Light harvesting in eukaryotic algae

Where light meets life

Radek Litvín

Habilitation thesis

Faculty of Science
University of South Bohemia in České Budějovice
Czech Republic
2024

"In fact, no one who is in the habit of using a prism could suppose for a moment that the two [biliverdin and chlorophyll] *were identical; for an observation which can be made in a few seconds, which requires no apparatus beyond a small prism, to be used with the naked eye, and which as a matter of course would be made by any chemist working at the subject, had the use of the prism made its way into the chemical world, is sufficient to show that chlorophyll and biliverdin are quite distinct."* [1]

George Gabriel Stokes, 1864

Contents

Preface

An atom located in the outer layers of a star transitions into an excited state by collisions with its neighbours. The atom relaxes back to its ground state. During the relaxation, a quantum of energy is emitted into the void around the star. In no time whatsoever, the quantum of energy is absorbed by an electron on a distant planet. This electron is shared among many atoms, bound together into a molecule which cannot exist in the conditions present on the star. The quantum of energy journeys around in the soft tissue where the molecule is located, finally reaching another molecule not far away. The energy is now used to eject an electron from this molecule, creating a very positive hole. Another electron is sourced from nearby to plug the hole. Ultimately, a very small fraction of the original quantum of energy is stored as a new chemical bond formed not by highly energetic events but in very gentle conditions within a fragile living cell. Eons pass, but the chemical bond remains in place. The molecule holding the bond is removed from its long dead maternal cell, modified, transported to the planet's interior and out again by powerful forces acting extremely slowly. The molecule is then released from its underground resting place, violently reacted with a small diatomic gas and the stored energy is finally released, used in a device which did not exist at the beginning of the story, built by short-living creatures which now occupy the planet.

What holds the story together? Where and how was the diatomic gas created? How can something as weak and intangible as light change the fate of a planet, create rocks, even continents? How does light power our lives? How do we use light to understand the story?

The official purpose of this text is to "put the results of the author's work into wider context". While this is technically possible, the text has to be put together in short or, preferably, very short time. Trade-offs abound and decisions have to be made. Some easy to make, some more difficult. This text concerns itself with the process of photosynthesis, as it happens in living organisms. Understanding at least a little bit of the photosynthesis process requires knowledge from biology, chemistry and physics. Some even advocate for the presence of exotic quantum effects in parts of the photosynthetic process. In wobbly, slimy, wet, weak, soft, living tissues. Odd. Regardless, even without advanced quantum physics topics, the breadth of required knowledge is considerable. 'Wider context' is complicated to delineate. My personal 'context' has always been very wide. Before moving into biophysics, I did a bachelor thesis on plant ecology while writing neural network code in Pascal during the evenings. Even staying within the photosynthesis and plant science/biophysics field, my interests have always been quite diverse. In the end, I've planned the text to provide key important anchor points for my perspective on the topic, without

being *too* wide in scope. The text is personal in topic selection yet tries to be impartial as much as possible in order to stay within some mildly conservative ideas about similar texts and their optimal composition. It is my hope that an interesting personal perspective can be found in the final form of this work.

Many obviously relevant topics are not included here, often because I could not provide better or newer insights than already available elsewhere. I did not include bioenergetic and thermodynamic considerations (the Z-scheme!). I'm not particularly well versed in these but also did not contribute to them at all. I did not include a chapter on pigments. While plant pigments, not only chlorophylls and carotenoids but also betalains and phycobilins, are one of my passions, I only contributed small bits to the first two and just a few unpublished experiments to the latter. Doing the pigments justice would double the length of the text (and quadruple the writing time, I'm afraid). I would have loved to discuss more of the photosystem mechanisms or many more odd algae groups but I was hard on myself and kept the text within reason (I hope!). I originally expected to write a bit about the methods I use, and love (fluorescence!), but in the end decided to omit them. Perhaps a better opportunity will arise in the future. I have also realized during writing that I'd love to prepare a version of it in my native Czech. Again, the time might come when it will be possible. It may not appear such to the reader but I did keep myself strict on side stories and avoided branching off in tangents as much as possible. Entire chapters could be made from some of the footnotes (and you haven't even seen all the deleted ones!).

A tremendous number of people influenced my work and made this text possible. My two teachers who are no longer with us shall be mentioned foremost. Pavel Šiffel was my supervisor during master studies. An extremely kind and knowledgeable man, I think of him every time I try to explain chlorophyll triplet state to somebody. In fact, I've been thinking of him daily for more than a decade after he'd left us forever (and still remember him often even after two decades). Ivan Šetlík was the same age as my grandfather yet one of the funniest and enjoyable scientists I've ever met. He knew about everything in the field of photosynthesis and his attention to detail as well as laser focus on quality were legendary.

I have two great mentors who shaped my professional life. František Vácha accepted me as a PhD student after I'd lost my supervisor. His kindness and skill in the lab as well as in other aspects of science were very inspirational and influential on me. Tomáš Polívka suggested that I write this text but, foremost, he is probably the best person I've met in science. Knowledgeable, kind, respected, good manager, hard-working, any positive characteristic you know, he has it in abundance. I wish I'd provide more satisfaction to the efforts of these men than I did in my career.

As is obvious from Chapters 2-9, I did not do any of the work presented there alone. I believe that my best work was produced in collaboration with Miroslava Herbstová, Zdenko Gardian and David Bína. We were a good team, I think. We were also supported by our great technicians Ivana Hunalová and František Matoušek. They deserve more credit than the brief notices in acknowledgements. Without their hands, there would not be much science done in our lab. Many others contributed to our science work, published or not. Some of them are listed as co-authors on papers, others contributed in ways which are often difficult to quantify or pinpoint. Life has convoluted ways sometimes. I hope they will forgive me for not mentioning their names here.

I have had the good luck to be always fully supported by my family. My parents and grandparents loved and love me unconditionally, I only wish I had a good way of paying the debt back. My dear wife and daughters have to put up with me even when I'm not at

my best and I'll be eternally grateful for their kindness and good spirits.

Regarding the following text, hopefully someone will find at least an infinitesimal inspiration in it. There are certainly many errors. I admit that I'm aware of some of them. Some simplifications even I consider too excessive. Sometimes the text would have to be too detailed or dry in order to be more accurate. Some (hopefully) smaller errors are left in the text to tease the reader. However, many errors are unintentional, hopefully they're not too extreme or embarrassing. As was the case often in the past, also here have I struggled with finding a positive approach to speculations, far-going discussions and hypotheses. I've always loved a good dataset and still prefer a nice 'trivial' spectrum or chromatogram to a hundred pages of speculation ('discussion'). I'd also like to extend my most sincere apologies to all wise readers for the last few paragraphs of Introduction. You see, I couldn't help myself, this is my text after all. I hope that the final text contains sufficient amount of comparatively solid information and not much of the wild speculation I resent. Some of the information in the following pages was quite hard to find, enjoy!

In České Budějovice, on January 15, 2024. Radek Litvín

Chapter 1

Introduction

1.1 Photosynthesis

Earth is a unique planet in the solar system, immediately recognized in a picture even by laymen. The major features of our home planet are the presence of atmosphere with clouds and the vast oceans covering its surface. Two other key differences from all the other planets known to humanity are active plate tectonics and the presence of a biosphere. Plate tectonics is hidden to the untrained eye and until the mid-20th century even to the experts [2]. On the other hand, the biosphere is obvious from the distance of space and much more so, of course, upon closer inspection. Even from outer space, one can recognize the green belts of boreal forests, the tropical rainforests of Africa and South America, or the coral reefs of the tropical Pacific ocean and along the coast of Australia. A careful look also reveals seasonal massive algae blooms in the polar seas [3]. In fact, the whole surface of the planet is covered in living matter, someplace perhaps hidden by its diminutive size, as in the deserts or polar regions [4], yet in other places prominently displayed, like in the world's forests. A substantial part of the planet's surface layer, crust, is also formed by biosphere products, effects and remnants, as is the case in many carbonate rocks, coal or even the iron ore deposits [5]. All of this mineral mass is, or was, formed directly or indirectly by the biosphere. Most of the organisms forming the biosphere now or in the past are ultimately driven by energy provided by our only star, the Sun, in the form of visible light. The process of conversion of light energy, carbon dioxide and water into the biomass of living organisms is called photosynthesis[1].

The overall process of photosynthesis can be described by a deceptively simple summary equation:

$$6\,\mathrm{CO_2} + 6\,\mathrm{H_2O} \xrightarrow{\text{light}} \mathrm{C_6H_{12}O_6} + 6\,\mathrm{O_2}$$

which does not really do justice to the convoluted process as it actually happens in cells. Like many other cellular processes, photosynthesis is highly complex. It involves a coordinated effort of tens of enzymes, catalysts which facilitate the otherwise thermodynamically highly unfavourable chemical reactions. These enzymes and their extremely important spatial arrangements cannot be easily summarized and written above the arrow in the summary equation unless one puts the word *chloroplast* there[2]. Such attempt is not

[1] The term *photosynthesis* is sometimes used in a general meaning of "synthesis driven by light" or even "mixing of light (colours)". Only the *sensu stricto* biological meaning will be used here.

[2] This would also be a simplification because photosynthesis runs also in cyanobacterial cells which do not have chloroplasts.

very useful because it does not really help the reader to understand what is going on in the reaction. However, it is the experience of the author that providing good, short and insightful explanations of what really is photosynthesis to the unitiated public or even to fellow researchers from other fields is *difficult*. Thus, the equation above needs to be accompanied by at least a textbook chapter to provide detailed explanation of what it actually means.[3]

Traditional descriptions of photosynthesis usually cover the process from absorption of light quanta in the cell to the production of stable six-carbon carbohydrate products. Photosynthesis is then further delineated into two stages. The first stage, which is powered directly by light energy and takes place in or around a membrane, is called *light reactions*. The second stage of the process is then powered by the products of the light reactions and includes the biochemical modifications needed to produce the abovementioned carbohydrates. This second stage of photosynthesis used to be called the dark reactions [10, 11]. However, it is now well established that the dark reactions actually depend on light as well and it is therefore more accurate to use some other name like *carbon reactions* [12]. Dissecting the summary equation, the light reactions deal with water on the reactant side and molecular oxygen on the product side and the carbon reactions deal with carbon dioxide on the reactant side and with carbohydrates on the product side (Fig.1.1). This text deals in detail with some of the processes directly involved with utilization of light energy, i.e. the light reactions.

