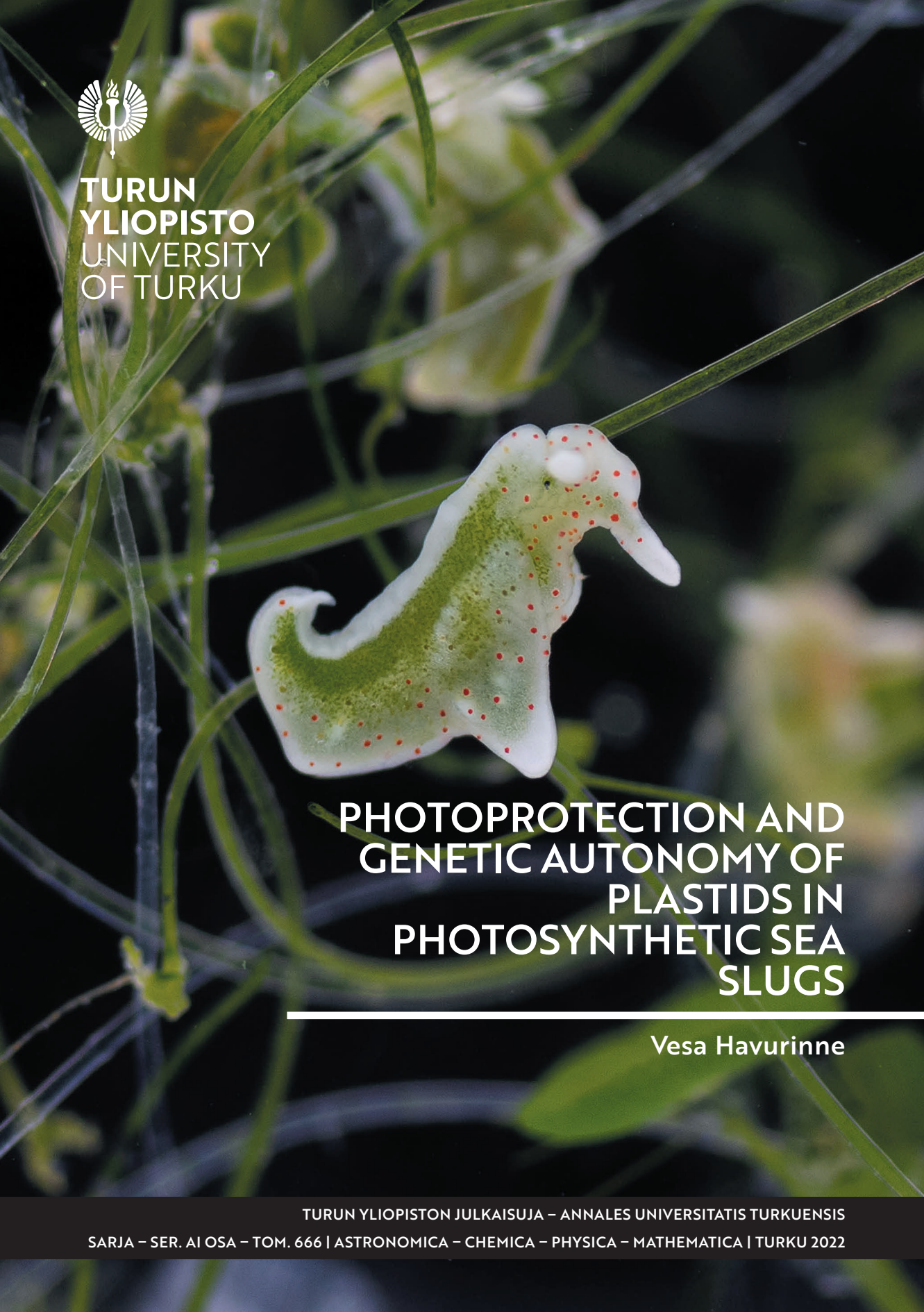

TURUN YLIOPISTO
UNIVERSITY OF TURKU

PHOTOPROTECTION AND GENETIC AUTONOMY OF PLASTIDS IN PHOTOSYNTHETIC SEA SLUGS

Vesa Havurinne

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. AI OSA – TOM. 666 | ASTRONOMICA – CHEMICA – PHYSICA – MATHEMATICA | TURKU 2022

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

PHOTOPROTECTION AND GENETIC AUTONOMY OF PLASTIDS IN PHOTOSYNTHETIC SEA SLUGS

Vesa Havurinne

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. AI OSA – TOM. 666 | ASTRONOMICA – CHEMICA – PHYSICA – MATHEMATICA | TURKU 2022

University of Turku

Faculty of Technology
Department of Life Technologies
Molecular Plant Biology
Doctoral Programme in Technology

Supervised by

Adjunct Professor, Esa Tyystjärvi
University of Turku
Faculty of Technology
Department of Life Technologies
Molecular Plant Biology unit

Reviewed by

Professor, Fikret Mamedov
University of Uppsala
Department of Chemistry
Sweden

Assistant Professor, João Serôdio
University of Aveiro
Department of Biology
Portugal

Opponent

Professor, Peter Jahns
Heinrich Heine University Düsseldorf
Department of Biology
Germany

The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

Cover Image: Vesa Havurinne

ISBN 978-951-29-8797-9 (PRINT)
ISBN 978-951-29-8798-6 (PDF)
ISSN 0082-7002 (Print)
ISSN 2343-3175 (Online)
Painosalama, Turku, Finland 2022

“If only I'd listened to my Uncle Poo-poo and gone into dentistry," whined Pepsi.
-The Harvard Lampoon, Bored of The Rings

UNIVERSITY OF TURKU
Faculty of Technology
Department of Life Technologies
Molecular Plant Biology
VESA HAVURINNE: Photoprotection and Genetic Autonomy of Plastids in Photosynthetic Sea Slugs
Doctoral Dissertation, 188 pp.
Doctoral Programme in Technology
February 2022

ABSTRACT

Certain sea slugs "steal" the photosynthetic cellular organelles, the plastids, from their prey algae and incorporate them, still functional, inside their own cells. These animals can then remain photosynthetic for months. The redox reactions of photosynthesis are associated with inevitable damage that needs to be constantly repaired. Running photosynthesis with plastids isolated from their algal cell should not be possible, as the algal nucleus that encodes essential maintenance proteins of the photosynthetic machinery is absent in the slug cells. How do photosynthetic sea slugs then avoid or repair the oxidative damage that their plastids should be facing? In my thesis, I have tackled this question by comparing the differences in photosynthetic electron transfer between the photosynthetic sea slug *Elysia timida* and the source of its plastids, the alga *Acetabularia acetabulum*. In addition, I compared the rates of photodamage to the plastids in the slugs and in their prey algae. I used the alga *Vaucheria litorea,* the prey of the slug *Elysia chlorotica*, to investigate the intrinsic properties of *V. litorea* plastids that could help explain how these plastids tolerate isolation.

I demonstrate that the slugs maintain the plastoquinone pool of the plastids in an oxidized state in the dark, which can reduce electron pressure and oxidative damage in the photosynthetic electron transfer chain during dark-to-light transitions. The plastids in the slugs also have an increased capacity to dissipate excessive excitation energy as heat compared to the algae. Secondly, I show that most plastids in the slugs are protected against excessive light and UV radiation; tightly packed plastids in the outer layers of the slugs shield the inner ones, and UV radiation is blocked by the slug tissue. Finally, I reveal that the plastids of *V. litorea* are genetically very autonomous in recovering from photodamage. The relative transcript levels of the plastid encoded translation elongation factor EF-Tu and the plastid maintenance protease FtsH increased in lab-isolated *V. litorea* plastids during a seven-day incubation period, supporting the view that they likely aid in the longevity of the plastids also inside the slugs. Furthermore, *V. litorea* plastids produce only low amounts of singlet oxygen, a reactive oxygen species that is known to inhibit the repair machinery of the plastids.

KEYWORDS: Algae, kleptoplasty, photoinhibition, photosynthetic sea slugs, plastids, PSII repair cycle, ROS, symbiosis

TURUN YLIOPISTO
Teknillinen tiedekunta
Bioteknologian laitos
Molekulaarinen kasvibiologia
VESA HAVURINNE: Photoprotection and Genetic Autonomy of Plastids in Photosynthetic Sea Slugs
Väitöskirja, 188 s.
Teknologian tohtoriohjelma
Helmikuu 2022

TIIVISTELMÄ

Tietyt merietanat ”varastavat” levien viherhiukkasia, ja sisällyttävät ne toimintakykyisinä omiin soluihinsa. Tämän jälkeen etanat pysyvät yhteytyskykyisinä kuukausia. Leväsoluista eristettyjen viherhiukkasten jatkuvan yhteyttämisen ei pitäisi olla mahdollista, sillä yhteyttämisen hapetus-pelkistysreaktiot vaurioittavat yhteytyskoneistoa itseään, vaatien jatkuvaa korjausta. Etanoiden solut eivät kuitenkaan sisällä leväsolun tumaa, joka koodaa viherhiukkasen ylläpidon kannalta välttämättömiä proteiineja. Miten yhteyttävät merietanat välttelevät valon aiheuttamaa vauriota tai korjaavat viherhiukkasiaan? Vastatakseni tähän kysymykseen, vertasin aluksi yhteyttämisen elektroninsiirtoreaktioita *Elysia timida* -merietanan ja sen viherhiukkasten lähteen, *Acetabularia acetabulum* -levän, välillä. Näitä kahta lajia käytin myös verratakseni valovaurion etenemistä merietanoissa ja niiden saalislevissä. Tutkin myös *Elysia chlorotica* -merietanan *Vaucheria litorea* -saalislevää selvittääkseni, ovatko kyseisen levän viherhiukkaset syntyjään valmiita selviämään eristyksissä.

Tulokseni osoittavat, että merietanoiden viherhiukkasten plastokinonivaranto pysyy hapettuneena pimeässä, mikä voi alentaa yhteyttämisen elektroninsiirtoketjun elektronipainetta ja vaurioitumista etanoiden siirtyessä valoon. Etanoiden viherhiukkasten kyky haihduttaa liiallista viritysenergiaa lämmöksi on myös voimakkaampi kuin saalislevillä. Lisäksi näytän, että etanoiden ulkokerrosten tiukkaan pakatut viherhiukkaset toimivat valokilpenä alemmille viherhiukkasille, ja etanan oma kudos suojaa viherhiukkasia UV-säteilyltä. Molemmat seikat vähentävät viherhiukkasten vaurioitumista. Lopuksi osoitan, että *V. litorea* -levän viherhiukkaset kykenevät palautumaan valovauriosta itsenäisesti ilman tumaa. Viherhiukkasten genomin koodaamien translaation elongaatiotekijä EF-Tu:n ja FtsH-ylläpitoproteaasin transkriptien suhteelliset määrät kasvoivat, kun *V. litorea* -levän viherhiukkasia pidettiin eristyksissä seitsemän päivää, mikä tukee aiempaa ehdotusta näiden kahden tekijän tärkeydestä viherhiukkasten pitkäkestoisuudelle. Tämän lisäksi nämä viherhiukkaset tuottavat vain vähän singlettihappea, jonka tiedetään estävän viherhiukkasten korjausmekanismeja toimimasta.

ASIASANAT: Fotoinhibitio, kleptoplastismi, levät, PSII:n korjauskierto, ROS, symbioosi, viherhiukkanen, yhteyttävät merietanat

Table of Contents

Abbreviations

(P)PFD	(Photosynthetic) photon flux density
$^1O_2$	Singlet oxygen
^{3}Chl	Triplet Chl
$^3O_2$	Triplet oxygen; the ground state (common form) of molecular oxygen
^{3}P680	Triplet Chl of PSII reaction centre
A_{0A}/A_{0B}	Primary electron acceptors from P700 in PSI
Acc_A/Acc_B	"Accessory" Chls in PSI reaction centre
ADP	Adenosine diphosphate
ASW	Artificial sea water
ATP	Adenosine triphosphate
ATPase	ATP synthase
CBB	Calvin-Benson-Bassham cycle
Chl	Chlorophyll
Chl_{D1}/Chl_{D2}	"Accessory" PSII reaction centre Chls
CP26/CP29	PSII antenna proteins
CP43/CP47	Intrinsic antenna proteins of PSII core
CTPA	Carboxyl terminal peptidase
Cyt *b6f*	Cytochrome *b6f* complex
Cyt *c6*	Cytochrome *c6*
D1/D2	PSII core proteins
DCBQ	2,6-dichloro-1,4-benzoquinone
DCMU	3-(3, 4-dichlorophenyl)−1, 1-dimethylurea
DCPIP	dichlorophenolindophenol
DDE	Diadinoxanthin de-epoxidase
DHA	Dehydroascorbate
DTE	Diatoxanthin epoxidase
ECS	Electrochromic shift
EF-Tu	Translation elongation factor Tu
EGT	Endosymbiotic gene transfer
ER	Endoplasmic reticulum
F_0	Minimum Chl fluorescence

FCP	Fucoxanthin-Chl *a*/*c*-binding protein
Fd	Ferredoxin
FLV	Flavodiiron protein
F_M	Maximum Chl fluorescence after dark acclimation
F_M'	Maximum Chl fluorescence of during illumination
FNR	Ferredoxin NAD(P)H oxidoreductase
FtsH	Plastid maintenance protease
F_V	Variable Chl fluorescence; F_M-F_0
F_V/F_M	Maximum quantum yield of PSII photochemistry
F_X, F_A, F_B	4Fe-4S clusters of PSI
G3P	Glyceraldehyde-3-phosphate
H_2O_2	Hydrogen peroxide
$HO^{\bullet}$	Hydroxyl radical
k_{PI}	Rate constant of photoinhibition of PSII
LHC	Light harvesting complex
LHCI	Light harvesting complex of PSI
LHCII	Light harvesting complex of PSII
LHCSR	Stress-related LHC proteins
LHCX	Stress-related LHC proteins of stramenopile algae
MDA	Monodehydroascorbate radical
NAD(P)H	Nicotinamide adenine dinucleotide (phosphate)
NDA2	Type 2 NDH protein
NDH	NADH-dehydrogenase-like complex
NPQ	Non-photochemical quenching (of Chl fluorescence)
$O_2^{\bullet-}$	Superoxide anion radical
OEC	Oxygen evolving complex of PSII
OJIP	Polyphasic fluorescence induction curve
P680	PSII reaction centre Chl conglomerate
P680*	Excited state PSII reaction centre Chl conglomerate
$P680^+$	Oxidized PSII reaction centre Chl conglomerate
P700	PSI reaction centre Chl conglomerate
P700*	Excited state PSI reaction centre Chl conglomerate
$P700^+$	Oxidized PSI reaction centre Chl conglomerate
P_A/P_B	PSI reaction centre Chls
PC	Plastocyanin
P_{D1}/P_{D2}	PSII reaction centre Chls
pERM	Plastid endoplasmic reticulum membrane
PGR5	Proton gradient regulation 5 -protein
PGRL-1	PGR5-like photosynthetic phenotype 1 -protein
Phe_{D1}	Pheophytin in the D1 protein of PSI

PhQ_A/PhQ_B	Phylloquinones of PSI
P_M	Maximum oxidation of P700
pmf	Proton motive force
PPM	Periplastidial membrane
PQ	Plastoquinone
PQH_2	Plastoquinol
PsaA/PsaB	PSI core proteins
PSI	Photosystem I
PSII	Photosystem II
PTOX	Plastid terminal oxidase
Q_A/Q_B	Primary/secondary stable electron acceptors of PSII
Q-cycle	Quinol oxidation cycle
qE	Energy-dependent component of NPQ
qI	Photoinhibition-related component of NPQ
Q_o/Q_i	PQ binding sites of Cyt *b6f*
qT	State transition component of NPQ
rETR	Relative electron transfer rate
ROS	Reactive oxygen species
RT-qPCR	Reverse transcription quantitative real-time PCR
Rubisco	Ribulose-1,5-bisphosphate carboxylase oxygenase
RuBP	Ribulose-1,5-bisphosphate
S0-S4	Different oxidation states of the OEC
SOD	Superoxide dismutase
TGA	Triacylglyserol
Tic	Translocator of the inner envelope membrane of the plastid
Toc	Translocator of the outer envelope membrane of the plastid
Trx	Thioredoxin
UTR	Untranslated region of mRNA
UV	Ultraviolet radiation (200-400 nm)
UVA	UV radiation in the 315-400 nm range
UVB	UV radiation in the 280-315 nm range
UVC	UV radiation in the 200-280 nm range
VIPP1	Vesicle inducing protein in plastids
Y_Z	Tyrosine residue involved in linear electron transfer in PSII
ΔpH	pH gradient across the thylakoid membrane
Φ_{II}	Effective quantum yield of PSII photochemistry in the light

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Havurinne V, Tyystjärvi E. Photosynthetic sea slugs induce protective changes to the light reactions of the chloroplasts they steal from algae. *eLife*, 2020; 9: e57389.

II Havurinne V, Aitokari R, Mattila H, Käpylä V, Tyystjärvi E. Ultraviolet screening by slug tissue and tight packing of plastids protect photosynthetic sea slugs from photoinhibition. *Photosynthesis Research*, 2021; doi: 10.1007/s11120-021-00883-7.

III Havurinne V, Handrich M, Antinluoma M, Khorobrykh S, Gould SB, Tyystjärvi E. Genetic autonomy and low singlet oxygen yield support kleptoplast functionality in photosynthetic sea slugs. *Journal of Experimental Botany*, 2021; 15: 5553-5568.

1 Introduction

The term kleptoplasty was originally coined in several publications more than 30 years ago (Gilyarov 1983; Wauch and Clark 1986; Clark et al. 1990). It refers to the phenomenon where heterotrophic organisms are not content with the powerhouse of the cell, the mitochondrion, but steal the organelle that is truly powering most life on Earth, the chloroplast (plastid), from their photosynthetic prey, and utilize it for their own gains, energetic or otherwise. The idea of short cutting the energy delivery straight from the Sun is by no means a singular endeavor. There are many creatures that subscribe to this ideology by way of harboring photosynthetic organisms in their immediate vicinity, like corals, lichen, salamanders and even humans to a degree that is up for philological debate (Kerney et al. 2011; Davy et al. 2012; Grimm et al. 2021). The symbiotic relationship between these organisms is generally a form of mutuliasm or commensalism. Kleptoplasty is often described as a special form of symbiosis, but this really isn't the case; in kleptoplasty the relationship between the animal and the photosynthetic organism is that of a herbivore and its prey, and the herbivore simply gains additional benefits by stealing the plastids. Kleptoplasty is not limited to a single group of organisms, and it is known to occur widely in single celled eukaryotes such as dinoflagellates, ciliates and foraminiferans (Minnhagen et al. 2008; Stoecker et al. 2009; Stamatakis et al. 2016). Indeed, even in animals this feat is not limited to a single group; a recent study showed that certain marine flatworms can steal plastids from algae, bringing the number of known kleptoplastic animal groups to two (Van Steenkiste et al. 2019). This work concentrates on describing kleptoplasty in the other group, photosynthetic sea slugs.

1.1 What are photosynthetic sea slugs?

"Sea slug" is an unofficial blanket term that describes a wide variety of marine invertebrates, but it is commonly used synonymously with nudibranchs (Nudibranchia), a specific order of marine mollusks. This preference likely stems from the fact that arguably some of the most eye-catching marine animals in the world belong to this clade (Fig. 1A). Therefore, often nudibranch is the correct name for the weird and colourful slug encountered during a trip to the reef. If the encounter

was with a slug of green complexion, however, there is a high chance that this mollusk belongs to the superorder Sacoglossa (Fig. 1B-C).

All photosynthetic sea slugs are sacoglossan, but not all sacoglossans are photosynthetic. The superorder Sacoglossa consist of 250-300 described species of sea slugs all over the world, but only a handful of them can maintain plastids stolen from their algal prey (kleptoplasts) photosynthetically active long enough to justify contemplating whether they might be utilizing the plastids for other beneficial purposes apart from simple digestion (Jensen 1997; Händeler et al. 2009). Sacoglossans are further divided into multiple clades, and the art of stealing functional plastids can be narrowed down to one superfamily, Plakobranchoidea (Bouchet et al. 2017). Within this taxon, functional kleptoplasty has been described in two separate families, Costasiellidae and Plakobranchidae, the latter one containing the majority of the known kleptoplastic species (Clark et al. 1981; Händeler et al. 2009; Christa et al. 2014a).

Several attempts have been made to classify photosynthetic slug species according to the longevity of their plastids, or plastid retention time. Typically, the slugs are divided into three categories: no retention, short-term retention and long-term retention. Species in the first category digest their plastids immediately, whereas short-term retention species usually exhibit some signs of photosynthetic activity for approximately 10 days, and the select few species in the long-term retention category tend to maintain their plastids functional for more than 20 days, sometimes even for as long as a year (Clark et al. 1990; Evertsen et al. 2007; Händeler et al. 2009; Middlebrooks et al. 2011; Christa et al. 2014a; de Vries et al. 2015). Although the criteria for inclusion in these different categories tends to vary slightly between different authors, certain species are generally accepted in the long-term retention category: *Elysia chlorotica*, *Elysia timida*, *Elysia crispata*, *Elysia clarki*, *Elysia viridis* and *Plakobranchus ocellatus*. All these slugs belong to the family Plakobranchidae, and from here on the terms photosynthetic or kleptoplastic (sea) slug refer explicitly to these slugs, unless otherwise mentioned.

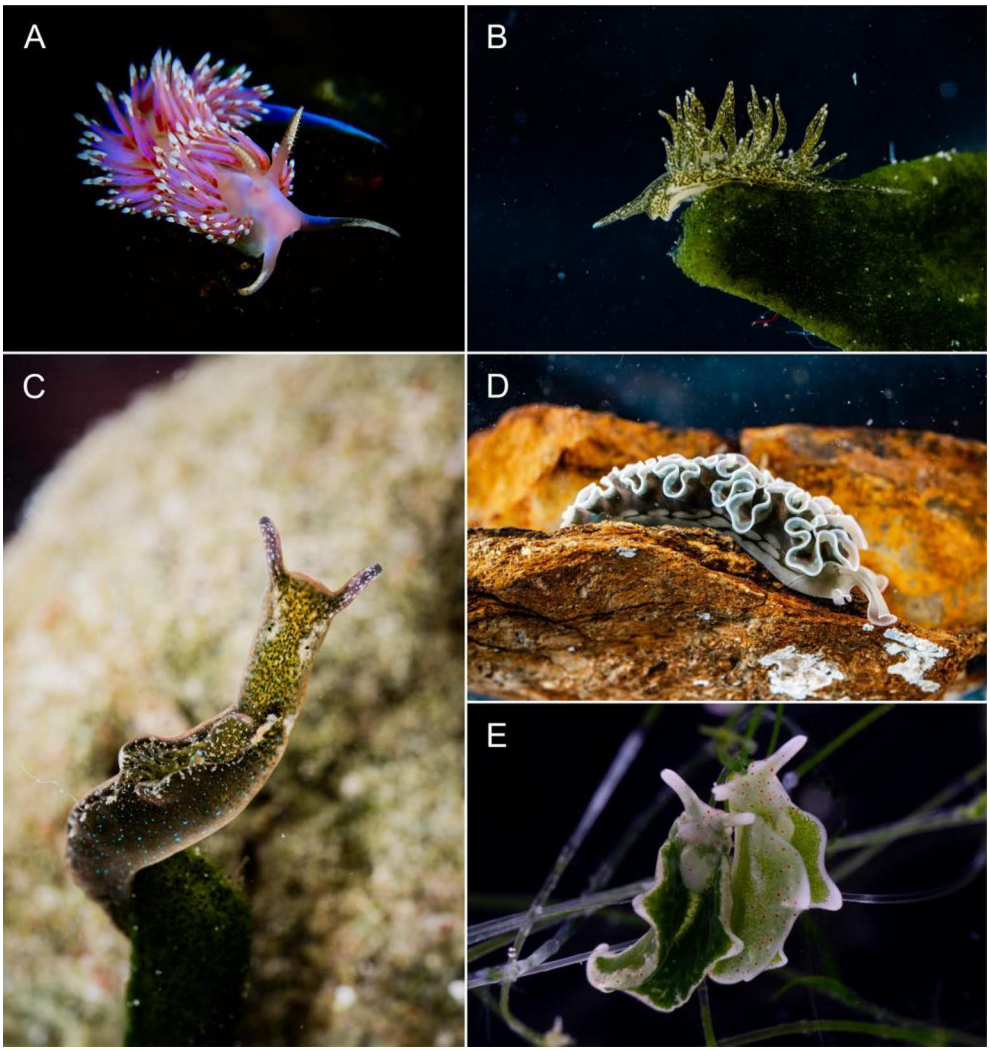

Figure 1. Sea slugs of the world. **(A)** A flamboyant nudibranch sea slug *Facelina auriculata*. **(B)** Despite its green color, the sacoglossan sea slug *Placida dendritica* is not able to retain plastids functional in its tissues. **(C)** *Elysia viridis* is often referred to as an example of a sacoglossan slug capable of long-term retention of plastids, but the longevity of its plastids depends heavily on the algal species it steals the plastids from. **(D, E)** Both *Elysia crispata* (the "sea lettuce"; **D**) and *Elysia timida* (**E**) are prime examples of photosynthetic sea slugs, as they both retain isolated plastids functional inside their own cells for more than 20 days. Two *E. timida* individuals are shown feeding on their prey green alga *Acetabularia acetabulum* in panel **E.** Photo credits: **(A)** Cessa Rauch, **(B-D)** Paulo Cartaxana, **(E)** Vesa Havurinne.

1.1.1 Anatomy

Photosynthetic sea slugs are sometimes referred to as “leaves that crawl” (Trench 1975). This is indeed an apt general description, and not just because of their green colour; they also possess wing-like appendices on their sides, the parapodia, that increase their surface area and do resemble the blades of a leaf (Clark 1994). The adult body length of these animals is usually 0.5-2 cm or shorter, rarely exceeding 3 cm (Jensen 1997). Overall, there are not too many external features that are shared completely unequivocally among Plakobranchidae, as even the parapodia can be either smooth, rough, or even frilled in some cases. In addition to the parapodia, and similarly different between species, most Plakobranchidae have two chemosensory rhinophores extended from the head, and the eyes are situated in their vicinity, often behind the base of the rhinophores (Krug et al. 2016). Without the plastids the colour of the slugs is pale grey with a brownish hue, although most species do have pigmented spots of altered size and colour covering their bodies (Krug et al. 2016). The role of these spots is unknown, although they have been suggested to function as additional light receptors that could supplement the light perception of the primitive eyes of the slugs (Rahat and Monselise 1978).

Plakobranchidae do not have distinct gills that facilitate oxygen uptake. Instead, their relatively large surface area allows sufficient gas exchange for their respiration, and their bodies are also covered in microvilli that can help the slugs obtain nutrients and amino acids directly from their environment (De Freese and Clark 1991; Clark 1994). The function of the distinct large vessels spread over the dorsal side of the slugs has not been elucidated, but the vessels are connected to the pericardium (a thin sac that surrounds the heart) and might be involved in circulation/respiration (Jensen 1992). Being simultaneous hermaphrodites, each individual slug has both male and female reproductive systems producing sperm and eggs at the same time (Jensen 1999; Schmitt et al. 2007). While many of the anatomical features of different slug species are certainly interesting, especially for taxonomic purposes, in terms of plastid acquisition the most interesting parts of the slugs’ anatomy are related to their digestive system.

Thieving, crawling, solar powered leaves. One more layman’s term that needs to be added to this informative list of attributes that describe Plakobranchidae is “sap sucking”. In fact, all Sacoglossa are called sap sucking sea slugs. This term has its origins in the feeding habits of these slugs; they first puncture the algal cell wall with their radula, a chitinous “tongue” with a single row of sharp teeth, and quite literally suck out the cellular contents (“sap”) of the algae they eat (Jensen 1997; Wägele et al. 2010). A unique feature that the Sacoglossa have evolved is a specialized pouch on the surface of their pharynx that stores all used worn out teeth of the radula (Jensen 1996). This “pouch” is the etymological origin of the name Sacoglossa (originally Ascoglossa, from the Greek words “askos”, a bag, and “glossa”, a

tongue). The food travels through the oesophagus of the slugs into the stomach, which is a small, thin-walled structure in most Plakobranchidae slugs. The intestine connects to the stomach on the dorsal side, and leads to the anus, located behind the rhinophores. The stomach is also the central point where all the ducts of the digestive gland are connected through two openings (Jensen 1992; Wägele 2010). The digestive gland tubules are extremely branched all over the bodies of photosynthetic sea slugs, but particularly aggregated in the parapodia. The tubules consist of a lumen that is surrounded by tissue of specialized digestive gland cells, that selectively digest all other algal components except the plastids (Graves et al. 1979; Marín and Ros 1993; Rumpho et al. 2000; Martin et al. 2013). Functional plastids are bound strictly to the digestive tissue cells surrounding them, even though the overall green colour of the slugs might suggest otherwise (Laetz et al. 2017).

During the uptake process, the plastids in the lumen of the digestive tubules seem to be first engulfed by phagosomes, and then released into the surrounding cells. Often the outer membrane of the plastids seems to be in direct contact with the cytosol, but then again, also observations of a possible host phagosomal membrane surrounding the plastids have been made, not to mention plastids that have seemingly lost their inner and outer membranes during the intake process (Martin et al. 2013; Wägele and Martin 2014). One major source of discrepancy between different studies, mostly relying on electron microscopy, is likely linked to the fact that even the long-term photosynthetic slug species do start to digest the plastids when deprived of their food (starved), and the phagosomal engulfment of the plastids marked for degradation is to be expected (Laetz et al. 2017; Laetz and Wägele 2017).

One of the biggest mysteries regarding photosynthetic sea slugs is how are they able to identify and select the plastids for incorporation into their cells. Recent studies have started to scratch the surface of this topic, and it is becoming evident that the selection/incorporation process involves elements of the innate immune system of the slugs, suggesting that the slugs might utilize similar mechanisms as corals (Cnidaria) during the establishment of symbiosis with algae (dinoflagellates) (Davy et al. 2012). For example, when juvenile *E. chlorotica* individuals start eating their prey algae for the first time, the transcription of multiple genes encoding proteins related to recognition of microbe-associated molecular patterns, like scavenger receptors, are upregulated (Chan et al. 2018). Such pattern recognition receptors might be crucial for identifying the specific glycan or lipopolysaccharide composition of the plastid outer membrane and lead to signalling cascades that prevent plastid digestion. Indeed, long-term photosynthetic sea slugs seem to have evolved a wide array of pattern recognition receptors, which supports the notion that they are involved in also plastid recognition, but how exactly remains open (Melo Clavijo et al. 2020; Maeda et al. 2021).

1.1.2 Life cycle

The detailed life cycles of most of the ~300 sacoglossan sea slug species are not known. However, the Plakobranchidae species *E. chlorotica, E. viridis* and *E. timida* have all been cultured in the lab to a degree that has enabled detailing parts of their life cycles (Trowbridge 2000; Rumpho et al. 2011; Pelletreau et al. 2012; Schmitt et al. 2014;). The larger species like *E. chlorotica* and *E. viridis* (adult slugs often more than 1.5 cm) have been shown to live for approximately 10-15 months, whereas the record lifetime of the smaller slug *E. timida* (usually well below 1.5 cm) is currently 8 months (Trowbridge 2000; Pelletrau et al. 2012; Schmitt et al. 2014).

There are three main strategies of larval development in marine invertebrates: planktotrophic, lecithotropic and direct development (Marín and Ros 1993; Yamaguchi et al. 2021). Planktotrophic larvae, called veligers, feed on unicellular microalgae during early development, whereas lecithotrophically developing veligers survive and develop by using the material in the egg capsules of the egg masses (Marín and Ros 1993). Both *E. chlorotica* and *E. viridis* have been shown to develop planktotrophically, and the veligers filter and digest microalgae from their surroundings for 12-46 days after hatching, before metamorphosing into juvenile slugs (Trowbridge 2000; Pelletreau et al. 2014).

The life cycle of *E. timida* is known to consist of both lecithotrophic and direct development (Marín and Ros 1993). This poecilogony, or life cycle polymorphism, is rare in marine animals, but known to exist quite widely in Sacoglossa (Yamaguchi et al. 2021). In the lecithotrophic mode, *E. timida* embryos develop inside the egg capsules for 16-18 days, before they hatch as free-swimming veligers. The free-swimming veliger stage lasts 3-4 days, until the veligers metamorphose into crawling juvenile slugs. The other development type in *E. timida* is direct development, where the development inside the eggs takes a few days longer, allowing the slugs enough time to metamorphose directly into crawling juvenile slugs before hatching (Marín and Ros 1993; Schmitt et al. 2014). In *E. timida* the main developments during the metamorphosis from a veliger to a juvenile slug include an increase in size, the loss of the shell, the formation of a functional radula and rudimentary rhinophores. Perhaps most importantly, it is also at this stage where the digestive gland is formed as an asymmetrical sack (Marín and Ros 1993). Metamorphosed *E. timida* juveniles then latch onto the large filamentous cells of the main prey alga of the adult slugs, *Acetabularia acetabulum*, and start feeding (Schmitt et al. 2014).

The plastids are not incorporated into the cells of juvenile *E. timida* slugs for long-term retention after initial feeding; it can take as long as 25 days of feeding after hatching for this to happen in *E. timida*. By this time the slugs have already developed many of the adult characteristics (Marín and Ros 1993; Schmitt et al. 2014; Laetz and Wägele 2017). It is therefore possible that for some photosynthetic slug species the initial contribution of the plastids to the slug's development is not

related to the photosynthetic functionality of the plastids inside their cells, but rather to digesting the plastids as food (Trowbridge 2000). The connection between *E. chlorotica* and its prey alga *Vaucheria litorea* is seemingly tighter, as *E. chlorotica* metamorphosis does not commence in the absence of *V. litorea* (Pelletreau et al. 2012; Pelletreau et al. 2014). Juvenile *E. chlorotica* individuals start feeding on *V. litorea* immediately after metamorphosis, which turns them green within hours, and intact plastids integrate into the developing digestive gland after two days of feeding. If *E. chlorotica* juveniles are taken away from their food source after seven days of feeding post metamorphosis, they continue their normal trajectory into adult slugs, and maintain the plastids in their digestive gland cells. Slugs fed for only 5 days do not develop, and deteriorate in starvation, indicating that in *E. chlorotica* functional kleptoplasty is established already after 6-7 days of feeding (Pelletreau et al. 2012). The development of the juveniles is hindered, and the life expectancy reduced when *E. chlorotica* juveniles are kept in the dark, suggesting that photosynthesis of the functional plastids may influence the normal life cycle of this slug (Pelletreau et al. 2012). The development from juveniles to adults generally entails an increase in size and the full emergence of the rudimentary organs that were already present in the juveniles after metamorphosis (Marín and Ros 1993; Trowbridge 2000).

Estimates of the time it takes for photosynthetic sea slugs to reach sexual maturity are rare in the literature. Some reports do, however, exist of 106 days old (post hatching) *E. timida* individuals reproducing in captivity, and *E. viridis* is expected to be sexually mature only after approximately 30 days (Trowbridge 2000; Schmitt et al. 2014). Photosynthetic sea slugs seem to inseminate one another mainly through the skin with their penises, as described in *E. timida* (Schmitt et al. 2007). However, contrary to the quite common "hit-and-run" strategy of hermaphrodites, where the mating individuals compete for the opportunity to inseminate the other without reciprocation, the copulation of *E. timida* involves an orchestrated courtship dance that ensures a high degree of sperm trading (Michiels and Newman 1998; Schmitt et al. 2007). A common occurrence in many sea slugs is the storage of allosperm (received sperm) to wait for egg cell maturation before fertilization, as is also the absorption of allosperm for nutritive benefits (Jensen 1999; Schmitt et al. 2007). The spawning (laying of egg masses) in Sacoglossa typically takes place within a week after mating. The individual fertilized eggs are enriched with albumin, encapsulated, and then covered in mucus one by one, eventually forming a spiral shaped egg mass that can contain thousands of eggs (Jensen 1999). One speciality of the slugs in the superfamily Plakobranchoidea is the extracapsular yolk material embedded in the egg masses. Its function and chemical composition are unclear, but it might serve as an additional nutritional reserve or as a toxic deterrent for predators (Marín and Ros 1993; Jensen 1999).

