzalka, K. (2020). The xanthophyll cycle in diatom Phaeodactylum tricornutum in response to light stress. *Plant Physiology and Biochemistry*, *152*, 125–137. https://doi.org/10.1016/J.PLAPHY.2020.04.043

Kumazawa, M., Nishide, H., Nagao, | Ryo, Inoue-Kashino, N., Shen, J.-R., Nakano, T., Uchiyama, I., Kashino, Y., & Ifuku, K. (2021). *Molecular phylogeny of fucoxanthin-chlorophyll a/c proteins from Chaetoceros gracilis and Lhcq/Lhcf diversity*. https://doi.org/10.1111/ppl.13598

Kurita, T., Iwai, M., Ohta, H., Sakuma, T., & Yamamoto, T. (2023). Genome editing for biodiesel production in oleaginous microalga, Nannochloropsis species. *Gene and Genome Editing*, *6*, 100027. https://doi.org/10.1016/J.GGEDIT.2023.100027

Kvernvik, A. C., Hoppe, C. J. M., Lawrenz, E., Prášil, O., Greenacre, M., Wiktor, J. M., & Leu, E. (2018). Fast reactivation of photosynthesis in arctic phytoplankton during the polar night. *Journal of Phycology*, *54*(4), 461–470. https://doi.org/10.1111/JPY.12750

Kvíderová, J., Souquieres, C.-E., & Elster, J. (2018). *Ecophysiology of photosynthesis of Vaucheria sp. mats in a Svalbard tidal flat*. https://doi.org/10.1016/j.polar.2018.11.006

Lacour, T., Babin, M., & Lavaud, J. (2020). Diversity in Xanthophyll Cycle Pigments Content and Related Nonphotochemical Quenching (NPQ) Among Microalgae: Implications for Growth Strategy and Ecology. *Journal of Phycology*, *56*(2), 245–263. https://doi.org/10.1111/JPY.12944

Lacour, T., Morin, P. I., Sciandra, T., Donaher, N., Campbell, D. A., Ferland, J., & Babin, M. (2019). Decoupling light harvesting, electron transport and carbon fixation during prolonged darkness supports rapid recovery upon re-illumination in the Arctic diatom Chaetoceros neogracilis. *Polar Biology*, *42*(10), 1787–1799. https://doi.org/10.1007/S00300-019-02507-2/FIGURES/7

Lavaud, J., & Goss, R. (2014). The Peculiar Features of Non-Photochemical Fluorescence Quenching in Diatoms and Brown Algae. *Advances in Photosynthesis and Respiration*, *40*, 421–443. https://doi.org/10.1007/978-94-017-9032-1_20

Lavaud, J., & Kroth, P. G. (2006). In diatoms, the transthylakoid proton gradient regulates the photoprotective non-photochemical fluorescence quenching beyond its control on the xanthophyll cycle. *Plant and Cell Physiology*, *47*(7), 1010–1016. https://doi.org/10.1093/PCP/PCJ058

Lavaud, J., Materna, A. C., Sturm, S., Vugrinec, S., & Kroth, P. G. (2012). Silencing of the Violaxanthin De-Epoxidase Gene in the Diatom Phaeodactylum tricornutum

Reduces Diatoxanthin Synthesis and Non-Photochemical Quenching. *PLOS ONE*, *7*(5), e36806. https://doi.org/10.1371/JOURNAL.PONE.0036806

Lavaud, J., Rousseau, B., & Etienne, A. (2004). General features of photoprotection by energy dissipation in planktonic diatoms (Bacillariophyceae). *J Phycol*, *40*.

Lavaud, J., Rousseau, B., Van Gorkom, H. J., & Etienne, A. L. (2002). Influence of the Diadinoxanthin Pool Size on Photoprotection in the Marine Planktonic Diatom Phaeodactylum tricornutum. *Plant Physiology*, *129*(3), 1398–1406. https://doi.org/10.1104/PP.002014

Leonelli, L., Erickson, E., Lyska, D., & Niyogi, K. K. (2016). Transient expression in Nicotiana benthamiana for rapid functional analysis of genes involved in non-photochemical quenching and carotenoid biosynthesis. *The Plant Journal*, *88*(3), 375–386. https://doi.org/10.1111/TPJ.13268

Lepetit, B., Gélin, G., Lepetit, M., Sturm, S., Vugrinec, S., Rogato, A., Kroth, P. G., Falciatore, A., & Lavaud, J. (2017). The diatom Phaeodactylum tricornutum adjusts nonphotochemical fluorescence quenching capacity in response to dynamic light via fine-tuned Lhcx and xanthophyll cycle pigment synthesis. *New Phytologist*, *214*(1), 205–218. https://doi.org/10.1111/NPH.14337

Li, D. H., Wilson, S., Mastroianni, G., & Ruban, A. V. (2023). Altered lipid acyl chain length controls energy dissipation in light-harvesting complex II proteoliposomes by hydrophobic mismatch. *Journal of Photochemistry and Photobiology B: Biology*, *246*, 112758. https://doi.org/10.1016/J.JPHOTOBIOL.2023.112758