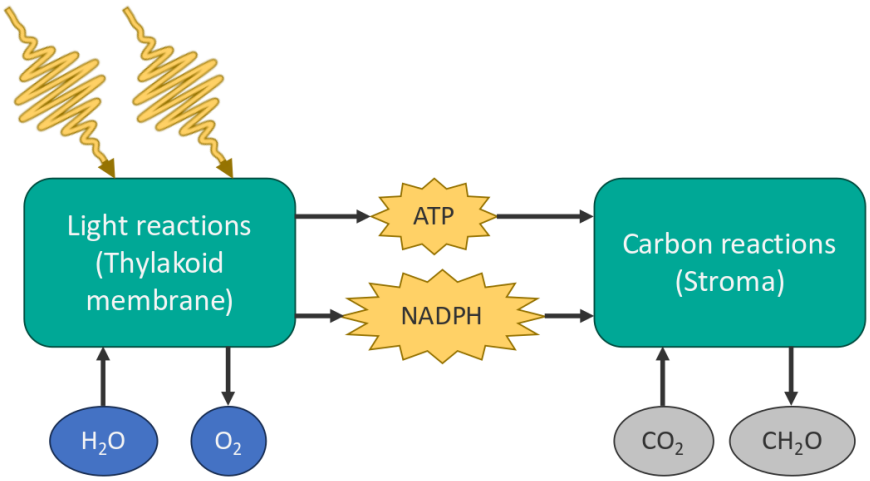

Figure 1.1: *Reactants and products of the light and carbon reactions of photosynthesis. Inspired by [12].*

Absorption of light quanta for photosynthesis and utilization or dissipation of their energy always happens in or near a biological membrane, a lipid-water interface often called a photosynthetic membrane. The nonpolar environment within the photosynthetic membrane hosts a number of protein supercomplexes of which two are the key energy-processing enzymes called photosystems. It is these enzymes which carry out the conversion of light energy into electrochemical potentials. The photosystems are large assemblies of different protein building blocks supplemented with a number of cofactors. Light-absorbing pigment molecules are the most prominent of these cofactors. The green colour of plant leaves is given by the pigment cofactors present in the photosystems. In oxygenic

[3] Of many sources for this text, these influenced me perhaps the most: [6–9].

photosynthesis, two photosystem types are present—photosystem I (PSI) and photosystem II (PSII) [13]. Photosystem I produces reduced electron carrier ferredoxin and, in extension, NADPH+H and can be described in enzymology terms as a plastocyanin-ferredoxin oxidoreductase. Photosystem II transfers electrons from water to plastoquinone and can be therefore denoted as water-plastoquinone oxidoreductase. Many other proteins are needed to complete the photosynthetic engine, some of which will be covered briefly later in this text. Even laymen usually know that photosynthesis produces molecular oxygen which is necessary for animals to breathe. Molecular oxygen is a byproduct of water oxidation [14] in PSII and, in terms of the light reactions of photosynthesis, it is not of particular interest, being just what remains after electrons have been liberated from water. Due to its relatively high reactivity, oxygen is otherwise an extremely important molecule in the discussion of photoprotective processes.

Light is a highly variable energy source. The intensity of incoming sunlight changes daily by at least two orders of magnitude. A major source of this variability is of course the daily movement of the Sun in the sky but weather also plays an important role[4]. Photosynthetic organisms therefore need to solve two key trade-offs in production and maintenance of the photosynthetic apparatus. First, sufficient amount of energy has to be harvested at minimum cost. Second, the collection of light energy has to be limited when there is no useful way of utilizing it (or the harvested energy has to be safely discarded). A solution to the first problem is to prepare only so many of the light-harvesting molecules that they're utilized most of the time at close to full capacity. The building cost of such molecules should also be minimized. In terms of cell economy, the amount of ATP, NAD(P)H, carbon and other elements needed to assemble the molecules is minimized. Research of the mechanisms of *light harvesting* is a very active part of the photosynthesis field of study and also a significant part of this work. The second problem then arises when more than the optimum amount of light is hitting the photosynthetic membrane. Most photosynthetic organisms, being fixed to the ground or having limited mobility, cannot move and hide in the shade if sunshine is at its maximum. The pigments of the photosynthetic membrane also cannot be shut down or choose not to absorb incoming light energy. An extremely important part of the photosynthetic machinery is therefore concerned with excess energy dissipation rather than utilization. One of the key mechanisms of energy dissipation is called *nonphotochemical quenching* (NPQ) [16–18]. This perhaps a bit confusing name is based on the fact that an excited pigment molecule can be de-excited or quenched either by a primary photochemical process, what we usually mean by 'photo-synthesis', or by some other and therefore 'non-photochemical' process.

The origin of oxygenic photosynthesis is hidden in very distant geological past and represents one of the big mysteries of biology [19–21]. There are no known unambiguously primarily primitive forms of photosynthesis on Earth today. It appears that all present photosynthetic organisms share the same oxygenic photosynthesis apparatus which can be traced back to the cyanobacterial form. Eukaryotic cells likely acquired photosynthesis via the process of endosymbiosis [22]. Endosymbiosis—in effect a domestication of cyanobacteria by eukaryotic cells—is a beautifully elegant hypothesis [23, 24]. Yet it again hides the true difficulty of permanent incorporation of one free-living organism into another organism and all its complexity. Putting a wild wolf into your living room will not produce a friendly retriever pet dog—a rather complicated and prolonged process is needed

[4]The stated range of two orders of magnitude is the difference between light of sufficient intensity to support low light-demanding plants and full sunshine. About one order of magnitude is the potential effect of clouds and approximately the same is true for the effect of solar elevation for most of the day.[15]

inbetween. Likewise, the differences between extant cyanobacteria and the chloroplast in the cells of wheat or oak tree leaves are massive [25] and therefore the process of taming of wild cyanobacterium by a proto-alga is difficult to comprehend. Surprisingly, it is now even believed that the process likely happened several times during evolution in a way described as serial endosymbiosis [26]. Eukaryotic organisms build the photosynthetic machinery by using genetic information located both in the chloroplast and in the cell nucleus. The presumed serial endosymbiotic events leading to some algae classes therefore make for a very complex and in essence a chimeric system where components originating from different organisms work together and produce a well-functioning photosynthetic apparatus. These processes provide the basis for the present diversity of photosynthetic processes in a very wide sampling of organisms as well as the major motivation for studying photosynthesis in non-model organisms as presented in the following chapters of this text.

1.2 The process of oxygenic photosynthesis

Photosynthesis provides organic carbon and energy for life in the form of glucose, a six-carbon carbohydrate. Glucose has multiple uses in the metabolism. It can be decomposed back to CO_2 and water via glycolysis and aerobic respiration, producing ATP and NADH, to be used in further energy-demanding cellular processes. In addition to this bioenergetic use, glucose can be used to build key structural polymer cellulose. Several other structural polymers such as hemicellulose, pectin, or agarose, are also made from carbohydrates. Pyruvate, the product of glycolysis, is the starting point for the synthesis of lipids and amino acids. Lipids most importantly form cellular membranes, but also serve other structural and energy storage roles. Amino acids are needed for the synthesis of proteins that are used either as enzymes or as building blocks for cell- and organism-level structures. Glucose can also be converted by the pentose phosphate pathway to a five-carbon carbohydrate ribose. Ribose is the key element of nucleic acids DNA and RNA, which store and process genetic information in cells. Thus, all known structures of life are linked to a single point of origin—glucose generated in the photosynthetic process.

Photosynthetic processes run in the cells of photosynthetic cyanobacteria and in the organelles of eukaryotic cells called chloroplasts. The two reactions of photosynthesis are running spatially close together. The light reactions are localized in a photosynthetic membrane that is separate from the outer membrane of cyanobacteria or the outer membrane of the chloroplast. Carbon reactions take place in the water phase near the photosynthetic membranes, either in cyanobacterial cells or in the inside space of the chloroplast.[5] The photosynthetic membrane is usually folded to a significant degree in order to substantially increase the area of the membrane and enable its packing in a small volume. The folded membrane forms a labyrinth of interconnected vesicles called thylakoids [28]. The proteins embedded in the thylakoid membranes are oriented in a specific way, and two terms are used to describe the spatial orientation in descriptions of photosynthesis. The inner space of the thylakoids is called the thylakoid lumen. The opposite—outside—space, which is connected to the cytoplasm in cyanobacteria, is called the stroma.

[5] In the words of Wilhelm Menke, who introduced the term 'thylakoid' in 1961: *"It follows from the investigations of Arnon and collaborators on the photosynthesis of isolated chloroplasts that the enzymes and coenzymes involved in the light phase of photosynthesis are contained in the lipoprotein complex. The enzymes of the dark phase can be extracted with aqueous solvents."* [27]

The light reactions of photosynthesis couple the transport of electrons through a chain of electron transport molecules with the transport of protons across the thylakoid membrane. Protons are transported to the lumenal side of the thylakoid membrane, resulting in different proton concentrations on each side of the membrane. Such created proton motive force between the two sides of the thylakoid membrane is used to power the synthesis of ATP.[6] This process of ATP production by the use of light energy is often called photophosphorylation or photosynthetic photophosphorylation [29]. To produce reduced electron carrier NADPH, an electron source is needed. In oxygenic photosynthesis, the electron source is water.