1.1.3 Are the slugs photosynthetic?

When the slugs have reached full adulthood and functional kleptoplasty has been established, they can survive in starvation for a few months or in some cases up to a year. The lifetimes of starved and fed *E. chlorotica* individuals, as well as the exceptional longevity of the stolen plastids in this slug, seem to correlate well, making it plausible that these slugs can indeed survive starvation for their natural one-year lifetime with the help of their plastids (Green et al. 2000; Rumpho et al. 2011; Pelletreau et al. 2012). However, *E. timida* and *E. viridis* seem to be only able to survive starvation periods that correlate with the longevity of their plastids, i.e., a few months, whereas their maximal reported life expectancies are known to be as long as 8 and 15 months, respectively (Trowbridge 2000; Vieira et al. 2009; Schmitt et al. 2014; Laetz and Wägele 2017). Suffice it to say, that after stealing the plastids from their prey algae the slugs can live without additional food for the rest of their lives, but the rest of their lives might be a short stint.

The plastids inside the slug *E. viridis* have been shown to be continuously replaced with new ones when the slugs are feeding (Frankenbach et al. 2021). This suggests that when food is available, the slugs tend to simply digest the plastids, and do not rely on photosynthesis provided by the plastids. Some slugs do face times of food scarcity in the nature; the food source of *E. timida*, *A. acetabulum,* becomes calcified and therefore impenetrable for the slugs during the autumn and spring (Marín and Ros 1992). The slugs do benefit from the plastids at such times, but there is no consensus regarding the modus how this benefit presents itself. The literature is riddled with contrasting reports on whether light, and therefore photosynthesis, is beneficial for adult slug survival or not (Christa et al. 2014a, b; Cartaxana et al. 2017; Laetz et al. 2017; Donohoo et al. 2020; Frankenbach et al. 2021; Maeda et al. 2021). The matter is further complicated by the fact that the absence of light likely affects the slug itself, not just the plastids, and recently this has been taken into account by utilizing low light, not darkness, or chemical inhibitors of photosynthesis to probe the effect of photosynthesis on slug survival (Christa et al. 2014b; Cartaxana et al. 2017; Donohoo et al. 2020).

The plastids of long-term retention slugs do fix carbon in a light-dependent manner and radiolabeled carbon can be found in the slug tissue and mucus after starvation, but the unanswered question is whether the carbon products are released for slug metabolism through active transport of photosynthates or by simple slow digestion of the plastids, or perhaps both (Trench et al. 1972; Christa et al. 2014b; Laetz et al. 2017; Cruz et al. 2020; Cartaxana et al. 2021). Recently, it was shown that the slugs *E. viridis* and *E. timida* fix and assimilate radiolabeled carbon and nitrogen in a light-dependent manner, and the labelled compounds tend to accumulate in the reproductive tissues of the slugs. Furthermore, the slugs also lay more eggs in moderate light than in the dark or very low light conditions (Cruz et al.

2020; Shiroyama et al. 2020; Cartaxana et al. 2021). This may indicate that the benefits from the plastids are not necessarily directed for adult slug survival per se, but rather for reproduction. Whether these slugs should be called photosynthetic, or photoautotrophic, is still up for debate.

1.2 Plastids in algae

Adult photosynthetic sea slugs are often very stenophagous, meaning that they only eat one specific species of macroalgae. This is also reflected in the different shapes of the radular teeth of the slugs; specialized shapes of the teeth are an adaptation that allows the slugs to be very efficient in puncturing specific cell walls (composed of e.g. mannan, xylan or cellulose) of their food algae (Jensen 1997). Even though they specialize in different species of macroalgae, most of the slugs steal their plastids exclusively from a single class of siphonaceous green algae, the Ulvophyceae (de Vries et al. 2013). To understand some of the fundamental problems isolated plastids should be facing inside animal cells, a brief description of the early life events that underpins the evolution and origin of plastids in algae is in order. Fitting to the main topic of this thesis, the early life events in question also include heterotrophic organisms that incorporate photosynthetic entities, cyanobacteria, into their own cells.

1.2.1 Primary endosymbiosis

The initial event of plastid acquisition by eukaryotes, or primary endosymbiosis, led to the emergence of the Archaeplastida, the group containing the Rhodophyta (red algae), Chlorophyta (green algae and land plants) and the Glaucophyta, approximately 1.5 billion years ago (Yoon et al. 2004). Although primary endosymbiosis is a rare event, it seems to have taken place twice in the history of life. Most plastids in contemporary algae and plants can be traced back to the original endosymbiosis that gave rise to the Archaeplastida, but a more recent primary endosymbiotic event has taken/is taking place in the cercozoan *Paulinella* (Ponce-Toledo et al. 2019; Stephens et al. 2021). The endosymbiont cyanobacterium of Archaeplastida was likely related to the *Gloeomargarita* cyanobacteria and taken in by the eukaryotic host cell through phagocytosis during primary endosymbiosis (Archibald 2015; Ponce-Toledo et al. 2019). All contemporary primary plastids of Archaeplastida have retained both the outer and inner membranes of the original cyanobacterium, known as the outer and inner envelope membranes of the plastids (Gould et al. 2008).

The enslavement of the endosymbiont by the host cell into an obligate organelle entailed a massive reduction in the genome size of the endosymbiont, partly owing

to endosymbiotic gene transfer (EGT) from the endosymbiont's genome into the nuclear genome of the host, but also due to a simple loss of genes from the endosymbiont (Ponce-Toledo et al. 2019). For reference, the genome size of the model cyanobacterium *Synechocystis* sp. PCC 6803 is 3.96 Mbp, whereas plastid genomes are usually 100-200 kbp, and even the largest ones are generally smaller than 2000 kpb (Zerulla et al. 2016; de Vries and Archibald 2018). Even when the genome is large, the gene content of the plastid genome (plastome) is still less than 5% of the gene content of a typical cyanobacterial genome (Green 2011; Simm et al. 2015; Ponce-Toledo et al. 2019). The plastomes of the prey algal species of the slugs are seemingly no exception to this rule, although they do encode for some interesting genes that are not ubiquitously distributed in the plastomes of algae and plants (Rumpho et al. 2008; de Vries et al. 2013; Maeda et al. 2021).

The plastid proteome contains approximately 500-2300 proteins, but the combined proteins encoded by the plastome and the EGT genes in the nuclear genome of Archaeplastida sum up to only approximately 200-600 proteins (Ponce-Toledo et al. 2019). The sizeable remainder of proteins in the plastid proteome are encoded by e.g., duplicated host genes that have been re-purposed to serve the plastid, or non-cyanobacterial bacterial genes in the nuclear genome. The origin of the non-cyanobacterial bacterial genes remains debated, but they might have been transferred to the nuclear genome of the ancestral Archaeplastida through horizontal gene transfer, possibly from Chlamydiales bacteria (Price et al. 2012; Qiu et al. 2013; Ponce-Toledo et al. 2019). The remarkable contribution of non-cyanobacterial genes to the function of plastids showcases the evolution of alterations that have shifted the once cyanobacterial endosymbiont into a eukaryote-specific energetic organelle.

Since most of the genes contributing to the proteome of the plastids eventually either moved to or emerged within the nucleus, a way of directing the gene products into the plastids must have evolved concurrently or subsequently. There are altogether six different locations where a protein can be targeted to within primary plastids: the outer envelope membrane, the intermembrane space, the inner envelope membrane, the plastid stroma, the thylakoid membrane and the thylakoid lumen (Gould et al. 2008). All Archaeplastida share a stringent targeting system for plastid proteins synthesized in the cytosol, which involves the use of transit peptides and the protein translocator machineries known as the translocators of the inner and outer envelope membranes of the chloroplast (Tic and Toc), but there are differences between the lineages (Price et al. 2012; Chen et al. 2018). The details of guiding the cytosolically translated precursor proteins to the correct compartment in the plastids are complicated in algae, and this underlines one major mystery regarding photosynthetic sea slugs. If there are genes in the nuclear genome of the slugs that have acquired a role in the plastids of the slugs, how are the gene products guided inside such a foreign organelle?

1.2.2 Secondary endosymbiosis

The only known photosynthetic slug that does not feed on primary plastid bearing ulvophytes is *E. chlorotica*. In fact, this slug does not feed on green algae at all; its culinary taste is more sophisticated, and it only enjoys a meal of *V. litorea* (Pelletreau et al. 2012; de Vries et al. 2013). Primary endosymbiotic events are rare, but secondary endosymbiosis is more common. It has been the driving force behind the spread of photosynthesis into the major secondary green algal groups Chlorarachniophyta and Euglenezoa, as well as the secondary red algal lineages in the groups Cryptophyta, Alveolata, Haptophyta and Stramenopiles (Stephens et al. 2021). The last one of these lineages contains the yellow-green algae (Xanthophyceae), to which also *V. litorea* belongs. In secondary endosymbiosis a eukaryote engulfed another photosynthetic eukaryote, and this endosymbiont was then merged into the cell ultimately as a plastid (Gould et al. 2008; Archibald 2015).

Just like during primary endosymbiosis, the genomes of the secondary endosymbionts went through a dramatic reduction in size and gene content during secondary endosymbiosis. This time, however, the EGT events took place largely between the nucleus of the endosymbiont and the nucleus of the host (Gould et al. 2008; Gagat et al. 2014; Archibald 2015; Füssy and Oborník 2018; Ponce-Toledo et al. 2019). Secondary endosymbiosis occurred at least three times independently; twice to account for the two separate secondary green algal lineages Chlorarachniophyta and Euglenezoa and once during the origin of the secondary red algae (Archibald 2015; Jackson et al. 2018). After this, determining the exact sequence and number of plastid acquisitions across the tree of life becomes a matter of what characteristics are emphasized in the analyses, mainly because several lineages of the secondary plastid bearing algae are known to have acquired plastids from yet other eukaryotic algae through tertiary endosymbiosis (Gagat et al. 2014; Füssy and Oborník 2018).

Secondary, or complex, plastids are typically surrounded by four membranes (Gould et al. 2015; Tomečková et al. 2020). The two innermost are essentially the same inner and outer envelope membranes also found in primary plastids. The outermost one is called the plastid endoplasmic reticulum membrane (pERM). This membrane is thought to have derived from the original phagocytotic membrane surrounding the endosymbiont, that over time fused with the endoplasmic reticulum (ER) and the nuclear envelope of the host (Cavalier-Smith 2000; Flori et al. 2016). Beneath the pERM is the periplastidial membrane (PPM), often stated to be the vestigial plasma membrane of the endosymbiont, although a hypothesis stating that PPM originated from the ER of the host has been put forward (Gould et al. 2015). The remnants of the endosymbiont's cytoplasm, including nucleomorphs, the vestigial nuclei found in chlorarachniophytes and cryptophytes, still exist between

the PPM and the outer envelope of the plastid in what is known as the periplastidal compartment (Archibald and Lane 2009; Sheiner and Striepen 2013).

The translocation of nucleus encoded proteins across the four membranes surrounding complex plastids was yet another evolutionary hurdle that secondary algal lineages had to overcome to become autotrophic. Like primary plastids, complex plastids also contain the major translocator complexes Toc and Tic of the outer and inner envelopes, and protein translocation through them is similar to primary plastids. However, the two additional membranes pERM and PPM require specific signal and transit peptides and machineries for correct protein translocation to complex plastids (Gould et al. 2008; Hempel et al. 2009; Sheiner and Striepen 2013; Gould et al. 2015; Flori et al. 2016). Interestingly, the complex plastids of *V. litorea* are stripped of the two outermost membranes during incorporation to the slug cells of *E. chlorotica*, suggesting that if any nucleus encoded gene products of *E. chlorotica* were to be targeted to the plastids, their transit and signal peptides should not be the same as the ones in *V. litorea* (Rumpho et al. 2001).

1.3 Photosynthesis

Photosynthesis is complicated, and it is an outright miracle that the plastids inside the slugs remain photosynthetically active at all, let alone for months. Briefly, the two major protein complexes facilitating photosynthetic electron transfer are Photosystem II and Photosystem I (PSII and PSI), situated in the thylakoid membrane. The main chain links connecting these two are lipid soluble plastoquinone (PQ) molecules inside the thylakoid membrane, the membrane embedded cytochrome *b6f* complex (Cyt *b6f*) and lumenal electron carrier proteins plastocyanin (PC) or cytochrome *c6* (Cyt *c6*). The concerted efforts of this linear electron transfer chain result in the reduction of $NADP^+$ to NADPH via the stromal electron carrier ferredoxin (Fd), and the formation of a proton/pH gradient across the thylakoid membrane. The electrochemical energy contained in the proton gradient is vented through a thylakoidal ATPase to (photo)phosphorylate ADP to ATP. Both NADPH and ATP are then used to fix CO_2 as sugars in the Calvin-Benson-Bassham (CBB) cycle (Vecchi et al. 2020). Unfortunately, studies focusing on photosynthesis at a molecular biology level in the prey macroalgal species of the slugs are rare (Handrich et al. 2017). Therefore, to get an idea about photosynthesis in green and stramenopile algae, most of the following discourse will be navigated with information from other chlorophytes, largely the green alga *Chlamydomonas reinhardtii*, and several stramenopile diatoms.

1.3.1 Photosystem II and plastoquinone

Before the photosynthetic electron transfer reactions within PSII and PSI can take place, light energy needs to be captured via photosynthetic pigments. Chl *a* is utilized by all algae and plants in light harvesting, but the other common Chl in green algae and plants, Chl *b,* is not. Instead, different forms of Chl *c* (mainly c_1, c_2 and c_3) are widespread in secondary red algae, including *V. litorea* (Büchel 2020). Most of the light harvesting in algae is facilitated by conserved membrane intrinsic light harvesting complex (LHC) proteins (Kühlbrandt et al. 1994; Ballottari et al. 2012). All LHC proteins bind Chl *a*, but they can also contain either Chl *b* or *c*, depending on the lineage. Typical carotenoids in the green lineage LHCs are lutein, neoxanthin, violaxanthin or zeaxanthin, whereas in the stramenopile diatoms the main carotenoids are fucoxanthin, diadinoxanthin or diatoxanthin. The LHCs of diatoms are, in fact, called fucoxanthin-Chl *a*/*c*-binding proteins (FCP), but here the term LHC will be used also to include FCPs, unless specific monomeric FCP proteins are discussed (Ballottari et al. 2012).

Two internal antenna proteins, CP43 and CP47, are part of the monomeric core of PSII and only bind Chl *a* (Su et al. 2017; Shen et al. 2019). Two PSII cores form a functional dimer complex in a conserved manner, but the composition and binding of the peripheral light harvesting complexes of PSII (LHCII) in the so called PSII supercomplexes is highly variable. In *C. reinhardtii* the PSII peripheral antennae system can be divided to two layers. The inner layer consists of monomeric LHC proteins CP26 and CP29 as well as a trimeric LHCII that is strongly bound to PSII. The outer layer contains only trimeric LHCIIs, that are either moderately or loosely bound to the PSII complex (Tokutsu et al. 2012; Shen et al. 2019; Cazzaniga et al. 2020). The LHC complexes forming a major part of the peripheral PSII antenna in the diatom *Chaetoceros gracilis* are tetramers. Altogether the peripheral antenna of PSII supercomplex in *C. gracilis* contains four LHCII tetramers, two strongly bound directly to the PSII cores and two moderately bound monomeric FCPs lining the PSII cores. Two additional monomeric FCPs are located at the periphery of the moderately bound LHCIIs (Nagao et al. 2019). It should be remembered, that even though *V. litorea* is a stramenopile alga, like diatoms, the pigment compositions and LHC arrangements might be completely different in this prey algal species of the slugs. And the same goes for drawing too strong conclusions from the green alga *C. reinhardtii* regarding the details in the green algal prey species of the slugs.

After a photon has excited a Chl molecule in the LHCs, the excitation energy is usually delocalized amongst a conglomerate of pigments that share the excitation, forming an exciton. The transfer of excitation energy from the LHCs to PSII (or PSI) is fast, but the physics involved are still being debated. The energy transfer is often described by semiclassical Förster resonant energy transfer models, where the energy is assumed to hop downhill along the different energy levels of donor and acceptor

pigments, but whether this is an accurate description of the events without taking into account different quantum effects is still unclear (Sumi 1999; Şener et al. 2011; Fassioli et al. 2014; Jumper et al. 2018). The reaction centre of PSII comprises the conserved D1 and D2 protein subunits containing four Chl *a* molecules. Two of these (P_{D1} and P_{D2}, where P stands for pigment and the subscript denotes their binding subunit) are part of the P680 reaction centre dimer that, according to classical interpretation, contains the primary electron donor in charge separation (P_{D1}), while the other two (Chl_{D1} and Chl_{D2}) are stated to have an accessory role. However, according to more recent takes on the topic, both P_{D1} and Chl_{D1} can function as electron donors in charge separation after P680 excitation to P680*, but the exact reactions leading to forward electron donation remain open (Mamedov et al. 2015; Romero et al. 2017). Nevertheless, excitation of the reaction centre Chls leads to charge separation, where the pheophytin (Phe) of D1 is the electron acceptor, creating the radical pair $P680^+Phe^-$ (Nagao et al. 2017). Soon after the electron from Phe^- transfers to the stable electron carrier PQ molecule Q_A, residing in the D2 subunit of PSII (Nadtochenko et al. 2014). Electrons return to the D1 branch when Q_A^- reduces another PQ molecule, Q_B, in a reaction that is regulated by a stromal bicarbonate ligated to a non-heme iron situated next to Q_A and Q_B (Brinkert et al. 2016). Two charge separations are required to fully reduce Q_B to Q_B^{2-}, followed by the protonation of Q_B^{2-} to plastoquinol (PQH_2) by two protons from the thylakoid stroma. PQH_2 is then released into the PQ pool of the thylakoid membrane (Guskov et al. 2009) (Fig. 2).

The electrons sent forward into the electron transfer chain are replaced by electrons from water molecules. Water oxidation takes place in the oxygen evolving complex (OEC) of PSII, and it is powered by $P680^+$. Four charge separations are required to extract four electrons from two substrate water molecules bound to the Mn_4CaO_5 cluster of the OEC (Fig. 2). This also results in the release of four protons and one oxygen molecule to the thylakoid lumen. After charge separation, $P680^+$ oxidizes a specific tyrosine residue of D1 (Y_Z), and the resulting radical $Y_Z^{\bullet}$, in turn, oxidizes the Mn_4CaO_5 cluster in the Kok-cycle (Kok 1970). The four Mn ions of the Mn_4CaO_5 cluster have different oxidation states, and their combined oxidation state determines the S-state of the OEC. In its most reduced state (S0) all but one of the ions are Mn^{3+}, whereas the fourth one is Mn^{4+}. After a charge separation event, one of the Mn^{3+} ions is oxidized to Mn^{4+}, marking the transition of the OEC from S0 to S1 state. The oxidation of the Mn^{3+} ions proceeds after subsequent charge separations until the S3 state, where all Mn ions are Mn^{4+}. The next charge separation induced state, S4, reduces very rapidly back to S0 without charge separation, simultaneously splitting the substrate water and releasing oxygen. Proton release is usually associated with electron donation to Y_Z in the Kok cycle, except for S1→S2, where only an electron is donated without proton release, and S4→S0, where only a proton

is released (Kern et al. 2018; Reiss et al. 2019; Mandal et al. 2020). It should be noted that even though the most reduced state is S0, the OEC tends to equilibrate towards S1 in the dark. This leads to the classical flash-induced oxygen evolution pattern, where the third flash after dark acclimation causes the transition S3→(S4)→S0, noticeable as a spike in oxygen evolution, whereafter the oxygen evolution follows a period-four flash oscillation pattern (Vass and Styring 1991; Joliot 2003).

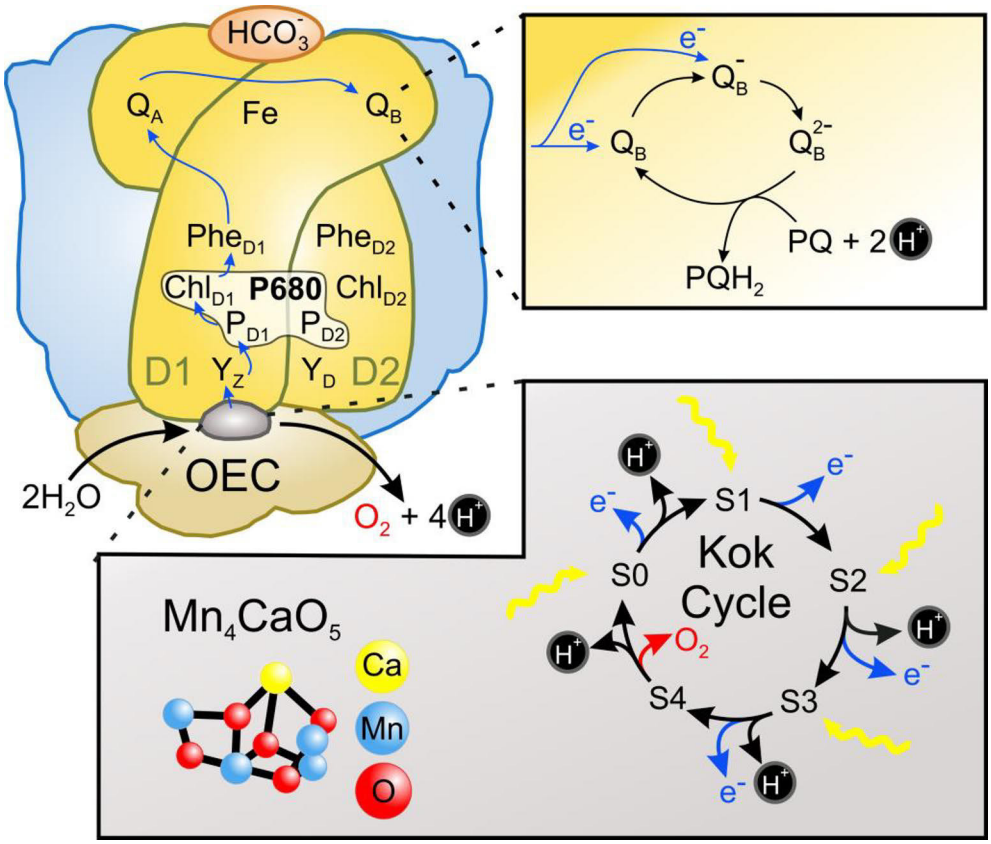

Figure 2. Light-induced linear electron transfer reactions in PSII. The top left scheme illustrates the main electron transfer pathway along the D1 and D2 proteins of PSII. P680 is depicted here as a conglomerate of P_{D1}, P_{D2} and Chl_{D1}.The top right panel shows the two-electron reduction of Q_B, which leads to its protonation and the release of a PQH_2 molecule into the thylakoid membrane. The cubane structure of the Mn_4CaO_5 cluster is shown in the bottom panel, as well as the H_2O oxidation cycle (Kok cycle) it catalyzes.

1.3.2 Cytochrome *b6f* and PC/Cyt *c6*

The basic structure of Cyt *b6f* is conserved, although changes in the minor subunit composition have been recorded in different lineages of algae. Any major deviations from the Cyt *b6f* functionality described below are not expected to exist in the prey algae of the slugs. The two core subunits forming a functional dimer have four major subunits each, that include the essential haem iron containing Cyt *f* and Cyt *b6*, the Rieske iron-sulfur protein and a fourth subunit binding both Chl *a* and β-carotene, as well as four minor subunits (Stroebel et al. 2003; Hasan et al. 2013; Malone et al. 2019; Malone et al. 2021). The oxidation of PQH_2 takes place in the Q-cycle (quinol oxidation cycle), where electrons are bifurcated into two pathways with chains of either high or low redox potential electron carriers (Mitchell 1975; Crofts et al. 1983).

There are two PQ binding sites in Cyt *b6f*, Q_o and Q_i, that are situated closer to the lumen or the stroma, respectively. In the the Q-cycle, one PQH_2 is first bound to the Q_o site, and one electron derived from PQH_2 oxidation is transferred to the lumenal electron carrier PC via the high-potential chain. This is accompanied by the release of the two protons from PQH_2 into the lumen, resulting in a $PQ^{\bullet-}$ radical in the Q_o site, and transfer of an electron from $PQ^{\bullet-}$ along the low-potential chain haems of Cyt *b6* to haem *c,* close to the Q_i site. The original PQH_2 in the Q_o site is now in its oxidized PQ form and diffuses into the thylakoid membrane, marking the end of the first half of the Q-cycle. To initiate the second cycle, another PQH_2 binds to the Q_o site, and the oxidation via the high-potential chain proceeds as before. The low-potential oxidation proceeds along the Cyt *b6* haems, this time creating a redox pair that includes a reduced haem *b* and the previously reduced haem *c* close to the Q_i site housing a PQ molecule. The two electrons from the haems are then used in reduction and protonation of the PQ molecule to PQH_2, which diffuses back into the thylakoid membrane. Altogether the passing of the four electrons from water oxidation through the Q-cycle to PC results in the release of eight protons into the thylakoid lumen (Alric et al. 2005; Cramer et al. 2011; Tikhonov 2014; Malone et al. 2021). The oxidation of PQH_2 by Cyt *b6f* is the rate limiting step of linear electron transfer (Rochaix 2011). It is also at Cyt *b6f* where the so-called photosynthetic control takes place; acidification of the lumen slows down PQH_2 oxidation in the Q_o site, possibly due to protonation of the proton accepting amino acid residues inside, before PQH_2 has the chance to burden the Q-cycle with its own electrons and protons (Tikhonov 2014; Colombo et al. 2016).

In most studied photosynthetic organisms, the main electron carrier between Cyt *b6f* and PSI is the copper containing lumenal protein PC. However, green algae (and cyanobacteria) can switch to an alternative, haem iron binding Cyt *c6* electron carrier as a response to copper limitation (Merchant et al. 1991; Torrado et al. 2019). On the other hand, in the secondary red stramenopiles the only electron carrier between Cyt

b6f and PSI is Cyt *c6* (Peers and Price 2006; Akazaki et al. 2009). It is therefore likely that the green algae eaten by the slugs use PC as the electron carrier, whereas *V. litorea* uses Cyt *c6*. Organisms that rely solely on Cyt *c6* show slightly less efficient electron donation to PSI than green algae relying on PC, but the mechanism is similar between the two electron carriers. Both carriers rely on electrostatic attraction to interact with the lumenal hydrophobic docking site of PSI, and in photosynthetic eukaryotes this interaction is thought to be enhanced by the positively charged lumenal loops of the PsaF subunit of PSI (Finazzi et al. 2005; Bernal-Bayard et al. 2013; Bernal-Bayard et al. 2015; Castell et al. 2021).

1.3.3 Photosystem I, ATP synthase and carbon fixation

In eukaryotes, PSI cores exist as functional monomers that form lineage-specific supercomplexes by attaching different PSI LHCs (LHCI) (Blankenship 1992; Jordan et al. 2001; Suga et al. 2019; Xu et al. 2020). PSI core does not have separate intrinsic antenna proteins; light absorption for photosynthesis in the PSI core is limited to the Chl *a* located in PsaA and PsaB proteins that also form the reaction centre of PSI (Jordan et al. 2001; Alboresi et al. 2017). The most recent structural studies indicate that the PSI supercomplex in *C. reinhardtii* contains two heterotetrameric "belts" of LHCIs on one side of PSI, with different compositions of the nine LHCI subunits. In addition, a heterodimeric LHCI is bound on the other side of PSI (Suga et al. 2019). The complete LHCI antenna of the diatom *C. gracilis* is made up of 24 FCP proteins. The FCP proteins form three layers; the innermost layer completely circles PSI, the middle layer forms a semi-circle, and the small outermost layer is only on the PsaA side of the PSI core (Xu et al. 2020). Once again, the LHCI compositions of the green algae and the stramenopile *V. litorea* eaten by the slugs might be completely different than LHCIs in the model green alga *C. reinhardtii* or stramenopile diatoms.

The evolutionarily conserved core of PSI contains two Chl *a* molecules that form the dimeric P700, located at the opposite branches, PsaA or PsaB, of the PSI reaction centre. Here, the P700 Chls will be called P_A or P_B (pigment in the A or B branch). Each branch also contains one accessory Chl *a* (Acc_A or Acc_B) and one Chl *a* that is part of the electron transfer chain (A_{0A} or A_{0B}), as well as a phylloquinone electron acceptor (PhQ_A or PhQ_B). Two mechanisms for the primary charge separation events in PSI have been proposed. In the classical one, excited P700 (P700*) donates an electron to A_{0A} or A_{0B}, and Acc_A and Acc_B have roles only as intermediates that facilitate the primary charge separation (Gobets et al. 2001; Shuvalov et al. 2007). The other mechanism proposes that the primary charge separation takes place between Acc_A or Acc_B and their respective A_0 electron acceptors, and P700 only donates an electron to Acc_A or Acc_B in a secondary reaction (Müller et al. 2003;

Müller et al. 2010; Di Donato et al. 2011; Santabarbara et al. 2015). According to both mechanisms, the electron hole is eventually located in P700 ($P700^+$) after charge separation and the electron is donated to either of the A_0 primary electron acceptors (Fig. 3).

Electron donation from PC or Cyt *c6* replaces the missing electron in $P700^+$, and the electron in A_0^- transfers first to the PhQ molecule in its corresponding branch and then to the first 4Fe-4S cluster, F_X, which is located at the cross-section between the two branches. Electron transfer continues along the next two 4Fe-4S clusters, F_A and F_B, located in the PsaC subunit of PSI, until the reduction of the stromal electron carrier Fd. After two electron reduction of Fds, ferredoxin NAD(P)H oxidoreductase (FNR) oxidizes the two Fds and reduces $NADP^+$ to NADPH, that can then be used in carbon fixation in the CBB cycle (Brettel 1997; Santabarbara et al. 2005; Vorphal et al. 2017) (Fig. 3). Alternatively, the electrons from Fd can be diverted to multiple different pathways in the plastid; perhaps most importantly to thioredoxins via ferredoxin:thioredoxin reductase. Thioredoxins are small dithiol:disulfide oxidoreductases that serve as a major regulatory hub in the plastids, and they have been shown to contribute to almost all aspects of plastid functionality by affecting the disulfide bridges in the cysteine residues of redox regulated proteins (Huppe et al. 1990; Nikkanen et al. 2017).

In addition to NADPH, ATP is formed during photosynthesis by photophosphorylation. Here, the accumulation of 12 protons in the thylakoid lumen during the transfer of four electrons along the linear electron transfer chain creates a proton motive force (*pmf*), which is utilized by the plastid F_1F_o ATP synthase (cF_1F_o, where c stands for chloroplast) to create ATP from ADP and inorganic phosphate. The membrane-intrinsic *pmf* driven motor (F_o) provides the rotational force for ADP photophosphorylation to ATP by the stromal F_1 catalytic site (Hahn et al. 2018). It is often stated that an average of 4.7 protons need to pass through the F_o to produce one ATP, but according to other estimates this can already be achieved by passing only four protons along the proton gradient (Seelert et al. 2000; Steigmiller et al. 2008; Vollmar et al. 2009; Petersen et al. 2012). In terms of CO_2 fixation in the CBB cycle, the four protons per ATP would support the ideal ATP/NADPH ratio (3/2) of 1.5 as a result of linear electron transfer, whereas the 4.7 protons per ATP would result in an ATP deficit with respect to NADPH ($2.6/2 \approx 1.3$). The matter is still somewhat open for interpretation, but one of the main reasons for the existence of alternative cyclic electron transfer routes in the electron transfer chain is thought to be to correct for the ATP deficiency (Kramer and Evans 2010; Burlacot et al. 2019; Walker et al. 2020).

The CBB cycle is traditionally divided to three distinct phases: carbon fixation, reduction of the carbon intermediates and regeneration of the five-carbon backbone ribulose-1,5-bisphosphate (RuBP). In the carbon fixation phase ribulose-1,5-

bisphosphate carboxylase oxygenase (Rubisco) catalyzes the reaction between CO_2 and RuBP, forming two 3-phosphoglycerates, that are taken into the reduction phase when they are phosphorylated by phosphoglycerate kinase using ATP. The resulting two 1,3-bisphosphoglycerates are processed further by glyceraldehyde-3-phosphate dehydrogenase that reduces the two 1,3-bisphosphoglycerates molecules to glyceraldehyde-3-phosphate (G3P) using NADPH. One G3P exits the cycle and can be further processed to suit the metabolic needs of the cell, while the other G3P enters the regeneration phase, where G3P goes through several enzymatic steps to regenerate RuBP (Calvin and Benson, 1948; Bassham et al. 1950; Michelet et al. 2013; Küken et al. 2018; Schreier and Hibberd 2019).

Generally, the two main organic carbon reserves of green algae are starch in the plastids and oils (mainly triacylglyserols; TGA) in the cytosol. During normal growth, starch is synthesized and stored during the day and degraded during the night. Although stress conditions tend to lead to the accumulation of oils in green algae, some conditions, like nitrogen limitation can result in starch accumulation (Siaut et al. 2011; Allen et al. 2018; Burlacot et al. 2019). Secondary red stramenopiles like diatoms do not possess the metabolic pathways for starch synthesis (Ball et al. 2011). Instead, stramenopiles have been shown to store the glucose polymers chrysolaminarin and laminarin in their vacuoles, as well as TGA as oil droplets mainly in their cytosol. Diatoms tend to accumulate oils in many different conditions, suggesting that carbon allocation to TGA is their prevalent form of carbon storage (Chiovitti et al. 2004; Michel et al. 2010; Wagner et al. 2017; Jensen et al. 2020). It is therefore not surprising that the green algal plastids in the slugs have been shown to accumulate starch, whereas the plastids of the stramenopile *V. litorea* seem to “leak” oil droplets into the cytosol of the slug’s cell (Pelletreau et al. 2014; Laetz et al. 2017).

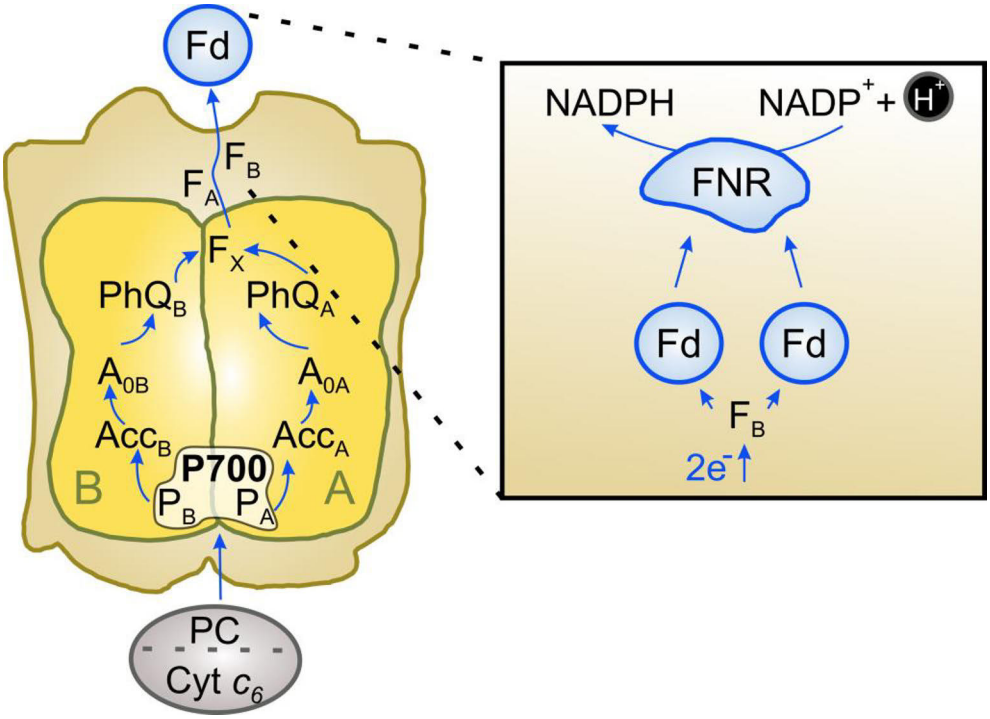

Figure 3. Light-induced linear electron transfer reactions in PSI. The scheme on the left illustrates the main electron transfer reactions along the A and B branches of PSI. The panel on the right shows the electron transfer reactions leading to NADPH production at the acceptor side of PSI.