Li, X. M., Zhang, Q. S., Tang, Y. Z., Yu, Y. Q., Liu, H. L., & Li, L. X. (2014). Highly efficient photoprotective responses to high light stress in Sargassum thunbergii germlings, a representative brown macroalga of intertidal zone. *Journal of Sea Research*, *85*, 491–498. https://doi.org/10.1016/J.SEARES.2013.08.004

Li, X. P., Björkman, O., Shih, C., Grossman, A. R., Rosenquist, M., Jansson, S., & Niyogi, K. K. (2000). A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature 2000 403:6768*, *403*(6768), 391–395. https://doi.org/10.1038/35000131

Li, X. P., Gilmore, A. M., Caffarri, S., Bassi, R., Golan, T., Kramer, D., & Niyogi, K. K. (2004). Regulation of Photosynthetic Light Harvesting Involves Intrathylakoid Lumen pH Sensing by the PsbS Protein. *Journal of Biological Chemistry*, *279*(22), 22866–22874. https://doi.org/10.1074/JBC.M402461200

Li, Z., Peers, G., Dent, R. M., Bai, Y., Yang, S. Y., Apel, W., Leonelli, L., & Niyogi, K. K. (2016). Evolution of an atypical de-epoxidase for photoprotection in the green lineage. *Nature Plants 2016 2:10*, *2*(10), 1–6. https://doi.org/10.1038/nplants.2016.140

Lichtlé, C., Arsalane, W., Duval, J. C., & Passaquet, C. (1995). CHARACTERIZATION OF THE LIGHT-HARVESTING COMPLEX OF GIRAUDYOPSIS STELLIFER

(CHRYSOPHYCEAE) AND EFFECTS OF LIGHT STRESS1. *Journal of Phycology*, *31*(3), 380–387. https://doi.org/10.1111/J.0022-3646.1995.00380.X

Lie, A. A. Y., Liu, Z., Terrado, R., Tatters, A. O., Heidelberg, K. B., & Caron, D. A. (2018). *A tale of two mixotrophic chrysophytes: Insights into the metabolisms of two Ochromonas species (Chrysophyceae) through a comparison of gene expression*. https://doi.org/10.1371/journal.pone.0192439

Liesner, D., Fouqueau, L., Valero, M., Roleda, M. Y., Pearson, G. A., Bischof, K., Valentin, K., & Bartsch, I. (2020). Heat stress responses and population genetics of the kelp Laminaria digitata (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations. *Ecology and Evolution*, *10*(17), 9144–9177. https://doi.org/10.1002/ECE3.6569

Litvín, R., Bína, D., Herbstová, M., & Gardian, Z. (2016). Architecture of the light-harvesting apparatus of the eustigmatophyte alga Nannochloropsis oceanica. *Photosynthesis Research*, *130*(1–3), 137–150. https://doi.org/10.1007/S11120-016-0234-1

Litvín, R., David Bína, ·, Miroslava Herbstová, ·, Pazderník, M., Kotabová, E., Gardian, Z., Trtílek, M., Prášil, · Ondřej, & Vácha, F. (2019). *Red-shifted light-harvesting system of freshwater eukaryotic alga Trachydiscus minutus (Eustigmatophyta, Stramenopila)*. *142*, 137–151. https://doi.org/10.1007/s11120-019-00662-5

Liu, J., Song, Y., & Qiu, W. (2017a). Oleaginous microalgae Nannochloropsis as a new model for biofuel production: Review & analysis. *Renewable and Sustainable Energy Reviews*, *72*, 154–162. https://doi.org/10.1016/J.RSER.2016.12.120

Liu, J., Song, Y., & Qiu, W. (2017b). Oleaginous microalgae Nannochloropsis as a new model for biofuel production: Review & analysis. *Renewable and Sustainable Energy Reviews*, *72*, 154–162. https://doi.org/10.1016/J.RSER.2016.12.120

Liu, M., Ding, W., Pan, Y., Hu, H., & Liu, J. (2023). Zeaxanthin epoxidase is involved in the carotenoid biosynthesis and light-dependent growth of the marine alga Nannochloropsis oceanica. *Biotechnology for Biofuels and Bioproducts*, *16*(1), 74. https://doi.org/10.1186/S13068-023-02326-Y

Lohr, M., & Wilhelm, C. (1999a). Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(15), 8784–8789. https://doi.org/10.1073/PNAS.96.15.8784

Lohr, M., & Wilhelm, C. (1999b). Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(15), 8784–8789. https://doi.org/10.1073/PNAS.96.15.8784

Malnoë, A., Schultink, A., Shahrasbi, S., Rumeau, D., Havaux, M., & Niyogi, K. K. (2018). The Plastid Lipocalin LCNP Is Required for Sustained Photoprotective