Although the two photosystems can be considered key in the light-powered machinery of the photosynthetic membrane, other protein complexes are needed to complete the energy conversion engine. The electron transport chain of the photosynthetic light reactions thus starts on the lumenal side of the photosystem II where electrons are extracted from water molecules. After passing through a series of PSII cofactors, the electron is handed over to an intramembrane carrier plastoquinone[7], which carries it in the membrane to another supercomplex, cytochrome b_6f. Cytochrome b_6f oxidizes the reduced plastoquinone and reduces another mobile electron carrier, plastocyanin. Unlike plastoquinone, which is a small hydrophobic molecule located in the membrane, plastocyanin is a water-soluble protein and carries electrons alongside the lumenal side of the embrane to the second photosystem, photosystem I. Photosystem I hands the electron over to the water-soluble electron carrier ferredoxin, located in the stroma, and ultimately the last enzyme of the electron transport chain, water-soluble ferredoxin-NADP oxidoreductase. Protons are translocated in two sites of the chain: PSII and cytochrome b_6f. In PSII, every electron liberated from water causes one proton to be added to the thylakoid lumen and one proton to be removed from the stroma (to protonate the reduced plastoquinone). Oxidation of plastoquinone in cytochrome b_6f is also accompanied by the release of protons into the lumen. Due to the so-called Q-cycle, two protons are transported across the membrane for every electron passing through the b_6f complex to plastocyanin. Overall, three protons are transported from the stroma to the lumen per each electron passing all the way from water to $NADP^+$. Such a path is called linear electron transport and requires two quanta of adequate energy to power the process. This linear electron transport is supplemented by another, cyclic, electron transport pathway in which electrons cycle from PSI back to cytochrome b_6f via additional protein complexes. The cyclic electron pathway is thus powered only by PSI and produces additional translocated protons but no NADPH.

1.2.1 Photosystem II

Photosystem II is a very large membrane-embedded protein complex present in all oxygen-evolving photosynthetic organisms. Structurally and functionally related complexes of anoxygenic bacteria, that do not produce molecular oxygen, are called type-II photosystems. The cannonical plant PSII complex has a molecular mass of about 1500 kDa[8] and

[6] Proton concentration is described by pH so often instead of proton concentration difference one speaks of difference of pH or ΔpH. Often one can also see the use of term "proton gradient" but it is difficult to visualize a smooth gradient as there is a membrane between the lumen and stroma, forming a well-defined barrier between the two compartments.

[7] Plastoquinone is a two-electron carrier. Upon reduction it becomes plastoquinol — the two keto groups of plastoquinone become hydroxy groups. For simplicity, the text does not distinguish between the two molecules.

[8] About a third of the mass of the eukaryotic ribosome [30].

a size of about 28 nm[9] (Fig. 1.2) [32–34]. The complex is a homodimer where each monomer consists in total of 29 protein subunits with ~200 pigment cofactors as well as other molecules. The electron transfer machinery is housed in the center of the complex formed predominantly by a heterodimer of D1/D2 proteins and called the reaction center (RC). The RC is surrounded by associated inner and outer light-harvesting antenna complexes. The electron transport chain components in the RC include in total 10 pigment molecules (six chlorophylls *a*, two pheophytins *a* (Pheo), and two β-carotenes) as well as other cofactors — two plastoquinones, one haem, one atom of each iron and calcium, and four atoms of manganese [32, 35]. The complex of inner antennas and RC of plant PSII is very similar to the same structure of cyanobacteria and is called the PSII core [36]. Overall, the PSII core consists of four major membrane-embedded proteins denoted D1, D2, CP43 and CP47[10], four extrinsic (water-soluble) proteins on the lumenal side — PsbO, PsbP, PsbQ and PsbTn[11] — and 12 smaller proteins surrounding the RC. The very center of PSII RC is a heterodimer of D1/D2 proteins which houses most of the electron transport cofactors. The extrinsic part of the RC contains a so-called oxygen-evolving complex with a cluster of atoms in Mn_4CaO_5 stoichiometry [40]. The two 'CP' proteins form the inner light-harvesting antenna of the PSII core, together binding 29 Chls *a* and seven β-carotenes (β-car).

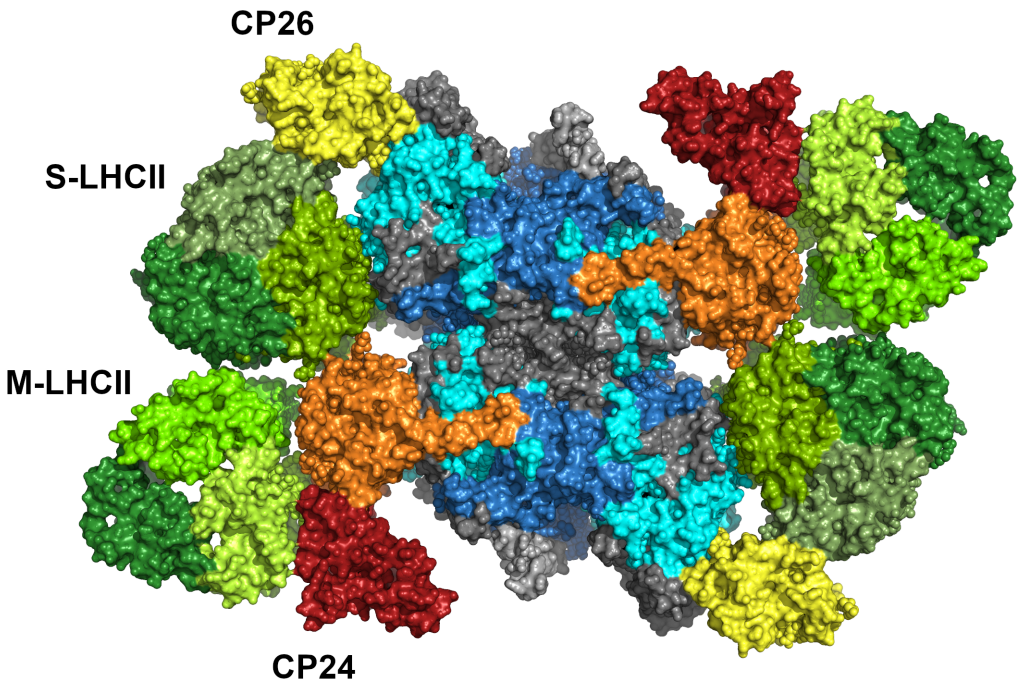

Figure 1.2: *Arrangement of the PSII supercomplex, as viewed from the stromal side of the membrane. The D1 and D2 proteins of the RC are in dark blue. Core antenna proteins CP43 and CP47 are in light blue. Monomeric Lhc antenna proteins are in red (CP24), orange (CP29) and yellow (CP26). Trimeric LHCII complexes are in green hues and labeled in the figure. Minor subunits of the supercomplex are rendered gray. Image was created from the cryo-EM structure of Arabidopsis PSII in PDB ID: 7OUI [34].*

[9] Approximately the size of a small virus like e.g. hepatitis A virus [31].

[10] The 'D' letters of D1 and D2 come from 'diffuse' (band in protein analysis) [37, 38] while 'CP' is an abbreviation of 'chlorophyll-protein' [39].

[11] Psb* - photosystem II proteins; proteins of photosystem I use the prefix Psa*.

The PSII core is surrounded by the outer antenna proteins. The outer antenna proteins have different pigment cofactors than the core complex and bind, in addition to Chl *a*, also Chl *b* molecules and carotenoids other than β-car (in plants there are neoxanthin, violaxanthin and lutein). Three monomeric antenna proteins, CP24, CP26, and CP29[12], are in direct contact with the core. The monomeric antenna proteins are supplemented by trimeric LHCII (Light-Harvesting Complex of PSII, LHCII as well as the monomeric antennas are formed by Lhcb proteins) complexes [41, 42]. One LHCII complex is relatively strongly bound to PSII and is in contact with CP43 and CP26 proteins, this complex is thus denoted as S-LHCII ('S' for 'strongly bound' [43]). A second LHCII complex is only connected to the core via the CP24 and CP29 proteins and is less strongly bound and therefore denoted M-LHCII ('moderately bound' [43]). More trimers of LHCII such as the 'loosely' bound L-LHCII [44] can be attached to the PSII if acclimation to low light intensities is needed [45]. The proteins of the PSII core are generally encoded in the chloroplast genome. Extrinsic PSII proteins (PsbO, PsbP, PsbQ and PsbTn) as well as the proteins forming the outer antenna (CP24, CP26, CP29 and LHCII) are encoded in the cell nucleus.

Electron transport starts in the PSII RC by one of the central chlorophyll molecules accepting an exciton from the light-harvesting complexes (Fig. 1.3).[13] In order to be able to extract electrons from water, a very large positive potential has to be created [46]. The heart of PSII, and the reason it is able to oxidize water, is the so-called primary electron donor. The primary donor is a pair of Chl *a* molecules in close proximity, often called a special pair. Due to its characteristic absorption maximum at 680 nm, the primary donor is denoted P680.[14] The counterpart to the primary donor is a primary acceptor, formed by one of the pheophytins *a* (the one in the D1 protein, $Pheo_{D1}$[15]). Following the initial excitation, a radical pair with oxidized P680 and reduced $Pheo_{D1}$ ($P680^{+}Pheo_{D1}^{-}$) is formed within tens of picoseconds [52]. This charge separation is then stabilized by an electron transfer to one of the quinone cofactors (Q_A) in $\sim$ 400 ps. The oxidized P680 extracts an electron from a side chain of a tyrosine from the D1 protein (TyrZ or Tyr160) in $\sim$ 50 ns. TyrZ, in turn, oxidizes the Mn_4CaO_5 cluster in 55 μs – 1 ms (the rate depends on the oxidation state of the cluster [54]). Meanwhile, on the opposite side of the RC, the electron is transferred between the two quinones from Q_A to Q_B in $\sim$ 0.3 ms (or $\sim$ 0.8 ms in the case that Q_B is already in Q_B^- state [55]). Upon double reduction, the plastoquinone from the Q_B pocket diffuses out of the protein complex (in the form of QH_2) and is replaced by another plastoquinone from the surrounding membrane. The Mn_4CaO_5 cluster binds two water molecules. Electrons are extracted from the cluster with a periodicity of four in a so-called Kok cycle [56, 57], starting from a fully reduced (dark-adapted) S_0 state via S_1, S_2, S_3 and S_4 and back to S_0.[16] The manganese cluster releases one molecule of

[12]The numbers '24', '26' and '29' are apparent molecular masses from the first detailed analyses of PSII where these proteins were detected [38].

[13]The central chlorophylls can of course be also excited directly by absorption of a photon but, considering the thylakoid membrane and PSII composition and typical light intensities, these events are quite rare *in vivo*.

[14]P680 comes from 'pigment' and characteristic absorption change observed upon oxidation of the primary donor [47–50].

[15]Despite the apparent symmetry of the cofactor arrangement in D1 and D2 proteins, only one path is active. It is not clear why is the other path via $Pheo_{D2}$ inactive. It appears to be a rudiment of early evolutionary stages of PSII and perhaps suitable for protection of the system in case of a blockade of linear electron flow [51–53].