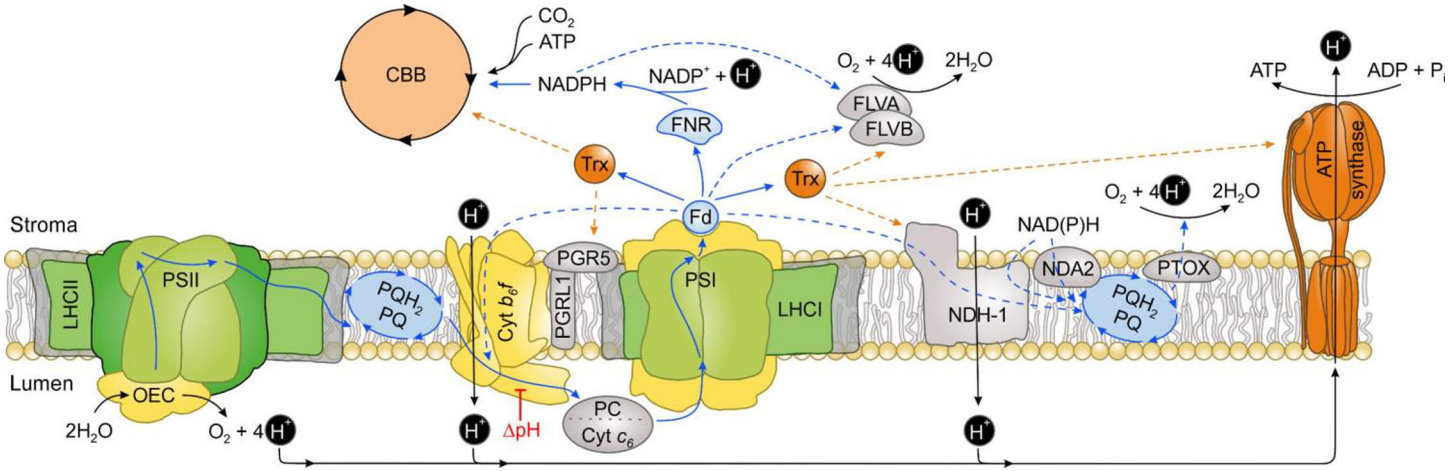

Figure 4. A simplified scheme of electron transfer routes in algae. Blue solid arrows depict the most studied linear electron transfer routes, whereas the routes pointed out by dashed blue arrows are less studied and their existence in different algae remains to be clarified. Orange dashed arrows indicate possible regulatory pathways of reduced thioredoxins (Trx). Components marked with gray color might exhibit differences in functionality between evolutionarily divergent lineages or be lost in certain algae. The gray shading around LHCII and LHCI portray the differences in LHCs in algae. Some components, mainly flavodiiron proteins (FLVA and FLVB) and plastid terminal oxidase (PTOX) are discussed in Chapter 1.5.2. Alternative electron sinks. The scheme is modified from Nikkanen et al. (2021).

1.3.4 Cyclic electron transfer

Cyclic electron transfer usually refers to the electron flow recirculating electrons around PSI. Generally, the electrons from Fd follow two main routes back to the PQ pool; either via the NADH-dehydrogenase-like (NDH) complex or a pathway that is dependent on the proteins proton gradient regulation 5 (PGR5) and PGR5-like photosynthetic phenotype 1 (PGRL-1) (Shikanai et al. 1998; Munekage et al. 2002; DalCorso et al. 2008). These pathways may or may not exist in different combinations in algae from different lineages, and their existence in both the green and the stramenopile prey algae of the slugs is completely unknown (Grouneva et al. 2013; Shimakawa and Miyake 2018). Some green algae, like *C. reinhardtii,* do not have a NDH complex, but they do utilize a type 2 NDH protein (NDA2) in their thylakoid membrane. Unlike the NDH complexes of cyanobacteria and plants (and possibly some green algae), NDA2 does not pump protons to the lumen while reducing PQ, and it seems to be mostly involved in reducing the PQ pool in anaerobic conditions in the dark by accepting electrons from stromal metabolites derived from fermentation (or mitochondria), not necessarily from Fd (Jans et al. 2008; Krishna et al. 2019; Zhang et al. 2020). If diatoms use NDH proteins, they are likely similar to the *C. reinhardtii* NDA2 (Grouneva et al. 2011).

Although it is still unclear what is the actual Fd-PQ reductase in the PRG5/PGRL-1 dependent pathway, it is a prominent cyclic electron transfer route in *C. reinhardtii* (Jokel et al. 2018). PGR5/PGRL-1 might not be part of the actual electron exchange, but they have been suggested to be essential modulators of FNR recruitment to the vicinity of PSI and Cyt *b6f*, creating a cyclic electron transfer supercomplex where the electrons from Fd may be be utilized by Cyt *b6f* to reduce the PQ pool (Mosebach et al 2017; Nikkanen et al. 2021). When the electrons from the reduced PQ pool are subsequently shunted through the normal Q-cycle of Cyt *b6f*, protons are released into the lumen without producing NADPH, which may help correct the ATP/NADPH ratio of linear electron transfer. Homologues for both PGR5 and PGRL-1 have been shown to exist in the diatoms *P. tricornutum* and *Thalassiosira pseudonana* (Grouneva et al. 2011).

1.4 Reactive oxygen species and photoinhibition

Not only is photosynthesis complicated, but it is fragile as well. The inevitable consequence of oxygen evolution near the redox active components of the photosynthetic electron transfer chain is the eventual production of reactive oxygen species (ROS). The main ROS related to photosynthesis are singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$) (Mattila et al. 2015; Foyer 2018; Khorobrykh et al. 2020). Uncontrolled production of ROS can be deleterious to the photosynthetic apparatus, and ROS are

heavily linked to light induced damage to PSII and PSI, or photoinhibition. It would therefore be in the best interest of a photosynthetic slug to avoid such ROS bursts for the sake of its plastids, but also to protect its own hide.

1.4.1 Photoinhibition of photosystem I

The primary ROS produced by reduction of oxygen is $O_2^{\bullet-}$. In photosynthesis $O_2^{\bullet-}$ is mainly produced by one-electron reduction of oxygen by PSI in the first step of the Mehler-reaction (Mehler 1951; Asada and Kiso 1973). The bulk of oxygen reduction by PSI associated electron transfer seems to involve redox cofactors inside PSI, possibly the iron-sulphur clusters F_A/F_B and F_X. However, recent studies suggest that PhQ_A is the main participant in oxygen reduction in PSI, especially during excessive light conditions (Takahashi and Asada 1988; Kruk et al. 2003; Kozuleva and Ivanov 2010; Kozuleva and Ivanov 2016; Kozuleva et al. 2021). The final products of the Mehler-reaction, H_2O_2 and oxygen, form via spontaneous or superoxide dismutase (SOD) catalyzed dismutation of $O_2^{\bullet-}$ in the stroma (Gray and Carmichael 1992; Asada 1996) (Fig. 5). The iron-sulphur clusters of PSI can be harmful if H_2O_2 production is out of control, as H_2O_2 can react with them and lead to the formation of the highly reactive $HO^{\bullet}$ via the Fenton reaction. Here, Fe^{2+} ion reduces H_2O_2, yielding Fe^{3+}, $HO^{\bullet}$ and HO^- (hydroxide ion) (Mizuta et al, 1997; Šnyrychová et al. 2006).

Photoinhibition of PSI can occur during excessive light conditions, especially in fluctuating light (Terashima et al. 1994; Allahverdiyeva et al. 2013; Shimakawa and Miyake 2018; Lima-Melo et al. 2019; Shimakawa et al. 2019). Electron donation from PSII and oxygen are prerequisites for photoinhibition of PSI, suggesting that ROS production in the vicinity of PSI is a major contributor (Sonoike 2011). Whether the main PSI damaging ROS is $O_2^{\bullet-}$, H_2O_2 or $HO^{\bullet}$ remains unclear, but it has been suggested that the initial targets of photoinhibition are F_A/F_B, and full photoinhibition to the level where charge separation does not occur involves the destruction of the F_X cluster and possibly also the PhQs (Inoue et al. 1986; Sonoike et al. 1995; Tiwari et al. 2016). The repair process of damaged PSI is metabolically expensive and slow, because it involves the complete degradation and resynthesis of the reaction centre (Zhang and Scheller 2004; Zhang et al. 2011).

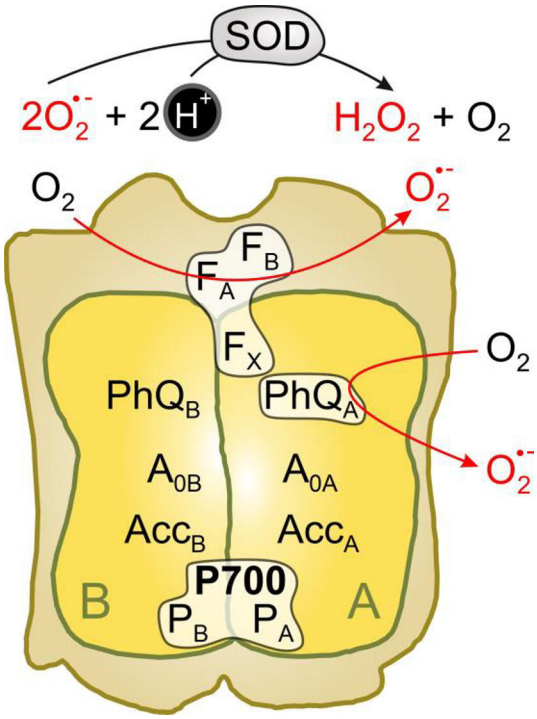

Figure 5. ROS production in PSI. One electron reduction of oxygen to $O_2^{\bullet-}$ by PSI (the first step of the Mehler-reaction) likely happens via either the iron-sulphur clusters F_X, F_A and F_B, or via the phylloquinone PhQ_A. The Mehler-reaction is complete after $O_2^{\bullet-}$ dismutates to H_2O_2 and oxygen in a reaction that can be catalyzed by the stromal SOD.

1.4.2 Photoinhibition of photosystem II

The main ROS produced in PSII is $^1O_2$. The production of $^1O_2$ entails a spin inversion of an outer orbital electron of oxygen (molecular oxygen is a triplet, $^3O_2$, and therefore actually a di-radical). In photosynthetic material this can occur when a triplet excited state Chl molecule (^{3}Chl) reacts with oxygen (Krasnovsky 1994). The main $^1O_2$ production pathway involves ^{3}Chl formation in the PSII reaction centre, specifically in the P680 Chl P_{D1} or in the accessory Chl_{D1} (collectively nominated as ^{3}P680 for clarity) (Diner et al. 2001; Noguchi et al. 2001). If forward electron transfer to Q_A is blocked due to, for example, fully reduced PQ pool in high light, the probability of charge recombination between $P680^+$ and Phe^- increases (Krieger-Liszkay 2005). This charge recombination can result in either re-excited P680*, ground state (singlet) P680 or the dangerous ^{3}P680. Oxygen can react with ^{3}P680, resulting in the formation of $^1O_2$ near the D1 protein of PSII and reversion of ^{3}P680 back to the ground state P680 (Rappaport and Lavergne 2009; Fischer et al. 2013; Pospíšil 2016) (Fig. 6). It has been shown that an increase in the electrochemical component of *pmf* across the thylakoid membrane during photosynthesis may

enhance $^1O_2$ production in PSII, possibly because the increased negative charge in the stromal side of the thylakoid membrane can force the electrons in Q_A^- back towards the lumen, or Phe_{D1}. This would also favour the charge recombination between the primary charge pair $P680^+Phe^-$ (Davis et al. 2016).

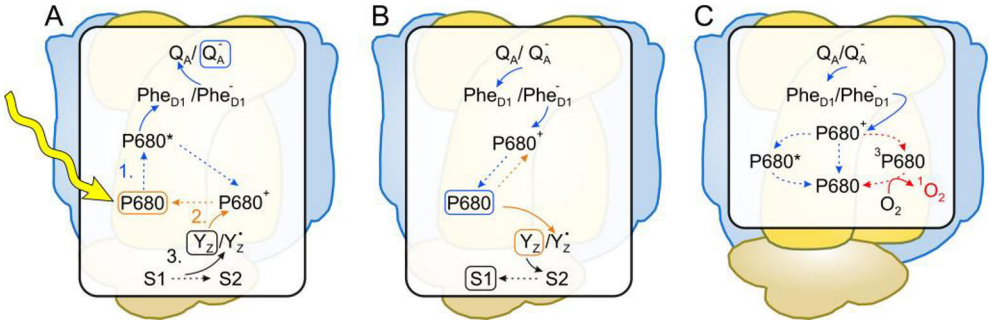

Figure 6. The main events leading to $^1O_2$ production in PSII. **(A)** Linear electron transfer to Q_A in PSII after P680 excitation. First, P680* reduces Phe_{D1} during charge separation, creating $P680^+$ and Phe_{D1}^- (the primary charge pair), which subsequently reduces Q_A to Q_A^-. (blue arrows). Next, $P680^+$ oxidizes Y_Z, creating P680 and $Y_Z^\bullet$ (orange arrows). Finally, the OEC donates an electron to $Y_Z^\bullet$, causing the OEC to shift to S2 and creating Y_Z (black arrows). The blue, orange and black boxes indicate the positions of the electrons from the reactions marked with the same colors after charge separation. **(B)** A schematic of the charge recombination reactions that may take place after charge separation, involving Q_A, Phe_{D1} and P680 in PSII. The order of the reactions both in the reducing side (blue arrows) and in the oxidizing side (black and orange arrows) is currently unknown. In the oxidizing side, the electron in Y_Z recombines with the S2 state of the OEC, reverting the S-state back to S1 and creating $Y_Z^\bullet$ (black arrows). Next, the electron from P680 may return to $Y_Z^\bullet$, creating Y_Z and $P680^+$ (orange arrows). $P680^+$ might accept the electron coming back from Q_A^- via Phe_{D1}, restoring P680 (blue arrows). The colored boxes show the position of the electrons after these reactions. **(C)** The charge recombination reactions from Q_A^- to $P680^+$ via Phe_{D1} in more detail. If the energy of the primary charge pair is high enough to re-excite P680 during charge recombination, it can lead to the formation of either P680* or the dangerous $^3P680$. While P680* may return back to its ground state P680 via photon emission (actually one of the surrounding Chls emits the photon), $^3P680$ reversion back to ground state often entails a reaction with oxygen, producing $^1O_2$. If the energy of the primary charge pair is insufficient to re-excite P680, the charge recombination between Phe_{D1} and $P680^+$ may produce P680 safely without dangerous intermediates.

PSII is very susceptible to photoinhibition, and an undeniable link exists between $^1O_2$ and photoinhibition of PSII. However, the primary mechanisms of PSII photoinhibition are still under debate. UV radiation (200-400 nm) is highly damaging to PSII, and a consensus exists that it causes photoinhibition by directly damaging the Mn_4CaO_5 cluster of the OEC (Hakala et al. 2005; Ohnishi et al. 2005). The primary damaging reaction in visible light (~400-700 nm) has been suggested to entail the absorption of a photon by the OEC that would damage PSII in a similar

fashion as UV radiation, but other views on the topic insist that the damage is caused by charge recombination and the associated $^1O_2$ production (Vass 2011; Tyystjärvi 2013; Nishiyama and Murata 2014). Other ROS that could be damaging PSII could be $O_2^{\bullet-}$ and $HO^{\bullet}$, as suggested recently (Kale et al. 2017). In addition to charge recombination and ROS, the highly reactive intermediates of PSII electron transfer chain, such as $P680^+$ and $Y_Z^{\bullet}$, have been suggested to contribute to PSII photoinhibition (Jegerschöld et al. 1990; Anderson et al. 1998). The degradation of the D1 protein ensues photoinhibition, and D1 has been shown to be subject to oxidation during photoinhibition, but the exact damaging process leading to a photoinhibited PSII that is unable to split water and donate electrons forward to the PQ pool is not clear (Aro et al. 1993; Tyystjärvi 2013; Kale et al. 2017).

1.4.3 Photosystem II repair cycle

Since photoinhibition of PSII is inevitable during photosynthesis, photosynthetic organisms have an efficient repair cycle of PSII (Aro et al. 1993). PSII repair in plants takes place only in the granal margins and the stromal lamellae of the thylakoids, whereas in *C. reinhardtii* it proceeds in multiple locations throughout the thylakoid membrane, likely due to less strict thylakoid stacking (Uniacke and Zerges 2007). The thylakoids of stramenopile diatoms are organised as long three-layer grana stacks throughout the plastid, and translocation of a damaged PSII from the middle of the stack to stroma-exposed regions for repair can be time-consuming. Diatoms can, however, accumulate large pools of inactive PSII in specific thylakoid zones to wait for re-assembly that occurs also during the night, unlike in cyanobacteria and plants where the repair is light dependent (Li et al. 2016a; Levitan et al. 2019). Since the prey algae species of the slugs are cohorts of green algae and stramenopiles, it is plausible to think that the PSII repair cycle in the prey algae follows the same basic principles as in the model algae species from both groups. However, since most studies on PSII repair cycle have been done with plants and cyanobacteria, care should be taken not to make too strong conclusions regarding the inner workings of the prey algae of the slugs.

The first step in the repair cycle is the monomerization of the dimeric PSII supercomplex and the localization of the monomeric PSII to specific repair zones. In plants this involves phosphorylation of several PSII subunits, including D1, but whether similar phosphorylation patterns govern PSII repair cycle in algae is not known (Tikkanen et al. 2008; Goral et al. 2010; Nath et al. 2013; Grieco et al. 2016). The disassembly of the PSII monomer continues with the release of LHCII, CP43 and the OEC, exposing the D1 protein for subsequent degradation (Theis and Schroda 2016). Removal of the phosphoryl group by phosphatases precedes the D1

degradation process in plants (Aro et al. 1992; Rintamäki et al. 1996; Kato and Sakamoto 2014).

The D1 protein of a damaged PSII is degraded either only by FtsH proteases or cooperatively by FtsH and Deg proteases (Kato and Sakamoto 2018). FtsH proteases are essential in protein quality control and degradation in almost all lifeforms, but photosynthetic organisms have a need for a wide repertoire of FtsH isoforms; for example, *Escherichia coli* only has one *ftsH* gene, whereas the plant *Arabidopsis thaliana* has 12 (Wagner et al. 2012). All known FtsH isoforms involved in protein degradation share an N-terminal transmembrane region, a C-terminal region containing an AAA-ATPase domain and a proteolytic zinc binding M41 protease domain (Kato and Sakamoto 2018). Thylakoidal FtsH isoforms are divided to type A and type B based on sequence similarity, and a functional FtsH complex in the thylakoids is a heterohexamer containing both A and B type subunits in a 1:1 (cyanobacteria) or 1:2 (plants) stoichiometry (Lindahl et al. 2000; Rodrigues et al. 2011; Boehm et al. 2012; Moldavski et al. 2012). *C. reinhardtii* only has one isomer of each subunit that likely also form a heterohexameric FtsH complex (Malnoë, et al. 2014; Wang et al. 2017). ATP hydrolysis provides the driving force that pulls the D1 protein out of the PSII monomer into the proteolytic zinc binding chamber of FtsH where D1 is degraded (Wagner et al. 2012). In addition to D1 degradation, FtsH proteases have been proposed to have a significant role in the maintenance of several other thylakoid proteins, including PSI, LHCI and Cyt *b6f* (Kato and Sakamoto 2018). The contribution of ATP independent Deg proteases to the PSII repair cycle in cyanobacteria is negligible, whereas in the green alga *C. reinhardtii* their role in the PSII repair cycle during photoinhibition remains uncertain (Barker et al. 2006; Malnoë et al. 2014; Theis et al. 2019).

The PSII re-assembly starts with de novo synthesis of D1 proteins in ribosomes near the PSII repair zones. The pool of *psbA* (D1) transcripts remains stable in the plastids, and transcription of *psbA* has been shown to be constitutive even in the dark, especially in plants (Rapp et al. 1992). Consequently, the expression of the plastid encoded *psbA* is thought to be mostly regulated post transcriptionally (Mulo et al. 2012). For example, in *C. reinhardtii* the maturation of the *psbA* transcript has been suggested to involve splicing and 5' UTR processing of the *psbA* transcript, controlled by active photosynthetic electron transfer. This enhances the binding of the transcripts with the ribosomes and is important for the initiation of translation (Deshpande et al. 1997; Bruick and Mayfield 1998; Mulo et al. 2012). Both 5' and 3' UTR of the *psbA* transcripts of plants seem to be important targets for possible proteins regulating ribosome recruitment and translation initiation (Memon et al. 1996; Gawroński et al. 2021).

In cyanobacteria, one main regulation point of *psbA* expression is the translation elongation step, which has been shown to be negatively affected by ROS,

particularly $^1O_2$ and H_2O_2 (Allakhverdiev and Murata 2004; Nishiyama et al. 2004). Here, specific cysteine residues of the translation elongation factors EF-G and EF-Tu can be oxidized by H_2O_2, which results in their inactivation and the cessation of protein synthesis (Kojima et al. 2009; Yutthanasirikul et al. 2016). The role of EF-Tu in the PSII repair cycle of eukaryotes has not been studied extensively, but it has been shown to be important for plants during heat stress (Ristic et al. 2004; Fu et al. 2008; Li et al. 2018). PSII repair cycle is also negatively affected by heat stress, and this has led to postulations about the importance of EF-Tu in PSII repair also in plants, especially since the oxidizable cysteine residue of EF-Tu exhibits high evolutionary conservation (De Laurentiis et al. 2011; Jimbo et al. 2019). Translation elongation in plants is also known to be light regulated, as it requires a transthylakoidal proton gradient (Muhlbauer and Eichacker 1998; Mulo et al. 2012). The PSII repair cycle includes the co-translational insertion of the new D1 into the thylakoid membrane, and the ribosomes are known to pause at distinct pause sites during translation, allowing the proper folding and insertion of cofactors into the protein (Järvi et al. 2015; Gawroński et al. 2018).

The highly complex PSII reassembly steps involve a plethora of auxiliary proteins, most of which are nucleus encoded in the plant *A. thaliana.* In plants, the new D1 peptide is targeted to the thylakoid membrane via a signal recognition particle and its receptor. At the thylakoid membrane, a translocase inserts the D1 into the PSII monomer, assisted by a membrane insertase and multiple other proteins like chaperones (Zhang et al. 2001; Nilsson and van Wijk 2002; Järvi et al. 2015; Theis and Schroda 2016). During the insertion process, the new D1 is processed to full maturity by a lumenal carboxyl terminal peptidase (CTPA) that excises a short C-terminal extension out of the D1 in cyanobacteria and primary green and red endosymbiotic lineages. This extension is interestingly missing in most secondary algae like the stramenopiles, which could be also important for the functionality of the repair cycle in *V. litorea* plastids inside the slugs (Satoh and Yamamoto 2007; Chang et al. 2021). The highly conserved vesicle inducing protein in plastids (VIPP1) has been suggested to be important for creating specific lipid microdomains in the thylakoid membrane near the PSII repair cites, which may attract the auxiliary proteins and ensure their proper function (Nordhues et al. 2012; Rutgers and Schroda 2013; Theis and Schroda 2016; Theis et al. 2019).

The piecing together of PSII continues with the attachment of CP43 to the PSII monomer, followed by the reassembly of the lumenal subunits of the OEC. This step is severely blocked unless the C-terminal extension of the new D1 has been excised by CTPA. The coordinator that might prevent premature assembly of PSII is likely PSB27 protein in *A. thaliana* and cyanobacteria. PSB27 binds to the C-terminus of the mature D1 after excision by CTPA and to the lumenal side of CP43, transiently occupying a site reserved for the OEC subunits. This facilitates the formation of the

rest of the OEC, including the Mn_4CaO_5 cluster (Chen et al. 2006; Roose and Pakrasi 2008; Wei et al. 2010; Cormann et al. 2014; 2016; Theis and Schroda 2016). Finally, the fully reassembled PSII monomer needs to find a partner to re-establish a functional PSII dimer supercomplex. For this, the repaired PSII monomers relocate to the appressed regions of the thylakoid membrane where the LHCII proteins are re-attached prior to dimerization (Mulo et al. 2008; Järvi et al. 2015; Theis and Schroda 2016). Even though the details of the PSII repair cycle might be different in the prey algae of the slugs, completing such a complicated process in its entirety day after day with plastids isolated from nuclear regulation is a seemingly impossible task.

1.5 Protection against light and ROS

Photosynthetic sea slugs are not sessile creatures and can move to the shade to escape excessive light conditions. Nevertheless, they do inhabit shallow coastal waters and are expected to occasionally face harsh illumination. During such times, it would be beneficial to make use of the inbuilt photoprotective mechanisms of the plastids to maintain them functional. Long-term acclimation responses of photosynthetic organisms to high light include decreasing the antennae size and altering the stoichiometry of the photosystems (Chow et al. 1990; Bonente et al. 2012). Because natural sunlight contains UV radiation, a common response to strong sunlight is the production of UV screening compounds, most common ones being mycosporine-like amino acids in algae (Sinha et al. 1998; Sun et al. 2020). More short term, dynamic photoprotection mechanisms fall into three strategic categories. The first strategy is to dissipate and redistribute the excess excitation energy at the level of light harvesting complexes. Secondly, electrons filling the photosynthetic electron transfer chain can be diverted to safe electron sinks. The third option is to utilize specific compounds that serve as safe targets for ROS and can be sacrificed and recycled instead of the photosynthetic apparatus.

1.5.1 Non-photochemical quenching

Several photoprotective mechanisms function by enhancing the safe thermal dissipation of excitation energy in the antennae. Because thermal dissipation also competes with Chl fluorescence for excitation energy, these mechanisms result in the quenching of Chl fluorescence originating from PSII. For this reason, these protective mechanisms are collectively called non-photochemical quenching (of fluorescence; NPQ) (Genty et al. 1989; Krause and Jahns 2004; Niyogi and Truong 2013; Kalaji et al. 2014; Kalaji et al. 2017). Here, only the three most basic components of NPQ, qT, qE and qI, are discussed (Müller et al. 2001).

Certain NPQ mechanisms function mainly during times of moderate to low illumination. State transitions, constituting the qT component of NPQ, are one such mechanism, although qT might have a role also during high light acclimation in *C. reinhardtii* (Allorent et al. 2013). State transitions re-distribute the light energy favourably between PSII and PSI as a response to altered light conditions. Conditions that reduce the PQ pool, like light preferentially exciting PSII, force PQH_2 to bind to the Q_o site of Cyt *b6f*, which activates the Stt7/STN7 kinase (Zito et al. 1999; Bellafiore et al. 2005). This threonine kinase phosphorylates specific subunits of the LHCII complex, initiating a reorganization of the thylakoid membrane to state II, where the phosphorylated LHCII dissociates from PSII and interacts with PSI (Depège et al. 2003; Longoni et al. 2015). Conversely, oxidation of the PQ pool by light that preferentially excites PSI deactivates Stt7/STN7 and allows the dephosphorylation of LHCII by the PPH1/TAP38 phosphatase, returning the light acclimation state back to state I (Pribil et al. 2010; Cariti et al. 2020; Mattila et al. 2020). According to a widespread notion, diatoms do not utilize state transitions as a part of their photoacclimatory repertoire (Owens 1986; Lepetit et al. 2012; Büchel 2015). However, other secondary red algae, like the stramenopile *Ochromonas Danica* and the haptophyte *Phaeocystis antarctica* seem to be capable of state transitions, but their physiological significance remains to be tested (Gibbs and Biggins 1989; Stamatakis et al. 2016).

The photoprotection mechanism prevalent in high light is the energy-dependent component of NPQ, qE. Once active, qE lowers the excitation energy delivery to the photosystems, mainly PSII, but also decreases the production of $^1O_2$ in the LHCs (Carbonera et al. 2012; Girolomoni et al. 2017). Acidification of the thylakoid lumen during photosynthesis is the main driving force of qE. The main pH sensor of plants, PsbS, has been shown to have a role in photoprotection also in *C. reinhardtii*, but not necessarily directly via NPQ (Redekop et al. 2020). Instead, *C. reinhardtii* utilizes specific stress-related LHC proteins (LHCSR), mainly LHCSR3, to induce qE (Allorent et al. 2013). LHCSR3 acts as a pH sensor of the thylakoid lumen via protonation, which activates and associates LHCSR3 with multiple antenna proteins of PSII and likely also PSI (Bonente et al. 2011; Ballottari et al. 2016; Girolomoni et al. 2019; Cazzaniga et al. 2020). Unlike PsbS, LHCSR3 binds Chl and xanthophylls, allowing it to act as the possible excitation energy quencher in qE (Perozeni et al. 2020; Troiano et al. 2021). Diatoms possess stress related LHC proteins, LHCXs, that share similarities with the LHCSR proteins (Bailleul et al. 2010). Some of the protonatable amino acid residues of LHCSRs are present also in LHCXs, but their protonation does not seem to be enough to trigger qE in diatoms (Buck et al. 2021). Inference from structural data suggests that pigment binding by LHCXs is likely (Ballottari et al. 2016; Wang et al. 2019). Importantly, LHCXs have

been suggested to bind the pigments of the xanthophyll cycle, another key element of qE (Buck et al. 2021).

In diatoms and other stramenopiles, including the prey alga of the slugs, *V. litorea* the main pigments involved in the xanthophyll cycle are diadinoxanthin and diatoxanthin (Cruz et al. 2015). The conversion of diadinoxanthin to diatoxanthin via diadinoxanthin de-epoxidase (DDE) is a mandatory factor in qE induction in many diatoms; in fact, the amount of diatoxanthin is directly proportional to the extent of qE in most diatoms (Goss and Jakob 2010; Goss and Lepetit 2015; Blommaert et al. 2017). The acidification of the thylakoid lumen has been suggested to activate the lumenal DDE by protonation, allowing the enzyme to bind to the thylakoid membrane and interact with diadinoxanthin (Goss and Jakob 2010; Lavaud et al. 2012). However, DDE has been shown to function even in almost neutral pH of 7.2, which is likely the reason why non-photochemical proton gradient formation, possibly via NDH protein(s), is enough to induce diatoxanthin formation and qE in the dark in diatoms (Jakob et al. 2001). It is possible that a diatoxanthin molecule bound to LHCX proteins might serve as sites for thermal dissipation in a similar vein as LHCSR3 in *C. reinhardtii* (Park et al. 2019). Alternatively, the conversion of diadinoxanthin to diatoxanthin could result in oligomerization of the LHCX proteins with the LHCII proteins, and the quenching would take place in the LHCII that also binds diatoxanthin (Goss and Jakob 2010; Buck et al. 2021).

The reversal of the xanthophyll cycle proceeds via diatoxanthin epoxidase (DTE) catalyzed epoxidation of diatoxanthin back to diadinoxanthin in a reaction that consumes NADPH and oxygen. DTE is very efficient in low to moderate light but is inhibited in the dark and in high light by unknown mechanisms (Goss et al. 2006a; Goss and Jakob 2010; Buck et al. 2021). The activity of DDE, on the other hand, increases linearly with light intensity, but the overall de-epoxidation by DDE is modest compared to the more efficient epoxidation by DTE (Blommaert et al. 2021). This points to a system where the photoprotective diatoxanthin is produced by default, and only its removal by the efficient DTE keeps the antenna system in a light-harvesting configuration during suitable light periods.

The basic idea of the xanthophyll cycle in green algae is the same as in diatoms, but the xanthophylls are different: violaxanthin and zeaxanthin are interconverted via de-epoxidation/epoxidation reactions in response to different light conditions (Yamamoto 1979; Jahns et al. 2009). The functional pH range for the violaxanthin de-epoxidase is approximately 5-6, which links its activity to electron transfer, whereas the zeaxanthin epoxidase is not affected by the pH gradient (Goss and Jakob 2010). Zeaxanthin is an important modulator of qE but is not strictly necessary for the induction of qE in *C. reinhardtii* or plants (Johnson et al. 2009; Bonente et al. 2011; Kress and Jahns 2017; Tian et al. 2019). However, *C. reinhardtii* seems to rely on a stromal lycopene cyclase homologue that is analogous to the violaxanthin de-

epoxidase found in many other green algae, suggesting that zeaxanthin might have a specific role in *C. reinhardtii* (Li et al. 2016b; Handrich et al. 2017). Recent studies in this alga have shown that the quenching associated directly with lumen acidification and protonation of LHCSR3 could be a completely different qE pathway that overlaps with the qE associated with zeaxanthin (Troiano et al. 2021). The exact details of how zeaxanthin allows the quenching to proceed in *C. reinhardtii* are not known, but PSII antennae and LHCSR proteins bind violaxanthin/zeaxanthin and are potential quenching sites (Troiano et al. 2021; Cazzaniga et al. 2020).

The last one of the traditional three NPQ components is qI that has much slower induction and relaxation kinetics than qE (seconds to minutes) or qT (minutes to tens of minutes). Classically, qI is the only component ascribed to the quenching mechanisms occurring during extended periods of high light illumination, and it is largely attributed to the peculiar quenching of fluorescence by photoinhibited PSII reaction centres (Müller et al. 2001; Matsubara and Chow 2004; Zavafer et al. 2019; Nawrocki et al. 2021). The contribution of qI to diatom NPQ is usually estimated to be negligible due to their efficient photoprotection (Lepetit et al. 2012). In addition to photoinhibition of PSII, other processes that have long-lasting and slow kinetics include a specific zeaxanthin-dependent quenching that is known to occur in plants even in the absence of a transthylakoid pH gradient and the PsbS protein (Nilkens et al. 2010; Malnoë 2018). Finally, a new form of sustained quenching was discovered in plants, which involves a plastid lipocalin. The current model for this lipocalin quenching entails the removal of the inhibition of lipocalin activity during stress conditions, allowing it to interact with the antennae of PSII, resulting in their energy dissipative state (Malnoë 2018; Malnoë et al. 2018).

1.5.2 Alternative electron sinks

Sometimes the quenching of excitation energy is not enough, and excessive amounts of electrons flood the photosynthetic electron transfer chain. In addition to linear and cyclic electron transfer, multiple alternative electron transfer routes exist in the plastids, and they can vent out the excessive electrons. Some of them manage to do this safer than the others.

The water-water cycle, where electrons originating from water in PSII are directed to reduce oxygen, ultimately yielding water, is most often touted to be an extension of the Mehler reaction (see chapter 1.4.1). Here, the product of the Mehler reaction, H_2O_2, is detoxified by ascorbate peroxidase to H_2O. Since the intermediates of the water-water cycle involving the Mehler reaction include $O_2^{\bullet-}$ and H_2O_2, it is not considered the safest of valves. However, because the oxygen reduction in this reaction series takes place only at PSI acceptor side, the electrons contributing to it

might at least enhance the ΔpH when they traverse the electron transfer chain all the way from PSII, which releases protons into the lumen. Therefore, they may also contribute to NPQ (Asada 1999; Makino et al. 2002). The plastid terminal oxidase (PTOX) that facilitates chlororespiration is another water-water -type exit point for electrons in the thylakoids of algae, as PTOX oxidizes the PQ pool and reduces oxygen to water. PTOX might be relevant for maintaining a suitable redox poise of the PQ pool during stress conditions in *C. reinhardtii,* for example to induce state transitions, whereas the role of PTOX in diatoms is unclear (Grouneva et al. 2011; Houille-Vernes et al. 2011; Fisher et al. 2020).

The Mehler-like reaction facilitated by flavodiiron proteins (FLVs) consumes electrons from NADPH and/or Fd to reduce oxygen to H_2O without the bothersome production of ROS. FLVs are widespread throughout the evolutionary tree, but seem to be curiously missing in angiosperm plants, primary red algae and most secondary red algae (Allahverdiyeva et al. 2015; Alboresi et al. 2019a; Nikkanen et al. 2021). The only secondary red lineage where they have been shown to exist to date is the dinoflagellate family Symbiodiniaceae that often form a symbiosis with corals (Cnidaria) (Shimakawa et al. 2021). FLVs work in pairs, and the active heterodimer in *C. reinhardtii* consists of FLVA and FLVB proteins that are similar to the Flv1 and Flv3 proteins of cyanobacteria (Allahverdiyeva et al. 2015; Jokel et al. 2015; Chaux et al. 2017). The alternative electron sink capacity of FLVs is extremely important for safeguarding PSI from photoinhibition during a transition from dark to light or in fluctuating light conditions in general; a sudden burst of light may temporarily flood the electron sink capacity of the CBB cycle, which can lead to an increase in the Mehler reaction if FLVs are not present (Jokel et al. 2018).