Energy Dissipation in Arabidopsis. *The Plant Cell*, *30*(1), 196–208. https://doi.org/10.1105/tpc.17.00536

Marshall, J. A., & Newman, S. (2002). Differences in photoprotective pigment production between Japanese and Australian strains of Chattonella marina (Raphidophyceae). *Journal of Experimental Marine Biology and Ecology*, *272*, 13–27. www.elsevier.com/locate/jembe

Mazor, Y., Borovikova, A., Caspy, I., & Nelson, N. (2017). *Structure of the plant photosystem I supercomplex at 2.6 Å resolution*. https://doi.org/10.1038/nplants.2017.14

Meng, E. C., Goddard, T. D., Pettersen, E. F., Couch, G. S., Pearson, Z. J., Morris, J. H., Ferrin, T. E., & Thomas Ferrin, C. E. (2023). UCSF ChimeraX: Tools for structure building and analysis. *Protein Science*, *32*(11), e4792. https://doi.org/10.1002/PRO.4792

Michel, G., Tonon, T., Scornet, D., Cock, J. M., & Kloareg, B. (2010). Central and storage carbon metabolism of the brown alga Ectocarpus siliculosus: Insights into the origin and evolution of storage carbohydrates in Eukaryotes. *New Phytologist*, *188*(1), 67–81. https://doi.org/10.1111/J.1469-8137.2010.03345.X

Mock, T., Otillar, R. P., Strauss, J., McMullan, M., Paajanen, P., Schmutz, J., Salamov, A., Sanges, R., Toseland, A., Ward, B. J., Allen, A. E., Dupont, C. L., Frickenhaus, S., Maumus, F., Veluchamy, A., Wu, T., Barry, K. W., Falciatore, A., Ferrante, M. I., … Grigoriev, I. V. (2017). Evolutionary genomics of the cold-adapted diatom Fragilariopsis cylindrus. *Nature 2017 541:7638*, *541*(7638), 536–540. https://doi.org/10.1038/nature20803

Müller, P., Li, X.-P., & Niyogi, K. K. (2001). *Update on Photosynthesis Non-Photochemical Quenching. A Response to Excess Light Energy*. www.plantphysiol.org

Müller-Moulé, P., Conklin, P. L., & Niyogi, K. K. (2002). Ascorbate Deficiency Can Limit Violaxanthin De-Epoxidase Activity in Vivo. *Plant Physiology*, *128*(3), 970–977. https://doi.org/10.1104/PP.010924

Murakami', S., & Packer, L. (1970). Light-induced Changes in the Conformation and Configuration of the Thylakoid Membrane of Ulva and Porphyra Chloroplasts in Vivo. *Plant Physiol*, *45*, 289–299. https://academic.oup.com/plphys/article/45/3/289/6090237

Murchie, E. H., & Ruban, A. V. (2020). Dynamic non-photochemical quenching in plants: from molecular mechanism to productivity. *The Plant Journal*, *101*(4), 885–896. https://doi.org/10.1111/TPJ.14601

Nagao, R., Kato, K., Ifuku, K., Suzuki, T., Kumazawa, M., Uchiyama, I., Kashino, Y., Dohmae, N., Akimoto, S., Shen, J.-R., Miyazaki, N., & Akita, F. (2020). Structural basis for assembly and function of a diatom photosystem I-light-harvesting

supercomplex. *Nature Communications*. https://doi.org/10.1038/s41467-020-16324-3

Nagao, R., Kato, K., Suzuki, T., Ifuku, K., Uchiyama, I., Kashino, Y., Dohmae, N., Akimoto, S., Shen, J. R., Miyazaki, N., & Akita, F. (2019). Structural basis for energy harvesting and dissipation in a diatom PSII–FCPII supercomplex. *Nature Plants*, *5*(8), 890–901. https://doi.org/10.1038/s41477-019-0477-x

Nan, G. N., Zhou, X. Q., Zhang, X. M., Zhang, Q. S., Hu, Z. M., Huang, R. P., & Zhang, D. (2022). Xanthophyll cycle-related non-photochemical quenching protects Sargassum thunbergii from high light-induced photoinhibition. *Frontiers in Marine Science*, *9*, 1067596. https://doi.org/10.3389/FMARS.2022.1067596/BIBTEX

Nawrocki, W. J., Liu, X., Raber, B., Hu, C., De Vitry, C., Bennett, D. I. G., & Croce, R. (2021). Molecular origins of induction and loss of photoinhibition-related energy dissipation qI. *Science Advances*, *7*(52), 55. https://doi.org/10.1126/SCIADV.ABJ0055/SUPPL_FILE/SCIADV.ABJ0055_SM.PDF

Nepper-Davidsen, J. N., Andersen, D. T., & Foldager, M. F. (2019). Exposure to simulated heatwave scenarios causes long-term reductions in performance in Saccharina latissima. *Marine Ecology Progress Series*, *630*(630), 25–39. https://doi.org/10.3354/meps13133