[16]'S' stands for State. Besides indicating the order of steps, the numbers also indicate the number of extracted electrons or accumulated positive charges [57].

molecular oxygen per four accumulated positive charges and, therefore, per four excitons reaching the primary donor. The electron extraction becomes progressively slower as the cycle proceeds, starting from about 0.04 ms for the $S_0 \rightarrow S_1$ step up to 1.6 ms for the $S_3 \rightarrow S_4$ step [54, 58]. Accumulation of an excessively strong positive charge of four protons is prevented by the release of protons from the Mn_4CaO_5 cluster after each electron donation step [59]. Thus, as a rule of thumb, it takes on average about 1 ms for delivery of one electron by PSII, powered by one photon.

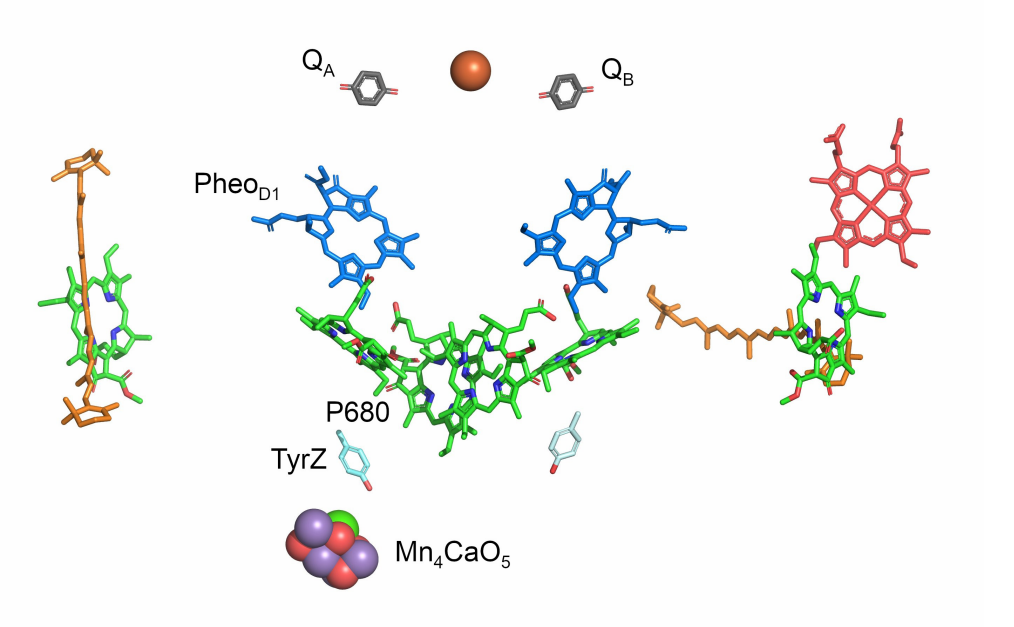

Figure 1.3: *Electron transfer chain cofactors of Photosystem II reaction center viewed within the membrane plane with stroma to the top. Chlorophylls a are in green, Pheophytins a in blue, β-carotenes in orange, tyrosine side-chains in light blue, plastoquinone heads in gray, heme molecule is in red. Atoms of the non-heme iron and of the Mn_4CaO_5 cluster are shown as space-filling volumes. Phytyl tails of the chlorophylls and pheophytins as well as the central magnesium atoms of chlorophyll were omitted for clarity. Image was created from the crystal structure of cyanobacterial PSII in PDB ID: 3WU2 [35].*

1.2.2 Cytochrome b_6f

Doubly reduced plastoquinones QH_2 produced by PSII diffuse within the membrane space and are subsequently processed by cytochrome b_6f (Cyt b_6f) [60–63]. Native Cyt b_6f is dimeric, with each monomer assembled from eight protein subunits and a number of co-factors. The total mass of the dimeric complex is about 230 kDa, with a size of 12 nm along the longest axis [64–66]. The major subunits include, as the name suggests, cytochrome f (PetA), cytochrome b_6 (PetB) and the PetC protein, which contains an iron-sulphur cluster (also called the 'Rieske' protein [61, 67]). Substantial part of the complex, specifically most of the cytochrome f and PetC proteins, extend out of the membrane into the thylakoid lumen. In terms of electron transport cofactors, each Cyt b_6f monomer contains four hemes and one [2Fe-2S] cluster as well as at least two plastoquinone-binding sites (Fig. 1.4) [68]. On a path through the complex cut approximately perpendicular to the membrane plane from the stroma to the lumen, one of the two quinone-binding sites, Q_i,

is close to two hemes denoted heme b_H and heme c_i.[17] Further towards the lumen, next to the second quinone-binding site, Q_o, is another heme — heme b_L. From heme b_L out towards the lumenal part of the complex is the [2Fe-2S] cluster and finally heme *f* (which is a type-*c* heme), located in the water-soluble part of the cytochrome f protein.[18] Of the eight Cyt b_6f proteins, two (PetC and small subunit PetM) are encoded in the cell nucleus, the other six proteins are encoded in the chloroplast genome.

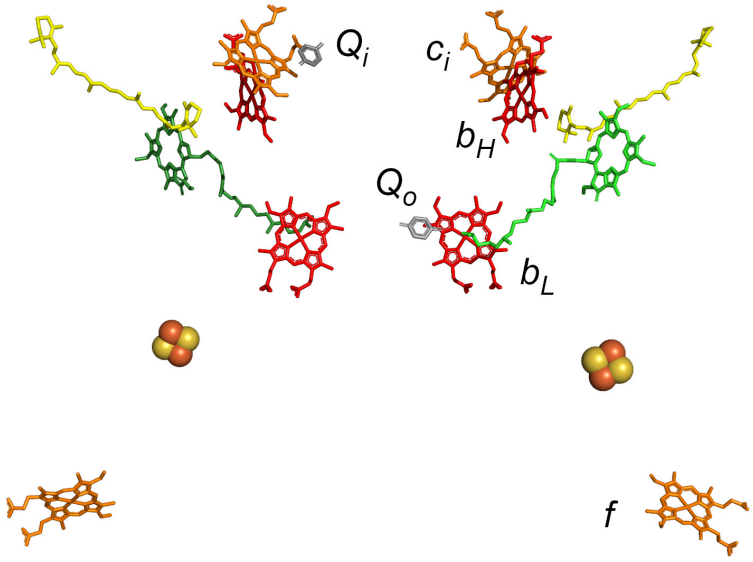

Figure 1.4: *Electron transfer chain cofactors of Cytochrome b_6f complex viewed in the membrane plane with stroma to the top and lumen to the bottom. Hemes c are in orange, hemes b in red, plastoquinone heads in gray, chlorophyll in green and β-carotene in yellow. Atoms of the [2Fe-2S] cluster are shown as space-filling volumes. Image was created from cryo-EM structure of spinach Cyt b_6f in PDB ID: 6RQF [68].*

During electron transport, reduced plastoquinone QH_2 is docked in the Q_o pocket and one of its electrons is carried away via the heme b_L and the [2Fe-2S] cluster to the heme *f*. This transport possibly requires considerable conformational change of either PetC or cytochrome f protein [75–77]. Cytochrome f ultimately hands the electron over to water soluble electron carrier plastocyanin, which carries it to PSI. At the same time, two protons formerly associated with QH_2 are released into the lumen. The second QH_2 electron is transported towards the stromal side of the membrane to the hemes b_H and c_i and finally to another plastoquinone docked nearby in the Q_i pocket [77, 78]. After another such cycle, two electrons have been sent by plastocyanin towards PSI, and two electrons were used to

[17] The literature on Cyt b_6f uses at least two parallel signing conventions [69]. Here the convention from crystal structure of Stroebel et al. [66, 70] is used. The other convention [63, 65] uses the following labels: heme b_H is heme b_n, heme c_i is heme c_n, heme b_L is heme b_p, site Q_o is Q_p whereas Q_i is Q_n. Indices *p* and *n* come from 'positive' (lumen) and 'negative' (stroma) side of the membrane whereas 'o' and 'i' come from 'outer' (also 'oxidizing') and 'inner' (side of the mitochondrial energy conversion membrane) [71]. Indices 'H' and 'L' come from 'high' and 'low' electron potential in such denoted sites.

[18] Hemes *b* and *c* most importantly differ by the method of attachment to proteins. The most common heme type is heme *c*, bound to proteins via two covalent thioether bonds (but heme c_i is apparently bound by only one thioether bond [66]). In contrast, hemes *b* are bound to protein by coordination to the central iron atom. The third basic heme type is heme *a*, which is derived from heme *b* by replacement of one methyl side chain by a formyl group and by the addition of a nonpolar 16-carbon alkyl chain in place of one of the vinyl side chains. [72–74].

reduce a plastoquinone molecule in the Q_i pocket. This reduction is accompanied by an uptake of two protons from the stroma to complete the formation of QH_2, which is then released from the Q_i binding site and can be later oxidized at the Q_o site. This mechanism, called the Q-cycle (Q for quinone), is similar to the process with the same name, operating in the mitochondrial cytochrome bc_1 complex (also called complex III), which shares homology with the chloroplast Cyt b_6f [63, 79–81]. Due to the Q-cycle, every electron passing from QH_2 to plastocyanin is accompanied by the translocation of two protons from the stroma to the lumen.