Although most of the algae in the red lineage do not seem to possess FLVs, many of them do exhibit oxygen dependent fast oxidation of P700 in PSI during dark to light transition (Shimakawa et al. 2019). This type of P700 oxidation has been designated as a sign of FLV activity, as, for example, cyanobacterial FLV deletion mutants lack the capability to maintain P700 oxidized during such treatments (Ilík et al. 2017; Jokel et al. 2018). The fact that FLV-like P700 oxidation kinetics exist even in algae where FLVs are not likely present underlines the importance of alternative electron sinks downstream of PSI, whatever they may be. Indeed, algae of the red lineage that are unable to efficiently oxidize P700 are susceptible to fluctuating light photoinhibition of PSI. However, many secondary red algae, like the diatom *P. tricornutum*, can oxidize P700 during a sudden light pulse even without oxygen, implying that multiple different mechanisms for this purpose have evolved throughout the tree of life (Shimakawa et al. 2019). Of course, the existence of FLVs or other types of alternative electron sinks downstream of PSI in the prey algae of the slugs has remained an open question.

1.5.3 ROS detoxification

Try as they might to prevent it, plastids of algae will have to deal with ROS. This is not always a bad thing, as ROS can also potentially contribute to signalling pathways that are required to respond to the metabolic state of the plastid (Foyer 2018). It is unclear how well the slug cell would/could respond to these signals, and they are probably better off trying to maintain the ROS levels to the minimum in their plastids. The following brief descriptions of ROS detoxification concern the processes as they are known to function mainly in plant plastids, and some of these mechanisms might be applicable to also slug plastids (Khorobrykh et al. 2020).

As mentioned earlier, the detoxification of $O_2^{\bullet-}$ and H_2O_2 involves reactions catalyzed by the enzymes SOD and ascorbate peroxidase, ultimately leading to the production of H_2O and an oxidized form of ascorbate, monodehydroascorbate radical (MDA). Reduced ascorbate (shortened here to ascorbate) is a very central molecule in almost all ROS detoxification, and it is therefore not surprising that a system known as the ascorbate-glutathione cycle is in place to reduce MDA back to ascorbate. Strictly speaking, glutathione is not necessary to regenerate ascorbate, as MDA can be reduced directly by Fd or by NADPH in a catalytic reaction involving MDA reductase. If not reduced immediately, however, MDA can react with another MDA, producing one ascorbate and one dehydroascorbate (DHA). Here, reduced glutathione reduces DHA to ascorbate with DHA reductase, and oxidized glutathione is then regenerated catalytically by glutathione reductase and NADPH (Asada 1999; Khorobrykh et al. 2020). Two-cysteine peroxiredoxins are known to complement ascorbate peroxidase in H_2O_2 detoxification in plastids by utilizing electrons from either NADPH or thioredoxins (Dayer et al. 2008; Pulido et al. 2010).

Carotenoids bound to the LHCs, like lutein and xanthophylls, are important agents of $^1O_2$ detoxification (Triantaphylidès and Havaux 2009). They can prevent the formation of $^1O_2$ in the first place by quenching both singlet excited Chl (in NPQ) and the more deleterious ^{3}Chl in the antennae, preventing the ^{3}Chl from reacting with oxygen (Ruban 2016; Malnoë 2018; Khorobryk et al. 2020). If $^1O_2$ still manages to form, the abundant carotenoids in the LHC and surrounding them can take the hit and react with it, mainly by physical quenching. This results in the triplet state of the carotenoid, and the triplet carotenoid dissipates the excess energy non-radiatively and safely (Di Mascio et al. 1990; Conn et al. 1991; Triantaphylidès and Havaux 2009). Carotenoids in the reaction centre of PSII are not thought to be able to quench the ^{3}P680 but can possibly quench other ^{3}Chls in the intrinsic antennae. Additionally, β-carotene inside PSII may take part in chemical scavenging of $^1O_2$, where β-carotene is oxidized to β-cyclocitral (Umena et al. 2011; Ramel et al. 2012; Fischer et al. 2013). LHCs are not the only place that contain antioxidants against $^1O_2$; free carotenoids and especially tocopherols (α-tocopherol) in the thylakoid membrane have an important role in detoxification of $^1O_2$. α-tocopherol can detoxify $^1O_2$ either

by chemical scavenging or physical quenching, although the latter one is thought to be rare (Triantaphylidès and Havaux 2009). The product of the chemical reaction between α-tocopherol and $^1O_2$ is 8-hydroperoxy-tocopherone, which can be reduced and recycled by ascorbate. This may tie the detoxification of also $^1O_2$ to the enzymatic ascorbate-glutathione cycle of ROS detoxification (Di Mascio et al. 1990; Kramarenko et al. 2006; Krieger-Liszkay and Trebst 2006; Triantaphylidès and Havaux 2009; Khorobrykh et al. 2020).

1.6 Plastids inside sea slugs, how does it work?

No one knows. That is not to say that nothing is known about plastid functionality inside the slugs, on the contrary. It is clear that it takes a specific type of slug species and a specific type of plastid to make functional kleptoplasty work. For example, both *E. timida* and *Elysia cornigera* feed on *A. acetabulum*, but only *E. timida* is able to store the plastids long-term in its body (de Vries et al. 2015). Conversely, both *P. ocellatus* and *E. viridis* feed on multiple algae species, but long-term retention of plastids is possible only if the plastids are stolen from specific algae, like the green ulvophyte algae *Halimeda macroloba* and *Bryopsis hypnoides*, respectively (Christa et al. 2013; Rauch et al. 2018). The plastomes of the algae that have their plastids incorporated by the slugs have not retained thousands of genes that would explain how they can autonomously take care of themselves without the algal nucleus, but there are certain interesting genes related to PSII repair cycle and plastid maintenance that are specific to these plastomes; the genes for FtsH and EF-Tu are common factors in the long-term plastid sources of the slugs, although only *V. litorea* contains *ftsH* with the proteolytic zinc binding protease domain (Mujer et al. 1996; Rumpho et al. 2008; de Vries et al. 2013; Christa et al. 2018; Maeda et al. 2021). Whether the plastids can maintain a functional PSII repair cycle in the slugs is, however, questionable. The plastids inside *E. chlorotica*, derived from the stramenopile *V. litorea* do synthesize D1 and other plastid proteins during the slug's starvation, but if this is the case in the rest of the slugs that steal green algal plastids is unclear (Mujer et al. 1996; Vieira et al. 2009).

The remarkable robustness of lab-isolated plastids of some ulvophytes and the stramenopile alga *V. litorea* has also been known for decades, and this is likely a factor that allows the plastids to withstand the physical stress and possible osmotic changes they face during the feeding process of the slugs (Giles and Sarafis 1972; Trench et al. 1973a,b; Green et al. 2005). The plastids can incorporate inorganic carbon in a light dependent manner once inside the slugs, and even at comparable rates to the algae themselves, which indicates that the CBB cycle is in working order (Rumpho et al. 2001; Christa et al. 2014b; Rauch et al. 2018; Cruz et al. 2020; Cartaxana et al. 2021). However, most reports on the photosynthetic functionality of

the plastids in the slugs come from Chl fluorescence measurements that are an indicator of mainly PSII activity. The xanthophyll cycle, involving either violaxanthin or diadinoxanthin, and qE are functional in the slug plastids, but the contribution of LHCSR and/or PsbS to qE in the slugs (and the macroalgae themselves) is not clear (Cruz et al. 2015; Handrich et al. 2017; Christa et al. 2018; Cartaxana et al. 2019).

Having the correct plastid inside the cells of a photosynthetic slug is only half the answer to functional kleptoplasty. The genetic details of the photosynthetic sea slugs themselves have only recently begun to unravel. While it is still too early to point to any one specific direction too confidently, the findings that several genes related to oxidative stress management and synthesis of sacoglossa-specific complex polyketides have undergone multiple gene duplications in long-term retention slugs, not to mention their upregulation during different stages of kleptoplasty, support the notion that the slugs are actively trying to accommodate their plastids (de Vries et al. 2015; Chan et al. 2018; Cai et al. 2019; Torres et al. 2020; Maeda et al. 2021). Whether the animal cell products make their way into the plastids remains to be seen. In the meantime, we can continue inspecting the basic properties of the plastids inside the slugs to determine the bottlenecks of plastid maintenance, where possible aid from the animal would be appreciated.

2 Aims of the study

It is currently not known how sacoglossan sea slugs can remain photosynthetic for weeks and months. The present work aims to reveal some of the photosynthetic and genetic factors contributing to the photoinhibition tolerance of photosynthetic sea slugs and their plastids. The specific goals of the project were the following:

1. Using the slug *E. timida* and its prey green alga *A. acetabulum*, I wanted to examine whether plastid incorporation into slug cells results in photoprotective alterations in the photosynthetic electron transfer chain of the plastids.
2. I intended to find out the extent of passive photoprotection provided by the slug tissue to the plastids against both visible light and UV radiation, again using *E. timida* and *A. acetabulum*.
3. The final goal was to pinpoint the inherent photosynthetic and genetic properties contributing to the longevity of the plastids of *V. litorea*, the only stramenopile prey alga of the slugs.

3 Materials and Methods

3.1 Organisms and culture conditions

Both *E. timida* and *A. acetabulum* were grown in 10 l plastic tanks at photosynthetic photon flux density (PPFD) 40-50 µmol $m^{-2}s^{-1}$ (12/12h light/dark cycle) at 22-25 °C temperature. The growth medium for *Acetabularia* was f/2 medium (Guillard and Ryther, 1962) made into 3.7 % artificial sea water (ASW; Sea Salt Classic, Tropic Marin, Montague, MA, USA), whereas the slugs were grown in plain 3.7 % ASW that was aerated with aquarium air spargers. A detailed description of the culturing methodology used for both species is given in Paper I of this thesis. Mainly freshly fed slugs and healthy green algae were used in the work, and exceptions to this are indicated in the text.

The stramenopile alga *V. litorea* C.Agardh 1823 (strain SCCAP K-0379) used in Paper III of this thesis was grown in f/2 medium made into 1% ASW (Tropic Marin) at PPFD 40 µmol $m^{-2}s^{-1}$ (12/12h light/dark) in 250 ml flasks without shaking. *V. litorea* cells were routinely maintained by detaching an inoculate cell mass (1-4 g wet weight) from older cultures, and then placing the mass into a new flask containing 50-100 ml fresh f/2 media. Only healthy green cultures that were 7-14 days old were used in the experiments. Approximately two months old spinach plants, routinely grown in PPFD 40 µmol $m^{-2}s^{-1}$ (8/16h dark/night), were also used in Paper III of this thesis. The growth temperatures for *V. litorea* and spinach were 17 and 22 °C, respectively.

3.1.1 *E. timida* feeding and starvation experiments

To induce changes to the photosynthetic machinery of *A. acetabulum* plastids prior to feeding them to the slugs in Paper I, the algae were grown in two separate conditions: elevated CO_2 (1% of air volume; high CO_2) and under strong illumination (high light) in combination with cold temperature. In the high CO_2 experiment the algae were acclimated to the altered gas composition for three days inside a separate growth cabinet (Algaetron AG230; Photon Systems Instruments, Drásov, Czech Republic), while maintaining the temperature and light conditions as identical as possible to the normal growth environment. The goal of the high light conditions

was to produce the red morphotype of *A. acetabulum*, that is known to emerge when these algae are put under a combination of high light and cold stress (Costa et al. 2012). In our conditions (continuous light, PPFD 600 µmol $m^{-2}s^{-1}$, 10 °C) truly red morphotypes of *A. acetabulum* were only noticeable after 31 days in the growth cabinet (Algaetron AG230).

Once the algal acclimation period to the new conditions had been carried out, the newly acclimatized *A. acetabulum* were fed to *E. timida* slugs. In the case of high CO_2 algae, the slugs were fed in cycles, where the tanks holding both the algae and the slugs were taken in and out of the high CO_2 chamber at set intervals to avoid suffocating the slugs with the excess CO_2. The time periods out of the chamber were kept short, approximately 1-2 h per cycle, to avoid losing the high CO_2 acclimation of the algae. This feeding cycle was continued for 4-6 days to ensure that the slugs had incorporated specifically acclimated plastids into their own cells. The high light acclimated red morphotype *A. acetabulum* cells were taken out of their acclimation chamber and presented to *E. timida* slugs for feeding inside Petri dishes in the normal growth conditions of the slugs. The slugs were allowed to incorporate the high light acclimated red *A. acetabulum* plastids for two days, and the red coloration of the slugs was taken as a sign that most green plastids inside the slugs had been replaced with the red ones.

Both slugs that had been feeding on high CO_2 acclimated algae and slugs feeding on algae in regular growth conditions were used in the starvation experiments, where the slugs were deprived of their food. Two different starvation conditions in ambient air were used in the present work: normal growth conditions and a fluctuating light regime. In the fluctuating light experiment the day/night cycle was normal (12/12 h), and the base level PPFD was also 40 µmol $m^{-2}s^{-}1$, but this background illumination was interrupted with a strong 10 s light pulse (PPFD 1500 µmol $m^{-2}s^{-1}$; Heliospectra L4A, Göteborg, Sweden) every 10 min during the day.

3.2 Isolation of plastids and thylakoid membranes

In Paper III, intact plastids were isolated from *V. litorea* using the protocol described in Green et al. (2005) with slight modifications. The cells were homogenized with ULTRA-TURRAX (IKA, Staufen, Germany) in plastid isolation buffer (0.2 % BSA, 1 mM EDTA, 50 mM Hepes-KOH pH 7.6, 1 mM $MgCl_2$, 330 mM sorbitol), and the homogenate was filtrated through Miracloth (Calbiochem, Darmstadt, Germany). The filtrate was pelleted (1900 x g, 5 min), resuspended in the isolation buffer, and pipetted between 75 and 30 % Percoll solutions. Plastids were collected from the interface after centrifugation (3500 x g, 20 min; swing-out rotor) without deceleration, and washed two times (2200 x g, 3 min) with the isolation buffer without BSA.

Thylakoid isolation procedure followed the protocol by Hakala et al. (2005), after keeping the plants and algae in the dark overnight prior to grinding them in a porcelain mortar in isolation buffer (1 % BSA, 1 mM EDTA, 1 mM glycine betaine, 40 mM HEPES-KOH (pH 7.4), 10 mM $MgCl_2$, 0.3 M sorbitol). The homogenate was filtered through Miracloth, pelleted and resuspended in osmotic shock buffer (10 mM HEPES-KOH (pH 7.4), 10 mM $MgCl_2$, 5 mM sorbitol). After the osmotic shock treatment, the samples were again pelleted and resuspended in a buffer containing 10 mM HEPES-KOH (pH 7.4), 10 mM $MgCl_2$, 5 mM NaCl, 500 mM sorbitol, where they were stored until use. All centrifugation steps used for pelleting the samples were done at 5000 x g for 5 min. All isolation procedures, including the plastid isolation, took place in dimly lit rooms at 4 °C.

3.3 Spectroscopic methods to probe photosynthesis

3.3.1 *E. timida* immobilization

The movement of photosynthetic sea slugs can seriously affect the spectroscopic measurements from these slugs (Cruz et al. 2012). To counteract this, in Paper I *E. timida* slug bodies were immobilized with either a swift decapitation using a razor or by alginate fixation (1 % alginate in H_2O, polymerized with 0.5 mM $CaCl_2$) for long (>1 s) or highly error-prone measurements, such as rapid light curves and P700 measurements, respectively. When Chl *a* fluorescence was measured with only a single saturating pulse lasting approximately one second, no other immobilization procedures apart from placing the slug individuals on a dry Petri dish were required.

3.3.2 Chl *a* fluorescence

The ratio of variable to maximum Chl *a* fluorescence (F_V/F_M; i.e. $(F_M-F_0)/F_M$) was routinely measured with pulse amplitude modulated (PAM) fluorometers (Dual-PAM 100 and PAM-2000; Heinz Walz GmbH, Effeltrich, Germany) to estimate PSII activity in Papers I, II and III. It should be noted, however, that the theoretical basis of the F_V/F_M parameter to represent the maximum quantum yield of PSII photochemistry (Genty et al. 1989) was recently disputed (Sipka et al. 2021), and this can affect also other parameters derived from fluorescence analyses, such as NPQ and relative electron transfer rate (rETR), that were also estimated in the current work. NPQ was calculated as F_M/F_M'-1, where F_M and F_M' stand for maximum fluorescence measured from dark acclimated sample and during illumination, respectively, and rETR as $0.42*\Phi_{II}*PPFD$, where 0.42 is the estimated

fraction of photons absorbed by PSII and Φ_{II} the effective quantum yield of PSII in the light.

More specific methods aimed for analysing electron transfer reactions within PSII were also used in Papers I and III. The polyphasic Chl *a* fluorescence rise kinetics (OJIP curves) were measured with AquaPen-P AP 110 P fluorometer (Photon Systems Instruments), whereas the decay of Chl *a* fluorescence after a single-turnover flash was measured with FL 200 fluorometer (Photon Systems Instruments, Drásov, Czech Republic). Because the work by Sipka et al. (2021) demonstrates that conformational changes in PSII are behind the rise of Chl fluorescence originating from PSII to the maximum level, the relationship of the OJIP fluorescence induction curves to Q_A reduction and electron transfer in general is unclear, and the data from OJIP analyses presented in this work should be treated with caution. The decay kinetics of Chl *a* fluorescence, however, can still provide information about electron transfer reactions related to Q_A^- oxidation by charge recombination reactions and forward electron transfer, especially when also measurements in the presence of DCMU (3-(3, 4-dichlorophenyl)−1, 1-dimethylurea; 10-20 µM), a PSII electron transfer inhibitor, are included. Also thermoluminescence was measured to estimate charge recombination reactions in isolated thylakoid membranes in Paper III. Thylakoids were pipetted on a filter paper, flashed with a Xenon flash (EGandG, Gaithersburg, MD, USA) at -20 °C, then heated at a rate of 0.47 °C s^{-1} and luminescence emitted by the samples during heating was recorded. The measurements were conducted in the absence and presence of DCMU.

In Paper II, fluorescence emission spectra after excitation with different wavelengths of light were measured both at room temperature using QEPro spectrometer (Ocean Optics, Largo, FL, USA). Corion band pass filters (Newport, Irvine, CA, USA) were used to get different excitation wavelengths. The room temperature fluorescence was measured from dark acclimated *E. timida* and *A. acetabulum* by placing them on a matte black cardboard, approximately 5 mm away from the light guide head (probe) of the spectrometer. The probe was positioned at a 45 ° angle regarding the sample. The light guide was bifurcated, and all visible light excitation wavelengths were guided to the sample through it, but the 390 nm UV light source had to be positioned directly above the sample. Care was taken so that the light guide did not shade the sample from UV-light during these measurements. The excitation light (P)PFD was always 4 µmol $m^{-2}s^{-1}$ for the visible light wavelengths, and 3 µmol $m^{-2}s^{-1}$ for the 390 nm UV-light.

3.3.3 *In vivo* absorptance and reflectance

Absorptance of whole *A. acetabulum* cells and *E. timida* individuals was measured in an integrating sphere (Labsphere, North Sutton, NH, USA) in Paper II. For this, 5-10 *A. acetabulum* cells or 30 live slugs in their culture media were placed into a glass tube and put into the integrating sphere. The sphere was illuminated with white light from a 1000 W Xenon lamp (Sciencetech Inc., London, Canada) and absorptance was measured with STS-VIS spectrometer. The absorptance of the samples was corrected with absorptance measurements from absolutely calibrated matte black surface.

The QE Pro spectrometer was used for the reflectance measurements from *E. timida* individuals and *A. acetabulum* placed on a matte black cardboard in Paper II. The probe was set perpendicular to the samples, with approximately 5 mm of space between the probe and a sample. Only white light from a slide projector was used for the reflectance measurements, and a white reflectance standard was used to get a full reflectance reading for calibration.

3.3.4 P700 and electrochromic shift

In Papers I and III, absorption changes at 830 nm (referenced with 870 nm) related to P700 oxidation were measured with Dual-PAM 100 (Heinz Walz GmbH) to inspect PSI activity (Schreiber and Klughammer 2008a; Klughammer and Schreiber 2016). When possible, the maximum oxidation of P700 (P_M) was estimated according to Klughammer and Schreiber (2008a), but in most cases PSI activity was estimated by inspecting the kinetics of P700 redox changes during dark-to-light transition with a light pulse (<1 s, 635 nm, PPFD 10 000 µmol $m^{-2}s^{-1}$), as described by Shimakawa et al. (2019). P700 redox kinetics were measured in aerobic and anaerobic conditions to probe the oxygen dependent electron sinks downstream of PSI. Oxygen was removed from the samples either by flushing them with nitrogen (spinach leaves) or by a combination of glucose oxidase (8 units ml^{-1}), glucose (6 mM) and catalase (800 units ml^{-1}) in the measurement media.

In Paper I, the electrochromic shift (ECS) signal was measured using the P515 module of Dual-PAM 100 that records the absorbance changes at 515 nm (550 nm used as a reference). These changes are indicative of modifications to the carotenoids in the sample, that are affected by the electric field component of *pmf* generated during thylakoid membrane energization in the light (Schreiber and Klughammer 2008b; Klughammer et al. 2013). The kinetics of ECS signal during a dark-to-light transition were recorded using a 780 ms light pulse (635 nm, PPFD 10 000 µmol $m^{-2}s^{-1}$).

3.4 Oxygen measurements

3.4.1 PSII and PSI activity

PSII and PSI activities of isolated thylakoids were also estimated under strong light (PPFD 3200 µmol $m^{-2}s^{-1}$) using a Clark-type oxygen electrode (Hansatech Instruments, King's Lynn, England) from samples containing 20 µg Chl ml^{-1} in Paper III. The same basic buffer solution was used for measuring both activities (1M glycine betaine, 40 mM HEPES-KOH (pH 7.4), 1 mM KH_2PO_4, 5 mM $MgCl_2$, 5 mM NaCl, 5 mM NH_4Cl and 330 mM sorbitol), but with small additions. For PSII activity measurements, where the activity was estimated as oxygen evolution, 0.5 mM 2,6-dichloro-1,4-benzoquinone (DCBQ) and 0.5 mM hexacyanoferrate(III) were added into the solution; DCBQ accepts electrons from PSII and hexacyanoferrate(III) keeps DCBQ oxidized. The additions into the buffer for PSI activity measurements, where the activity was measured as oxygen consumption, were 0.3 mM dichlorophenolindophenol (DCPIP), 0.01 mM DCMU, 0.12 mM methyl viologen, 32 mM Na-ascorbate, 0.6 mM NaN_3; DCMU blocks electron traffic from PSII, DCPIP donates electrons to Cyt *b6f* and Na-ascorbate keeps DCPIP reduced, whereas methyl viologen accepts electrons from PSI, which consumes oxygen and produces H_2O_2, and NaN_3 inhibits possible catalase activity, preventing oxygen from being produced in the breakdown of H_2O_2. Temperature was maintained at 22 °C for the measurements, and the samples were constantly stirred with a magnet.

In Paper III, a bare platinum Joliot-type oxygen electrode (Photon Systems Instruments) was used to estimate charge recombination reactions within PSII in isolated thylakoids diluted to 50 µg Chl ml^{-1} in the same base buffer that was used for PSII and PSI activity measurements (without the specific additions), supplemented with 50 mM KCl. After keeping the thylakoids on the electrode in the dark for 10 min, they were exposed to a laser flash train (1 flash/s, 532 nm Nd:YAG laser; Minilite, Continuum, San Jose, CA, USA) and the oxygen evolved by each 4 ns flash was recorded. To probe the charge recombination reactions with this setup, some of the samples were exposed to a pre-flash and different dark times prior to proceeding with the flash train.

3.4.2 $^1O_2$ measurements

In paper III, $^1O_2$ was measured from isolated thylakoids diluted to 100 µg Chl ml^{-1} using a method that relies on the efficient reaction between $^1O_2$ and histidine (rate constant 5 x 10^7 $M^{-1}s^{-1}$; Bisby et al. 1999), as described earlier (Telfer et al. 1994; Rehman et al. 2013). The addition of histidine (20 mM) results in faster oxygen

consumption from the sample due to the additional oxygen depletion when $^1O_2$ reacts with the imidazole ring of histidine, and the difference in oxygen consumption rates in the absence and presence of histidine was taken as a measure of $^1O_2$ production in the samples. For this, the thylakoid samples in the same basic buffer as was used for PSII and PSI activity measurements were illuminated with a strong light (PPFD 3200 µmol $m^{-2}s^{-1}$) in the cuvette of the oxygen electrode (Hansatech), while being constantly stirred by a magnet at 22 °C.

3.5 Photoinhibition of PSII and PSI

3.5.1 *In vivo* photoinhibition of PSII

Photoinhibition of PSII in intact organisms in Papers II and III was evaluated by measuring the decrease in F_V/F_M in response to different light treatments. F_V/F_M is known to correlate well with photoinhibition of PSII, even though the theoretical basis for F_V/F_M to present the maximum quantum yield of PSII photochemistry no longer holds true (Tyystjärvi 2013; Sipka et al. 2021). In most cases, the samples were exposed to lincomycin, a plastid specific translation inhibitor, prior to the light treatments, to block PSII repair cycle (Mulo et al. 2003). This is essential when evaluating the damaging reaction of photoinhibition without interference from the simultaneous repair mechanisms. The lincomycin concentration was 10 mg/ml, unless specified otherwise. In Paper III photoinhibition was also measured from samples that had been exposed to cycloheximide (1 mM), a cytosolic translation inhibitor. When inhibitors were used, the samples were always incubated in their presence overnight in the dark prior to the light treatments. Temperature was maintained at 17-23 °C during the photoinhibition treatments, depending on the experiment. Artificial Sunlight Module lamp (SLHolland, Breda, The Netherlands) was used as a white light source for the photoinhibition treatments, whereas monochromatic light was obtained with multiple LEDs and filters (Newport, Irvine, CA, USA).

3.5.2 *In vitro* photoinhibition of PSII and PSI

In paper III, photoinhibition was estimated *in vitro* in isolated thylakoid membranes. When photoinhibition of PSII and PSI was estimated with an oxygen electrode, the isolated thylakoids were diluted to 100 µg Chl ml^{-1} in a buffer solution containing 1 M glycine betaine, 40 mM HEPES-KOH (pH 7.4), 5 mM $MgCl_2$, 5 mM NaCl, 330 mM sorbitol, and exposed to photoinhibitory light treatment (PPFD 1000 µmol $m^{-2}s^{-1}$) while being constantly stirred with a magnet. Temperature was maintained at 22 °C during the light treatment. Aliquots were taken at set intervals during the

photoinhibition treatment, and PSII or PSI activities were measured as described above (section 3.4.1.).

To measure photoinhibition using spectroscopic methods *in vitro*, 50 µl of thylakoid suspension containing 25 µg Chl was pipetted on to a filter paper disk, which was then pinned between an inverted lid and bottom of a plastic Petri dish. The filter paper assemblage was then moistened with a buffer solution (1 M glycine betaine, 40 mM HEPES-KOH (pH 7.4), 5 mM $MgCl_2$, 5 mM NaCl, 330 mM sorbitol) from the opening on the side of the assemblage. Thylakoids inside this contraption were then exposed to high light and activities of both photosystems (F_V/F_M, PSII; P_M, PSI) were measured with Dual-PAM 100 (Heinz Walz GmbH) at set intervals. Temperature was maintained at 22 °C during the light treatment. Dark control treatments were always performed for all *in vitro* photoinhibition treatments.

3.5.3 Rate constant of PSII photoinhibition

The rate of PSII photoinhibition was estimated by calculating its rate constant (k_{PI}) from the loss of PSII activity during a light treatment using Sigmaplot (v.13.0 and v.14.0; Systat Software, San Jose, CA, USA) in Papers II and III. Here the original data were modelled according to first-order reaction kinetics using the exponential decay equation $A(t)=\exp(-k_{PI}^{t})$. Here, A stands for PSII activity at time t, and k_{PI} is the rate constant of PSII photoinhibition. The value of A at the onset of the experiments was always pre-set according to the data. Dark inactivation of PSII was also estimated for the *in vitro* photoinhibition experiments and the rate constant of dark inactivation was subtracted from the raw k_{PI} obtained from the light treatments.

3.6 General ROS assays

In Paper III, lipid peroxidation during *in vitro* photoinhibition treatments was estimated using the method described by Heath and Packer (1968). Malondialdehyde, a product of lipid peroxidation, reacts with thiobarbituric acid, leading to colorimetric changes that are observable as an increase in the absorbance at 532 nm. Here, thylakoid aliquots from the photoinhibition treatment were mixed with 20 % trichloroacetic acid solution containing 0.5 % thiobarbituric acid. The mixture was incubated at 80 °C for 30 min, put on ice for 5 min and centrifuged (13500 x g, 5 min) to separate the precipitate from the supernatant. The increase in absorbance, measured from the supernatant at 532 nm using 600 nm absorbance to correct for unspecific turbidity ($Abs_{532-600}$) was taken as an indicator of the relative amount of malondialdehyde in the samples. Malondialdehyde formation in the dark was estimated separately, and malondialdehyde formation during the light treatment was corrected by subtracting the dark values.

Oxyblot™ Protein Oxidation Detection Kit (MilliporeSigma, Burlington, MA, USA) was used to measure protein carbonylation, an indicator of protein oxidation, during the *in vitro* photoinhibition treatments in Paper III. Thylakoid aliquots containing 45 μg protein were treated with dithiotreitol and prepared according to the manufacturer's protocol. Proteins were separated with 10 % SDS-PAGE and Immobilon Western Chemiluminescent HRP Substrate (MilliporeSigma) was used to detect carbonylated proteins.

3.7 Molecular biology

3.7.1 Western blot

Depending on the experiment in Papers I and III, samples for SDS-PAGE (Next Gel; VWR, Radnor, PA, USA) were loaded into the wells of a 10 % polyacrylamide gel on a total Chl or protein basis. Protein concentration was estimated using DC Protein Assay (Bio-Rad, Hercules, CA, USA). After SDS-PAGE, the proteins were transferred on to Immobilon-P PVDF membranes (MilliporeSigma) and immunodetected with different primary antibodies, using goat anti-rabbit IgG (H+L) alkaline phosphatase conjugate (Life Technologies, Carlsbad, CA, USA) in conjunction with CDP-star Chemiluminescence Reagent (Perkin-Elmer, Waltham, MA, USA) for imaging. Protein band quantification was done using Fiji (Schindelin et al. 2012).

3.7.2 RT-qPCR

Transcript levels of a set of genes in isolated plastids were estimated with RT-qPCR in Paper III. RNA was isolated using Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO, USA), and samples containing 10 ng RNA were utilized for cDNA synthesis (iScript™ cDNA Synthesis Kit, BioRad, Hercules, CA, USA) after DNAse treatment (Thermo Scientific, Waltham, MA, USA). RT-qPCR in StepOnePlus (Applied Biosystems, Foster City, CA, USA) was done using reagents from Bio-rad and primers designed using Primer3. Analysis of the results was done using the ΔΔCt method (Pfaffl 2001) by normalizing the data to time point 0 and to two reference genes, that were selected based on their similar behaviour during the incubation of isolated plastids.

3.8 Pigment analyses

Chl concentration of different samples was routinely estimated spectrophotometrically using different solvents. In Paper III, where Chl

concentration was measured from spinach, containing Chls *a* and *b*, and *V. litorea,* containing Chls *a* and *c*, Chl was extracted in 90 % acetone from both organisms and quantified using the appropriate wavelengths, extinction coefficients and equations described earlier (Jeffrey and Humphrey 1975). In Paper II Chl was quantified from *E. timida* individuals and *A. acetabulum* after extraction in N,N-dimethylformamide according to Porra et al. (1989).

Carotenoid and α-tocopherol content of spinach and *V. litorea* thylakoids was estimated with HPLC in Paper III. Pigments were extracted in 100 % methanol, centrifuged (12000 x g, 15 min) and the resulting supernatant was collected and filtrated (0.2 µm). Pigment separation was performed essentially as described by Gilmore and Yamamoto (1991), using a series 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) with a reverse phase C18 column (LiChroCART 125-4; Hewlett Packard, Palo Alto, CA, USA). Pigments were detected based on their absorbance at 440 nm, whereas α-tocopherol detection relied on fluorescence (excitation wavelength 295 nm, emission 340 nm). Pigments were identified and quantified using standards from DHI Lab Products (Hørsholm, Denmark) when possible.

4 Main results

4.1 Photosynthetic light reactions in *E. timida* and *A. acetabulum*

4.1.1 The PQ pool of *E. timida* is not strongly reduced in the dark

To compare the details of electron transfer in PSII and electron donation to the PQ pool in photosynthetic sea slugs and their prey algae, the Q_A^- reoxidation kinetics were measured by recording the decay of Chl fluorescence after a single turnover flash in *E. timida* and *A. acetabulum* (Fig. 2 in Paper I).

The Q_A^- reoxidation kinetics between these two organisms were nearly identical when the plastids inside *E. timida* individuals originated from *A. acetabulum* grown in normal growth conditions, and both the slugs and the algae used in the experiments were nice and green. The similarity in the oxidation kinetics was present both without and with DCMU, showing that the slugs do not seem to alter the fundamentals of electron transfer within PSII (Fig. 2A, B in Paper I). However, when samples from the same conditions were deprived of oxygen, Q_A^- reoxidation slowed down in both species, but was noticeably faster in *E. timida* than in the algae (Fig. 2C in Paper I). Non-photochemical reduction of the PQ pool is known to occur in many photosynthetic organisms, and this can cause slower Q_A^- reoxidation due to PQH_2 binding to the Q_B pocket of PSII (de Wijn and van Gorkom 2001). Additionally, the so-called wave-like kinetics of fluorescence decay were very clear in *A. acetabulum* cells that had been acclimated to high light and cold temperature to induce the red morphotype of this alga (González-Wangüemert et al. 2006; Costa et al. 2012). The wave-phenomenon is known to be a consequence of strong reduction of the PQ pool in the dark in cyanobacteria and green algae (Deák et al. 2014; Krishna et al. 2019). However, *E. timida* individuals that had incorporated the red morphotype plastids to the level of turning the slugs red did not show any sign of the wave phenomenon (Fig. 2D in Paper I).

To check whether the differences in the dark redox state of the PQ pool have an effect also on the full photochemical reduction of the PQ pool during a dark to light transition, Chl fluorescence rise kinetics during a strong multiple turnover light pulse

were measured from healthy green *E. timida* and *A. acetabulum* taken from their normal growth conditions (Fig. 3A in Paper I). The most notable finding from these measurements was that the fluorescence rise to the maximum fluorescence level took ~300 ms longer in the slugs than in the algae, possibly simply because it took longer to fully reduce the initially more oxidized PQ pool of the slugs.

4.1.2 NPQ is stronger in *E. timida* than in *A. acetabulum*

Growing *A. acetabulum* in high CO_2 conditions (1% CO_2) resulted in elevated NPQ levels in comparison to algae grown in normal, ambient air growth conditions, when the NPQ levels of dark acclimated algae were monitored during rapid light curve measurements (Fig. 5H in Paper I). In these measurements, the samples were exposed to successively stronger light treatments, each lasting 90 s, prior to a saturating pulse analysis. The NPQ levels of *E. timida* slugs that were allowed to incorporate plastids from high CO_2 acclimatized algae were also higher than of slugs that had only been fed algae from ambient air conditions, suggesting that the light protection phenotype of the algae is transferred to the slugs to some extent (Fig. 5G in Paper I). However, during the rapid light curve measurements both ambient air and high CO_2 slugs showed higher levels and faster onset of NPQ than the algae they stole their plastids from.

To investigate the reasons behind the differences in NPQ between the slugs and the algae, the ECS signal during a strong light pulse was measured from ambient air grown samples of both organisms (Fig. 4A in Paper I). The short ECS measurements did not allow to break the signal down to its two individual components (electric field and ΔpH) and the ECS signal during the light pulse was taken as an indicator of the total *pmf* in the thylakoid membranes (Kramer et al. 2003). The *pmf* kept on building up during most of the strong light pulse in the slugs, whereas the *pmf* in *A. acetabulum* dissipated to a stable level after the initial spike in thylakoid membrane energization caused by the sudden light burst.