Neubauer, C. (1993). Multiple Effects of Dithiothreitol on Nonphotochemical Fluorescence Quenching in Intact Chloroplasts (Influence on Violaxanthin De-epoxidase and Ascorbate Peroxidase Activity). *Plant Physiology*, *103*(2), 575–583. https://doi.org/10.1104/PP.103.2.575

Nilkens, M., Kress, E., Lambrev, P., Miloslavina, Y., Müller, M., Holzwarth, A. R., & Jahns, P. (2010). Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in Arabidopsis. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1797*(4), 466–475. https://doi.org/10.1016/J.BBABIO.2010.01.001

Niyogi, K. K., Björkman, O., & Grossman, A. R. (1997a). Chlamydomonas Xanthophyll Cycle Mutants Identified by Video Imaging of Chlorophyll Fluorescence Quenching. *The Plant Cell*, *9*(8), 1369–1380. https://doi.org/10.1105/TPC.9.8.1369

Niyogi, K. K., Björkman, O., & Grossman, A. R. (1997b). The roles of specific xanthophylls in photoprotection. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(25), 14162–14167. https://doi.org/10.1073/PNAS.94.25.14162

Niyogi, K. K., Grossman, A. R., & Björkman, O. (1998). Arabidopsis mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. *The Plant Cell*, *10*(7), 1121–1134. https://doi.org/10.1105/TPC.10.7.1121

Niyogi, K. K., Shih, C., Soon Chow, W., Pogson, B. J., DellaPenna, D., & Björkman, O. (2001). Photoprotection in a zeaxanthin- and lutein-deficient double mutant of Arabidopsis. *Photosynthesis Research 2001 67:1*, *67*(1), 139–145. https://doi.org/10.1023/A:1010661102365

Niyogi, K. K., & Truong, T. B. (2013). Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Current Opinion in Plant Biology*, *16*(3), 307–314. https://doi.org/10.1016/J.PBI.2013.03.011

Nymark, M., Valle, K. C., Brembu, T., Hancke, K., Winge, P., Andresen, K., Johnsen, G., & Bones, A. M. (2009). An Integrated Analysis of Molecular Acclimation to High Light in the Marine Diatom Phaeodactylum tricornutum. *PLOS ONE*, *4*(11), e7743. https://doi.org/10.1371/JOURNAL.PONE.0007743

Nymark, M., Valle, K. C., Hancke, K., Winge, P., Andresen, K., Johnsen, G., Bones, A. M., & Brembu, T. (2013). Molecular and Photosynthetic Responses to Prolonged Darkness and Subsequent Acclimation to Re-Illumination in the Diatom Phaeodactylum tricornutum. *PLOS ONE*, *8*(3), e58722. https://doi.org/10.1371/JOURNAL.PONE.0058722

Ocampo-Alvarez, H., & García-Mendoza, E. (2013). Antagonist effect between violaxanthin and de-epoxidated pigments in nonphotochemical quenching induction in the qE deficient brown alga Macrocystis pyrifera. *Biochimica et Biophysica Acta*, *1827*, 427–437. https://doi.org/10.1016/j.bbabio.2012.12.006

Ohi, M., Li, Y., Cheng, Y., & Walz, T. (2004). Negative Staining and Image Classification - Powerful Tools in Modern Electron Microscopy. *Biological Procedures Online*, *6*(1), 23–34. https://doi.org/10.1251/BPO70

Olaizola, M., La Roche, J., Kolber, Z., & Falkowski, P. G. (1994). Non-photochemical fluorescence quenching and the diadinoxanthin cycle in a marine diatom. *Photosynthesis Research 1994 41:2*, *41*(2), 357–370. https://doi.org/10.1007/BF00019413

Owens, T. G., Gallagher, J. C., & Alberte, R. S. (1987). PHOTOSYNTHETIC LIGHT-HARVESTING FUNCTION OF VIOLAXANTHIN IN NANNOCHLOROPSIS SPP. (EUSTIGMATOPHYCEAE). *Journal of Phycology*, *23*(2), 79–85. https://doi.org/10.1111/J.1529-8817.1987.TB04429.X

Park, S., Steen, C. J., Lyska, D., Fischer, A. L., Endelman, B., Iwai, M., Niyogi, K. K., & Fleming, G. R. (2019). Chlorophyll-carotenoid excitation energy transfer and charge transfer in Nannochloropsis oceanica for the regulation of photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(9), 3385–3390. https://doi.org/10.1073/pnas.1819011116

Peers, G., Truong, T. B., Ostendorf, E., Busch, A., Elrad, D., Grossman, A. R., Hippler, M., & Niyogi, K. K. (2009). An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. *Nature*, *462*(7272), 518–521. https://doi.org/10.1038/NATURE08587

Perin, G., Bellan, A., Lyska, D., Niyogi, K. K., & Morosinotto, T. (2022). *Modulation of xanthophyll cycle impacts biomass productivity in the marine microalga Nannochloropsis 2 3*. https://doi.org/10.1101/2022.08.16.504082