In terms of the electron transfer rate, the diffusion of plastoquinone from PSII to Cyt b_6f depends on the specific arrangement of the supercomplexes (see below), but generally takes units of milliseconds [82]. The donation of the electrons by QH_2 at the Q_o site takes about 10-20 ms and is considered to be the slowest step of the whole photosynthetic electron transport chain [63, 77, 83]. Cytochrome f is then reduced in about 4 ms [76].[19]

In addition to the above-described structural features and mechanics, Cyt b_6f also binds one chlorophyll *a* and one β-carotene molecule. These pigments are most likely not used as light-harvesting agents or as redox-active components, but rather as structural and regulatory elements. Structural information indicates that the nonpolar tail of the Chl *a* acts as a gatekeeper for the Q_o site and prevents partially oxidized plastoquinone from escaping the pocket [65, 66, 68]. The single β-car molecule is not close enough to the Chl *a* to provide photoprotective function and it was suggested that it has a role in the assembly or stabilization of a proposed Cyt b_6f-PSI supercomplex [63].[20]

1.2.3 Plastocyanin and cytochrome c_6

Electron transfer from Cyt b_6f to PSI is mediated by the mobile electron carrier plastocyanin [89, 90]. Plastocyanin is a small (4 nm along the longest axis, 10 kDa mass) water-soluble protein localized in the thylakoid lumen. The electron-carrying capacity is provided by a single copper atom coordinated by four ligands in an asymmetric position, less than 1 nm from the nearest protein surface [91]. The copper atom is in the Cu^{II} state in an oxidized plastocyanin.[21] The protein is overall net negatively charged at neutral pH, but its surface shows significant charge anisotropy to enable the specific binding of the oxidized plastocyanin to Cyt b_6f and of the reduced plastocyanin to PSI. Part of the protein surface is also hydrophobic. Interaction with Cyt b_6f likely involves an electrostatic interaction between negative charges on plastocyanin and positive charges on the cytochrome f [94]. Likewise, the interaction with PSI is guided by similar principles and aided by the hydrophobic patch as well [95, 96]. The electron transfer rate from the cytochrome f to plastocyanin is on the order of 200-500 μs [97] while electron donation from plastocyanin to the oxidized primary donor of PSI (see below) is much faster at about 12 μs [98, 99]. Plastocyanin diffusion time between the two membrane-bound complexes depends on the membrane architecture and supercomplex distribution, but is comparable to its reduction time, i.e. $\sim$300 μs [100, 101].

[19] Unlike the situation in e.g. PSII, detailed up-to-date kinetic information on Cyt $b_6f$function seems hard to come by.

[20] It appears that many aspects of Cyt b_6f function are not fully understood. For example, there are conflicting reports of energy transfer from the β-car to Chl *a* and back [84–86] as well as lack of such processes, in some species at least [87, 88].

[21] Plastocyanin is a member of a large family of "blue copper proteins". Other members include for example azurin, stellacyanin or umecyanin [92, 93]

Plastocyanin is generally found in cyanobacteria and in land plants. The protein has been lost in the red algae and is missing in most of the organisms that inherited photosynthesis from them as well [102, 103]. When plastocyanin is missing, it is replaced by another, functionally very similar, electron transporter cytochrome c_6 [95, 104]. As the name suggests, the electron-carrying cofactor of cytochrome c_6 is a single heme. It is known that organisms carrying genes for both electron carriers can preferably use one or the other carrier according to the availability of iron and copper [105]. Therefore, it has been hypothesized that the original electron carrier was an iron-containing cyt c_6[22] that was replaced by copper-binding plastocyanin when iron became rare following the Great Oxidation Event [106]. Both plastocyanin and cytochrome c_6 are encoded by nuclear genes.

1.2.4 Photosystem I

Like PSII, Photosystem I is also a very large multi-subunit protein supercomplex, giving name to related 'type-I' photosystems of anoxygenic bacteria. Most of the PSI is hosted within the thylakoid membrane space, with a small part extending to the stromal space and the plastocyanin docking area exposed in the luminal space. The plant PSI supercomplex is monomeric, about 17 nm along the longest axis with a molecular weight of about 600 kDa [107–109].[23] The supercomplex contains about 16 protein subunits with $\sim$ 200 pigment cofactors. The heart of the supercomplex is a heterodimer of PsaA and PsaB subunits[24]. The PsaA and PsaB proteins include elements of RC and of the inner light-harvesting antenna. Thus, instead of the four key subunits of the PSII core (D1, D2, CP43 and CP47) there are only two major subunits of the PSI core.[25] Besides the PsaA/PsaB dimer, the PSI core is formed by 10 additional protein subunits, the most interesting of which are PsaC, PsaD, PsaE, and PsaF proteins. Subunits PsaC, PsaD, and PsaE form a ridge exposed to the stroma and provide a docking site for ferredoxin. Subunit PsaF, on the other hand, is exposed to the lumen and provides a key part of the plastocyanin docking site [117, 118]. The PSI core is supplemented by an outer light-harvesting antenna, formed by four LHCI proteins (Lhca1-4), which are assembled in a crescent shape on one side of the complex (Figure 1.5a). If required, additional light-harvesting capacity is provided by attachment of LHCII trimers [119, 120]. The latest plant PSI structure shows in total 192 pigment cofactors: 156 chlorophylls and 36 carotenoids [121]. Of these pigments, the outer LHCI antenna itself contributes 56 chlorophylls, which include all 13 Chls *b* present in the complex, and 13 carotenoids. In contrast to PSII, only five PSI proteins are encoded in the chloroplast genome (PsaA, PsaB, PsaC, PsaI and PsaJ), the other 11 are provided by the nuclear genome.

In terms of electron transport chain, the PSI RC contains six Chl *a* molecules, two phylloquinones[26], and three [4Fe-4S] clusters. The spatial arrangement of the chlorophyll and

[22] C-type soluble cytochromes are abundant in bioenergetics [104].

[23] Cyanobacterial PSI is typically trimeric but mono-, di- and tetramers have been observed as well [110–114].

[24] Like in the case of PSII the two RC subunits are very similar in structure, forming an 'almost' homodimer. It is speculated that in the very origins of photosynthesis both photosystems were 'true' homodimers [20, 53, 115, 116].

[25] The PsaA and PsaB proteins each have 11 transmembrane helices. In PSII, the D1 and D2 proteins have five transmembrane helices while CP43 and CP 47 have six transmembrane helices. Due to the apparent conservation of overall RC structure and positions of the cofactors, it is assumed that the hypothetical primordial photosystem was a homodimer of proteins with 11 transmembrane helices [20, 53].

[26] Phylloquinone, known as vitamin K_1, plays an important role in human health. Most well-known is its role in production of blood coagulation factors. The most important source of the vitamin are PSI complexes

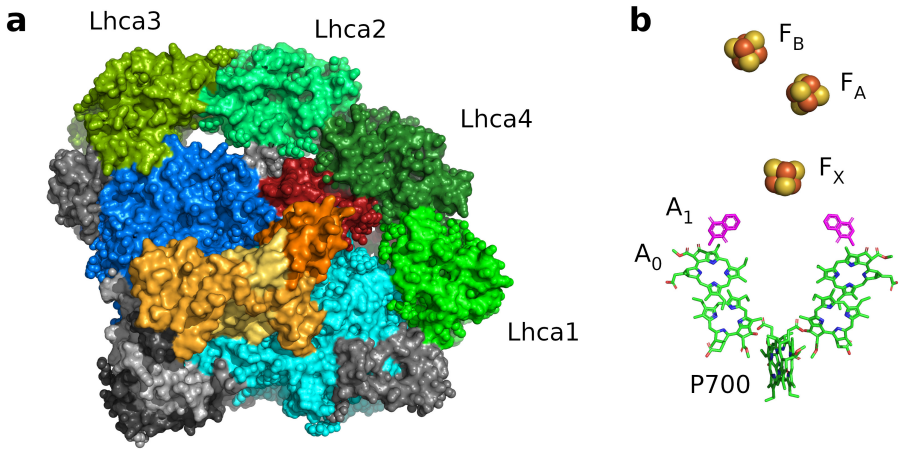

Figure 1.5: *Structure of Photosystem I.* ***a)*** *PSI as viewed from the stromal side. Subunits of the RC are in blue (PsaA is dark, PsaB light blue), ferredoxin binding ridge subunits PsaC-E are in hues of orange, PsaF is in dark red, Lhc proteins of LHCI are in green hues, other subunits are in gray.* ***b)*** *Electron transfer chain cofactors of PSI reaction center viewed within the membrane plane with stroma to the top. Chlorophylls a are in green, phylloquinone heads in magenta, atoms of the [4Fe-4S] clusters are shown as space-filling volumes. Phytyl tails of chlorophylls and phylloquinones as well as chlorophyll central magnesium atoms were omitted for clarity. Image was created from the crystal structure of pea PSI in PDB ID: 5L8R [121].*

quinone cofactors pf PSI RC closely resembles the situation in PSII RC (Figure 1.5b). The primary donor P700[27] is formed by a dimer of chlorophylls located close to the lumenal side of the membrane and to the plastocyanin docking site.[28] There are two parallel electron transfer pathways running in the direction from lumen to stroma between P700 and the first iron-sulfur cluster called F_X. Each of the two pathways is formed by an unlabelled chlorophyll, then by primary acceptor chlorophyll denoted A_0 and by secondary acceptor phylloquinone (A_1) [129–131].[29] The two pathways join again on the F_X cluster, and the electron further proceeds from the F_X via F_A and F_B [4Fe-4S] clusters to ferredoxin, a stromal soluble electron carrier. Following an excitation reaching P700, the charge separation to state $P700^+A_0^-$ appears in less than 1 ps. Further electron transfer to state $P700^+A_0A_1^-$ happens in about 30 ps. Reduction of F_X by A_1^- then takes about 200 ns. Electron transfer between the [4Fe-4S] clusters is assumed to be faster than 500 ns, and finally the reduction of ferredoxin by F_B^- happens in about 500 ns [130–132]. Both of the paths, via PsaA and PsaB branch, are presently believed to be active in electron transport, though it seems that they operate at slightly different rates [133–135]. The slowest step of electron transfer in PSI is the reduction of $P700^+$ by plastocyanin in about 12 μs (see above). The PSI catalytic function is thus almost two orders of magnitude faster than that of PSII.

---

of green foliage present in food [122–125].

[27] Like P680 in PSII, also P700 is named after characteristic absorbance spectrum of the spectral species. The letter 'P' stands for 'pigment' [126, 127].

[28] One of the P700 chlorophylls, located in PsaA in site eC-A_1, is an epimer of Chl *a* denoted Chl *a'* [121, 128, 129].

[29] The cofactor sites can be also found labelled in literature as eC-A_1 and eC-B_1 (P700), eC-B_2 and eC-A_2 (no other label, the Chl binding proteins PsaA and PsaB are flipped here versus the rest of the pathway), eC-A_3 and eC-B_3 (two A_0 sites) and Q_K-A and Q_K-B (two A_1 sites) [110].