4.1.3 Oxygen-dependent electron sinks are functional in *E. timida* plastids

Next, the contribution of alternative electron sinks to plastid longevity in the slug *E. timida* was put under scrutiny. The existence of one of the main photoprotective electron sinks downstream of PSI in green algae, FLVs, was confirmed by a Western blot in *A. acetabulum* (Fig. 6 in Paper I). The functionality of FLVs was probed by measuring the oxidation kinetics of P700 during a transition from dark to light by exposing the samples to a strong light pulse. *A. acetabulum* samples taken from ambient air conditions exhibited clear oxidation of P700 during the pulse, and the

oxidation was even stronger when a second pulse was fired 10 s after the first one. P700 oxidation in *E. timida* individuals whose plastids derived from ambient air grown algae was not nearly as strong as in the algae during the initial light pulse, but P700 oxidation did start to increase towards the end of the pulse. However, the second pulse after 10s of darkness noticeably increased the P700 oxidation capacity also in the slugs. Still, a clear dip in the oxidation was noticeable almost immediately after turning on the light, whereafter the slugs retained a stable level of P700 oxidation for the rest of the pulse. This implies that the alternative electron sink downstream of PSI, be it FLVs or not, was not as efficient in maintaining P700 oxidized in the slugs as it was in the algae (Fig. 4B in Paper I).

Since FLVs are an oxygen dependent electron sink, P700 oxidation kinetics were also measured in anaerobic conditions, again from samples grown in ambient air conditions (Fig. 4D in Paper I). The absence of oxygen had a dramatic effect in the algae, as the P700 oxidation towards the end of the light pulse was almost completely inhibited, and even with the second pulse the oxidation was severely inefficient. The effect of anaerobic conditions was not nearly as drastic in the slugs as it was in the algae. However, exposing the slugs to anaerobic conditions removed the slight oxidation at the end of the first light pulse, and also the second pulse oxidation kinetics did not show signs of increased oxidation towards the end, but remained stable.

Growing *A. acetabulum* in high CO_2 conditions decreased their P700 oxidation capacity compared to ambient air grown algae, both during the initial and the second high light pulse (Fig. 5B, D in Paper I). This decrease could not, however, be appointed to a simple decrease in the FLV content of the algae, as the amount of FLVs did not seem to decrease in high CO_2 according to Western blotting (Fig. 6 in Paper I). Nevertheless, allowing *E. timida* individuals to incorporate plastids from high CO_2 algae did result in similar changes in the P700 oxidation of the slugs as witnessed in the algae (Fig. 5A, C in Paper I).

To inspect whether the altered P700 oxidation capacity affects the longevity of the plastids inside *E. timida*, two starvation experiments were conducted using slugs whose plastids were derived from either ambient air or high CO_2 grown algae. In the first one, the slugs were deprived of their food in their normal growth conditions, where the only fluctuations in light were due to the 12/12h day/night cycle. Here, the PSII activities of both groups declined almost identically, as probed by the Chl fluorescence-based index F_V/F_M, even though the differences in P700 oxidation capacity were still measurable at day 5 of the starvation experiment (Fig. 7 in Paper I). However, when these two slug groups were starved in fluctuating light conditions, where the background illumination (PPFD 40 µmol $m^{-2}s^{-1}$) during the day was supplemented with a 10 s high light pulse (1500 µmol $m^{-2}s^{-1}$) every 10

minutes, the rapid phase of F_V/F_M decrease was initiated approximately 6-10 days earlier in the high CO_2 slugs than in the ambient air slugs (Fig. 8 in Paper I).

4.2 Passive photoprotection of the plastids in photosynthetic sea slugs

4.2.1 Photoinhibition of PSII is slower in *E. timida* than in *A. acetabulum*

Light response curves of photoinhibition of PSII were constructed by exposing the slugs and the algae to seven different light intensities of white light. The decrease in F_V/F_M in all tested light intensities followed the first-order reaction kinetics of photoinhibition of PSII well, which allowed determining the rate constant of photoinhibition for both organisms (Fig. 1A, B in Paper II). Plotting the rate constants against the PPFD of the treatment light revealed that photoinhibition of PSII is directly proportional to PPFD in both *E. timida* and *A. acetabulum*. However, the plastids inside *A. acetabulum* were considerably more susceptible to photoinhibition in all tested white light intensities (Fig. 1C in Paper II).

The visible light action spectra of photoinhibition of PSII were measured to distinguish whether the slugs were simply blocking part of the visible light spectrum, which could have affected their susceptibility to white light photoinhibition. Once again, the slugs were more resilient against photoinhibition caused by the monochromatic visible light treatments than the algae, but the overall shape of the visible light action spectrum of photoinhibition was similar in both species. The minor differences between the shapes of the action spectra were most pronounced in the blue-to-violet treatment light wavelengths 470 and 425 nm; 470 nm light resulted in an increase in photoinhibition compared to the damage caused by green 560 nm light in the slugs, but not so much in the algae, whereas 425 nm light was already showing the well-known damaging signs of short wavelength blue light in the algae, but not in the slugs (Fig. 2 inset in Paper II).

4.2.2 Tight packing of plastids protects them from photoinhibition of PSII in *E. timida*

The existence of passive photoprotection mechanisms that the slugs might provide to their plastids against excessive irradiance were inspected by multiple spectroscopic measurements in *E. timida* individuals and *A. acetabulum* cells. The simplest evidence for passive photoprotection against visible light offered by the slugs to their plastids came from absorptance spectra measurements. The visible light absorptance spectra were dominated by photosynthetic pigments; both organisms

showed absorption peaks in the red and blue light regions and low absorption in the green light region, which is typical for photosynthetic material (Fig. 4B in Paper II). When the absorptance spectra were normalized to the total Chl of the samples, it became evident that the slugs absorb considerably less light per Chl than their algal counterparts, indicating that the plastids in the slugs are more concentrated than in the algae (Fig. 4C).

Light penetration into the slug tissue or algal cells was also estimated by inspecting their room temperature Chl fluorescence emission spectra excited by different monochromatic visible light wavelengths (Fig. 3 in Paper II). An enrichment of 750 nm fluorescence was noticeable with all tested excitation wavelengths in the slugs compared to the algae, which is a common feature in samples that contain a high concentration of Chl (Lichtenthaler et al. 1981; Weis 1985). This also supported the suggestion that the slugs fill their bodies up to a high concentration of plastids.

When photosynthetic sea slugs are put into starvation, they start to lose their plastids almost immediately (Serôdio et al. 2010; Laetz et al. 2017). This was also clear in the reflectance spectra measured from freshly fed slugs and slugs starved for nine and 21 days, or until the slugs were completely devoid of plastids, which showed that the dips in the reflectance caused by Chl absorption smoothened out as the starvation proceeded (Fig. 4A in Paper II). To test the protective effect of high concentration of plastids against photoinhibition of PSII, slugs that had been freshly fed or kept in starvation for nine days were exposed to a white light photoinhibition treatment. The starved slugs were significantly more susceptible to white light photoinhibition than freshly fed slugs, possibly because the loss of plastids led to an increased exposure of the remaining plastids during the photoinhibition treatment (Fig. 6 in Paper II).

4.2.3 The plastids inside *E. timida* are protected from UV radiation

In the complete action spectra of photoinhibition of PSII in *E. timida* and *A. acetabulum,* also photoinhibition caused by UV radiation was measured (Fig. 2 in Paper II). As is common in photosynthetic organisms, UV radiation was a lot more damaging to PSII than any of the tested monochromatic visible light wavelengths. This was true for both *E. timida* and *A. acetabulum*, but the increase in the rate constant of photoinhibition going from the 425 nm blue/violet light to UVA radiation (365 nm) was more dramatic in the algae than in the slugs. Furthermore, when the wavelength of the UV radiation treatment shortened from UVA to UVB (312 nm) and UVC (254 nm), the rate constant of photoinhibition increased to a larger degree

in the algae than in the slugs, making it clear that something slug specific was protecting the plastids from UV radiation in *E. timida*.

The penetration of UVA into the slug tissue and algal cells was inspected using the same Chl fluorescence excitation method as with the monochromatic visible light wavelengths, but this time the samples were excited with 390 nm UVA radiation. In the algae, UVA excitation resulted in only a slightly lower level of Chl fluorescence emission than with 470 nm visible light excitation. UVA exposure of the slugs, on the other hand, did not excite almost any Chl to emit fluorescence, showing that UVA was screened by the slug tissue, therefore preventing it from efficiently reaching the plastids inside the slugs (Fig. 3A, B in Paper II). To check if the mechanisms protecting the plastids inside the slugs against visible light and UV radiation were completely different, also UVA photoinhibition experiments were done with slugs that had been starved for nine days. Although there was no statistical significance between the rate constant of UVA photoinhibition of freshly fed slugs and starved slugs, UVA photoinhibition was stronger in the starved individuals (Fig. 6 in Paper II). It is likely that the same mechanisms that protected the slug plastids from visible light were also contributing to UVA protection, making it difficult to estimate the individual contribution of both at the same time.

4.3 Innate characteristics of the plastids contributing to their longevity

In Paper III, the focus of the research shifted from the *E. timida*/*A. acetabulum* system to *V. litorea,* the stramenopile prey alga of *E. chlorotica,* to uncover innate factors of the plastids contributing to long-term photosynthesis in the slugs. Since the specifics of *V. litorea* needed to be compared to something, spinach was selected, somewhat arbitrarily, to represent the primary endosymbiotic green lineage in the measurements whenever possible. *E. timida* was used only as a proof of concept for experiments that required an actual photosynthetic sea slug, since getting *E. chlorotica* was unfortunately impossible.

4.3.1 Plastids of *V. litorea* are highly autonomous in recovering from photoinhibition of PSII

Lab-isolated *V. litorea* plastids were incubated in the growth conditions of the algae for seven days, and expression of nine plastid encoded genes was analysed with qPCR at different time points of the incubation. Two of these genes, *rbcL* and *psaA* were used as reference genes. The transcripts of all tested genes decreased during the incubation period, but in comparison to the reference genes, the relative transcript levels of *ftsH* and *tufA* increased throughout the incubation period. These genes

encode the FtsH protease and EF-Tu, respectively. The relative amounts of the transcripts of other tested genes decreased or remained stable in comparison to the reference genes (Fig. 2B in Paper III).

The autonomy of the plastids of the prey algae of the slugs in recovering from photoinhibition of PSII was first tested in an actual photosynthetic slug using *E. timida*. The plastids of *E. timida*, originating from the green alga *A. acetabulum*, do also contain the genes for FtsH and EF-Tu, although the *ftsH* gene in *A. acetabulum* plastome was shown not to contain the metalloprotease domain that is required for D1 degradation (Christa et al. 2018). Nevertheless, *E. timida* slugs were photoinhibited by high light both in the absence and presence of lincomycin, and the slugs that had been treated with lincomycin were not able to recover their PSII activity (F_V/F_M) almost at all during an overnight recovery period, whereas the F_V/F_M in the absence of lincomycin recovered back to 78% capacity of the initial level (Fig. 2C in Paper III).

The autonomy of *V. litorea* plastids to repair PSII was tested by functionally isolating them from nucleus encoded proteins using cycloheximide, a cytosolic translation inhibitor. The cycloheximide poisoned algae were subjected to a 60 min photoinhibitory high light treatment, and the F_V/F_M of the samples was recorded at different timepoints of the light treatment, as well as during a subsequent 250 min recovery in the dark and in low light. Cycloheximide had almost no effect on photoinhibition of PSII or the recovery process in *V. litorea* (Fig. 3B in Paper III). The experiments were also done with spinach leaves in the presence and absence of cycloheximide, where the introduction of cycloheximide made the leaves highly susceptible to photoinhibition of PSII. The F_V/F_M of spinach leaves did recover in the presence of cycloheximide, but not to the same extent as in untreated leaves put through the same process (Fig. 3A in Paper III).

The expression of both A- and B-type FtsH proteins was also inspected during the photoinhibition treatment with Western blot. In *V. litorea,* cycloheximide did not have any significant effect on the FtsH levels during the 60 min high light treatment or the first 60 min recovery period in the dark, but both FtsH A and B protein levels were up at the end of the recovery period compared to the samples that had not been subjected to cycloheximide (Fig. 3D in paper III). Cycloheximide did not affect the FtsH levels of spinach leaves (Fig. 3C in paper III).

4.3.2 Isolated *V. litorea* thylakoids produce a lot of ROS in general, but not $^1O_2$

Next, photoinhibition of isolated thylakoids of spinach and *V. litorea* was estimated during a 60 min high light treatment. The rate constant of PSII photoinhibition was higher in spinach thylakoids (0.03 min^{-1}) than in *V. litorea* (0.01 min^{-1}) (Table 2 in

Paper III) when PSII activity at different timepoints during the high light treatment was estimated by separate oxygen evolution measurements in strong light (Fig 4A in Paper III). Also F_V/F_M decay was faster in spinach thylakoids, although the rate constants were not calculated from the F_V/F_M decay experiments (Fig. 6C, D in Paper III). However, the PSI side of the electron transfer chain exhibited signs of more dramatic high light induced damage in *V. litorea* thylakoids than in spinach when PSI activity was estimated by measuring the oxygen consumption separately in strong light. The oxygen consumption by PSI of spinach thylakoids was almost unaffected by the high light treatment, whereas the oxygen consumption by *V. litorea* PSI decreased with almost the same kinetics as PSII oxygen evolution (Fig. 6A, B in Paper III). PSI damage during the high light treatment was also estimated by measuring the decrease in the maximum P700 oxidation (P_M) in both species. According to this index, the damage to PSI proceeded similarly in both, although the decrease in P_M in spinach thylakoids reached a stationary phase only after 30 min in high light, whereas the decrease in P_M was rapid only during the first 15 min of the treatment in *V. litorea* thylakoids (Fig. 6C, D in Paper III).

The effect of the thylakoid isolation procedure on the P700 oxidation kinetics was also inspected to estimate the possible causes behind the differences in PSI susceptibility to photoinhibition between spinach and *V. litorea* thylakoids. As expected, the P700 oxidation kinetics of intact spinach leaves were not affected by anaerobic conditions, and P700 remained largely reduced during a strong light pulse after dark acclimation both in the presence and absence of oxygen. Furthermore, the overall P700 oxidation kinetics of isolated spinach thylakoids in the presence of oxygen were similar to the kinetics of intact leaves (Fig. 6E in Paper III). The capability of intact *V. litorea* cells to maintain P700 oxidized during a strong light pulse was strongly inhibited in anaerobic conditions, and the isolated thylakoids of *V. litorea* exhibited a noticeable decrease in their P700 oxidation capacity even in the presence of oxygen compared to the situation in intact cells (Fig. 6F in Paper III).

ROS production during a 60 min high light treatment was estimated indirectly from the isolated thylakoids by measuring the production of malondialdehyde, an indicator of lipid peroxidation, and protein carbonylation, an indicator of protein oxidation. The thylakoids of *V. litorea* seemed to produce more ROS in high light than spinach according to both general ROS assays (Fig. 4B, C in Paper III). However, the production of $^1O_2$, the only ROS that was specifically measured, in freshly isolated *V. litorea* thylakoids was lower than in spinach thylakoids even though other photosynthetic parameters between the thylakoids isolated from these two species were highly comparable (Fig. 5A, Table 2 in Paper III).

4.3.3 *V. litorea* thylakoids contain efficient $^1O_2$ antioxidants and exhibit slow PSII charge recombination

The main antioxidants against $^1O_2$ are α-tocopherol and carotenoids (Khorobrykh et al. 2020). An HPLC analysis of the pigments in spinach and *V. litorea* thylakoids revealed that the spinach thylakoids did not contain any α-tocopherol, whereas α-tocopherol was readily available (36.29 μg ml-1) for ROS detoxification in *V. litorea* thylakoids even after the overnight dark period that preceded the thylakoid isolation (Table 3 in Paper III). The carotenoid content per Chl was also seemingly higher in *V. litorea* than in spinach thylakoids, as the normalized absorption in the 460-520 nm range was stronger in the absorption spectra of dimethylformamide extracted pigments from *V. litorea* thylakoids (Fig. 5E in Paper III).

Next, the charge recombination reactions within PSII were inspected in spinach and *V. litorea* thylakoids using three different methods: flash oxygen evolution, fluorescence decay kinetics after a single turnover flash (Q_A^- reoxidation) and thermoluminescence. When spinach thylakoids were subjected to a single preflash prior to the flash train of the actual flash oxygen evolution measurements, the S-state equilibration back to the dark stable distribution via charge recombination was complete after a 100 s dark period between the preflash and the flash train. In *V. litorea*, however, the 100 s dark period between the preflash and the flash train was not enough to fully equilibrate the S-states back to the original distribution. A clear indicator of this was the fact that the second flash of the flash train after the 100 s dark period in *V. litorea* thylakoids still resulted in considerably more oxygen being released by PSII than originally (after a 10 min darkness with no preflash) (Fig. 5B in Paper III). Chl fluorescence decay kinetics after a single turnover flash also indicated that the charge recombination reactions were slower in *V. litorea* than in spinach; when forward electron transfer from Q_A^- was blocked by DCMU, the fluorescence decay was noticeably slower in *V. litorea* (Fig. 5C in Paper III).

The data obtained from the thermoluminescence measurements were not as unambiguous, perhaps because anti-freeze substances like glycerol were not used in the measurements. In fact, the finding that thermoluminescence measured in the presence and absence of DCMU (the Q and B band of thermoluminescence, respectively) exhibited peaks at lower temperatures in *V. litorea* than in spinach rather showed that at least the minor charge recombination pathway leading to luminescence emission was more favourable in *V. litorea* (Fig. 5D in Paper III). The temperature gap between the Q and B bands was, however, smaller in *V. litorea* (10 °C) than in spinach (13 °C), which indicates a narrower energy gap between Q_A and Q_B in *V. litorea*.

5 Discussion

5.1 Active photoprotection of the slug's plastids at a molecular level

5.1.1 Oxidized PQ pool alleviates electron pressure in PSII

One thing that a photosynthetic sea slug would likely want to do is to maintain the PQ pool of its plastids in a state where the PQ pool can accept as much electrons as possible, should there be a sudden burst of excessive light. This could alleviate the electron pressure in PSII, and therefore also reduce the risk of undesired charge recombination reactions leading up to $^1O_2$ production in PSII. According to the results shown in Paper I, this is something that indeed does likely occur in the plastids of the slug *E. timida*. The results from three separate measurements support this; the Q_A^- reoxidation is faster in *E. timida* than in *A. acetabulum* in anaerobic conditions (Fig. 2C in Paper I), the fluorescence decay in the Q_A^- reoxidation measurements exhibits completely different kinetics in the red morphotype *A. acetabulum* than in the red morphotype slugs (Fig. 2D in Paper I) and the fluorescence rise to the maximum level during a strong light pulse takes considerably longer in the slugs than in the algae (Fig. 3A in Paper I).

Perhaps the most convincing data concerning the differences in the non-photochemical reduction of the PQ pool in the dark came from the Q_A^- reoxidation measurements performed on the red morphotype *A. acetabulum* and *E. timida*. When the dark reduction of the PQ pool is very strong, like in sulphur deprived *C. reinhardtii* cells kept in anaerobic conditions in the dark, it can lead to wave-like kinetics of Chl fluorescence decay in Q_A^- reoxidation measurements (Deák et al. 2014; Krishna et al. 2019). The wave-like kinetics were very clear in *A. acetabulum* cells that had been acclimated to high light and cold temperature to induce the red morphotype of this alga (González-Wangüemert et al. 2006; Costa et al. 2012). However, *E. timida* individuals that had incorporated the red morphotype plastids to the level of turning the slugs red did not show any sign of the wave phenomenon (Fig. 2D in Paper I).

Unfortunately (or fortunately), the science regarding the PSII Chl fluorescence rise to its maximum level has moved forward after Paper I was published, casting

doubt to the conclusions that were derived from the fluorescence rise kinetics in Paper I. It is true, that full reduction of the PQ pool is often referred to as a prerequisite for maximum fluorescence of PSII, which might suggest that the photochemical reduction of the entire PQ pool takes longer in the slug plastids (Kramer et al. 1995; Yaakoubd et al. 2002). However, a recent study showed that the maximum fluorescence emitted by the PSIIs of a sample is not due to the full closure of the reaction centres (all Q_A molecules are reduced), but rather the result of structural changes within PSII that occur during continuous illumination of the sample (Sipka et al. 2021). Therefore, the full photochemical reduction of the PQ pool was poorly estimated in Fig. 3A of Paper I. Nowadays the redox state of the PQ pool can be assessed directly by HPLC in photosynthetic organisms, and this should also be considered for future studies of the slugs as well, although applying the HPLC method to the slugs is likely an endless swamp of new challenges (Kruk and Karpinski 2006; Khorobrykh et al. 2020; Mattila et al. 2020).

Altogether, the data in Fig 2 and Fig. 3A of Paper I do, however, suggest that the dark reduction of the PQ pool is weaker in the plastids inside the slug *E. timida* than in the original algal host of the plastids, *A. acetabulum*. It is unclear what the mechanisms contributing to the dark reduction of the PQ pool are in *A. acetabulum* and why they are not working to the same extent in the plastids of the slug. One explanation could be that the stromal reductants that are utilized to reduce the PQ pool, likely via NDH, are of cytosolic or mitochondrial origin in the alga. Of course, also photosynthetic sea slugs have mitochondrial metabolism, but whether the slugs are willing or able to invest their own energetic currency to suit the needs of a stolen organelle is questionable. Another possibility is that degradation of starch is not efficient in the plastids inside the slugs, therefore decreasing the number of reductants available for PQ reduction in the dark in the slugs. In *C. reinhardtii,* fermentative degradation of starch is thought to be the main source of electrons for PQ reduction in the dark in anaerobic conditions (Krishna et al. 2019). Indeed, the plastids inside *E. timida* have been shown to mainly accumulate, not degrade, starch during starvation, making this a reasonable suggestion (Laetz et al. 2017). It is also reasonable to think that, in comparison to *A. acetabulum*, the more oxidized PQ pool of the slugs would simply take longer to become fully reduced in the light, but this was only tentatively shown to occur by analysing the enigmatic fluorescence rise kinetics (Fig 3A in Paper I).

5.1.2 NPQ is enhanced due to a build-up of *pmf*

Even if maintained in an oxidized state, the PQ pool and the rest of the electron transfer chain can only accommodate a finite number of electrons. Therefore, electron pressure is to be expected even in slug plastids if excessive light conditions

persist. It would therefore be beneficial for the plastids inside the slugs to be able to efficiently dissipate excessive light energy already at the level of light harvesting antennae. According to the data shown in Fig. 5 G, H of Paper I, the slugs not only maintain the original NPQ capacity of the plastids, but they also seem to enhance it. The amplification of NPQ in the plastids of *E. timida* was noticeable both as a faster induction and generally higher levels of NPQ than what was witnessed in the plastids inside *A. acetabulum.*

Admittedly, the differences in the behaviour of NPQ between the slugs and the algae are somewhat minute. Therefore, one possible explanation for the differences could simply be the highly different optical properties of these two organisms, which might affect the saturating pulse analysis of Chl fluorescence. The ECS measurements from the slugs and the algae do, however, point to a difference in the build-up of *pmf* between the two; in the alga *A. acetabulum pmf* dissipates to a steady level within <1 s after a transition from dark to light, whereas in the slug *E. timida pmf* seems to be mainly building up during the same time period in the light (Fig. 4A in Paper I). This suggests that the acidification of the thylakoid lumen is stronger in the slug's plastids, making it plausible that the enhanced NPQ of the slugs is a real physiological phenomenon, not just an artefact of the fluorescence measurements.

Paper I of this thesis is not the first study where the differences between the NPQ in the plastids of the slugs and the algae have been noticed. Indeed, both *E. timida* and *E. chlorotica* have been shown to induce NPQ faster and to higher levels than their prey algae, but these differences were not discussed in detail in the studies where the data were presented, possibly to avoid too strong conclusions based on the Chl fluorescence data (Cruz et al. 2015; Christa et al. 2018). The short- to long-term retention sea slug *E. viridis* steals plastids from green algae belonging to the group Bryopsidales. Many algae in this group do not possess a xanthophyll cycle and have seemingly completely lost the ability to induce qE type NPQ; they seem to rather rely on some form of sustained quenching (Cruz et al. 2015; Christa et al. 2017). Although there are slight differences in the NPQ induction between the Bryopsidales plastids inside *E. viridis* and inside the algae, the differences are not nearly as clear as in *E. timida* or *E. chlorotica* and their prey algae. Most importantly, the Bryopsidales plastids inside *E. viridis* do not seem to exhibit faster induction of NPQ than the algae themselves (Cruz et al. 2015; Christa et al. 2018). This might suggest that if NPQ is enhanced in the slugs, the enhancement works mainly at the level of the energy dependent qE component of NPQ, which agrees with the *pmf* build-up witnessed in the plastids of *E. timida* (Fig. 4A in Paper I). The mechanisms contributing to NPQ in the green alga *A. acetabulum* on a molecular level are largely unknown, but fine-tuning NPQ to be more efficient has potential in being very beneficial for maintaining the plastids functional inside slug cells.

5.1.3 Alternative electron sinks protect the plastids during fluctuating light

Even with a more oxidized PQ pool and enhanced NPQ, excessive light reaching the photosynthetic machinery can flood the electron transfer chain with electrons in the plastids of the slugs. This is a real possibility especially during sudden fluctuations in light intensity, when carbon fixation might not be able to consume all the electrons gushing from PSI. A last-ditch effort of guiding the excess electrons to safe alternative electron sinks has proven a successful strategy in many photosynthetic organisms. Not making use of such innate sinks of the plastids would be a terrible strategy for the slugs, as these sinks, most notably FLVs, protect one of the most precious components of the electron transfer chain, PSI, from photoinhibition (Allahverdiyeva et al. 2013; Jokel et al. 2018). Being a sensible creature, the slug *E. timida* does indeed seem to be holding on to the alternative electron sinks downstream of PSI that are in place in the plastids of *A. acetabulum* (Fig. 4B-D in Paper I).

The capability of these alternative electron sinks to oxidize P700 during a transition from dark to light does, however, seem to decrease once the plastids are incorporated into the cells of *E. timida* (Fig. 4B in Paper I). The FLV-specific antibody that reacts with both FLVA and FLVB proteins of *C. reinhardtii* did recognize FLVs in *A. acetabulum* (Fig. 6 in Paper I), and the P700 oxidation in *A. acetabulum* was severely hindered and of smaller amplitude in anaerobic conditions (Fig. 4D in Paper I). These data suggest, that at least in *A. acetabulum* the electron sinks facilitating P700 oxidation during a strong light pulse are FLVs. The P700 oxidation in the plastids of *E. timida* did also respond to anaerobic conditions; the small increase in P700 oxidation that was noticeable in aerobic conditions was not present in the absence of oxygen, and the $P700^+$ signal in general was a lot weaker than in aerobic conditions (Fig. 4D in Paper I). It is possible that in the dark the respiration of the slugs removes some of the oxygen that would otherwise be reduced by FLVs during a transition from dark to light, even in aerobic conditions. This could be behind the weaker P700 oxidation witnessed in the slugs than in the algae during a strong light pulse.

Switching the growth conditions of *A. acetabulum* from ambient air to high CO_2 lowered their P700 oxidation capacity (Fig. 5B, D in Paper I). Originally, this experiment was designed to lower the FLV content in the plastids on the basis that elevated CO_2 environment has been shown to lead to downregulation of FLVs in cyanobacteria and *C. reinhardtii*, possibly because the excess inorganic carbon allows the CBB cycle to consume more electrons (Zhang et al. 2012; Jokel et al. 2015; Santana-Sanchez et al. 2019). However, the ambient air and high CO_2 grown *A. acetabulum* did not show any differences in the amount of FLVs (Fig. 6 in Paper I). The band that was detected by the FLV antibody in *A. acetabulum* samples was

relatively broad, and it is likely that it contained both FLVA and FLVB, but it is impossible to distinguish if either of these individual proteins were downregulated from the blot presented in Fig. 6 in Paper I. In *C. reinhardtii* FLVA and FLVB likely form a functional heterodimer (Allahverdiyeva et al. 2015; Nikkanen et al. 2021). If the FLV system is similar in *A. acetabulum*, a decrease in either one of these subunits could result in a decreased functionality of FLVs in general. The redox regulation of FLV activity has recently gained traction among researchers, as FLVs contain conserved cysteine residues that are potential targets for the thioredoxin systems of the plastids (Alboresi et al. 2019b). Altered redox regulation of FLVs could also be behind the differences in FLV activity between ambient air and high CO_2 grown *A. acetabulum*. Regardless of the exact reason behind the lowered P700 oxidation capacity of high CO_2 *A. acetabulum*, this lowered P700 oxidation capacity was also noticeable in *E. timida* individuals that ingested plastids from high CO_2 algae (Fig. 5A, C in Paper I).

As mentioned earlier, P700 oxidation by FLVs or other alternative electron sinks is an important photoprotective mechanism during fluctuating light. Therefore, it is not surprising that *E. timida* slugs whose plastids were of ambient air or high CO_2 origin did not show differences in the decay of their photosynthetic activity (F_V/F_M) when starved in steady light conditions, where the only fluctuations in light were due to the day/night cycle (Fig. 7C in Paper I). When the slugs were starved in fluctuating light, however, the fast phase of F_V/F_M decay did start earlier in the high CO_2 slugs, suggesting that the lowered P700 oxidation capacity led to a more severe damage to the photosynthetic apparatus in fluctuating light (Fig. 8A in Paper I). This indicates that also the slugs benefit from maintaining alternative electron sinks functional in their plastids, if they want to maintain these organelles in working order in the nature, where light fluctuations are unavoidable.

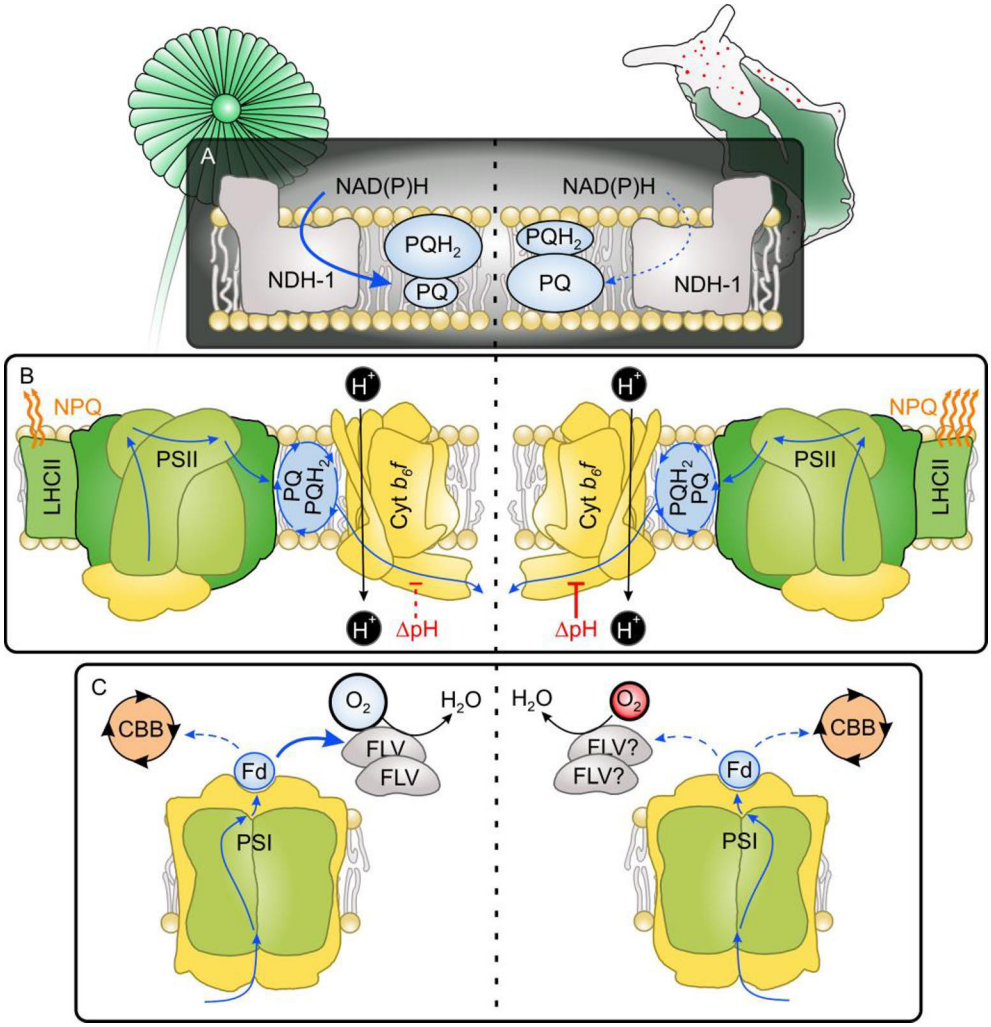

Figure 7. Differences in the electron transfer reactions between the alga *A. acetabulum* (left panels) and the slug *E. timida* (right panels) revealed in Paper I. **(A)** Dark reduction of the PQ pool is weak in the plastids of *E. timida* compared to *A. acetabulum*, which might help the slugs maintain efficient electron flow from PSII during sudden transitions from dark to light. NDH-1 is shown here as the facilitator of PQ dark reduction by donating electrons from NAD(P)H, but the details remain to be solved. **(B)** During a dark-to-light transition, the *pmf* of the slugs builds up, unlike in *A. acetabulum*. The build-up of *pmf* is likely at least partially due to a large ΔpH, which could result in stronger cessation of electron flow through the Cyt *b6f* complex to PSI, possibly decreasing the risk of ROS formation at PSI. The differences in ΔpH could also explain the stronger NPQ witnessed in the slugs. **(C)** Alternative, oxygen-dependent electron sinks do work and protect PSI of *E. timida* during sudden bursts of light, but their functionality seems weak compared to *A. acetabulum*. If said electron sinks are FLVs, the slug respiration in the dark could deprive them of the oxygen needed for their full functionality.

5.2 Photoprotection at the level of the slug's body and tissues

The molecular level photoprotective mechanisms discussed in the previous chapter might be important for decreasing the inherent susceptibility of the plastids to photoinhibition, but just how much photoinhibition, specifically of PSII, does the conglomerate of plastids face inside the body of the slugs? This was the leading research question in Paper II of this thesis. Different photosynthetic slugs have been shown to shun away or curl up by closing their parapodia in response to bright light, but instead of such active forms of photoprotection, Paper II focused more on passive strategies that might exist simply due to the fact the plastids are inside an animal body (Cruz et al. 2013; Cartaxana et al. 2018; Cartaxana et al. 2019) (Fig. 8).

5.2.1 Plastids inside the slugs are protected against both visible light and UV radiation

The sea slug *E. timida* is optically very different than the algal cell of *A. acetabulum*. One major difference between the two is the arrangement, or packing, of the plastids; *E. timida* is obviously a lot thicker than a single siphonaceous *A. acetabulum* cell, and the plastids are more concentrated throughout the slug's body than in the algal cell (Fig. 4 in Paper II). Being packed so tightly in the body of *E. timida,* especially in the parapodia, also prevents the plastids in the deeper layers from absorbing excessive light (or light in general), as most of the light is being absorbed by the thick concentration of plastids in the outer layers of the slug (Fig. 4C). This also explains the drastic differences in susceptibility to photoinhibition of PSII between *A. acetabulum* and *E. timida*, especially when PSII activity is estimated as the Chl fluorescence parameter F_V/F_M, which integrates the fluorescence from the deeper layers (within limits) with the fluorescence from the outer layers (Fig. 1 and 3 in Paper II) (Serôdio and Campbell 2021).

The finding that the plastids inside *E. timida* are not as susceptible to photoinhibition of PSII as the plastids inside *A. acetabulum* has been described previously, but the reasons for this were not discussed in detail (Christa et al. 2018). The lack of discussion could be due to the glaring obviousness of the situation, or to once again avoid too strong conclusions based on Chl fluorescence data from optically very different samples. Indeed, it should be noted that even though the bulk of the plastids inside *E. timida* are seemingly less susceptible to photoinhibition of PSII, the fluorescence measurements in Fig. 1 in Paper II do not allow to determine the inherent photoinhibition susceptibility of an individual PSII in the plastids of a slug. Also, even though the F_V/F_M parameter has been shown to correlate well with other PSII activity estimates during photoinhibition experiments, the fact is, that with the recent advances in PSII fluorescence emission research, the basis for F_V/F_M to

represent the maximum quantum yield of PSII photochemistry is questionable (Tyystjärvi 2013; Sipka et al. 2021).

Nevertheless, the data in especially Figs. 3 and 4 in Paper II do indicate that the slugs are more concentrated in terms of plastids, or Chl, than their algal counterparts, and thickening Chl concentrations have been shown to be an efficient means of photoprotection also in leaves of plants (Pätsikkä et al. 2002). Slug individuals that had lost some of their plastids during nine days of starvation were more susceptible to photoinhibition of PSII (Fig. 6 in Paper II). This supports the notion that a thick plastid concentration is indeed protecting the bulk of the plastids inside the slugs. Utilizing the plastids themselves as shields against excessive light suits the voracious feeding habits of the slugs well; given the opportunity, the slugs tend to fill themselves up with plastids, which provides them with a good starting point for a simple and effective photoprotective mechanism that allows the plastids to stay functional for longer periods of time during starvation.