Perin, G., Bellan, A., Michelberger, T., Lyska, D., Wakao, S., Niyogi, K. K., & Morosinotto, T. (2023). Modulation of xanthophyll cycle impacts biomass productivity in the marine microalga Nannochloropsis . *Proceedings of the National Academy of Sciences*, *120*(25), e2214119120. https://doi.org/10.1073/PNAS.2214119120/SUPPL_FILE/PNAS.2214119120.SAPP.PDF

Perin, G., Bellan, A., Segalla, A., Meneghesso, A., Alboresi, A., & Morosinotto, T. (2015). Generation of random mutants to improve light-use efficiency of Nannochloropsis gaditana cultures for biofuel production. *Biotechnology for Biofuels*, *8*(1), 1–13. https://doi.org/10.1186/S13068-015-0337-5/FIGURES/7

Pi, X., Tian, L., Dai, H. E., Qin, X., Cheng, L., Kuang, T., Sui, S. F., & Shen, J. R. (2018). Unique organization of photosystem I-light-harvesting supercomplex revealed by cryo-EM from a red alga. *Proc. Natl Acad. Sci. USA*, *115*(17), 4423–4428. https://doi.org/10.1073/pnas.1722482115

Pi, X., Zhao, S., Wang, W., Liu, D., Xu, C., Han, G., Kuang, T., Sui, S.-F., & Shen, J.-R. (2019). The pigment-protein network of a diatom photosystem II-light-harvesting antenna supercomplex. *Science (New York, N.Y.)*, *365*(6452), eaax4406. https://doi.org/10.1126/science.aax4406

Pinnola, A., Dall'Osto, L., Gerotto, C., Morosinotto, T., Bassi, R., & Alboresi, A. (2013). Zeaxanthin Binds to Light-Harvesting Complex Stress-Related Protein to Enhance Nonphotochemical Quenching in Physcomitrella patens. *The Plant Cell*, *25*(9), 3519–3534. https://doi.org/10.1105/TPC.113.114538

Porra, R. J., Thompson, W. A., & Kriedemann, P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *975*(3), 384–394. https://doi.org/10.1016/S0005-2728(89)80347-0

Qin, X., Pi, X., Wang, W., Han, G., Zhu, L., Liu, M., Cheng, L., Shen, J. R., Kuang, T., & Sui, S. F. (2019). Structure of a green algal photosystem I in complex with a large number of light-harvesting complex I subunits. *Nat. Plants*, *5*(3), 263–272. https://doi.org/10.1038/s41477-019-0379-y

Qin, X., Suga, M., Kuang, T., & Shen, J. R. (2015). Photosynthesis. Structural basis for energy transfer pathways in the plant PSI-LHCI supercomplex. *Science*, *348*(6238), 989–995. https://doi.org/10.1126/science.aab0214

Quaas, T., Berteotti, S., Ballottari, M., Flieger, K., Bassi, R., Wilhelm, C., & Goss, R. (2015). Non-photochemical quenching and xanthophyll cycle activities in six green

algal species suggest mechanistic differences in the process of excess energy dissipation. *Journal of Plant Physiology*, *172*, 92–103. https://doi.org/10.1016/J.JPLPH.2014.07.023

Rico, C., Matsuda, Y., Lin, X., Litvin, R., Blommaert, L., Vancaester, E., J Huysman, M. J., Osuna-Cruz, C. M., Lavaud, J., Lepetit, B., Winge, P., Bones, A. M., Vandepoele, K., Vyverman, W., & Sabbe, K. (2020). *Light Regulation of LHCX Genes in the Benthic Diatom Seminavis robusta*. https://doi.org/10.3389/fmars.2020.00192

Semchonok, D. A., Sathish Yadav, K. N., Xu, P., Drop, B., Croce, R., & Boekema, E. J. (2017). Interaction between the photoprotective protein LHCSR3 and C2S2 Photosystem II supercomplex in Chlamydomonas reinhardtii. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1858*(5), 379–385. https://doi.org/10.1016/J.BBABIO.2017.02.015

Shen, L., Huang, Z., Chang, S., Wang, W., Wang, J., Kuang, T., Han, G., Shen, J. R., & Zhang, X. (2019). Structure of a C2S2M2N2-type PSII–LHCII supercomplex from the green alga Chlamydomonas reinhardtii. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(42), 21246–21255. https://doi.org/10.1073/pnas.1912462116

Shimasaki, Y., Mukai, K., Takai, Y., Qiu, X., & Oshima, Y. (2021). *antioxidants Recent Progress in the Study of Peroxiredoxin in the Harmful Algal Bloom Species Chattonella marina*. https://doi.org/10.3390/antiox10020162

Short, A., Fay, T. P., Crisanto, T., Mangal, R., Niyogi, K. K., Limmer, D. T., & Fleming, G. R. (2023). Kinetics of the xanthophyll cycle and its role in photoprotective memory and response. *Nature Communications 2023 14:1*, *14*(1), 1–10. https://doi.org/10.1038/s41467-023-42281-8