1.2.5 Ferredoxin, FNR and other proteins of the thylakoid membrane

Electrons are carried away from the reduced F_B cluster of PSI by the stromal water-soluble carrier ferredoxin [136]. Ferredoxin is the key link between the thylakoid membrane, which produces reducing power, and downstream biochemical processes. Ferredoxin is a member of a large family of proteins that contain Fe-S clusters of different stoichiometries. Photosynthetic ferredoxin is a small, 10 kDa, protein with active site formed by a [2Fe-2S] cluster that carries one electron at a time [137, 138]. In chloroplasts, the primary counterpart of reduced ferredoxin is the ferredoxin-$NADP^+$ oxidoreductase, which provides reduced NADPH necessary for carbon assimilation reactions. Ferredoxin can also return the electron from PSI back to the thylakoid membrane in a cyclic electron transfer process [139]. However, ferredoxin is a promiscuous electron donor and also supplies electrons to other synthetic processes which include biosynthesis of chlorophyll *b*, phytochromes, and fatty acids [140–143]. The ferredoxin apoprotein, PetF, is encoded in the nuclear genome in plants. Red algae and groups that inherited their chloroplast generally encode ferredoxin in the chloroplast genome [144].

In analogy to plastocyanin, ferredoxin also has a functional doppelgänger, flavodoxin. Flavodoxin has been lost during the evolution of land plants, possibly as a consequence of an increased iron availability on dry land, but is found in cyanobacteria, red algae and some of their kin, and in chlorophyte green algae [145–147]. Flavodoxin is a ~21 kDa protein, encoded in the nuclear genome, with flavin mononucleotide as a prosthetic group [148, 149].[30]

Linear photosynthetic electron transport from water to $NADP^+$ terminates in the ferredoxin-$NADP^+$ oxidoreductase (FNR). FNR is a stromal water-soluble protein of about 35 kDa with two distinct domains. The N-terminal domain binds one flavin adenine dinucleotide (FAD) cofactor, while the C-terminal domain provides the $NADP^+$ binding site. At least two functionally distinct FNR isoforms, which potentially interact and form dimers, are known from plants [144]. Ferredoxin docks to FNR with its [2Fe-2S] cluster close to the FAD, while the protein is in extensive contact with both FNR domains [154–156]. During the catalytic process, $NADP^+$ is first bound to the FNR, followed by two consecutive ferredoxins to produce, ultimately, a doubly reduced FAD cofactor. The reduction of $NADP^+$ is believed to be carried out in a single step [157, 158]. The FNR protein is encoded by nuclear genes.

Besides linear electron transport, the existence of cyclic electron transport (CET) from PSI back to Cyt b_6f has been known for a very long time [159]. However, the molecular components of this process are only slowly becoming to be known in the last two decades and the details and specifics are still a mystery [139]. Two independent routes of CET are currently postulated. One route requires a protein supercomplex called NADH dehydrogenase-like complex (NDH) [160–163]. The other route requires small proteins PGR5 and PGRL1 [164–166]. NDH is a large protein complex of about 0.4 MDa, which is known to be able to bind ferredoxin and use the electron from it to reduce plastoquinone. NDH likely also couples the reduction of plastoquinone with proton pumping across the membrane [167]. The PGR5-PGRL1[31] route likely involves electron transport from ferredoxin to plastoquinone with the Cyt b_6f and FNR playing important roles [168].

[30] Ferredoxin and flavodoxin are two of the oldest existing protein structures. Ferredoxins with [*n*Fe-*n*S] active sites are hypothesised to have been among the first redox components of life on Earth [150–153].

[31] PGR5 stands for "proton gradient regulation 5" and consequently PGRL1 comes from "pgr5-like photosynthetic phenotype 1" [165, 166, 168].

1.2.6 F_oF_1-ATP synthase

The proton motive force generated via processes associated with the electron flow in the thylakoid membrane is finally utilized by the F_oF_1-ATPase (or ATP synthase), which uses it to produce ATP. The F_oF_1-ATPases are a large family of supercomplexes which are present in bacteria, archaea, mitochondria, and chloroplasts. The ATP synthase has the mass of about 550 kDa and size of approximately 22 nm along the longest axis (Fig. 1.6). The complex is formed by nine different proteins, predominantly encoded in the chloroplast. ATP synthase is anchored in the membrane by the F_o subsection, mainly consisting of a multimer of subunits c, so called c-ring. The stromal part of the enzyme is called F_1 and mostly composed of a hexamer of two building blocks, α and β subunits.[32] The two parts are connected by two 'stalks'. An external stalk is formed by long proteins of subunits b and b' while the central stalk is formed by subunits ε and γ [171].

The F_oF_1-ATPase is a molecular motor. Protons are translocated through the membrane via the c-ring of the F_o subsection. Passage of protons through the c-ring causes it to rotate, together with the central stalk, which induces torque forces on the catalytic part, the F_1 subsection. F_1 consequently changes conformation, which in turn triggers the synthesis of ATP from ADP and phosphate [172–174].

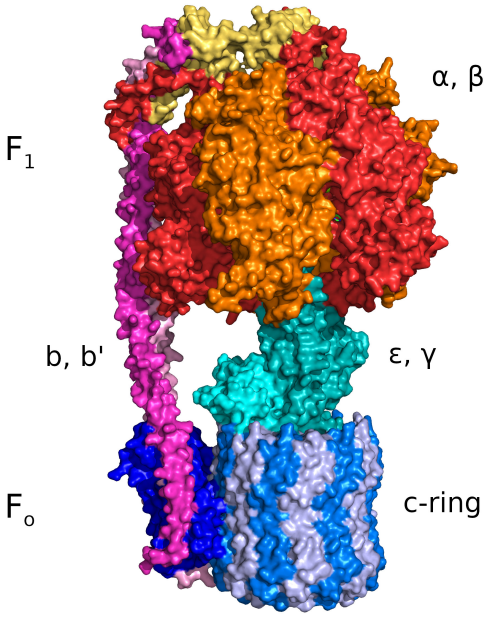

Figure 1.6: *Structure of the chloroplast ATP synthase viewed within the membrane plane. The bottom part of the supercomplex is embedded in the thylakoid membrane and the upper part sticks out into the chloroplast stroma. The upper F_1 subsection is prevented from rotation by the peripheral stalk, which also attaches to the F_o subsection. The individual subunits of the c-ring are colored in alternating blue hues but they are, in fact, identical. Subunits of the F_1 subsection are in red (α) and orange (β). Central stalk proteins are in cyan (ε) and teal (γ). The external stalk in magenta (subunit b) and pink (subunit b') is attached to the F_o subsection by subunit a (dark blue) and to the F_1 by subunit δ (yellow). Image was prepared from the cryo-electron microscopy structure of spinach ATPase PDB ID: 6FKF [171].*

The c-ring itself is a homooligomer of ion-binding proteins. One full rotation of the

[32] The labels come from 'coupling factor 1' for F_1 [169] and 'oligomycin sensitivity-conferring factor' for F_o [170].

c-ring requires the passage of a number of protons which is equal to the number of the monomers forming the c-ring. While the F_1 subunit is hexameric, able to synthesize three ATP per one full c-ring rotation, the F_o subunit can have different symmetries. Members of the F_oF_1 ATP synthase family can have between 8 and 15 subunits within the c-ring of F_o [175, 176]. The number of translocated protons required to produce one ATP molecule is therefore not the same for all ATP synthases. A higher required number of protons likely signifies an ability to produce ATP even with lower proton motive forces present on the membrane, at the cost of lower efficiency. Chloroplast ATP synthase has 14 c-ring components, thus, for one full catalytic cycle (three ATPs produced), 14 protons need to be translocated [171, 177].[33]

1.2.7 Higher organization of the photosynthetic membrane

The photosynthetic complexes described above assemble into higher order super- and mega-complex assemblies in the native thylakoid membrane. Furthermore, the membrane system itself can have a complex three-dimensional structure, contrary to the naïve notion of an effectively two-dimensional plane membrane arrangement. This spatial organization likely optimizes the distances and concentrations of electron transport chain cofactors, facilitating efficient electron transfer.

Starting from the level of the chloroplast and zooming in, it has been known for more than a hundred years that the thylakoid membrane in chloroplasts of higher plants aggregates and forms stacks of discoid thylakoid vesicles, which are called grana [178, 179]. Grana are formed by as much as tens of thylakoid vesicles tightly stacked on top of each other and interconnected by a network of so-called stromal lamellae [180, 181]. The thylakoid membranes forming the grana are closer together on their stromal side whereas the intermembrane space on the lumenal side (effectively the inside space of the thylakoid vesicles) is bigger. The thylakoids forming grana are about 300–600 nm in diameter, the stromal gap is about 3.5 nm wide and the lumenal gap is about 4.5 nm wide [182–184]. The lumenal gap provides sufficient space to accommodate the water-soluble (lumenal) parts of PSII and Cyt b_6f. The smaller stromal gap accommodates the stromal parts of the aforementioned complexes but not the stromal parts of the PSI and ATPase complexes (see below). The size of the grana both in terms of the thylakoid diameter as well as in terms of the number of thylakoids in the stacks is regulated in response to the light intensity available to the plant. In low light intensities, the grana become wider and contain more thylakoids [185–187]. The grana architecture also dynamically responds to short-term illumination intensity changes in order to acclimate to the present conditions and to provide optimal function of the photosynthetic electron transfer chain [184, 188]. Upon illumination, the lumenal gap increases (the thylakoid vesicles 'swell') and in high light stress the grana diameter decreases[34] and the stromal gap also increases (the grana stacks 'unstack') [190–192].

The photosynthetic membrane system is not only spatially complex as described above but also heterogeneous when examining the presence of electron transfer chain components, i.e. a lateral heterogeneity can be observed in the thylakoid membrane [193]. The grana regions contain mostly PSII and Cyt b_6f. The water-soluble stromal parts of PSI and ATPase are larger than the limited intermembrane space of the grana and these com-

[33] For comparison, the animal mitochondrial ATP synthase has only eight subunits [176].