The plastids inside *E. timida* were also found to be very well protected against UV radiation, based on the action spectra of photoinhibition of PSII in both the slugs and the algae (Fig. 2 in Paper II). The fluorescence emission from UVA excited slugs was also very weak compared to the algae, which indicates that the slug body or mucus functions as an efficient UV screen that prevents UV radiation from reaching the plastids (Fig. 3A, B in Paper II). This is a very probable cause for the UV resilience of the plastids inside *E. timida*. How exactly do the slug's screen UV radiation remains to be solved, but good candidates for UV screening compounds are the complex polyketides that are specific to long-term retention photosynthetic sea slugs, including *E. timida*. For example, the precursor of the polyketide tridachione absorbs already at 400 nm, and the absorption increases until reaching a peak at 335 nm, and has a strong shoulder in the UVB and UVC regions all the way down to 250-260 nm (Torres et al. 2020). The fact that these compounds may show stronger absorption in the UVC and UVB regions than in the UVA region could be behind the finding that the rate constant of photoinhibition did not increase noticeably from UVA to UVB in the slugs (Fig. 2 in Paper II). Photosynthetic sea slugs inhabit shallow marine environments, where they are likely exposed to at least some UV radiation, but the true significance of this UV screening to plastid longevity remains to be tested in conditions that mimic natural environments.

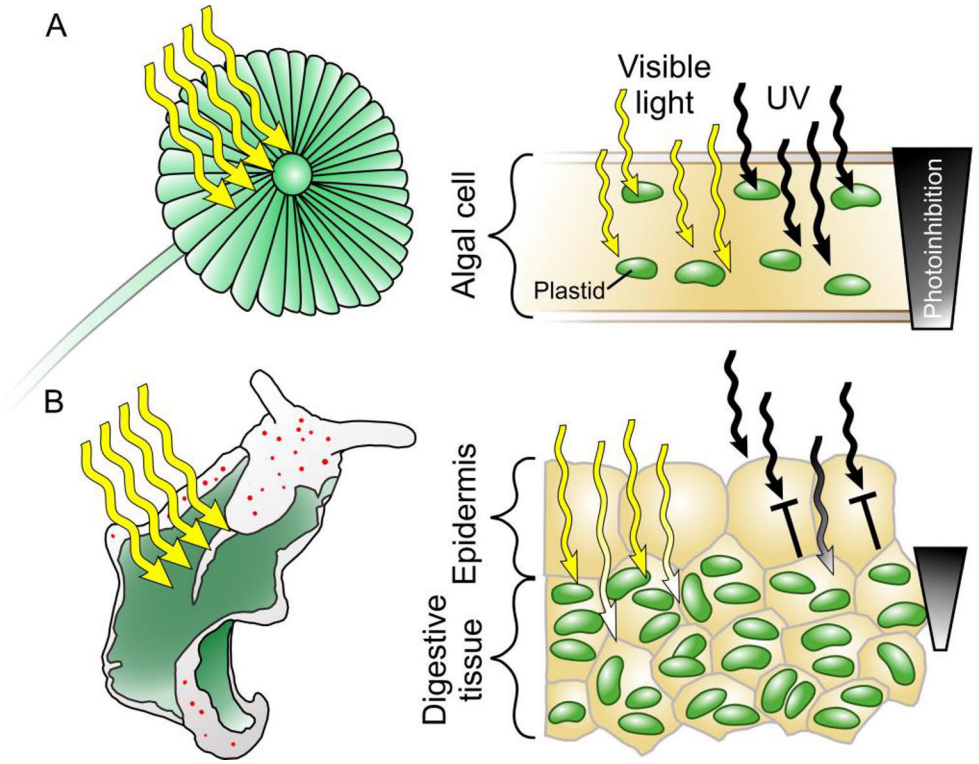

Figure 8. The body of the slug *E. timida* protects the plastids from photoinhibition. **(A)** The plastids are distributed along the narrow filamentous single cell of the green alga *A. acetabulum*, and visible light reaches most of the plastids. The algae might therefore be well-suited to perform photosynthesis even under suboptimal irradiance. The downside is that most of the plastids will also experience considerable photoinhibition when the algae are exposed to bright light. The algae are also highly susceptible to photoinhibition caused by UV radiation, suggesting that they do not synthesize UV screening compounds, at least not without UV acclimation. **(B)** The plastids are tightly packed in the digestive tissue cells of the slugs, effectively allowing them to use the plastids packed in the outer layers of the tissue as a shield that takes the brunt of the photoinhibitory damage caused by both visible light and UV radiation. Furthermore, the body of the slug shields the plastids from UV radiation, possibly by UV screening compounds in the epidermis (and other tissues) or in the mucus.

5.3 Photoinhibition and repair mechanisms in the plastids of the prey algae

What if excessive light energy still overflows the photosynthetic machinery of the stolen plastids inside the slugs, despite the fine tuning of the electron transfer and passive protection of the plastids discussed in the previous chapters? Are there any specific innate characteristics of the plastids in the specific prey algal species of the slugs that could further alleviate and repair the damage caused by photoinhibition?

These were the questions guiding the research effort in Paper III of this thesis, where the genetic autonomy and photoinhibition of the plastids of the secondary red alga *V. litorea,* the plastid source for *E. chlorotica*, were inspected in detail.

5.3.1 Genetic autonomy of the plastids is sufficient for PSII repair

The idea that a set of only three genes, *psbA* (D1), *ftsH* (thylakoid maintenance protease FtsH) and *tufA* (translation elongation factor EF-Tu), could be at the heart of plastid maintenance in photosynthetic sea slugs was originally put forward almost a decade ago (de Vries et al. 2013). In its original form, this idea postulated that the lack of *ftsH* in the plastome of *Bryopsis hypnoides* would prevent the long-term maintenance of the plastids from this green alga inside polyphagous slugs like *E. viridis*. However, more recent sequence analyses have revealed that also *B. hypnoides* plastome contains *ftsH,* suggesting that the situation might be more complicated (Christa et al. 2018). In fact, almost all algae, be they of the green or red lineage, do contain all three genes in their plastomes, unlike land plants, where both *ftsH* and *tufA* seem to have moved to the nuclear genome (de Vries et al. 2013). Even if *ftsH* and *tufa* were not plastid encoded in *V. litorea*, their prominent roles in plastid maintenance, especially in PSII repair cycle, would warrant an in-depth investigation about their possible involvement in plastid longevity in sea slugs (Kato and Sakamoto 2018; Jimbo et al. 2019).

When the transcript levels of seven plastome genes were inspected throughout a seven-day incubation of lab-isolated *V. litorea* plastids, it became evident that the trancripts of *ftsH* and *tufA* did not decay at the same rate as the transcripts of all other tested genes of the plastome (Fig. 2B in Paper III). This suggests that the expression of *ftsH* and *tufA* was active and possibly upregulated as the isolation proceeded, or at the very least these transcripts were more long-lived than the others. Furthermore, functional isolation of *V. litorea* plastids inside the algal cell by cycloheximide resulted in elevated levels of FtsH also at a protein level (normalized to total Chl of the samples) during recovery from photoinhibition, as inspected by Western blotting using antibodies for both A and B type FtsH proteins (Fig. 3D in Paper III). These data might indicate that isolating *V. litorea* plastids from the regulatory network of the algal cytosol leads to an upregulation of the plastid encoded *ftsH* and *tufA*, which would supply the plastids inside the slugs with proteases that can degrade D1 after photoinhibition, as well as support plastid translation that can replenish the D1-less PSII monomers with new D1 proteins (de Vries et al. 2013; Kato and Sakamoto 2018). Indeed, both photoinhibition and subsequent recovery proceeded almost identically in *V. litorea* cells in the absence and presence of cycloheximide, suggesting that the PSII repair cycle is not dependent on active translation of nucleus

encoded proteins in the plastids of *V. litorea* (Fig. 3B in Paper III). The contribution of FtsH to the resilience against the combination of photoinhibition and cycloheximide is still, however, somewhat questionable in *V. litorea;* the elevated FtsH levels were only noticeable at the very end of the recovery, and they did not enhance the recovery. On the contrary, the F_V/F_M of cycloheximide treated algae was lower at the end of the recovery than in samples without cycloheximide. Perhaps the slug *E. chlorotica* is a better host for the plastids than the barren ruins of a cycloheximide ravaged *V. litorea* cell.

Another problem exists with the hypothesis that plastid encoded FtsH is a major factor facilitating the longevity of plastids inside photosynthetic sea slugs. The plastid *ftsH* of *V. litorea* is likely functional based on sequence data, as it contains the critical M41 domain required for proteolytic degradation of proteins, including D1. On the other hand, the proteolytic capability of the plastid *ftsH* of all other prey (green) algae of the slugs is questionable, because they were recently shown not to contain the M41 peptidase metalloprotease domain (Christa et al. 2018). And even if the plastids encode for a single FtsH subunit with proteolytic capability, the functionality of the FtsH complex is still dubious, because in photosynthetic organisms the FtsH complex is known to be a heterohexamer containing both A and B type subunits (Moldavski et al. 2012). With all that said, the green algal *A. acetabulum* plastids inside the slug *E. timida* were able to recover from photoinhibition during an overnight recovery period, whereas inhibiting plastid translation with lincomycin prevented the recovery (Fig. 2C in Paper III). The recovery was not 100% without lincomycin, but it does indicate that even the plastids derived from green algae, like *A. acetabulum,* are sufficiently autonomous to facilitate PSII repair inside the slugs, at least when the plastids are fresh.

FtsH proteins that do not (presumably) contain proteolytic activity due to the lack of the M41 domain exist also in plants, named FtsHi (inactive), and they have been shown to be associated with both the plastid envelope and the thylakoid membrane with largely unknown functions (Mishra et al. 2019). Maybe the FtsH proteins in the plastids of the prey algae of the slugs can form functional homo-oligomers, like in mitochondria, which would suit *V. litorea* (and *E. chlorotica*) with its one proteolytic FtsH subunit (Janska et al. 2013). Or perhaps some of the FtsH subunits in the functional hetero-oligomers can be substituted by the non-proteolytic FtsH encoded by the plastomes of the green prey algae of the slugs.

5.3.2 Electron sinks downstream of PSI are important for preventing oxidative damage in *V. litorea*

Inspecting the photosynthetic properties of algae in isolated systems, like in isolated thylakoids, can be problematic, because the intricate details of the complex network

of redox reactions controlling photosynthesis in intact cells or plastids might be lost and easily misinterpreted. But this can also be considered an advantage of *in vitro* photosynthesis studies done with isolated thylakoids; the loss of some of the native electron transfer routes and sinks that were in place when the thylakoids were inside their native environment can offer clues about their importance in preventing oxidative stress during high light. *In vitro* studies can also even out some of the optical differences between different species and justify the comparisons between evolutionarily distant relatives, especially when the basic photosynthetic properties between the two are as similar as between the isolated thylakoid membranes from spinach and *V. litorea* (Table 2 in Paper III).

Compared to spinach thylakoids, *V. litorea* thylakoids seemed more susceptible to general oxidative stress in high light (Fig. 4B, C in Paper III). The finding that PSI showed signs of photoinhibition according to two different PSI activity estimates, oxygen consumption and P_M, might also suggest that photoinhibition of PSI was more severe in the isolated thylakoids of *V. litorea* than in spinach thylakoids, where only P_M showed any indications of decreased PSI activity in high light (Fig. 6A-D in Paper III). Isolated thylakoids of both species are presumably devoid of the main electron sink downstream of PSI, the CBB cycle. Unlike spinach leaves, intact *V. litorea* cells exhibit "FLV-like" P700 oxidation kinetics during a strong light pulse after darkness (Fig. 6E, F insets in Paper III). The photosynthetic machinery of *V. litorea* might therefore rely heavily on the combined sink capacity of both the CBB cycle and the mysterious proteins that are maintaining P700 oxidized in the secondary red algal lineage of photosynthetic organisms (Shimakawa et al. 2019).

According to the P700 oxidation kinetics of isolated *V. litorea* thylakoids, some of the alternative electron sink capacity is lost during the thylakoid isolation procedure (Fig. 6F in Paper III). This is a possible cause for the drastic general oxidative stress witnessed in the isolated thylakoids of *V. litorea* in high light, as electrons from PSI would be forced to create ROS in the Mehler reaction in the absence of safe electron sinks. A direct implication of this is, that photosynthetic sea slugs should maintain the CBB cycle and alternative electron sinks, like FLVs in the *E. timida*/*A. acetabulum* system, in working order to avoid massive oxidative damage to the plastids but also to their own cells. Future work should focus on detailing the molecular players behind P700 oxidation in *V. litorea* and secondary red algae in general; if this process is indeed not facilitated by FLV proteins, it could reveal a whole new means of photoprotection that could be useful as a tool to improve the photoprotection of crop plants (Wada et al. 2018).

5.3.3 Low $^1O_2$ yield in *V. litorea* is beneficial for an efficient PSII repair cycle

The isolated thylakoids of *V. litorea* were more resilient against photoinhibition of PSII than spinach thylakoids, even though the general oxidative stress was more rampant in *V. litorea* (Table 2, Figs. 4A and 6C, D in Paper III). One ROS that has an undeniable link to photoinhibition of PSII is $^1O_2$, even though its connection to the actual damaging reaction(s) of photoinhibition remains debated (Vass 2011; Tyystjärvi 2013). Therefore, it is conceivable that the measured lower $^1O_2$ production in *V. litorea* compared to spinach might have contributed to the slower decrease in PSII activity in high light in *V. litorea* thylakoids (Fig. 5A in Paper III). Based on the data in Fig. 5 B-F in Paper III, it seems like *V. litorea* is very reluctant to produce $^1O_2$; the charge recombination reactions within the PSII of *V. litorea* are slow compared to spinach, which reduces the risk of ^{3}P680 formation, but the thylakoids of this alga are also supplied with plenty of powerful antioxidants against $^1O_2$, α-tocopherol and carotenoids, even in the darkness that preceded the thylakoid isolation (Vass 2011; Treves et al. 2016; Khorobrykh et al. 2020). The measurable production of $^1O_2$ depends on its generation (mainly) via the charge recombination reactions within PSII and its detoxification by the available antioxidants, and it is impossible to determine which is the more prominent component behind the low $^1O_2$ yield in *V. litorea* based on the data in Fig. 5A in Paper III.

The differences in photoinhibition of PSII in the isolated thylakoids of spinach and *V. litorea* were not massive. The PSII of *V. litorea* is certainly not immune to photoinhibition, unlike the extremophile algae *Chlorella ohadii* (that also produces very little $^1O_2$) (Treves et al. 2016). The low inherent $^1O_2$ yield of *V. litorea* is likely more beneficial in terms of maintaining a functional PSII repair cycle, especially in the isolated plastids inside the slug *E. chlorotica*, as PSII repair has been shown to be inhibited by $^1O_2$ (Nishiyama et al. 2006). It is possible that the tendency of *V. litorea* to avoid $^1O_2$ production is something that helps *E. chlorotica* in maintaining the plastids in working order by alleviating stress from the repair machinery of the plastids. Based on the data in Paper I and on the work by other groups, both alternative (FLVs) and main sinks (CBB cycle) of photosynthesis are at least somewhat functional even in the plastids stolen by photosynthetic sea slugs (Rumpho et al. 2001; Christa et al. 2014b; Rauch et al. 2018). It is therefore not likely that the plastids would experience such drastic oxidative damage by ROS produced in the PSI side of the electron transfer chain as witnessed in the isolated thylakoids of *V.litorea* in high light. If that is the case, then the low yield of the main ROS produced by PSII, $^1O_2$, would be a major factor in supporting the longevity of *V. litorea* plastids inside *E. chlorotica*.

6 Concluding remarks

How do photosynthetic sea slugs maintain their stolen plastids photosynthetically active without the help of the algal nucleus for extensive time periods? Unfortunately, a comprehensive answer to this question remains elusive even after the publication of this thesis. However, the data presented in the pages of this book do describe novel photoprotective mechanisms that are only present after the plastids have been incorporated to the slug cells (Papers I and II) and deepen our knowledge concerning the role of the specific plastids themselves in both avoiding and repairing the light induced damage to the photosynthetic machinery (Paper III). The mechanisms described and discussed in this thesis can be divided to three categories: I) active and fast responding photoprotective measures of the plastids that can be enhanced upon incorporation to slug cells, II) passive protection of the plastids from UV radiation and excessive light, which is reliant on the packing of the plastids inside the slugs, and III) autonomous repair machinery of the plastids that is still functional even in the cellular environment of the slugs.

The research presented in the pages of this book was driven purely by curiosity. This kind of an opportunity for a PhD project is sadly becoming a rare commodity. There are no immediate gains to be reaped from the results of my thesis, and future research efforts regarding these incredible photosynthetic animals should focus on whatever sparks joy in the inquisitive mind of a scientist brave (or stupid) enough to study them with the limited number of resources available.

Acknowledgements

First off, I would like to thank all the financial supporters of my work, including Kone Foundation, Finnish Cultural Foundation, Finnish Academy of Science and Letters, UTUGS, Turku University Foundation and SmartBio. Buying bread has been a major motivator pushing me forward with this thesis, and without such adventurous foundations that are willing to fund projects with as little economical benefits to be immediately gained as with my project, this thesis would have stopped in its tracks years ago. Thank you!

Professor Eevi Rintamäki is thanked for the help with all the bureaucracy involved with actually graduating (and also for the oppressive feeling that I should get this thesis out of the way every time I passed you in Pharmacity, this really pushed me to get things done...eventually). Academician Eva-Mari Aro is thanked for being the major driving force in creating the photosynthesis research community in Turku. I am proud to have been part of it. Professor Albert Porcar-Castell and Professor Yagut Allahverdiyeva-Rinne are thanked for taking up the time to join my advisory committee meetings throughout the years and offering valuable advice for the continuation of my work. I would also like to express gratitude to Professor Fikret Mamedov and Assistant Professor João Serôdio for reviewing my thesis. And of course Professor Peter Jahns is thanked for accepting the invitation to be my opponent. The technical support in our unit is top notch, so cheers for the assistance Anniina, Eve and Mika. Tapio is especially acknowledged for bringing a fresh air of gallows humour in our midst and Kurt is thanked for instilling a fear of god in me for the rest of my life, I think.

Esa, I think you are propably the smartest person in my social circle (which is admittedly not large, but think of it more in the lines of being exclusive) and it has been an honour to have you supervise my work and co-author the papers. I don't think many supervisors would have been so open to me completely changing my topic on the fly to such a weird and niche research topic as photosynthetic sea slugs. Luckily it all worked out in the end, but really, thanks for the trust. I'm not exactly sure who brought up this topic initially, you or Taina, but I want to thank Taina for being the supervisor for my bachelor's thesis concentrating on the photosynthetic sea slug *E. chlorotica*. Even though it took some time to take effect, my bachelor's

thesis was the initial spark that eventually led me to where I am now, about to graduate as a slug doctor.

All co-authors of the papers in this thesis are very warmly thanked, but a few special mentions are in order. First, Sven, this thesis would not have been possible without you accepting me to visit your lab and later on letting me participate in a slug trip to Elba. I really appreciate it (also the sun and the sea in Elba... and the beers). Also Riina, Ville and Mikko deserve a special mention, you have all made this thesis a thousand times easier with the help in the lab. And Mikko is thanked for having some faith in me in supervising his master's thesis, it was a new experience.

The coffee/lunch/movie community in Pharmacity is exceptional. There are many like it, but this one is mine. Thanks to Hari, Olli, Otso, Lauri, Pasi, Daniel, Julia, Duncan and many others for this. Janne is thanked for being the alpha male that he is, but also for sharing a passion for shitty movies that led to the cesspit of political incorrectness in our unit, a.k.a the shitty movie night. Juha, you beat me to it, but I finally made it to graduation! Now can I please have the epiphany that has clearly driven you to be an exceptionally stable individual who can balance doing excellent research and a meaningful life? Thanks for the peer support (and friendly competition) that has lasted for *sigh* nearly 12 years now. Let that sink in. I think you could maybe improve your karaoke a bit, though (ask Janne for help). My friends back home have also been paramount for me in maintaining some level of sanity throughout the years. I would like to thank Pasi, Marika, Jore and Miikka for having open doors (and beer taps) whenever I was around, this really helped me vent out the frustrations of doing a PhD.

Heta. Before you I thought that I might be spending the rest of my life alone with my slugs (which is, of course, a perfectly honourable way of wasting a life). Thank you for putting up with my hermitous life style, especially during the last year. Also thank you for not putting up with my shit when it comes to vaguely describing ideas about science (or life in general); your sharp logic usually cuts through my neanderthal way of thinking like a hot knife through butter. I love you.

Olen aina voinut palata kotiin, oli tilanne mikä tahansa. Kotona käynti on aina myös palauttaunut voimiani ja luonut uskoa huomiseen. Tästä haluan kiittää sisaruksiani Hannaa, Villeä ja Hannua, mutta etenkin vanhempiani Aria ja Ullaa, jotka ovat aina tukeneet työtäni, vaikka työni tarkoitus on ollut ajoittain hakusessa itse kullakin. Tämä väitöskirja on omistettu isälleni ja äidilleni. Voikaa hyvin.

16.01.2022

Vesa Havurinne

List of References

Akazaki H, Kawai F, Hosokawa M, Hama T, Chida H, Hirano T, Lim BK, Sakurai N, Hakamata W, Park SY, Nishio T, Oku T (2009) Crystallization and structural analysis of cytochrome *c6* from the diatom *Phaeodactylum tricornutum* at 1.5 Å resolution. *Biosci Biotechnol Biochem* 73: 189–191

Alboresi A, Le Quiniou C, Yadav SKN, Scholz M, Meneghesso A, Gerotto C, Simionato D, Hippler M, Boekema EJ, 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 Phytol* 213: 714–726

Alboresi A, Storti M, Cendron L, Morosinotto T (2019b) Role and regulation of class-C flavodiiron proteins in photosynthetic organisms. *Biochem J* 476: 2487–2498

Alboresi A, Storti M, Morosinotto T (2019a) Balancing protection and efficiency in the regulation of photosynthetic electron transport across plant evolution. *New Phytol* 221: 105–109

Allahverdiyeva Y, Isojärvi J, Zhang P, Aro EM (2015) Cyanobacterial Oxygenic Photosynthesis is Protected by Flavodiiron Proteins. *Life* 5: 716–743

Allahverdiyeva Y, Mustila H, Ermakova M, Bersanini L, Richaud P, Ajlani G, Battchikova N, Cournac L, Aro EM (2013) Flavodiiron proteins Flv1 and Flv3 enable cyanobacterial growth and photosynthesis under fluctuating light. *Proc Natl Acad Sci USA* 110: 4111–4116

Allakhverdiev SI, Murata N (2004) Environmental stress inhibits the synthesis de novo of proteins involved in the photodamage–repair cycle of Photosystem II in *Synechocystis* sp. PCC 6803. *Biochim Biophys Acta Bioener* 1657: 23–32

Allen JW, Tevatia R, Demirel Y, DiRusso CC, Black PN (2018) Induction of oil accumulation by heat stress is metabolically distinct from N stress in the green microalgae *Coccomyxa subellipsoidea* C169. *PLoS One* 13: e0204505

Allorent G, Tokutsu R, Roach T, Peers G, Cardol P, Girard-Bascou J, Seigneurin-Berny D, Petroutsos D, Kuntz M, Breyton C, Franck F, Wollman FA, Niyogi KK, Krieger-Liszkay A, Minagawa J, Finazzi G (2013) A Dual Strategy to Cope with High Light in *Chlamydomonas reinhardtii*. *Plant Cell* 25: 545–557

Alric J, Pierre Y, Picot D, Lavergne J, Rappaport F (2005) Spectral and redox characterization of the heme Ci of the cytochrome *b6f* complex. *Proc Natl Acad Sci USA* 102: 15860–15865

Anderson JM, Park YI, Chow WS (1998) Unifying model for the photoinactivation of Photosystem II in vivo under steady-state photosynthesis. *Photosynth Res* 56: 1–13

Archibald JM (2015) Endosymbiosis and Eukaryotic Cell Evolution. *Curr Biol* 25: R911–R921

Archibald JM, Lane CE (2009) Going, Going, Not Quite Gone: Nucleomorphs as a Case Study in Nuclear Genome Reduction. *J Hered* 100: 582–590

Aro EM, Kettunen R, Tyystjärvi E (1992) ATP and light regulate D1 protein modification and degradation. Role of D1* in photoinhibition. *FEBS Lett* 297: 29–33

Aro EM, Virgin I, Andersson B (1993) Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. *Biochim Biophys Acta Bioener* 1143: 113–134

Asada K (1996) Radical production and scavenging in the chloroplasts. In: Baker NR (ed) Photosynthesis and the Environment, Kluwer Academic Publisher; Dordrecht, pp 128–150

Asada K (1999) The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. *Annu Rev Plant Physiol Plant Mol Biol* 50: 601–639

Asada K, Kiso K (1973). The photo-oxidation of epinephrine by spinach chloroplasts and its inhibition by superoxide dismutase: evidence for the formation of superoxide radicals in chloroplasts. *Agric Biol Chem* 37: 453–454

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. *Proc Natl Acad Sci USA* 107: 18214–18219

Ball S, Colleoni C, Cenci U, Raj JN, Tirtiaux C (2011) The evolution of glycogen and starch metabolism in eukaryotes gives molecular clues to understand the establishment of plastid endosymbiosis. J Exp Bot 62: 1775–1801

Ballottari M, Girardon J, Dall'Osto L, Bassi R (2012) Evolution and functional properties of Photosystem II light harvesting complexes in eukaryotes. *Biochim Biophys Acta Bioenerg* 1817: 143–157

Ballottari M, Truong TB, De Re E, Erickson E, Stella GR, Fleming GR, Bassi R, Niyogi KK (2016) Identification of pH-sensing Sites in the Light Harvesting Complex Stress-related 3 Protein Essential for Triggering Non-photochemical Quenching in *Chlamydomonas reinhardtii*. *J Biol Chem* 291: 7334–7346

Barker M, de Vries R, Nield J, Komenda J, Nixon PJ (2006) The Deg proteases protect *Synechocystis* sp. PCC 6803 during heat and light stresses but are not essential for removal of damaged D1 protein during the photosystem two repair cycle. *J Biol Chem* 281: 30347–30355

Bassham JA, Benson AA, Calvin M (1950) The path of carbon in photosynthesis. *J Biol Chem* 185: 781–787

Bellafiore S, Barneche F, Peltier G, Rochaix JD (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. *Nature* 433: 892–895

Bernal-Bayard P, Molina-Heredia FP, Hervás M, Navarro JA (2013) Photosystem I Reduction in Diatoms: As Complex as the Green Lineage Systems but Less Efficient. *Biochemistry* 52: 8687–8695

Bernal-Bayard P, Pallara C, Castell MC, Molina-Heredia FP, Fernández-Recio J, Hervás M, Navarro JA (2015) Interaction of photosystem I from *Phaeodactylum tricornutum* with plastocyanins as compared with its native cytochrome *c6*: Reunion with a lost donor. *Biochim Biophys Acta Bioener* 1847: 1549–1559

Bisby RH, Morgan CG, Hamblett I, Gorman AA (1999) Quenching of singlet oxygen by Trolox C, ascorbate, and amino acids: effects of pH and temperature. *J Phys Chem* 103: 7454–7459

Blankenship RE (1992) Origin and early evolution of photosynthesis. *Photosynth Res* 3: 91–111

Blommaert L, Chafai L, Bailleul B (2021) The fine-tuning of NPQ in diatoms relies on the regulation of both xanthophyll cycle enzymes. *Sci Rep* 11: 12750

Blommaert L, Huysman MJJ, Vyverman V, Lavaud J, Sabbe K (2017) Contrasting NPQ dynamics and xanthophyll cycling in a motile and a non-motile intertidal benthic diatom. *Limnol Oceanogr* 62: 1466–1479

Boehm M, Yu J, Krynicka V, Barker M, Tichy M, Komenda J, Nixon PJ, Nield J (2012) Subunit Organization of a Synechocystis Hetero-Oligomeric Thylakoid FtsH Complex Involved in Photosystem II Repair. *Plant Cell* 24: 3669–3683

Bonente G, Ballottari M, Truong TB, Morosinotto T, Ahn TK, Fleming GR, Niyogi KK, Bassi R (2011) Analysis of LhcSR3, a protein essential for feedback de-excitation in the green alga *Chlamydomonas reinhardtii*. *PLoS Biol* 9: e1000577

Bonente G, Pippa S, Castellano S, Bassi R, Ballottari M (2012) Acclimation of *Chlamydomonas reinhardtii* to Different Growth Irradiances. *J Biol Chem* 287: 5833–5847

Bouchet P, Rocroi JP, Hausdorf B, Kaim A, Kano Y, Nützel A, Parkhaev P, Schrödl M, Strong EE (2017) Revised classification, nomenclator and typification of gastropod and monoplacophoran families. *Malacologia* 61: 1-526

Brettel K (1997) Electron transfer and arrangement of the redox cofactors in photosystem I. *Biochim Biophys Acta* 1318: 322–373

Brinkert K, De Causmaecker S, Krieger-Liszkay A, Fantuzzi A, Rutherford AW (2016) Bicarbonate-induced redox tuning in Photosystem II for regulation and protection. *Proc Natl Acad Sci USA* 113: 12144–12149

Bruick RK, Mayfield SP (1998) Processing of the psbA 5′ Untranslated Region in *Chlamydomonas reinhardtii* Depends upon Factors Mediating Ribosome Association. *J Cell Biol* 143: 1145–1153

Büchel C (2015) Evolution and function of light harvesting proteins. *J Plant Physiol* 172: 62–75

Büchel C (2020) Light harvesting complexes in chlorophyll *c*-containing algae. *Biochim Biophys Acta Bioenerg* 1861: 148027

Buck JM, Kroth PG, Lepetit B (2021) Identification of sequence motifs in Lhcx proteins that confer qE-based photoprotection in the diatom *Phaeodactylum tricornutum*. *Plant J* 108: 1721–1734

Burlacot A, Peltier G, Li-Beisson Y (2019) Subcellular Energetics and Carbon Storage in *Chlamydomonas*. *Cells* 8: 1154

Cai H, Li Q, Fang X, Li J, Curtis NE, Altenburger A, Shibata T, Feng M, Maeda T, Schwartz JA, Shigenobu S, Lundholm N, Nishiyama T, Yang H, Hasebe M, Li S, Pierce SK, Wang J (2019) A draft genome assembly of the solar-powered sea slug *Elysia chlorotica*. *Sci Data* 6: 190022

Calvin M, Benson AA (1948) The Path of Carbon in Photosynthesis. *Science* 107: 476–480

Carbonera D, Gerotto C, Posocco B, Giacometti GM, Morosinotto T (2012) NPQ activation reduces chlorophyll triplet state formation in the moss *Physcomitrella patens*. *Biophys Acta Bioener* 1817: 1608–1615

Cariti F, Chazaux M, Legendre-Lefebvre L, Longoni P, Ghysels B, Johnson X, Goldschmidt-Clermont M (2020) Regulation of light harvesting in *Chlamydomonas reinhardtii*: Two protein phosphatases are involved in state transitions. *Plant Physiol 183*: 1749–1764

Cartaxana P, Morelli L, Jesus B, Calado G, Calado R, Cruz S (2019) The photon menace: kleptoplast protection in the photosynthetic sea slug *Elysia timida*. *J Exp Biol* 222: jeb202580

Cartaxana P, Morelli L, Quintaneiro C, Calado G, Calado R, Cruz S (2018) Kleptoplast photoacclimation state modulates the photobehaviour of the solar-powered sea slug *Elysia viridis*. *J Exp Biol* 221: jeb180463

Cartaxana P, Rey F, LeKieffre C, Lopes D, Hubas C, Spangenberg JE, Escrig S, Jesus B, Calado G, Domingues R, Kühl M, Calado R, Meibom A, Cruz S (2021) Photosynthesis from stolen chloroplasts can support sea slug reproductive fitness. *Proc R Soc B* 288: 20211779

Cartaxana P, Trampe E, Kühl M, Cruz S (2017) Kleptoplast photosynthesis is nutritionally relevant in the sea slug *Elysia viridis*. *Sci Rep* 7: 7714

Castell C, Bernal-Bayard P, Ortega JM, Roncel M, Hervás M, Navarro JA (2021) The heterologous expression of a plastocyanin in the diatom *Phaeodactylum tricornutum* improves cell growth under iron-deficient conditions. *Physiol Plant* 171: 277–290

Cavalier-Smith T (2000) Membrane heredity and early chloroplast evolution. *Trends Plant Sci* 5: 174-182

Cazzaniga S, Kim M, Bellamoli F, Jeong J, Lee S, Perozeni F, Pompa A, Jin ES, Ballottari M (2020) Photosystem II antenna complexes CP26 and CP29 are essential for nonphotochemical quenching in *Chlamydomonas reinhardtii*. *Plant Cell Environ* 43: 496–509

Chan CX, Vaysberg P, Price DC, Pelletreau KN, Rumpho ME, Bhattacharya D (2018) Active Host Response to Algal Symbionts in the Sea Slug *Elysia chlorotica*. *Mol Biol Evol* 35: 1706–1711

Chang W, Li C, Cui Z, Li W, Song H, Chang H, Fu W, Wang C, Huang T, Luo Y, Shan Y, Wang Y, Wang F, Xu M, Fu A (2021) Diverged Early From CtpB and CtpC, CtpA Has Evolved to Process D1 Precursor in Oxygenic Photosynthetic Organisms. *Front Plant Sci* 12: 780

Chaux F, Burlacot A, Mekhalfi M, Auroy P, Blangy S, Richaud P, Peltier G (2017) Flavodiiron proteins promote fast and transient O_2 photoreduction in *Chlamydomonas*. *Plant Physiol* 174: 1825–1836

Chen H, Zhang D, Guo J, Wu H, Jin M, Lu Q, Lu C, Zhang L (2006) A Psb27 homologue in *Arabidopsis thaliana* is required for efficient repair of photodamaged photosystem II. *Plant Mol Biol* 61: 567–575

Chen YL, Chen LJ, Chu CC, Huang PK, Wen JR, Li HM (2018) TIC236 links the outer and inner membrane translocons of the chloroplast. *Nature* 564: 125–129

Chiovitti A, Molino P, Crawford SA, Teng R, Spurck T, Wetherbee R (2004) The glucans extracted with warm water from diatoms are mainly derived from intracellular chrysolaminaran and not extracellular polysaccharides. *Eur J Phycol* 39: 117–128

Chow WS, Melis A, Anderson JM (1990) Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. *Proc Natl Acad Sci USA* 87: 7502–7506

Christa G, Cruz S, Jahns P, de Vries J, Cartaxana P, Esteves AC, Serôdio J, Gould SB (2017) Photoprotection in a monophyletic branch of chlorophyte algae is independent of energy-dependent quenching (qE). *New Phytol* 214: 1132–1144

Christa G, Gould SB, Franken J, Vleugels M, Karmeinski D, Händeler K, Martin WF, Wägele H (2014a) Functional kleptoplasty in a limapontioidean genus: phylogeny, food preferences and photosynthesis in *Costasiella*, with a focus on *C. ocellifera* (Gastropoda: Sacoglossa). *J Molluscan Stud* 80: 499–507

Christa G, Pütz L, Sickinger C, Melo Clavijo J, Laetz EM, Greve C, Serôdio J (2018) Photoprotective Non-photochemical Quenching Does Not Prevent Kleptoplasts From Net Photoinactivation. Front Ecol Evol 6: 121

Christa G, Wescott L, Schäberle TF, König GM, Wägele H (2013) What remains after 2 months of starvation? Analysis of sequestered algae in a photosynthetic slug, *Plakobranchus ocellatus* (Sacoglossa, Opisthobranchia), by barcoding. *Planta* 237: 559–572

Christa G, Zimorski V, Woehle C, Tielens AGM, Wägele H, Martin WF, Gould SB (2014b) Plastid-bearing sea slugs fix CO_2 in the light but do not require photosynthesis to survive. *Proc R Soc B* 281: 20132493

Clark KB (1994) Ascoglossan (=Sacoglossa) Molluscs in the Florida Keys: Rare Marine Invertebrates at Special Risk. *Bull Mar Sci* 54: 900–916