Short, A. H., Fay, T. P., Crisanto, T., Hall, J., Steen, C. J., Niyogi, K. K., Limmer, D. T., & Fleming, G. R. (2022). Xanthophyll-cycle based model of the rapid photoprotection of Nannochloropsis in response to regular and irregular light/dark sequences. *The Journal of Chemical Physics*, *156*(20), 205102. https://doi.org/10.1063/5.0089335

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology*, *7*. https://doi.org/10.1038/MSB.2011.75

Simionato, D., Basso, S., Giacometti, G. M., & Morosinotto, T. (2013). Optimization of light use efficiency for biofuel production in algae. *Biophys Chem*, *182*, 71–78. https://doi.org/10.1016/j.bpc.2013.06.017

Simionato, D., Block, M. A., la Rocca, N., Jouhet, J., Maréchal, E., Finazzi, G., & Morosinotto, T. (2013). The response of Nannochloropsis gaditana to nitrogen

starvation includes de novo biosynthesis of triacylglycerols, a decrease of chloroplast galactolipids, and reorganization of the photosynthetic apparatus. *Eukaryot Cell*, *12*(5), 665–676. https://doi.org/10.1128/ec.00363-12

Simionato, D., Sforza, E., Corteggiani Carpinelli, E., Bertucco, A., Giacometti, G. M., & Morosinotto, T. (2011a). Acclimation of Nannochloropsis gaditana to different illumination regimes: Effects on lipids accumulation. *Bioresource Technology*, *102*(10), 6026–6032. https://doi.org/10.1016/J.BIORTECH.2011.02.100

Simionato, D., Sforza, E., Corteggiani Carpinelli, E., Bertucco, A., Giacometti, G. M., & Morosinotto, T. (2011b). Acclimation of Nannochloropsis gaditana to different illumination regimes: Effects on lipids accumulation. *Bioresource Technology*, *102*(10), 6026–6032. https://doi.org/10.1016/J.BIORTECH.2011.02.100

Six, C., Finkel, Z. V., Rodriguez, F., Marie, D., Partensky, F., & Campbell, D. A. (2008). Contrasting photoacclimation costs in ecotypes of the marine eukaryotic picoplankter Ostreococcus. *Limnology and Oceanography*, *53*(1), 255–265. https://doi.org/10.4319/LO.2008.53.1.0255

Soule, K. M., & Rumpho, M. E. (2012). Light-regulated photosynthetic gene expression and phosphoribulokinase enzyme activity in the heterokont alga vaucheria litorea (xanthophyceae) and its symbiotic molluskan partner elysia chlorotica (gastropoda). *Journal of Phycology*, *48*(2), 373–383. https://doi.org/10.1111/J.1529-8817.2012.01111.X

Streckaite, S., Gardian, Z., Li, F., Pascal, A. A., Litvin, R., Robert, B., & Llansola-Portoles, M. J. (2018). Pigment configuration in the light-harvesting protein of the xanthophyte alga Xanthonema debile. *Photosynthesis Research*, *138*, 139–148. https://doi.org/10.1007/s11120-018-0557-1

Suga, M., Ozawa, S. I., Yoshida-Motomura, K., Akita, F., Miyazaki, N., & Takahashi, Y. (2019). Structure of the green algal photosystem I supercomplex with a decameric light-harvesting complex I. *Nature Plants 2019 5:6*, *5*(6), 626–636. https://doi.org/10.1038/s41477-019-0438-4

Sukenik, A., Livne, A., Apt, K. E., & Grossman, A. R. (2000). CHARACTERIZATION OF A GENE ENCODING THE LIGHT-HARVESTING VIOLAXANTHIN-CHLOROPHYLL PROTEIN OF NANNOCHLOROPSIS SP. (EUSTIGMATOPHYCEAE). *Journal of Phycology*, *36*(3), 563–570. https://doi.org/10.1046/J.1529-8817.2000.99115.X

Sun, K.-M., Gao, C., Zhang, J., Tang, · Xuexi, Wang, Z., Zhang, X., & Li, Y. (2020). Rapid formation of antheraxanthin and zeaxanthin in seconds in microalgae and its relation to non-photochemical quenching. *Photosynthesis Research*, *144*, 317–326. https://doi.org/10.1007/s11120-020-00739-6

Sylak-Glassman, E. J., Malnoe, A., De Re, E., Brooks, M. D., Fischer, A. L., Niyogi, K. K., & Fleming, G. R. (2014). Distinct roles of the photosystem II protein PsbS and zeaxanthin in the regulation of light harvesting in plants revealed by fluorescence

lifetime snapshots. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(49), 17498–17503. https://doi.org/10.1073/PNAS.1418317111

Taddei, L., Chukhutsina, V. U., Lepetit, B., Stella, G. R., Bassi, R., van Amerongen, H., Bouly, J. P., Jaubert, M., Finazzi, G., & Falciatore, A. (2018). Dynamic Changes between Two LHCX-Related Energy Quenching Sites Control Diatom Photoacclimation. *Plant Physiology*, *177*(3), 953–965. https://doi.org/10.1104/PP.18.00448