[34] The lumenal gap effectively doubles to about 9 nm [189] while the grana diameter decreases by about 20 % [187].

plexes cannot fit in the stacked grana membrane regions. PSI and ATPase complexes are therefore mostly found in the stromal lamellae [190, 194]. The complexes are generally very densely packed in the membranes. It has been estimated that proteins represent about 80 % of the area of the grana membranes and 70 % of the stromal lamellae area [183]. The mobile electron carriers plastoquinone and plastocyanin, which functionally interconnect the complexes and complete the electron transfer chain, therefore have to diffuse through a maze of tight spaces between the supercomplexes [101].

The thylakoid membrane protein supercomplexes are known to associate in larger assemblies, possibly to improve the diffusion-driven electron transport. In the grana of low-light-grown plants, besides possessing a higher number of the LHCII subunits, PSII can assemble into effectively crystalline arrays with hundreds of highly ordered PSII particles [182, 195–197]. PSI is known to form megacomplexes with Cyt b_6f [198, 199] and with the NDH complex [200–202]. A supercomplex of PSII and PSI has also been observed [203–206].

In contrast to present advanced knowledge of spatial arrangement of the photosynthetic membranes and the protein complexes within, the functional significance of grana for plant photosynthesis has been much harder to explain. It is understood that a flat thylakoid membrane filled haphazardly with the electron transport chain components (covering 80 % of its area, see above) is likely to suffer from significant inefficiencies: i) close contact of PSI and PSII will lead to spillover of excitation energy from the slower PSII to faster PSI; ii) it will be quite difficult to regulate relative energy flows to the photosystems; iii) likewise, regulation of the relative contributions of linear and cyclic energy flows will be difficult; and finally iv) the diffusion distances and times for the mobile electron carriers will be too long for efficient electron transport to happen [190, 207, 208]. Yet, somehow, cyanobacteria and most eukaryotic algae manage without grana. Thus, while the existence of the grana can be at least partially rationalized by the abovementioned issues, the emergence of grana in the evolution of land plants and full understanding of the issue remain elusive.

1.2.8 Light harvesting

The electron transfer chain machinery described above is powered by light energy. Despite its apparent power, sunlight is a dilute light source. How much energy is received by photosynthetic pigments on a molecular level? While full sunlight provides about 2 000 μmol photons.m^{-2}.s^{-1} of photosynthetically active radiation, the long term average light intensity is of course considerably smaller. The lower levels of dense crop canopies receive about a fifth of the intensity above the canopy [209]. The ground below dense canopy rainforests receives below 10 μmol photons.m^{-2}.s^{-1} on average. Nevertheless, some green plants survive in these comparatively dark environments. Light can be, of course, at a premium in aquatic habitats. In eutrophic waters with dense pelagic algal biomass, light intensities can drop to 10 μmol photons.m^{-2}.s^{-1} within less than the first 1 m of depth [210].

Zooming in to the molecular level now, the two millimoles of photons per square meter in the full sunlight intensity translate to about 12 photons per Å^2 (per second). The Chl *a* molecule has an absorption cross-section of about 0.8 Å^2.[35] Therefore, at full sunlight intensity, a single Chl *a* molecule is expected to absorb about 10 photons per second. The long term average in a partly shaded environment will thus be less than a photon per

[35] Using [8, 211] and an estimated Chl *a* molar extinction coefficient of 21 300 l·mol^{-1}·cm^{-1} (calculated for the purpose of this text as an average value of the Chl *a* absorption spectrum in the spectral range of 400–700 nm from unpublished data).

second. The primary donors of the photosystems are built by four Chl *a* molecules and without some light energy concentrating mechanism they would not receive more than a photon per second for most of the time. Therefore, the photosystem reaction centers would effectively sit idle and would not even be able to use the mechanisms described above. For example, PSII requires two absorbed photons to reduce the plastoquinone by two electrons. A recombination (backward) reaction competes with the plastoquinone reduction (forward) reaction. At low photon absorption frequency the forward reaction will not succeed and reduced plastoquinone will not be produced. Light harvesting systems solve this problem by greatly increasing the number of pigments connected to a single primary donor [8].

The light harvesting function is almost exclusively carried out by complexes of pigments and proteins[36] associated with the two photosystems. The pigment complement of these pigment-protein complexes can form more than a third of the total mass of the complex. Light-harvesting complexes can be broadly classified according to their external properties into water-soluble and membrane-intrinsic types. The cyanobacterial phycobiliproteins, which are found also in red and cryptophyte algae, are an example of the water-soluble type. The reaction centers of both photosystems in oxygenic photosynthesis are supplemented by inner light-harvesting proteins, which are present also in cyanobacteria. The inner and also outer antennas of most eukaryotic phototrophs are membrane-intrinsic. The PSII core is essentially formed by attaching two light-harvesting proteins, CP43 and CP47, to the PSII RC. These proteins add 29 Chls *a* to the RC, expanding the photon capture rate by a factor of six [35, 213]. Likewise, the PSI core complex contains about 100 Chl *a* molecules, an increase in light harvesting capability by a factor of 17 in comparison to the six Chls *a* of the PSI RC [121].

The light harvesting capacity of the photosystem cores is further enhanced by attaching outer antenna complexes. In most eukaryotes, the outer antenna is formed by monomeric, dimeric, trimeric or tetrameric arrangements of the proteins of the Lhc family. As described in the previous chapters, PSII includes three monomeric antennas of this type (CP24 (protein Lhcb6), CP 26 (Lhcb5) and CP29 (Lhcb4)) and up to three trimers of LHCII (built from proteins Lhcb1, 2 and 3), which increase the light harvesting capacity of PSII by more than a factor of six. PSI core is supplemented by four Lhca proteins and potentially also LHCII trimers, for a light harvesting capacity increase of about a factor of two.

Proteins of the Lhc family represent one of the great innovations of the eukaryotic phototrophs. All Lhcs share the same basic configuration of three transmembrane α-helices, denoted A, B and C.[37] The core structure of the Lhc monomer is formed by the helices A and B, which cross each other at an angle of about 55° and a distance of 1 nm (Fig. 1.7) [215, 216]. The Lhc proteins are built from about 230 amino acid monomers and have total masses around 20 kDa. The Lhc protein scaffold is densely packed with about 15 kDa of pigment cofactors.[38] Considering the size of the folded Lhc protein, the concentration of the pigment cofactors is considerable, close to 0.5 M.[39] Unsurprisingly, the pigment

[36]The only exceptions are the chlorosomes of some non-oxygenic bacteria which are mostly built from agregates of pigments and other small molecules [212].

[37]In the amino acid sequence of the Lhc proteins, the helices are in the order B-C-A [214].

[38]The mass of a chlorophyll molecule is about 900 g/mol, carotenoid molecules have an average mass of about 580 g/mol.

[39]Assuming the size of the LHCII monomer is about $5 \times 5 \times 3$ nm^3 [214] and that it contains 18 pigment cofactors, one gets the concentration of 0.4 M. Similar numbers can be obtained for other photosynthetic membrane complexes [217]. It is also insightful to look at the capability to capture light. The molar ex-

cofactors are not just decoration on the protein scaffold but rather an integral part of the overall structure. Indeed, the Lhc protein cannot fold properly without the presence of the pigments [218, 219].

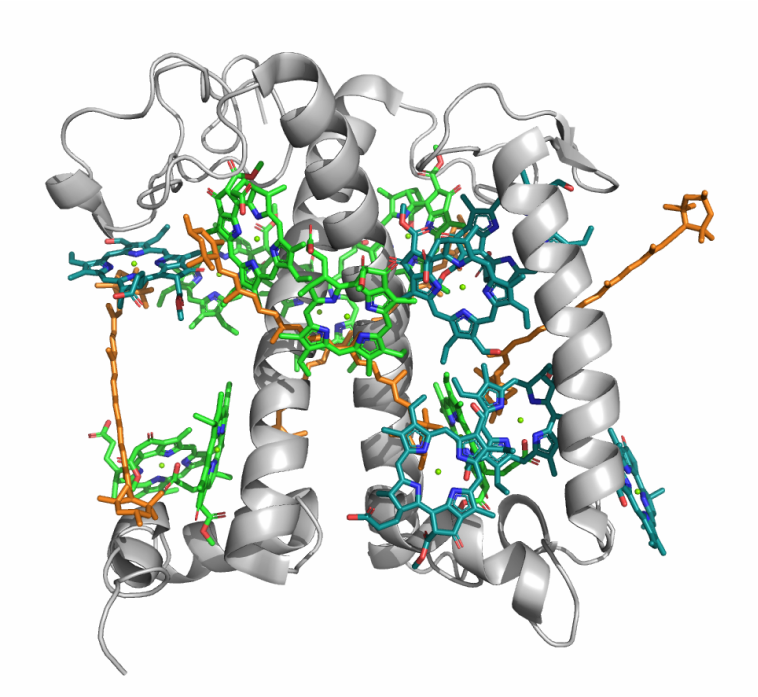

Figure 1.7: *Structure of the LHCII monomer from pea. Shown as a side view in the membrane plane. The lumenal surface is at the bottom, stromal surface at the top. The protein backbone is rendered in gray, Chls a are in green, Chls b in teal, carotenoids are in orange. Phytyl tails of the chlorophylls are omitted for clarity. Image was created using the crystal structure of pea LHCII in PDB ID: 2BHW [216].*

Having high concentration of pigment molecules in close vicinity to the reaction centers, how does the energy concentration mechanism work? Obviously, the absorbed light energy has to move from molecule to molecule, very efficiently, and be able to reach the reaction center before it is lost to the environment. It turns out that the excited pigment molecule can be viewed analogically to a harmonic oscillator which, via some sort of coupling, can transfer its energy to another oscillator. This is described at the molecular level by the Förster resonance energy transfer mechanism [220–222]. The coupling comes from the electrostatic interaction (dipole-dipole interaction) and depends on the magnitudes of transition dipole moments of the interacting molecules, their orientations and their distances. One other important condition for energy transfer is that the absorption and emission energies of interacting pigments are reasonably close.[40] In many cases, the energy flows from a molecule with a higher energy (shorter absorbance wavelength) to a molecule with a lower energy (longer absorbance wavelength) but this is not without exceptions [223, 224]. The pigment molecules present in many light-harvesting proteins are kept close to each other, at fairly precise positions, yet mostly prevented from getting

tinction coefficients of photosynthetic pigments are on the order of 100 000 $M^{-1}.cm^{-1}$. A common 1 cm spectroscopy cuvette filled with a solution of pigments with the same concentration as in the light-harvesting proteins would be pitch black even after 100× dilution.