Clark KB, Jensen KR, Stirts HM, Fermin C (1981) Chloroplast symbiosis in a non-elysiid mollusc, *Costasiella lilianae* Marcus (Hermaeidae: Ascoglossa = Sacoglossa): effects of temperature, light intensity, and starvation on carbon fixation rate. *Biol Bull* 160: 43–54

Clark KB, Jensen KR, Strits HM (1990) Survey for functional kleptoplasty among west atlantic Ascoglossa (=Sacoglossa) (Mollusca: Opisthobranchia). *Veliger* 33: 339–345

Colombo M, Suorsa M, Rossi F, Ferrari R, Tadini L, Barbato R, Pesaresi P (2016) Photosynthesis Control: An underrated short-term regulatory mechanism essential for plant viability. *Plant Signal Behav* 11: e1165382

Conn PF, Schalch W, Truscott TG (1991) The singlet oxygen and carotenoid interaction. *J Photochem Photobiol B Biol* 11: 41–47

Cormann KU, Bartsch M, Rögner M, Nowaczyk MM (2014) Localization of the CyanoP binding site on photosystem II by surface plasmon resonance spectroscopy. *Front Plant Sci* 5: 595

Cormann KU, Möller M, Nowaczyk MM (2016) Critical Assessment of Protein Cross-Linking and Molecular Docking: An Updated Model for the Interaction Between Photosystem II and Psb27. *Front Plant Sci* 7: 157

Costa J, Giménez-Casalduero F, Melo R, Jesus B (2012) Colour morphotypes of *Elysia timida* (Sacoglossa, gastropoda) are determined by light acclimation in food algae. *Aquat Biol* 17: 81–89

Cramer WA, Hasan SS, Yamashita E (2011) The Q cycle of cytochrome *bc* complexes: a structure perspective. *Biochim Biophys Acta Bioener* 7: 788–802

Crofts AR, Meinhardt SW, Jones KR, Snozzi M (1983) The role of the quinone pool in the cyclic electron-transfer chain of *Rhodopseudomonas sphaeroides* A modified Q-cycle mechanism. *Biochim Biophys Acta Bioener* 723: 202–218

Cruz S, Calado R, Serôdio J, Cartaxana P (2013) Crawling leaves: photosynthesis in sacoglossan sea slugs. *J Exp Bot* 64: 3999–4009

Cruz S, Cartaxana P, Newcomer R, Dionísio G, Calado R, Serôdio J, Pelletreau KN, Rumpho ME (2015) Photoprotection in sequestered plastids of sea slugs and respective algal sources. *Sci Rep* 5: 7904

Cruz S, LeKieffre C, Cartaxana P, Hubas C, Thiney N, Jakobsen S, Escrig S, Jesus B, Kühl M, Calado R, Meibom A (2020) Functional kleptoplasts intermediate incorporation of carbon and nitrogen in cells of the Sacoglossa sea slug *Elysia viridis*. *Sci Rep* 10: 10548

DalCorso G, Pesaresi P, Masiero S, Aseeva E, Schünemann D, Finazzi G, Joliot P, Barbato R, Leister D (2008) A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in *Arabidopsis*. *Cell* 132: 273–285

Davis GA, Kanazawa A, Schöttler MA, Kohzuma K, Froehlich JE, Rutherford AW, Satoh-Cruz M, Minhas D, Tietz S, Dhingra A, Kramer DM (2016) Limitations to photosynthesis by proton motive force-induced photosystem II photodamage. *eLife* 5: e16921

Davy SK, Allemand D, Weis VM (2012) Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiol Mol Biol Rev* 762: 229–261

Dayer R, Fischer BB, Eggen RIL, Lemaire SD (2008) The Peroxiredoxin and Glutathione Peroxidase Families in *Chlamydomonas reinhardtii*. *Genetics* 179: 41–57

De Freese DE, Clark KB (1991) Transepidermal Uptake of Dissolved Free Amino Acids from Seawater by Three Ascoglossan Opisthobranchs. *J Moll Stud* 57: 65–74

De Laurentiis EI, Mo F, Wieden HJ (2011) Construction of a fully active Cys-less elongation factor Tu: Functional role of conserved cysteine 81. *Biochim Biophys Acta Proteins Proteom* 1814: 684–692

de Vries J, Archibald JM (2018) Plastid genomes. *Curr Biol* 28: R329–R341

de Vries J, Habicht J, Woehle C, Huang C, Christa G, Wägele H, Nickelsen J, Martin WF, Gould SB (2013) Is ftsH the Key to Plastid Longevity in Sacoglossan Slugs? *Genome Biol Evol* 5: 2540–2548

de Vries J, Woehle C, Christa G, Wägele H, Tielens AGM, Jahns P, Gould SB (2015) Comparison of sister species identifies factors underpinning plastid compatibility in green sea slugs. *Proc R Soc B* 282: 20142519

de Wijn R, van Gorkom HJ (2001) Kinetics of electron transfer from Q_a to Q_b in photosystem II. *Biochemistry* 40: 11912–11922

Deák Z, Sass L, Éva K, Vass I (2014) Characterization of wave phenomena in the relaxation of flash-induced chlorophyll fluorescence yield in cyanobacteria. *Biochim Biophys Acta Bioener* 1837: 1522–1532

Depège N, Bellafiore S, Rochaix JD (2003) Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in *Chlamydomonas*. *Science* 299: 1572–1575

Deshpande N, Bao Y, Herrin D (1997). Evidence for light/redox-regulated splicing of psbA pre-RNAs in *Chlamydomonas* chloroplasts. *RNA* 3: 37–48

Di Donato M, Stahl AD, van Stokkum IHM, van Grondelle R, Groot ML (2011) Cofactors Involved in Light-Driven Charge Separation in Photosystem I Identified by Subpicosecond Infrared Spectroscopy. *Biochemistry* 50: 480–490

Di Mascio P, Devasagayam TP, Kaiser S, Sies H (1990) Carotenoids, tocopherols and thiols as biological singlet molecular oxygen quenchers. *Biochem Soc Trans* 18: 1054–1056

Diner BA, Schlodder E, Nixon PJ, Coleman WJ, Rappaport F, Lavergne J, Vermaas WFJ, Chisholm DA (2001) Site-Directed Mutations at D1-His198 and D2-His197 of Photosystem II in *Synechocystis* PCC 6803: Sites of Primary Charge Separation and Cation and Triplet Stabilization. *Biochemistry* 40: 9265–9281

Donohoo SA, Wade RM, Sherwood AR (2020) Finding the sweet spot: sub-ambient light increases fitness and kleptoplast survival in the sea slug *Plakobranchus* cf. *ianthobaptus* Gould, 1852. *Biol Bull* 238: 154–166

Evertsen J, Burghard I, Johnsen G, Wägele H (2007) Retention of functional chloroplasts in some sacoglossans from the Indo-Pacific and Mediterranean. *Mar Biol* 151: 2159–2166

Fassioli F, Dinshaw R, Arpin PC, Schole GD (2014) Photosynthetic light harvesting: excitons and coherence. *J R Soc Interface* 11: 20130901

Finazzi G, Sommer F, Hippler M (2005) Release of oxidized plastocyanin from photosystem I limits electron transfer between photosystem I and cytochrome *b6f* complex *in vivo*. *Proc Natl Acad Sci USA* 102: 7031–7036

Fischer BB, Hideg E, Krieger-Liszkay A (2013) Production, Detection, and Signaling of Singlet Oxygen in Photosynthetic Organisms. *Antioxid Redox Signal* 18: 2145–2162

Fisher NL, Campbell DA, Hughes DJ, Kuzhiumparambil U, Halsey KH, Ralph PJ, Suggett DJ (2020) Divergence of photosynthetic strategies amongst marine diatoms. *PLoS One* 15: e0244252

Flori S, Jouneau PH, Finazzi G, Maréchal E, Falconet D (2016) Ultrastructure of the Periplastidial Compartment of the Diatom *Phaeodactylum tricornutum*. *Protist* 167: 254–267

Foyer CH (2018) Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. *Environ Exp Bot* 154: 134–142

Frankenbach S, Luppa Q, Serôdio J, Greve C, Bleidissel S, Melo Clavijo J, Laetz EM, Preisfeld A, Christa G (2021) Kleptoplasts are continuously digested during feeding in the plastid-bearing sea slug *Elysia viridis*. *J Molluscan Stud* 87: eyab022

Fu J, Momcilović I, Clemente TE, Nersesian N, Trick HN, Ristic Z (2008) Heterologous expression of a plastid EF-Tu reduces protein thermal aggregation and enhances CO_2 fixation in wheat (*Triticum aestivum*) following heat stress. *Plant Mol Biol* 68: 277–288

Füssy Z, Oborník M (2018) Complex Endosymbioses I: From Primary to Complex Plastids, Multiple Independent Events. In: Maréchal E (ed) Plastids. Methods in Molecular Biology, vol 1829, Humana Press, New York, pp 17–35

Gagat P, Bodył A, Mackiewicz P, Stiller JW (2014) Tertiary Plastid Endosymbioses in Dinoflagellates. In: Löffelhardt W (ed) Endosymbiosis, Springer, Vienna, pp 233–290

Gawroński P, Enroth C, Kindgren P, Marquardt S, Karpiński S, Leister D, Jensen PE, Vinther J, Scharff LB (2021) Light-Dependent Translation Change of *Arabidopsis psbA* Correlates with RNA Structure Alterations at the Translation Initiation Region. *Cells* 10: 322

Gawroński P, Jensen PE, Karpiński S, Leister D, Scharff LB (2018) Pausing of Chloroplast Ribosomes Is Induced by Multiple Features and Is Linked to the Assembly of Photosynthetic Complexes. *Plant Physiol* 176: 2557–2569

Genty B, Briantais JM, Baker NR (1989) The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* 990: 87–92

Gibbs PB, Biggins J (1989) Regulation of the distribution of excitation energy of *Ochromonas danica*, an organism containing a chlorophyll-*a*/*c*/carotenoid light harvesting antenna. *Photosynth Res* 21: 81–91

Giles KL, Sarafis V (1972) Chloroplast survival and division in vitro. *Nature New Biol* 236: 56–58

Gilyarov MS (1983) Appropriation of functioning organelles of food organisms by phytophagous and predatory opistobranch mollusks as a specific category of food utilization. *Zh Obschch Biol* 44: 614-620

Girolomoni L, Cazzaniga S, Pinnola A, Perozeni F, Ballottari M, Bassi R (2019) LHCSR3 is a nonphotochemical quencher of both photosystems in *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci USA* 116: 4212–4217

Girolomoni L, Ferrante P, Berteotti S, Giuliano G, Bassi R, Ballottari M (2017) The function of LHCBM4/6/8 antenna proteins in *Chlamydomonas reinhardtii*. *J Exp Bot* 68: 627–641

Gobets B, van Grondelle R (2001) Energy transfer and trapping in photosystem I. *Biochim Biophys Acta* 1507: 80–99

González-Wangüemert M, Giménez-Casalduero F, Pérez-Ruzafa Á (2006) Genetic differentiation of *Elysia timida* (Risso, 1818) populations in the southwest mediterranean and mar menor coastal lagoon. *Biochem Syst Ecol* 34: 514–527

Goral TK, Johnson MP, Brain AP, Kirchhoff H, Ruban AV, Mullineaux CW (2010) Visualizing the mobility and distribution of chlorophyll proteins in higher plant thylakoid membranes: effects of photoinhibition and protein phosphorylation. *Plant J* 62: 948–959

Goss J, Jakob T (2010) Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynth Res* 106: 103–122

Goss R, Lepetit B (2015) Biodiversity of NPQ. *J Plant Physiol* 172: 13–32

Goss R, Pinto EA, Wilhelm C, Richter M (2006a) 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. *J Plant Physiol* 163: 1008–1021

Gould SB, Maier UG, Martin WF (2015) Protein Import and the Origin of Red Complex Plastids. *Curr Biol* 25: R515–R521

Gould SB, Waller RF, McFadden GI (2008) Plastid evolution. *Ann Rev Plant Biol* 59: 491–517

Graves DA, Gibson MA, Bleakney JS (1979) The digestive diverticula of *Alderia modesta* and *Elysia chlorotica* Opisthobranchia Sacoglossa. *Veliger* 21: 415-422

Gray B, Carmichael AJ (1992) Kinetics of superoxide scavenging by dismutase enzymes and manganese mimics determined by electron spin resonance. *Biochem J* 281: 795–802

Green BJ, Fox TC, Rumpho ME (2005) Stability of isolated algal chloroplasts that participate in a unique mollusc/kleptoplast association. *Symbiosis* 40: 31–40

Green BJ, Li WY, Manhart JR, Fox TC, Summer EJ, Kennedy RA, Pierce SK, Rumpho ME (2000) Mollusc-algal chloroplast endosymbiosis. Photosynthesis, thylakoid protein maintenance, and chloroplast gene expression continue for many months in the absence of the algal nucleus. *Plant Physiol* 124: 331–342

Green BR (2011) Chloroplast genomes of photosynthetic eukaryotes. *Plant J* 66: 34–44

Grieco M, Jain A, Ebersberger I, Teige M (2016) An evolutionary view on thylakoid protein phosphorylation uncovers novel phosphorylation hotspots with potential functional implications. J Exp Bot 67: 3883–3896

Grimm M, Grube M, Schiefelbein U, Zühlke D, Bernhardt J, Riedel K (2021) The lichens' microbiota, still a mystery? *Front Microbiol* doi: 10.3389/fmicb.2021.623839

Grouneva I, Gollan PJ, Kangasjärvi S, Suorsa M, Tikkanen M, Aro EM (2013) Phylogenetic viewpoints on regulation of light harvesting and electron transport in eukaryotic photosynthetic organisms. *Planta* 237: 399–412

Grouneva I, Rokka A, Aro EM (2011) The Thylakoid Membrane Proteome of Two Marine Diatoms Outlines Both Diatom-Specific and Species-Specific Features of the Photosynthetic Machinery. *J Proteome Res* 10: 5338–5353

Guskov A, Kern J, Gabdulkhakov A, Broser M, Zouni A, Saenger W (2009) Cyanobacterial photosystem II at 2.9-Å resolution and the role of quinones, lipids, channels and chloride. *Nat Struct Mol Biol* 16: 334–342

Hahn A, Vonck J, Mills DJ, Meier T, Kühlbrandt W (2018) Structure, mechanism, and regulation of the chloroplast ATP synthase. *Science* 360: eaat4318

Hakala M, Tuominen I, Keränen M, Tyystjärvi T, Tyystjärvi E (2005) Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of Photosystem II. *Biochim Biophys Acta* 1706: 68–80

Händeler K, Grzymbowski YP, Krug PJ, Wägele H (2009) Functional chloroplasts in metazoan cells - a unique evolutionary strategy in animal life. *Front Zool* 6: 28

Handrich M, de Vries J, Gould SB, Serôdio J, Christa G (2017) Ulvophyceaen photophysiology and research opportunities. *Perspect Phycol* 4: 83–92

Hasan SS, Yamashita E, Baniulis D, Cramer WA (2013) Quinone-dependent proton transfer pathways in the photosynthetic cytochrome *b6f* complex. *Proc Natl Acad Sci USA* 110: 4297–4302

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125: 189–198

Hempel F, Bullmann L, Lau J, Zauner S, Maier UG (2009) ERAD-Derived Preprotein Transport across the Second Outermost Plastid Membrane of Diatoms. *Mol Biol Evol* 26: 1781–1790

Houille-Vernes L, Rappaport F, Wollman FA, Alric J, Johnson X (2011) Plastid terminal oxidase 2 (PTOX2) is the major oxidase involved in chlororespiration in *Chlamydomonas*. *Proc Natl Acad Sci USA* 108: 20820–20825

Huppe HC, de Lamotte-Guéry F, Jacquot JP, Buchanan BB (1990) The ferredoxin-thioredoxin system of a green alga, *Chlamydomonas reinhardtii*. *Planta* 180: 341–351

Ilík P, Pavlovič A, Kouřil R, Alboresi A, Morosinotto T, Allahverdiyeva Y, Aro EM, Yamamoto H, Shikanai T (2017) Alternative electron transport mediated by flavodiiron proteins is operational in organisms from cyanobacteria up to gymnosperms. *New Phytol* 214: 967–972

Inoue K, Sakurai H, Hiyama T (1986) Photoinactivation Sites of Photosystem I in Isolated Chloroplasts. *Plant Cell Physiol* 27: 961–968

Jackson C, Knoll AH, Chan CX, Verbruggen H (2018) Plastid phylogenomics with broad taxon sampling further elucidates the distinct evolutionary origins and timing of secondary green plastids. *Sci Rep* 8: 1523

Jahns P, Latowski D, Strzalka K (2009) Mechanism and regulation of the violaxanthin cycle: The role of antenna proteins and membrane lipids. *Biochim Biophys Acta Bioener* 1787: 3–14

Jakob T, Goss R, Wilhelm C (2001) Unusual pH-dependence of diadinoxanthin de-epoxidase activation causes chlororespiratory induced accumulation of diatoxanthin in the diatom *Phaeodactylum tricornutum. J Plant Physiol* 158: 383–390

Jans F, Mignolet E, Houyoux PA, Cardol P, Ghysels B, Cuiné S, Cournac L, Peltier G, Remacle C, Franck F (2008) A type II NAD(P)H dehydrogenase mediates light-independent plastoquinone reduction in the chloroplast of *Chlamydomonas*. *Proc Natl Acad Sci USA* 105: 20546–20551

Järvi S, Suorsa M, Aro EM (2015) Photosystem II repair in plant chloroplasts – Regulation, assisting proteins and shared components with photosystem II biogenesis. *Biochim Biophys Acta Bioener* 1847: 900–909

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls *a*, *b*, *c1* and *c2* in higher plants, algae and natural phytoplankton. *Biochem Physiol Pflanz* 167: 191–194

Jegerschöld C, Virgin I, Styring S (1990) Light-dependent degradation of the D1 protein in photosystem II is accelerated after inhibition of the water splitting reaction. *Biochemistry* 29: 6179–6186

Jensen EL, Yangüez K, Carrière F, Gontero B (2020) Storage Compound Accumulation in Diatoms as Response to Elevated CO_2 Concentration. *Biology* 9: 5

Jensen KR (1992) Anatomy of some Indo-Pacific Elysiidae (Opistobranchia: Sacoglossa(=Ascoglossa)), with a discussion of the generic division and phylogeny. *J Moll Stud* 58: 257–296

Jensen KR (1996) Phylogenetic systematics and classification of the Sacoglossa (Mollusca, Gastropoda, Opisthobranchia). *Philos Trans R Soc Lond B Biol Sci* 351: 91–122

Jensen KR (1997) Evolution of the Sacoglossa (Mollusca, Opisthobranchia) and the ecological associations with their food plants. *Evol Ecol* 11: 301–335

Jensen KR (1999) Copulatory behaviour in three shelled and five non-shelled sacoglossans (Mollusca, Opistobranchia), with a discussion of the phylogenetic significance of copulatory behaviour. *Ophelia* 51: 93–106

Jimbo H, Izuhara T, Hihara Y, Hisabori T, Nishiyama Y (2019) Light-inducible expression of translation factor EF-Tu during acclimation to strong light enhances the repair of photosystem II. *Proc Natl Acad Sci USA* 116: 21268–21273

Johnson MP, Pérez-Bueno ML, Zia A, Horton P, Ruban AV (2009) The Zeaxanthin-Independent and Zeaxanthin-Dependent qE Components of Nonphotochemical Quenching Involve Common Conformational Changes within the Photosystem II Antenna in *Arabidopsis*. *Plant Physiol* 149: 1061–1075

Jokel M, Johnson X, Peltier G, Aro EM, Allahverdiyeva Y (2018) Hunting the main player enabling *Chlamydomonas reinhardtii* growth under fluctuating light. *Plant J* 94: 822–835

Jokel M, Kosourov S, Battchikova N, Tsygankov AA, Aro EM, Allahverdiyeva Y (2015) *Chlamydomonas* flavodiiron proteins facilitate acclimation to anoxia during sulfur deprivation. *Plant Cell Physiol* 56: 1598–1607

Joliot P (2003) Period-four oscillations of the flash-induced oxygen formation in photosynthesis. *Photosynth Res* 76: 65–72

Jordan P, Fromme P, Witt HT, Klukas O, Saenger W, Krauß N (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. *Nature* 411: 909–917

Jumper CC, Rafiq S, Wang S, Scholes GD (2018) From coherent to vibronic light harvesting in photosynthesis. *Curr Opin Chem Biol* 47: 39–46

Kalaji HM, Schansker G, Brestic M, Bussotti F, Calatayud A, Ferroni L, Goltsev V, Guidi L, Jajoo A, Li P, Losciale P, Mishra VK, Misra AN, Nebauer SG, Pancaldi S, Penella C, Pollastrini M, Suresh K, Tambussi E, Yanniccari M, Zivcak M, Cetner MD, Samborska IA, Stirbet A, Olsovska K, Kunderlikova K, Shelonzek H, Rusinowski S, Bąba W (2017) Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynth Res* 132: 13–66

Kalaji HM, Schansker G, Ladle RJ, Goltsev V, Bosa K, Allakhverdiev SI, Brestic M, Bussotti F, Calatayud A, Dąbrowski P, Elsheery NI, Ferroni L, Guidi L, Hogewoning SW, Jajoo A, Misra AN, Nebauer SG, Pancaldi S, Penella C, Poli DB, Pollastrini M, Romanowska-Duda ZB, Rutkowska B, Serôdio J, Suresh K, Szulc W, Tambussi E, Yanniccari M, Zivcak M (2014) Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynth Res* 122: 121–158

Kale R, Hebert AE, Frankel LK, Sallans L, Bricker TM, Pospíšil P (2017) Amino acid oxidation of the D1 and D2 proteins by oxygen radicals during photoinhibition of Photosystem II. *Proc Natl Acad Sci USA* 114: 2988–2993

Kato Y, Sakamoto W (2014) Phosphorylation of photosystem II core proteins prevents undesirable cleavage of D1 and contributes to the fine-tuned repair of photosystem II. *Plant J* 79: 312–321

Kato Y, Sakamoto W (2018) FtsH Protease in the Thylakoid Membrane: Physiological Functions and the Regulation of Protease Activity. *Front Plant Sci* 9: 855

Kern J, Chatterjee R, Young ID, Fuller FD, Lassalle L, Ibrahim M, Gul S, Fransson T, Brewster AS, Alonso-Mori R, Hussein R, Zhang M, Douthit L, de Lichtenberg C, Cheah MH, Shevela D, Wersig J, Seuffert I, Sokaras D, Pastor E, Weninger C, Kroll T, Sierra RG, Aller P, Butryn A, Orville AM, Liang M, Batyuk A, Koglin JE, Carbajo S, Boutet S, Moriarty NW, Holton JM, Dobbek H, Adams PD, Bergmann U, Sauter NK, Zouni A, Messinger J, Yano J, Yachandra VK (2018) Structures of the intermediates of Kok's photosynthetic water oxidation clock. *Nature* 563: 421–425

Kerney R, Kim E, Hangarter RP, Heiss AA, Bishop CD, Hall BK (2011) Intracellular invasion of green algae in a salamander host. *Proc Natl Acad Sci USA* 108: 6497–6502

Khorobrykh S, Havurinne V, Mattila H, Tyystjärvi E (2020) Oxygen and ROS in Photosynthesis. *Plants* 9: 91

Klughammer C, Schreiber U (2016) Deconvolution of ferredoxin, Plastocyanin, and P700 transmittance changes in intact leaves with a new type of kinetic LED array spectrophotometer. *Photosynth Res* 128: 195–214

Klughammer C, Siebke K, Schreiber U (2013) Continuous ECS-indicated recording of the proton-motive charge flux in leaves. *Photosynth Res* 117: 471–487

Kojima K, Motohashi K, Morota T, Oshita M, Hisabori T, Hayashi H, Nishiyama Y (2009) Regulation of Translation by the Redox State of Elongation Factor G in the Cyanobacterium *Synechocystis* sp. PCC 6803. *J Biol Chem* 284: 18685–18691

Kok B, Forbush B, McGloin M (1970) Cooperation of charges in photosynthetic O_2 evolution. I. A linear four step mechanism. *Photochem Photobiol* 11: 457–475

Kozuleva M, Ivanov B (2010) Evaluation of the participation of ferredoxin in oxygen reduction in the photosynthetic electron transport chain of isolated pea thylakoids. *Photosynth Res* 105: 51–61

Kozuleva M, Ivanov B (2016) The Mechanisms of Oxygen Reduction in the Terminal Reducing Segment of the Chloroplast Photosynthetic Electron Transport Chain. *Plant Cell Physiol* 57: 1397–1404

Kozuleva M, Petrova A, Milrad Y, Semenov A, Ivanov B, Redding KE, Yacoby I (2021) Phylloquinone is the principal Mehler reaction site within photosystem I in high light. *Plant Physiol* 186: 1848–1858

Kramarenko GG, Hummel SG, Martin SM, Buettner GR (2006) Ascorbate reacts with singlet oxygen to produce hydrogen peroxide. *Photochem Photobiol* 82: 1634–1637

Kramer D, Evans JR (2010) The Importance of Energy Balance in Improving Photosynthetic Productivity. *Plant Physiol* 155: 70–78

Kramer DM, Cruz JA, Kanazawa A (2003) Balancing the central roles of the thylakoid proton gradient. *Trends Plant Sci* 8: 27–32

Kramer DM, Di Marco G, Loreto F (1995) Contribution of plastoquinone quenching to saturation pulse-induced rise of chlorophyll fluorescence in leaves. In: Mathis P (ed) Photosynthesis: From Light to Biosphere, Kluwer Academic Publishers, Dordrecht, pp 147–150

Krasnovsky AA (1994) Singlet molecular oxygen and primary mechanisms of photo-oxidative damage of chloroplasts. Studies based on detection of oxygen and pigment phosphorescence. *Proc R Soc Edinb* 102: 219–235

Krause GH, Jahns P (2004) Non-photochemical energy dissipation determined by chlorophyll fluorescence quenching: characterization and function. In: GC Papageorgiou, Govindjee (eds) Chlorophyll a Fluorescence: A Signature of Photosynthesis, Kluwer Academic Publishers, Dordrecht, pp 463–495

Kress E, Jahns P (2017) The Dynamics of Energy Dissipation and Xanthophyll Conversion in *Arabidopsis* Indicate an Indirect Photoprotective Role of Zeaxanthin in Slowly Inducible and Relaxing Components of Non-photochemical Quenching of Excitation Energy. *Front Plant Sci* doi: 10.3389/fpls.2017.02094

Krieger-Liszkay A (2005) Singlet oxygen production in photosynthesis. *J Exp Bot* 56: 337–346

Krieger-Liszkay A, Trebst A (2006) Tocopherol is the scavenger of singlet oxygen produced by the triplet states of chlorophyll in the PSII reaction centre. J Exp Bot 57: 1677–1684

Krishna PS, Morello G, Mamedov F (2019) Characterization of the transient fluorescence wave phenomenon that occurs during H_2 production in *Chlamydomonas reinhardtii*. *J Exp Bot* 70: 6321–6336

Krug PJ, Vendetti JE, Valdés Á (2016) Molecular and morphological systematics of *Elysia* Risso, 1818 (Heterobranchia: Sacoglossa) from the Caribbean region. *Zootaxa* 4148: 1–137

Kruk J, Jemiola-Rzeminska M, Burda K, Schmid G, Strzalka K (2003) Scavenging of superoxide generated in photosystem I by plastoquinol and other prenyllipids in thylakoid membranes. *Biochemistry* 42: 8501–8505

Kruk J, Karpinski S (2006) An HPLC-based method of estimation of the total redox state of plastoquinone in chloroplasts, the size of the photochemically active plastoquinone-pool and its redox state in thylakoids of *Arabidopsis*. *Biochim Biophys Acta* 1757: 1669–1675

Kühlbrandt W, Wang DN, Fujiyoshi Y (1994) Atomic model of plant light-harvesting complex by electron crystallography. *Nature* 367: 614–621

Küken A, Sommer F, Yaneva-Roder L, Mackinder LCM, Höhne M, Geimer S, Jonikas MC, Schroda M, Stitt M, Nikoloski Z, Mettler-Altmann T (2018) Effects of microcompartmentation on flux distribution and metabolic pools in *Chlamydomonas reinhardtii* chloroplasts. *eLife* 7: e37960

Laetz EMJ, Rühr PT, Bartolomaeus T, Preisfeld A, Wägele H (2017) Examining the retention of functional kleptoplasts and digestive activity in sacoglossan sea slugs. *Org Divers Evol* 17: 87–99

Laetz EMJ, Wägele H (2017) Chloroplast digestion and the development of functional kleptoplasty in juvenile *Elysia timida* (Risso, 1818) as compared to short-term and non-chloroplast-retaining sacoglossan slugs. *PLoS One* 12: e0182910

Lavaud J, Materna AC, Sturm S, Vugrinec S, Kroth PG (2012) Silencing of the Violaxanthin De-Epoxidase Gene in the Diatom *Phaeodactylum tricornutum* Reduces Diatoxanthin Synthesis and Non-Photochemical Quenching. *PLoS One* 7: e36806

Lepetit B, Goss R, Jakob T, Wilhelm C (2012) Molecular dynamics of the diatom thylakoid membrane under different light conditions. *Photosynth Res* 111: 245–257

Levitan O, Chen M, Kuang X, Cheong KY, Jiang J, Banal M, Nambiar N, Gorbunov MY, Ludtke SJ, Falkowski PG, Dai W (2019) Structural and functional analyses of photosystem II in the marine diatom *Phaeodactylum tricornutum*. *Proc Natl Acad Sci USA* 116: 17316–17322

Li G, Woroch AD, Donaher NA, Cockshutt AM, Campbell DA (2016a) A Hard Day's Night: Diatoms Continue Recycling Photosystem II in the Dark. *Front Mar Sci* 3: 218

Li X, Cai C, Wang Z, Fan B, Zhu C, Chen Z (2018) Plastid Translation Elongation Factor Tu Is Prone to Heat-Induced Aggregation Despite Its Critical Role in Plant Heat Tolerance. *Plant Physiol* 176: 3027–3045

Li Z, Peers G, Dent RM, Bai Y, Yang SY, Apel W, Leonelli L, Niyogi KK (2016b): Evolution of an atypical de-epoxidase for photoprotection in the green lineage. *Nat Plants* 2: 16140

Lichtenthaler HK, Buschmann C, Döll M, Fietz HJ, Bach T, Kozel U, Meier D, Rahmsdorf U (1981) Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynth Res* 2: 115–141

Lima-Melo Y, Alencar VTCB, Lobo AKM, Sousa RHV, Tikkanen M, Aro EM, Silveira JAG, Gollan PJ (2019) Photoinhibition of Photosystem I Provides Oxidative Protection During Imbalanced Photosynthetic Electron Transport in *Arabidopsis thaliana*. *Front Plant Sci* 10: 916

Lindahl M, Spetea C, Hundal T, Oppenheim AB, Adam Z, Andersson B (2000) The thylakoid FtsH protease plays a role in the light-induced turnover of the photosystem II D1 protein. *Plant Cell* 12: 419–431

Longoni P, Douchi D, Cariti F, Fucile G, Goldschmidt-Clermont M (2015) Phosphorylation of the Light-Harvesting Complex II Isoform Lhcb2 Is Central to State Transitions. *Plant Physiol* 169: 2874–2883

Maeda T, Takahashi S, Yoshida T, Shimamura S, Takaki Y, Nagai Y, Toyoda A, Suzuki Y, Arimoto A (2021) Chloroplast acquisition without the gene transfer in kleptoplastic sea slugs, *Plakobranchus ocellatus*. *eLife* 10: e60176

Makino A, Miyake C, Yokota A (2002) Physiological Functions of the Water–Water Cycle (Mehler Reaction) and the Cyclic Electron Flow around PSI in Rice Leaves. *Plant Cell Physiol* 43: 1017–1026

Malnoë A (2018) Photoinhibition or photoprotection of photosynthesis? Update on the (newly termed) sustained quenching component qH. *Environ Exp Bot* 154: 123–133

Malnoë A, Schultink A, Shahrasbi S, Rumeau D, Havaux M, Niyogi KK (2018) The Plastid Lipocalin LCNP Is Required for Sustained Photoprotective Energy Dissipation in *Arabidopsis*. *Plant Cell* 30: 196–208

Malnoë A, Wang F, Girard-Bascou J, Wollman FA, de Vitry C (2014) Thylakoid FtsH Protease Contributes to Photosystem II and Cytochrome *b6f* Remodeling in *Chlamydomonas reinhardtii* under Stress Conditions. *Plant Cell* 26: 373–390

Malone LA, Proctor MS, Hitchcock A, Hunter CN, Johnson MP (2021) Cytochrome *b6f* – Orchestrator of photosynthetic electron transfer. *Biochim Biophys Acta Bioener* 1862: 148380

Malone LA, Qian P, Mayneord GE, Hitchcock A, Farmer DA, Thompson RF, Swainsbury DJK, Ranson NA, Hunter CN, Johnson MP (2019) Cryo-EM structure of the spinach cytochrome *b6f* complex at 36 Å resolution. *Nature* 575: 535–539

Mamedov M, Govindjee, Nadtochenko V, Semenov A (2015) Primary electron transfer processes in photosynthetic reaction centers from oxygenic organisms. *Photosynth Res* 125: 51–63

Mandal M, Kawashima K, Saito K, Ishikita H (2020) Redox Potential of the Oxygen-Evolving Complex in the Electron Transfer Cascade of Photosystem II. *J Phys Chem Lett* 11: 249–255

Marín A, Ros JD (1992) Dynamics of a peculiar plant-herbivore relationship: the photosynthetic ascoglossan *Elysia timida* and the chlorophycean *Acetabularia acetabulum*. *Mar Biol* 112: 677–682

Marín A, Ros JD (1993) Ultrastructural and ecological aspects of the development of chloroplast retention in the sacoglossan gastropod *Elysia timida*. *J Moll Stud* 59: 95-104

Martin R, Walther P, Tomaschko KH (2013) Phagocytosis of algal chloroplasts by digestive gland cells in the photosynthesis-capable slug Elysia timida (Mollusca, Opisthobranchia, Sacoglossa). *Zoomorphology* 132: 253–259

Matsubara S, Chow WS (2004) Populations of photoinactivated photosystem II reaction centers characterized by chlorophyll a fluorescence lifetime *in vivo*. *Proc Natl Acad Sci USA* 101: 18234–18239

Mattila H, Khorobrykh S, Hakala-Yatkin M, Havurinne V, Kuusisto I, Antal T, Tyystjärvi T, Tyystjärvi E (2020) Action spectrum of the redox state of the plastoquinone pool defines its function in plant acclimation. *Plant J* 104: 1088–1104

Mattila H, Khorobrykh S, Havurinne V, Tyystjärvi E (2015) Reactive oxygen species: Reactions and detection from photosynthetic tissues. *J Photochem Photobiol B Biol* 152: 176–214

Mehler AH (1951) Studies on reactions of illuminated chloroplasts: I. Mechanism of the reduction of oxygen and other hill reagents. *Arch Biochem Biophys* 33: 65–77

Melo Clavijo J, Frankenbach S, Fidalgo C, Serôdio J, Donath A, Preisfeld A, Christa G (2020) Identification of scavenger receptors and thrombospondin-type-1 repeat proteins potentially relevant for plastid recognition in Sacoglossa. *Ecol Evol* 10: 12348–12363

Memon AR, Meng B, Mullet JE (1996) RNA-binding proteins of 37/38 kDa bind specifically to the barley chloroplast psbA3′-end untranslated RNA. *Plant Mol Biol* 30: 1195–1205

Merchant S, Hill K, Howe G (1991) Dynamic interplay between two copper-titrating components in the transcriptional regulation of cyt *c6*. *EMBO J* 10: 1383–1389

Michel G, Tonon T, Scornet D, Cock JM, 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 Phytol* 188: 67–81

Michelet L, Zaffagnini M, Morisse S, Sparla F, Pérez-Pérez ME, Francia F, Danon A, Marchand CH, Fermani S, Trost P, Lemaire SD (2013) Redox regulation of the Calvin–Benson cycle: something old, something new. *Front Plant Sci* 4: 470

Michiels N, Newman L (1998) Sex and violence in hermaphrodites. *Nature* 391: 647