Taddei, L., Stella, G. R., Rogato, A., Bailleul, B., Fortunato, A. E., Annunziata, R., Sanges, R., Thaler, M., Lepetit, B., Lavaud, J., Jaubert, M., Finazzi, G., Bouly, J. P., & Falciatore, A. (2016). Multisignal control of expression of the LHCX protein family in the marine diatom Phaeodactylum tricornutum. *Journal of Experimental Botany*, *67*(13), 3939–3951. https://doi.org/10.1093/JXB/ERW198

Takahashi, H., Iwai, M., Takahashi, Y., & Minagawa, J. (2006). Identification of the mobile light-harvesting complex II polypeptides for state transitions in Chlamydomonas reinhardtii. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(2), 477–482. https://doi.org/10.1073/PNAS.0509952103

Tanabe, Y., Shitara, T., Kashino, Y., Hara, Y., & Kudoh, S. (2009). Utilizing the Effective Xanthophyll Cycle for Blooming of Ochromonas smithii and O. itoi (Chrysophyceae) on the Snow Surface. *Plos One*. https://doi.org/10.1371/journal.pone.0014690

Teo, C. L., Atta, M., Bukhari, A., Taisir, M., Yusuf, A. M., & Idris, A. (2014). Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths. *Bioresource Technology*, *162*, 38–44. https://doi.org/10.1016/J.BIORTECH.2014.03.113

Tian, L., Nawrocki, W. J., Liu, X., Polukhina, I., Van Stokkum, I. H. M., & Croce, R. (2019). PH dependence, kinetics and light-harvesting regulation of nonphotochemical quenching in Chlamydomonas. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(17), 8320–8325. https://doi.org/10.1073/PNAS.1817796116/SUPPL_FILE/PNAS.1817796116.SAPP.PDF

Tibiletti, T., Auroy, P., Peltier, G., & Caffarri, S. (2016). Chlamydomonas reinhardtii PsbS Protein Is Functional and Accumulates Rapidly and Transiently under High Light. *Plant Physiology*, *171*(4), 2717–2730. https://doi.org/10.1104/PP.16.00572

Uhrmacher, S., Hanelt, D., & Nultsch, W. (1995). Zeaxanthin content and the degree of photoinhibition are linearly correlated in the brown alga Dictyota dichotoma. *Marine Biology*, *123*, 159–165.

Umetani, I., Kunugi, M., Yokono, M., Takabayashi, A., & Tanaka, A. (2018). Evidence of the supercomplex organization of photosystem II and light-harvesting complexes in

Nannochloropsis granulata. *Photosynthesis Research*, *136*(1), 49–61. https://doi.org/10.1007/s11120-017-0438-z

Vieler, A., Wu, G., Tsai, C. H., Bullard, B., Cornish, A. J., Harvey, C., Reca, I. B., Thornburg, C., Achawanantakun, R., Buehl, C. J., Campbell, M. S., Cavalier, D., Childs, K. L., Clark, T. J., Deshpande, R., Erickson, E., Armenia Ferguson, A., Handee, W., Kong, Q., … Benning, C. (2012a). Genome, Functional Gene Annotation, and Nuclear Transformation of the Heterokont Oleaginous Alga Nannochloropsis oceanica CCMP1779. *PLoS Genetics*, *8*(11). https://doi.org/10.1371/journal.pgen.1003064

Vieler, A., Wu, G., Tsai, C.-H., Bullard, B., Cornish, A. J., Harvey, C., Reca, I.-B., Thornburg, C., Achawanantakun, R., Buehl, C. J., Campbell, M. S., Cavalier, D., Childs, K. L., Clark, T. J., Deshpande, R., Erickson, E., Armenia Ferguson, A., Handee, W., Kong, Q., … Benning, C. (2012b). Genome, Functional Gene Annotation, and Nuclear Transformation of the Heterokont Oleaginous Alga Nannochloropsis oceanica CCMP1779. *PLoS Genetics*, *8*(11), e1003064. https://doi.org/10.1371/journal.pgen.1003064

Walter, J., & Kromdijk, J. (2022). Here comes the sun: How optimization of photosynthetic light reactions can boost crop yields. *Journal of Integrative Plant Biology*, *64*(2), 564–591. https://doi.org/10.1111/JIPB.13206

Walters, R. G., Ruban, A. v., & Horton, P. (1996). Identification of proton-active residues in a higher plant light-harvesting complex. *Proceedings of the National Academy of Sciences*, *93*(24), 14204–14209. https://doi.org/10.1073/PNAS.93.24.14204

Wang, H., Ji, B., Wang, J., Guo, F., Zhou, W., Gao, L., & Liu, T. Z. (2014). Growth and biochemical composition of filamentous microalgae Tribonema sp. as potential biofuel feedstock. *Bioprocess and Biosystems Engineering*, *37*(12), 2607–2613. https://doi.org/10.1007/S00449-014-1238-X/TABLES/2

Wang, W., Yu, L. J., Xu, C., Tomizaki, T., Zhao, S., Umena, Y., Chen, X., Qin, X., Xin, Y., Suga, M., Han, G., Kuang, T., & Shen, J. R. (2019). Structural basis for blue-green light harvesting and energy dissipation in diatoms. *Science*, *363*(6427). https://doi.org/10.1126/SCIENCE.AAV0365/SUPPL_FILE/AAV0365-WANG-SM.PDF

Wang, W., Zhao, S., Pi, X., Kuang, T., Sui, S. F., & Shen, J. R. (2020). Structural features of the diatom photosystem II–light-harvesting antenna complex. *The FEBS Journal*, *287*(11), 2191–2200. https://doi.org/10.1111/FEBS.15183

Wang, W., Zhao, S., Shen, L., Li, X., Tao, Q., Li, Z., Xu, C., Zhou, C., Yang, Y., Sang, M., & Han, G. (2023). Structural insights into photosystem II supercomplex and trimeric FCP antennae of a centric diatom Cyclotella meneghiniana. *Preprint*. https://doi.org/10.21203/rs.3.rs-3094926/v1

Wetherbee, R., Jackson, C. J., Repetti, S. I., Clementson, L. A., Costa, J. F., van de Meene, A., Crawford, S., & Verbruggen, H. (2019). The golden paradox - a new

heterokont lineage with chloroplasts surrounded by two membranes. *Journal of Phycology*, *55*(2), 257–278. https://doi.org/10.1111/JPY.12822

Wilken, S., Choi, C. J., & Worden, A. Z. (2020). Contrasting Mixotrophic Lifestyles Reveal Different Ecological Niches in Two Closely Related Marine Protists. *Journal of Phycology*, *56*(1), 52–67. https://doi.org/10.1111/JPY.12920

Wilken, S., Huisman, J., Naus-Wiezer, S., & Van Donk, E. (2013). Mixotrophic organisms become more heterotrophic with rising temperature. *Ecology Letters*, *16*(2), 225–233. https://doi.org/10.1111/ELE.12033

Xu, C., Pi, X., Huang, Y., Han, G., Chen, X., Qin, X., Huang, G., Zhao, S., Yang, Y., Kuang, T., Wang, W., Sui, S. F., & Shen, J. R. (2020). Structural basis for energy transfer in a huge diatom PSI-FCPI supercomplex. *Nature Communications 2020 11:1*, *11*(1), 1–12. https://doi.org/10.1038/s41467-020-18867-x

Yang, R., Wei, D., & Xie, J. (2020). Diatoms as cell factories for high-value products: chrysolaminarin, eicosapentaenoic acid, and fucoxanthin. *Critical Reviews in Biotechnology*, *40*(7), 993–1009. https://doi.org/10.1080/07388551.2020.1805402

Yoon, H. S., Andersen, R. A., Boo, S. M., & Bhattacharya, D. (2009). Stramenopiles. *Encyclopedia of Microbiology, Third Edition*, 721–731. https://doi.org/10.1016/B978-012373944-5.00253-4

You, X., Zhang, X., Cheng, J., Xiao, Y., Ma, J., Sun, S., Zhang, X., Wang, H.-W., & Sui, S.-F. (2023). In situ structure of the red algal phycobilisome-PSII-PSI-LHC megacomplex. *Nature*, *616*. https://doi.org/10.1038/s41586-023-05831-0

Yuasa, K., Shikata, T., Kitatsuji, S., Yamasaki, Y., & Nishiyama, Y. (2020). *Extracellular secretion of superoxide is regulated by photosynthetic electron transport in the noxious red-tide-forming raphidophyte Chattonella antiqua*. https://doi.org/10.1016/j.jphotobiol.2020.111839

Zhang, X., Xu, D., Guan, Z., Wang, S., Zhang, Y., Wang, W., Zhang, X., Fan, X., Li, F., & Ye, N. (2020). Elevated CO 2 concentrations promote growth and photosynthesis of the brown alga Saccharina japonica. *Journal of Applied Phycology*, *32*, 1949–1959. https://doi.org/10.1007/s10811-020-02108-1

Zhao, L.-S., Wang, P., Li, K., Zhang, Q.-B., He, F.-Y., Li, C.-Y., Su, H.-N., Chen, X.-L., Liu, L.-N., & Zhang, Y.-Z. (2023). *Structural basis and evolution of the photosystem I-light-harvesting supercomplex of cryptophyte algae*. https://doi.org/10.1093/plcell/koad087

Zhu, S. H., & Green, B. R. (2010). Photoprotection in the diatom Thalassiosira pseudonana: Role of LI818-like proteins in response to high light stress. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1797*(8), 1449–1457. https://doi.org/10.1016/J.BBABIO.2010.04.003