Middlebrooks ML, Pierce SK, Bell SS (2011) Foraging behavior under starvation conditions is altered via photosynthesis by the marine gastropod, *Elysia clarki*. *PLoS One* 6: e22162

Minnhagen S, Carvalho WF, Salomon PS, Janson S (2008) Chloroplast DNA content in *Dinophysis (Dinophyceae)* from different cell cycle stages is consistent with kleptoplasty. *Environ Microbiol* 10: 2411–2417

Mishra LS, Mielke K, Wagner R, Funk C (2019) Reduced expression of the proteolytically inactive FtsH members has impacts on the Darwinian fitness of *Arabidopsis thaliana*. J Exp Bot 70: 2173–2184

Mitchell P (1975) The protonmotive Q cycle: a general formulation. *FEBS Lett* 59: 137–139

Mizuta Y, Masumizu T, Kohno M, Mori A, Packer L (1997) Kinetic analysis of the Fenton reaction by ESR-spin trapping. *IUBMB Life* 43: 1107–1120

Moldavski O, Levin-Kravets O, Ziv T, Adam Z, Prag G (2012) The Hetero-Hexameric Nature of a Chloroplast AAA+ FtsH Protease Contributes to Its Thermodynamic Stability. *PLoS One* 7: e36008

Mosebach L, Heilmann C, Mutoh R, Gäbelein P, Steinbeck J, Happe T, Ikegami T, Hanke G, Kurisu G, Hippler M (2017) Association of Ferredoxin:$NADP^+$ oxidoreductase with the photosynthetic apparatus modulates electron transfer in *Chlamydomonas reinhardtii*. *Photosynth Res* 134: 291–306

Muhlbauer SK, Eichacker LA (1998) Light-dependent formation of the photosynthetic proton gradient regulates translation elongation in chloroplasts. *J Biol Chem* 273: 20935–20940

Mujer CV, Andrews DL, Manhart JR, Pierce SK, Rumpho ME (1996) Chloroplast genes are expressed during intracellular symbiotic association of *Vaucheria litorea* plastids with the sea slug *Elysia chlorotica*. *Proc Natl Acad Sci USA* 93: 12333–1238

Müller MG, Niklas J, Lubitz W, Holzwarth AR (2003) Ultrafast Transient Absorption Studies on Photosystem I Reaction Centers from *Chlamydomonas reinhardtii*. 1. A New Interpretation of the Energy Trapping and Early Electron Transfer Steps in Photosystem I. *Biophys J* 85: 3899–3922

Müller MG, Slavov C, Luthra R, Redding KE, Holzwarth AR (2010) Independent initiation of primary electron transfer in the two branches of the photosystem I reaction center. *Proc Natl Acad Sci USA* 107: 4123–4128

Müller P, Li X, Niyogi KK (2001) Non-Photochemical Quenching. A Response to Excess Light Energy. *Plant Physiol* 125: 1558–1566

Mulo P, Pursiheimo S, Hou CX, Tyystjärvi T, Aro EM (2003) Multiple effects of antibiotics on chloroplast and nuclear gene expression. *Funct Plant Biol* 30: 1097–1103

Mulo P, Sakurai I, Aro EM (2012) Strategies for psbA gene expression in cyanobacteria, green algae and higher plants: From transcription to PSII repair. *Biochim Biophys Acta Bioener* 1817: 247–257

Mulo P, Sirpiö S, Suorsa M, Aro EM (2008) Auxiliary proteins involved in the assembly and sustenance of photosystem II. *Photosynth Res* 98: 489–501

Munekage Y, Hojo M, Meurer J, Endo T, Tasaka M, Shikanai T (2008) PGR5 Is Involved in Cyclic Electron Flow around Photosystem I and Is Essential for Photoprotection in *Arabidopsis*. *Cell* 110: 361–371

Nadtochenko VA, Semenov AY, Shuvalov VA (2014) Formation and decay of P680 (P_{D1}–P_{D2})$^{+}$$Pheo_{D1}^{-}$ radical ion pair in photosystem II core complexes. *Biochim Biophys Acta Bioenerg* 1837: 1384–1388

Nagao R, Kato K, Suzuki T, Ifuku K, Uchiyama I, Kashino Y, Dohmae N, Akimoto S, Shen JR, Miyazaki N, Akita F (2019) Structural basis for energy harvesting and dissipation in a diatom PSII–FCPII supercomplex. *Nat Plants* 5: 890–901

Nagao R, Yamaguchi M, Nakamura S, Ueoka-Nakanishi H, Noguchi T (2017) Genetically introduced hydrogen bond interactions reveal an asymmetric charge distribution on the radical cation of the special-pair chlorophyll P680. *J Biol Chem* 292: 7474–7486

Nath K, Poudyal RS, Eom JS, Park YS, Zulfugarov IS, Mishra SR, Tovuu A, Ryoo N, Yoon HS, Nam HG, An G, Jeon JS, Lee CH (2013) Loss-of-function of OsSTN8 suppresses the photosystem II core protein phosphorylation and interferes with the photosystem II repair mechanism in rice (*Oryza sativa*). *Plant J* 76:675–686

Nawrocki WJ, Liu X, Raber B, Hu C, de Vitry C, Bennett DIG, Croce R (2021) Molecular origins of induction and loss of photoinhibition-related energy dissipation qI. *Sci Adv* 7: eabj0055

Nikkanen L, Solymosi D, Jokel M, Allahverdiyeva Y (2021) Regulatory electron transport pathways of photosynthesis in cyanobacteria and microalgae: Recent advances and biotechnological prospects. *Physiol Plant* 173: 514–525

Nikkanen L, Toivola J, Guinea Diaz M, Rintamäki E (2017) Chloroplast thioredoxin systems: prospects for improving photosynthesis. *Phil Trans R Soc B* 372: 20160474

Nilkens M, Kress E, Lambrev P, Miloslavina Y, Müller M, Holzwarth AR, 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*. *Biochim Biophys Acta* 1797: 466–475

Nilsson R, van Wijk KJ (2002) Transient interaction of cpSRP54 with elongating nascent chains of the chloroplast-encoded D1 protein; 'cpSRP54 caught in the act'. *FEBS Lett* 524: 127–133

Nishiyama Y, Allakhverdiev SI, Murata N (2006) A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochim Biophys Acta* 1757: 742–749

Nishiyama Y, Allakhverdiev SI, Yamamoto H, Hayashi H, Murata N (2004) Singlet oxygen inhibits the repair of photosystem II by suppressing translation elongation of the D1 protein in *Synechocystis* sp. PCC 6803. *Biochemistry* 43: 11321–11330

Nishiyama Y, Murata N (2014) Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery. *Appl Microbiol Biotechnol* 98: 8777–8796

Niyogi KK, Truong TB (2013) Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Curr Opin Plant Biol* 16: 307–314

Noguchi T, Tomo T, Kato C (2001) Triplet Formation on a Monomeric Chlorophyll in the Photosystem II Reaction Center As Studied by Time-Resolved Infrared Spectroscopy. *Biochemistry* 40: 2176–2185

Nordhues A, Schottler MA, Unger AK, Geimer S, Schonfelder S, Schmollinger S, Rutgers M, Finazzi G, Soppa B, Sommer F, Mühlhaus T, Roach T, Krieger-Liszkay A, Lokstein H, Crespo JL, Schroda M (2012) Evidence for a role of VIPP1 in the structural organization of the photosynthetic apparatus in *Chlamydomonas*. *Plant Cell* 24: 637–659

Ohnishi N, Allakhverdiev SI, Takahashi S, Higashi S, Watanabe M, Nishiyama Y, Murata N (2005) Two-Step Mechanism of Photodamage to Photosystem II: Step 1 Occurs at the Oxygen-Evolving Complex and Step 2 Occurs at the Photochemical Reaction Center. *Biochemistry* 44: 8494–8499

Owens TG (1986) Light-Harvesting Function in the Diatom *Phaeodactylum tricornutum*: II. Distribution of Excitation Energy between the Photosystems. *Plant Physiol* 80: 739–746

Park S, Steen CJ, Lyska D, Fischer AL, Endelman B, Iwai M, Niyogi KK, Fleming GR (2019) Chlorophyll–carotenoid excitation energy transfer and charge transfer in *Nannochloropsis oceanica* for the regulation of photosynthesis. *Proc Natl Acad Sci USA* 116: 3385–3390

Pätsikkä E, Kairavuo M, Šeršen F, Aro EM, Tyystjärvi E (2002) Excess copper predisposes Photosystem II to photoinhibition *in vivo* by outcompeting iron and causing decrease in leaf chlorophyll. *Plant Physiol* 129: 1359–1367

Peers G, Price NM (2006) Copper-containing plastocyanin used for electron transport by an oceanic diatom. *Nature* 441: 341–344

Pelletreau KN, Weber APM, Weber KL, Rumpho ME (2014) Lipid Accumulation during the Establishment of Kleptoplasty in *Elysia chlorotica*. *PLoS One* 9: e97477

Pelletreau KN, Worful JM, Sarver KE, Rumpho ME (2012) Laboratory culturing of *Elysia chlorotica* reveals a shift from transient to permanent kleptoplasty. *Symbiosis* 58: 221–232

Perozeni F, Beghini G, Cazzaniga S, Ballottari M (2020) *Chlamydomonas reinhardtii* LHCSR1 and LHCSR3 proteins involved in photoprotective non-photochemical quenching have different quenching efficiency and different carotenoid affinity. *Sci Rep* 10: 21957

Petersen J, Förster K, Turina P, Gräber P (2012) Comparison of the H^+/ATP ratios of the H^+-ATP synthases from yeast and from chloroplast. *Proc Natl Acad Sci USA* 109: 11150–11155

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29: 16–21

Ponce-Toledo RI, López-García P, Moreira D (2019) Horizontal and endosymbiotic gene transfer in early plastid evolution. *New Phytol* 224: 618–624

Porra RJ, Thompson WA, Kriedemann PE (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. *Biochim Biophys Acta* 975: 384–394

Pospíšil P (2016) Production of Reactive Oxygen Species by Photosystem II as a Response to Light and Temperature Stress. *Front Plant Sci* 7: 1950

Pribil M, Pesaresi P, Hertle A, Barbato R, Leister D (2010) Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow. *PLoS Biol* 8: e1000288

Price DC, Chan CX, Yoon HS, Yang EC, Qiu H, Weber APM, Schwacke R, Gross J, Blouin NA, Lane C, Reyes-Prieto A, Durnford DG, Neilson JAD, Lang BF, Burger G, Steiner JM, Löffelhardt W,

Meuser JE, Posewitz MC, Ball S, Arias MC, Henrissat B, Coutinho PM, Rensing SA, Symeonidi A, Doddapaneni H, Green BR, Rajah VD, Boore J, Bhattacharya D (2012) *Cyanophora paradoxa* Genome Elucidates Origin of Photosynthesis in Algae and Plants. *Science* 335: 843–847

Pulido P, Spínola MC, Kirchsteiger K, Guinea M, Pascual MB, Sahrawy M, Sandalio LM, Dietz KJ, González M, Cejudo FJ (2010) Functional analysis of the pathways for 2-Cys peroxiredoxin reduction in *Arabidopsis thaliana* chloroplasts. *J Exp Bot* 61: 4043–4054

Qiu H, Price DC, Weber APM, Facchinelli F, Yoon HS, Bhattacharya D (2013) Assessing the bacterial contribution to the plastid proteome. *Trends Plant Sci* 18: 680–687

Rahat M, Monselise EBI (1978) Photobiology of the chloroplast hosting mollusc *Elysia timida* (Opistobranchia). *J Exp Biol* 79: 225–233

Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylidès C, Havaux M (2012) Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. *Proc Natl Acad Sci USA* 109: 5535–5540

Rapp JC, Baumgartner BJ, Mullet J (1992) Quantitative analysis of transcription and RNA levels of 15 barley chloroplast genes. Transcription rates and mRNA levels vary over 300-fold; predicted mRNA stabilities vary 30-fold. *J Biol Chem* 267: 21404–21411

Rappaport F, Lavergne J (2009) Thermoluminescence: theory. *Photosynth Res* 101: 205–216

Rauch C, Tielens AGM, Serôdio J, Gould SB, Christa G (2018) The ability to incorporate functional plastids by the sea slug *Elysia viridis* is governed by its food source. *Mar Biol* 165: 82

Redekop P, Rothhausen N, Rothhausen N, Melzer M, Mosebach L, Dülger E, Bovdilova A, Caffarri S, Hippler M, Jahns P (2020) PsbS contributes to photoprotection in *Chlamydomonas reinhardtii* independently of energy dissipation. *Biophys Acta Bioener* 1861: 5–6

Rehman AU, Cser K, Sass L, Vass I (2013) Characterization of singlet oxygen production and its involvement in photodamage of Photosystem II in the cyanobacterium *Synechocystis* PCC 6803 by histidine-mediated chemical trapping. *Biochim Biophys Acta* 1827: 689–698

Reiss K, Morzan UN, Grigas AT, Batista VS (2019) Water Network Dynamics Next to the Oxygen-Evolving Complex of Photosystem II. *Inorganics* 7: 39

Rintamäki E, Kettunen R, Aro EM (1996) Differential D1 dephosphorylation in functional and photodamaged photosystem II centers. Dephosphorylation is a prerequisite for degradation of damaged D1. *J Biol Chem* 271: 14870–14875

Ristic Z, Wilson K, Nelsen C, Momcilović, I, Kobayashi S, Meeley R, Muszynski M, Habben J (2004) A maize mutant with decreased capacity to accumulate chloroplast protein synthesis elongation factor (EF-Tu) displays reduced tolerance to heat stress. *Plant Sci* 167: 1367–1374

Rochaix JD (2011) Regulation of photosynthetic electron transport. *Biochim Biophys Acta Bioener* 1807: 375–383

Rodrigues RAO, Silva-Filho MC, Cline K (2011) FtsH2 and FtsH5: two homologous subunits use different integration mechanisms leading to the same thylakoid multimeric complex. *Plant J* 65: 600–609

Romero E, Novoderezhkin VI, van Grondelle R (2017) Quantum design of photosynthesis for bio-inspired solar-energy conversion. *Nature* 543: 355–365

Roose JL, Pakrasi HB (2008) The Psb27 protein facilitates manganese cluster assembly in photosystem II. *J Biol Chem* 283: 4044–4050

Ruban AV (2016) Nonphotochemical chlorophyll fluorescence quenching: Mechanism and effectiveness in protecting plants from photodamage. *Plant Physiol* 170: 1903–1916

Rumpho ME, Pelletreau KN, Moustafa A, Bhattacharya D (2011) The making of a photosynthetic animal. *J Exp Biol* 214: 303–311

Rumpho ME, Summer EJ, Green BJ, Fox TC, Manhart JR (2001) Mollusc/algal chloroplast symbiosis: how can isolated chloroplasts continue to function for months in the cytosol of a sea slug in the absence of an algal nucleus? *Zoology* 104: 303–312

Rumpho ME, Summer EJ, Manhart JR (2000) Solar-Powered Sea Slugs. Mollusc/Algal Chloroplast Symbiosis. *Plant Physiol* 123: 29–38

Rumpho ME, Worful JM, Lee J, Kannan K, Tyler MS, Bhattacharya D, Moustafa A, Manhart JR (2008) Horizontal gene transfer of the algal nuclear gene *psbO* to the photosynthetic sea slug *Elysia chlorotica*. *Proc Natl Acad Sci USA* 105: 17867–17871

Rutgers M, Schroda M (2013) A role of VIPP1 as a dynamic structure within thylakoid centers as sites of photosystem biogenesis? *Plant Signal Behav* 8: e27037

Santabarbara S, Bullock B, Rappaport F, Redding KE (2015) Controlling Electron Transfer between the Two Cofactor Chains of Photosystem I by the Redox State of One of Their Components. *Biophys J* 108: 1537–1547

Santabarbara S, Heathcote P, Evans MCW (2005) Modelling of the electron transfer reactions in Photosystem I by electron tunnelling theory: The phylloquinones bound to the PsaA and the PsaB reaction centre subunits of PS I are almost isoenergetic to the iron-sulfur cluster F_X. *Biochim Biophys Acta* 1708: 283–310

Santana-Sanchez A, Solymosi D, Mustila H, Bersanini L, Aro EM, Allahverdiyeva Y (2019) Flavodiiron proteins 1-to-4 function in versatile combinations in O_2 photoreduction in cyanobacteria. *eLife* 8: e45766

Satoh K, Yamamoto Y (2007) The carboxyl-terminal processing of precursor D1 protein of the photosystem II reaction center. *Photosynth Res* 94: 203–215

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9: 676–682

Schmitt V, Anthes N, Michiels NK (2007) Mating behaviour in the sea slug *Elysia timida* (Opisthobranchia, Sacoglossa): hypodermic injection, sperm transfer and balanced reciprocity. *Front Zool* 4: 17

Schmitt V, Händeler K, Gunkel S, Escande ML, Menzel D, Gould SB, Martin WF, Wägele H (2014) Chloroplast incorporation and long-term photosynthetic performance through the life cycle in laboratory cultures of *Elysia timida* (Sacoglossa, Heterobranchia). *Front Zool* 11: 5

Schreiber U, Klughammer C (2008a) Saturation pulse method for assessment of energy conversion in PSI. *PAM Application Notes* 1: 11–14

Schreiber U, Klughammer C (2008b) New accessory for the DUAL-PAM-100: the P515/535 module and examples of its application. *PAM Application Notes* 1: 1–10

Schreier TB, Hibberd JM (2019) Variations in the Calvin–Benson cycle: selection pressures and optimization? J Exp Bot 70: 1697–1701

Seelert H, Poetsch A, Dencher NA, Engel A, Stahlberg H, Müller DJ (2000) Structural biology. Proton-powered turbine of a plant motor. *Nature* 405:418–419

Şener M, Strümpfer J, Hsin J, Chandler D, Scheuring S, Hunter CN, Schulten K (2011). Förster energy transfer theory as reflected in the structures of photosynthetic light-harvesting systems. *Chemphyschem* 12: 518–531

Serôdio J, Campbell DA (2021) Photoinhibition in optically thick samples: effects of light attenuation on chlorophyll fluorescence-based parameters. *J Theor Biol* 513: 110580

Serôdio J, Pereira S, Furtado J, Silva R, Coelho H, Calado R (2010) *In vivo* quantification of kleptoplastic chlorophyll a content in the "solar-powered" sea slug *Elysia viridis* using optical methods: spectral reflectance analysis and PAM fluorometry. *Photochem Photobiol Sci* 9: 68–77

Sheiner L, Striepen B (2013) Protein sorting in complex plastids. *Biochim Biophys Acta* 1833: 352–359

Shen L, Huang Z, Chang S, Wang W, Wang J, Kuang T, Han G, Shen JR, Zhang X (2019) Structure of a C2S2M2N2-type PSII–LHCII supercomplex from the green alga *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci USA* 116: 21246–21255

Shikanai T, Endo T, Hashimoto T, Yamada Y, Asada K, Yokota A (1998) Directed disruption of the tobacco *ndhB* gene impairs cyclic electron flow around photosystem I. *Proc Natl Acad Sci USA* 95: 9705–9709

Shimakawa G, Miyake C (2018) Oxidation of P700 Ensures Robust Photosynthesis. *Front Plant Sci* 9: 1617

Shimakawa G, Murakami A, Niwa K, Matsuda Y, Wada A, Miyake C (2019) Comparative analysis of strategies to prepare electron sinks in aquatic photoautotrophs. *Photosynth Res* 139: 401–411

Shimakawa G, Shoguchi E, Burlacot A, Ifuku K, Che Y, Kumazawa M, Tanaka K, Nakanishi S (2021) Coral symbionts evolved a functional polycistronic flavodiiron gene. *Photosynth Res* doi: 10.1007/s11120-021-00867-7

Shiroyama H, Mitoh S, Ida TY, Yusa Y (2020) Adaptive significance of light and food for a kleptoplastic sea slug: implications for photosynthesis. *Oecologia* 194: 455–463

Shuvalov VA, Yakovlev AG, Vasilieva LG, Shkuropatov AY (2007) Primary charge separation between P700* and the primary electron acceptor complex A-A_O: A comparison with bacterial reaction centers. In: Golbeck JH (ed) Photosystem I, Springer, Dordrecht, pp 291–300

Siaut M, Cuiné S, Cagnon C, Fessler B, Nguyen M, Carrier P, Beyly A, Beisson F, Triantaphylidès C, Li-Beisson Y, Peltier G (2011) Oil accumulation in the model green alga *Chlamydomonas reinhardtii*: characterization, variability between common laboratory strains and relationship with starch reserves. *BMC Biotechnol* 11: 7

Simm S, Keller M, Selymesi M, Schleiff E (2015) The composition of the global and feature specific cyanobacterial core-genomes. *Front Microbiol* 6: 219

Sinha RP, Klisch M, Gröniger A, Häder DP (1998) Ultraviolet-absorbing/screening substances in cyanobacteria, phytoplankton and macroalgae. *J Photochem Photobiol B Biol* 47: 83–94

Sipka G, Magyar M, Mezzetti A, Akhtar P, Zhu Q, Xiao Y, Han G, Santabarbara S, Shen JR, Lambrev PH, Garab G (2021) Light-Adapted Charge-Separated State of Photosystem II: Structural and Functional Dynamics of the Closed Reaction Center. *Plant Cell* 33: 1286–1302

Šnyrychová I, Pospíšil P, Nauš J (2006) Reaction pathways involved in the production of hydroxyl radicals in thylakoid membrane: EPR spin-trapping study. *Photochem Photobiol Sci* 5: 472-476

Sonoike K (2011) Photoinhibition of photosystem I. *Physiol Plant* 142: 56–64

Sonoike K, Terashima I, Iwaki M, Itoh S (1995) Destruction of photosystem I iron-sulfur centers in leaves of *Cucumis sativus* L. by weak illumination at chilling temperatures. *FEBS Lett* 362: 235–238

Stamatakis K, Vayenos D, Kotakis C, Gast RJ, Papageorgiou GC (2016) The extraordinary longevity of kleptoplasts derived from the Ross Sea haptophyte *Phaeocystis antarctica* within dinoflagellate host cells relates to the diminished role of the oxygen-evolving Photosystem II and to supplementary light harvesting by mycosporine-like amino acids. *Biochim Biophys Acta Bioener* 1858: 189–195

Steigmiller S, Turina P, Gräber P (2008) The thermodynamic H^+/ATP ratios of the H^+-ATPsynthases from chloroplasts and *Escherichia coli*. *Proc Natl Acad Sci USA* 105: 3745–3750

Stephens TG, Gabr A, Calatrava V, Grossman AR, Bhattacharya D (2021) Why is primary endosymbiosis so rare? *New Phytol* 231: 1693–1699

Stoecker DK, Johnson MD, de Vargas C, Not F (2009) Acquired phototrophy in aquatic protists. *Aquat Microb Ecol* 57: 279–310

Stroebel D, Choquet Y, Popot JL, Picot D (2003) An atypical haem in the cytochrome *b6f* complex. *Nature* 426: 413–418

Su X, Ma J, Wei X, Cao P, Zhu D, Chang W, Liu Z, Zhang X, Li M (2017) Structure and assembly mechanism of plant C2S2M2-type PSII-LHCII supercomplex. *Science* 357: 815–820

Suga M, Ozawa SI, 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. *Nat Plants* 5: 626–636

Sumi H (1999) Theory on Rates of Excitation-Energy Transfer between Molecular Aggregates through Distributed Transition Dipoles with Application to the Antenna System in Bacterial Photosynthesis. *J Phys Chem B* 103: 252–260

Sun Y, Zhang N, Zhou J, Dong S, Zhang X, Guo L, Guo G (2020) Distribution, Contents, and Types of Mycosporine-Like Amino Acids (MAAs) in Marine Macroalgae and a Database for MAAs Based on These Characteristics. *Mar Drugs* 18: 43

Takahashi M, Asada K (1988) Superoxide production in aprotic interior of chloroplast thylakoids. *Arch Biochem Biophys* 267: 714–722

Telfer A, Bishop SM, Phillips D, Barber J (1994) Isolated photosynthetic reaction center of Photosystem II as a sensitizer for the formation of singlet oxygen. Detection and quantum yield determination using a chemical trapping technique. J Biol Chem 269: 13244–13253

Terashima I, Funayama S, Sonoike K (1994) The site of photoinhibition in leaves of *Cucumis sativus* L. at low temperature is photosystem I, not photosystem II. *Planta* 193: 300–306

Theis J, Lang J, Spaniol B, Ferté S, Niemeyer J, Sommer F, Zimmer D, Venn B, Mehr SF, Mühlhaus T, Wollman FA, Schroda M (2019) The *Chlamydomonas deg1c* Mutant Accumulates Proteins Involved in High Light Acclimation. *Plant Physiol* 181: 1480–1497

Theis J, Schroda M (2016) Revisiting the photosystem II repair cycle. *Plant Signal Behav* 11: e1218587

Tian L, Nawrocki WJ, Liu X, Polukhina I, van Stokkum IHM, Croce R (2019) pH dependence, kinetics and light-harvesting regulation of nonphotochemical quenching in *Chlamydomonas*. *Proc Natl Acad Sci USA* 116: 8320–8325

Tikhonov AN (2014) The cytochrome *b6f* complex at the crossroad of photosynthetic electron transport pathways. *Plant Physiol Biochem* 81: 163–183

Tikkanen M, Nurmi M, Kangasjärvi S, Aro EM (2008) Core protein phosphorylation facilitates the repair of photodamaged photosystem II at high light. *Biochim Biophys Acta* 1777: 1432–1437

Tiwari A, Mamedov F, Grieco M, Suorsa M, Jajoo A, Styring S, Tikkanen M, Aro EM (2016) Photodamage of iron–sulphur clusters in photosystem I induces non-photochemical energy dissipation. *Nat Plants* 2: 16035

Tokutsu R, Kato N, Bui KH, Ishikawa T, Minagawa J (2012) Revisiting the Supramolecular Organization of Photosystem II in *Chlamydomonas reinhardtii*. *J Biol Chem* 287: 31574–31581

Tomečková L, Tomčala A, Oborník M, Hampl V (2020) The Lipid Composition of *Euglena gracilis* Middle Plastid Membrane Resembles That of Primary Plastid Envelopes. *Plant Physiol* 184: 2052–2063

Torrado A, Ramírez-Moncayo C, Navarro JA, Mariscal V, Molina-Heredia FP (2019) Cytochrome *c6* is the main respiratory and photosynthetic soluble electron donor in heterocysts of the cyanobacterium *Anabaena* sp. PCC 7120. *Biochim Biophys Acta Bioener* 1860: 60–68

Torres JP, Lin Z, Winter JM, Krug PJ, Schmidt EW (2020) Animal biosynthesis of complex polyketides in a photosynthetic partnership. *Nat Commun* 11: 2882

Trench RK (1975) Of 'leaves that crawl': functional chloroplasts in animal cells. *Symp Soc Exp Biol* 29 :229–265

Trench RK, Boyle JE, Smith DC (1973a) The association between chloroplasts of *Codium fragile* and the mollusc *Elysia viridis* I. Characteristics of isolated *Codium* chloroplasts. *Proc Royal Soc B* 184: 51–61

Trench RK, Boyle JE, Smith DC (1973b) The association between chloroplasts of *Codium fragile* and the mollusc *Elysia viridis* II. Chloroplast ultrastructure and photosynthetic carbon fixation in *E. viridis*. *Proc Royal Soc B* 184: 63–81

Trench RK, Trench ME, Muscatine L (1972) Symbiotic chloroplasts; their photosynthetic products and contribution to mucus synthesis in two marine slugs. *Biol Bull* 142: 335–349

Treves H, Raanan H, Kedem I, Murik O, Keren N, Zer H, Berkowicz SM, Giordano M, Norici A, Shotland Y, Ohad I, Kaplan A (2016) The mechanisms whereby the green alga *Chlorella ohadii*, isolated from desert soil crust, exhibits unparalleled photodamage resistance. *New Phytol* 210: 1229–1243

Triantaphylidès C, Havaux M (2009) Singlet oxygen in plants: Production, detoxification and signaling. *Trends Plant Sci* 14: 219–228

Troiano JM, Perozeni F, Moya R, Zuliani L, Baek K, Jin ES, Cazzaniga S, Ballottari M, Schlau-Cohen GS (2021) Identification of distinct pH- and zeaxanthin-dependent quenching in LHCSR3 from *Chlamydomonas reinhardtii*. *eLife* 10: e60383

Trowbridge CD (2000) The missing links: Larval and post-larval development of the ascoglossan opisthobranch *Elysia viridis*. *J Mar Biol Ass UK* 80: 1087–1094

Tyystjärvi E (2013) Photoinhibition of Photosystem II. *Int Rev Cell Mol Biol* 300: 243–303

Umena Y, Kawakami K, Shen JR, Kamiya N (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* 473: 55–60

Uniacke J, Zerges W (2007) Photosystem II assembly and repair are differentially localized in *Chlamydomonas*. *Plant Cell* 19: 3640–3654

Van Steenkiste NWL, Stephenson I, Herranz M, Husnik F, Keeling PJ, Leander BS (2019) A new case of kleptoplasty in animals: Marine flatworms steal functional plastids from diatoms. *Sci Adv* 5: eaaw4337

Vass I (2011) Role of charge recombination processes in photodamage and photoprotection of the photosystem II complex. *Physiol Plant* 142: 6–16

Vass I, Styring S (1991) pH-dependent charge equilibria between tyrosine-D and the S states in photosystem II. Estimation of relative midpoint redox potentials. *Biochemistry* 30: 830–9

Vecchi V, Barera S, Bassi R, Dall'Osto L (2020) Potential and Challenges of Improving Photosynthesis in Algae. *Plants* 9: 67

Vieira S, Calado R, Coelho H, Serôdio J (2009) Effects of light exposure on the retention of kleptoplastic photosynthetic activity in the sacoglossan mollusc *Elysia viridis*. *Mar Biol* 156: 1007–1020

Vollmar M, Schlieper D, Winn M, Büchner C, Growth G (2009) Structure of the c_{14} rotor ring of the proton translocating chloroplast ATP synthase. *J Biol Chem* 284: 18228–18235

Vorphal MA, Bruna C, Wandersleben T, Dagnino-Leone J, Lobos-González F, Uribe E, Martínez-Oyanedel J, Bunster M (2017) Molecular and functional characterization of ferredoxin NADP(H) oxidoreductase from *Gracilaria chilensis* and its complex with ferredoxin. *Biol Res* 50: 39

Wada S, Yamamoto H, Suzuki Y, Yamori W, Shikanai T, Makino A (2018) Flavodiiron Protein Substitutes for Cyclic Electron Flow without Competing CO_2 Assimilation in Rice. *Plant Physiol* 176: 1509–1518

Wägele H, Martin WF (2014) Endosymbioses in Sacoglossan Sea slugs: Plastid-Bearing Animals that Keep Photosynthetic Organelles Without Borrowing Genes. In: Löffelhardt W (ed) Endosymbiosis, Springer, Vienna, pp 291–324

Wägele H, Stemmer K, Burghardt I, Händeler K (2010) Two new sacoglossan sea slug species (Opisthobranchia, Gastropoda): *Ercolania annelyleorum* sp. nov. (Limapontioidea) and *Elysia asbecki* sp. nov. (Plakobranchoidea), with notes on anatomy, histology and biology. *Zootaxa* 2676: 1–28

Wagner H, Jakob T, Fanesi A, Wilhelm C (2017) Towards an understanding of the molecular regulation of carbon allocation in diatoms: The interaction of energy and carbon allocation. *Philos Trans R Soc Lond B Biol Sci* 372: 1–10

Wagner R, Aigner H, Funk C (2012) FtsH proteases located in the plant chloroplast. *Physiol Plant* 145: 203–214

Walker BJ, Kramer DM, Fisher N, Fu X (2020) Flexibility in the Energy Balancing Network of Photosynthesis Enables Safe Operation under Changing Environmental Conditions. *Plants* 9: 301

Wang F, Qi Y, Malnoë A, Choquet Y, Wollman FA, de Vitry C (2017) The High Light Response and Redox Control of Thylakoid FtsH Protease in *Chlamydomonas reinhardtii*. *Mol Plant* 10: 99–114

Wang W, Yu LJ, Xu C, Tomizaki T, Zhao S, Umena Y, Chen X, Qin X, Xin Y, Suga M, Han G, Kuang T, Shen JR (2019) Structural basis for blue-green light harvesting and energy dissipation in diatoms. *Science* 363: eaav0365

Wauch GR, Clark KB (1986) Seasonal and geographic variation in chlorophyll level of *Elysia tuca* (Ascoglossa: Opisthobranchia). *Mar Biol* 92: 483–487

Wei L, Guo J, Ouyang M, Sun X, Ma J, Chi W, Lu C, Zhang L (2010) LPA19, a Psb27 homolog in *Arabidopsis thaliana*, facilitates D1 protein precursor processing during PSII biogenesis. *J Biol Chem* 285: 21391–21398

Weis E (1985) Chlorophyll fluorescence at 77 K in intact leaves: characterization of a technique to eliminate artifacts related to self-absorption. *Photosynths Res* 6: 73–86

Xu C, Pi X, Huang Y, Han G, Chen X, Qin X, Huang G, Zhao S, Yang Y, Kuang T, Wang W, Sui SF, Shen JR (2020) Structural basis for energy transfer in a huge diatom PSI-FCPI supercomplex. *Nat Commun* 11: 5081

Yaakoubd B, Andersen R, Desjardins Y, Samson G (2002) Contributions of the free oxidized and Q_B-bound plastoquinone molecules to the thermal phase of chlorophyll *a* fluorescence. *Photosynth Res* 74: 251–257

Yamaguchi S, Yusa Y, Iwasa Y (2021) Evolution of life cycle dimorphism: An example of sacoglossan sea slugs. *J Theor Biol* 525: 110760

Yamamoto HY (1979) Biochemistry of the violaxanthin cycle in higher-plants. *Pure Appl Chem* 51: 639–648

Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D (2004) A Molecular Timeline for the Origin of Photosynthetic Eukaryotes. *Mol Biol Evol* 21: 809–818

Yutthanasirikul R, Nagano T, Jimbo H, Hihara Y, Kanamori T, Ueda T, Haruyama T, Konno H, Yoshida K, Hisabori T, Nishiyama Y (2016) Oxidation of a Cysteine Residue in Elongation Factor EF-Tu Reversibly Inhibits Translation in the Cyanobacterium *Synechocystis* sp. PCC 6803. *J Biol Chem* 291: 5860–5870

Zavafer A, Iermak I, Cheah MH, Chow WS (2019) Two Quenchers Formed During Photodamage of Phostosystem II and The Role of One Quencher in Preemptive Photoprotection. *Sci Rep* 9: 17275

Zerulla K, Ludt K, Soppa J (2016) The ploidy level of *Synechocystis* sp. PCC 6803 is highly variable and is influenced by growth phase and by chemical and physical external parameters. *Microbiology* 162: 730–739

Zhang C, Shuai J, Ran Z, Zhao J, Wu Z, Liao R, Wu J, Ma W, Lei M (2020) Structural insights into NDH-1 mediated cyclic electron transfer. *Nat Commun* 11: 888

Zhang L, Paakkarinen V, Suorsa M, Aro EM (2001) A SecY homologue is involved in chloroplast-encoded D1 protein biogenesis. *J Biol Chem* 276: 37809–37814

Zhang P, Eisenhut M, Brandt AM, Carmel D, Silén HM, Vass I, Allahverdiyeva Y, Salminen TA, Aro EM (2012) Operon *flv4-flv2* provides cyanobacterial photosystem II with flexibility of electron transfer. *Plant Cell* 24: 1952–1971

Zhang S, Scheller HV (2004) Photoinhibition of Photosystem I at Chilling Temperature and Subsequent Recovery in *Arabidopsis thaliana*. *Plant Cell Physiol* 45: 1595–1602

Zhang Z, Jia Y, Gao H, Zhang L, Li H, Meng Q (2011) Characterization of PSI recovery after chilling-induced photoinhibition in cucumber (*Cucumis sativus* L.) leaves. *Planta* 234: 883–889

Zito F, Finazzi G, Delosme R, Nitschke W, Picot D, Wollman FA (1999) The Qo site of cytochrome b6f complexes controls the activation of the LHCII kinase. *EMBO J* 18: 2961–2969

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

Painosalama Oy, Turku, Finland 2022

ISBN 978-951-29-8797-9 (PRINT)
ISBN 978-951-29-8798-6 (PDF)
ISSN 0082-7002 (Print)
ISSN 2343-3175 (Online)