[40] I.e. there is a non-zero overlap of the donor molecule emission spectrum and acceptor molecule absorption spectrum.

too close. Upon close contact (with distances approximately below 1.0 nm) the energy transfer mechanism is better described by a mechanism called exciton coupling, where two or more molecules act effectively as a single supermolecule in which the excitation energy can be delocalized and transferred also by means of electron exchange [225, 226].

Excitation energy transfer, like many molecular-level events, is probabilistic. To assure high efficiency, energy transfer has to be much faster (have higher probability) than energy dissipation mechanisms. Chl *a*, which is the terminal excitation energy acceptor, has an excited state lifetime of about 6 ns in solution [227]. In contrast, the last light-harvesting step—transfer of the excitation energy to the reaction centers—has a rate of about 50 ps or faster, giving the efficiency of this final energy transfer step of at least 99 % [213, 217]. Energy transfer across the whole pool of light-harvesting pigments in the antenna, where multiple energy transfer steps have to occur, is of course slower. The first steps happen inside the protein monomers and involve transfer from carotenoids to Chl *a*, from Chl *b* to Chl *a* and from higher energy Chls *a* to lower energy Chls *a*. The transfer times are on the order of a few picoseconds to fractions of a picosecond. Typically, many pathways are active in parallel [228–231]. The excitation energy then migrates between light-harvesting monomers in order to reach the reaction center. The total migration time depends on the total size of the excitonically connected supercomplex unit and can reach more than 150 ps in the case of PSII [232, 233]. Due to the fast energy transfer within a network of coupled pigments, the quantum efficiency of the light-harvesting system is close to unity.[41]. The energy efficiency is lower though as some energy is lost in relaxation processes from higher excited states, it is estimated that this loss is about 7 % [234]. Some photons are also absorbed by pigment molecules which do not transfer energy to Chl *a* such as some β-carotenes in photosystem cores or 'free' carotenoids present in the photosynthetic membranes.

The light absorption capability of pigment molecules cannot be turned off. The regulation of light harvesting on the molecular level[42] is therefore limited to two options. First, light harvesting capability can be enhanced or diminished by protein synthesis or deconstruction. Plants growing in darker conditions contain more Lhc proteins than those from highly illuminated spots. This acclimation process is mostly achieved by synthesis of additional LHCII trimers. The number of LHCII can at least double in low light versus high light laboratory conditions. Plant LHCII is rich in Chl *b*, therefore, the amount of antennas can be easily indirectly observed also on the organism level via changes in Chl *a*/Chl *b* ratio [45, 196, 245].

Second, the relative light-harvesting capabilities of the two photosystems can be rebalanced by relocating some of the LHCII trimers from PSII to PSI and vice versa, this process is called state transition. The utility of state transition stems from the fact that the two photosystems differ slightly in their light absorption spectra and in their turnover rates. PSI is able to absorb more light above 680 nm than PSII, which, conversely, is more efficient in capturing light around 650 nm due to its higher content of Chl *b* [246]. State transitions are therefore able to compensate for light spectrum changes during the diurnal cycle, changes in shading etc. Adjusting the relative flow of energy into the photosystems is also likely needed during changes of the activity of the linear and cyclic electron

[41] Assuming 100 ps as an average time from an absorption of a photon to its delivery to the reaction center, the efficiency is 98 %.

[42] On an organism level, light can be prevented from reaching the photosynthetic membrane by changes in leaf orientation [235, 236], production of light-blocking devices like hairs or crystals [237–239], or movement of chloroplasts [240–242]. Some unicellular organisms are also able to change their location in the environment in order to optimize light harvesting rates [243, 244].

flows. Relocation of LHCII during state transition is a result of its phosphorylation status, controlled by the plastoquinone pool redox state [247, 248]. Excessive activity of PSII leads to overreduction of plastoquinone pool which triggers activation of a kinase STN7. STN7 in turn phosphorylates LHCII proteins Lhcb1 and Lhcb2 which afterwards causes migration of the LHCII trimers towards PSI [249, 250]. The reverse reaction is triggered when oxidized plastoquinone pool, generated by comparatively greater activity of PSI, no longer activates the STN7 kinase. A phosphatase called TAP38 or PPH1 dephosphorylates LHCII, which in turn migrates towards PSII [251, 252].[43] The described phosphorylation and dephosphorylation processes take a few minutes to reach equilibria at the organismal level whereas the migration of individual LHCII trimers can be as fast as milliseconds [246, 248].

1.2.9 Photoprotection

It is our common experience that many industrially produced materials, like car paint or plastics, and even more so our living compatriots, as well as our own bodies, are subject to damage and destruction when exposed to high light intensity (Fig. 1.8). Despite of the state transition and the acclimatory responses of protein synthesis and degradation, photosynthetic organisms can experience frequent periods when the amount of collected excitation energy is in excess and cannot be productively utilized by the electron transport chain. Energy not used by the photochemical reactions can power unwelcome side reactions which can damage the unfortunate organism.[44] Due to its slower turnover rate and its oxidative power, photosystem II is the prime target of this damage, generally called photoinhibition. As the options for the regulation of the amount of light energy collected by the light-harvesting system are limited, most of the photosynthetic regulatory capacity is focused on controlling the fate of the harvested energy. There are two basic modes of operation of these regulatory mechanisms, which convert the excess excitation energy to heat. First, there are likely several pathways which safely dump the excess harvested energy before it can initiate harmful reactions. Most or all of these mechanisms are known under the umbrella term nonphotochemical chlorophyll fluorescence quenching (NPQ). Second, there are mechanisms which deactivate some of the harmful photoproducts before thay can cause further harm to the cellular components. These mechanisms quench either triplet states of chlorophyll or reactive oxygen species created by the triplet states of chlorophyll.

Part of the energy harvested by the photosynthetic pigments is lost to the environment in the form of fluorescence. Effectively all of this fluorescence is coming from Chl *a* located in the light-harvesting complexes. It has been recognized early in the history of photosynthesis research that the yield of the chlorophyll fluorescence is not constant [18, 258–260]. When the electron transport chain of the thylakoid membrane is activated, it withdraws energy from the light-harvesting system and thus lowers the fluorescence yield.[45] This is to be expected and the observation and the process are called photoche-

[43] Interestingly, state transition was originally discovered from Chl *a* fluorescence yield changes in cells of red and green microalgae [253, 254]. The process is not known from other eukaryotic algae like diatoms or haptophytes, which have secondary endosymbiotic chloroplasts.

[44] Most of the destructive reactions involve molecular oxygen, which is activated either by transfer of electrons, creating superoxide, O_2^- [255], or by energy transfer from a photosensitizer (mostly Chl *a*), creating singlet oxygen, $^1O_2$ [256]. It thus appears that life truly was better in the past (before the Great Oxidation Event) [257].

[45] Conversely, when the electron transport chain is blocked, the fluorescence yield increases by a factor

Figure 1.8: *A Phalaenopsis orchid bleached by direct sunshine. The plant was grown in a south-facing yet shaded place and suffered after the solar elevation dropped in the autumn. A period of overcast weather prevented a gradual acclimation of the plant to the new conditions. Photoprotective mechanisms, like many other regulatory features, are tuned to the specific conditions, the plant does not maintain high protective capacity when it is not needed. While a multicellular organism like this orchid can cauterize around the wound, a unicellular alga does not have such an option. Plant and photo from the author's collection.*

mical quenching. There are, however, other quenching processes, which are not directly related to the electron transfer and are therefore called nonphotochemical [17, 263, 264]. Nonphotochemical processes dissipate excitation energy as heat and thus also cause a decrease of the yield of the Chl *a* fluorescence (see Fig. 1.9). The extent of the NPQ depends on the organism and its acclimation state [265]. As much as 80 % of harvested excitation energy can be quenched by NPQ [266–269]. The nature and mechanisms of the nonphotochemical quenching processes are debated to this day. There are, however, several players known to be active in the NPQ.

Foremost, it is clear that the major NPQ mechanisms are triggered by energy flow through the membrane as a result of actinic illumination and the accumulated protons in the lumen, i.e. they are 'energy-dependent'. NPQ then requires the presence of a protein called PsbS [270, 271] and chemical modification of some of the violaxanthin molecules present within the thylakoid membrane into zeaxanthin—so called xanthophyll cycle [272, 273]. PsbS is a 21 kDa protein of the extended Lhc family. Unlike most Lhcs, PsbS has four transmembrane helices and does not bind pigments [274, 275]. It is presumed that PsbS is dimeric in an inactive form and monomeric in an active form [276].

Formation of low pH in the thylakoid lumen upon intense illumination causes protonation of the PsbS protein, which activates a quenching state of the light harvesting antenna.

of three to five [261, 262].

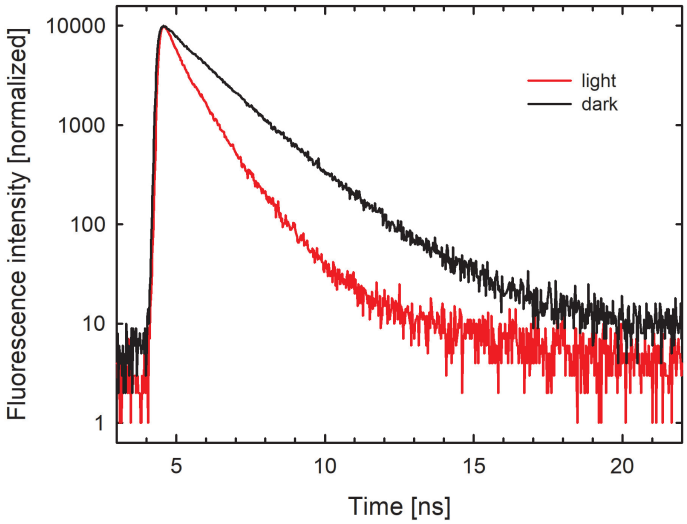

Figure 1.9: *Time-resolved chlorophyll fluorescence signal from cells of haptophyte alga Emiliania huxleyi. NPQ processes were activated by 1 h exposure to 1000 μmol photons.m^{-2}.s^{-1} light intensity (about 50 % of full sunlight). The area under the red curve (light-exposed cells) is 53 % of the area under the black curve (dark-adapted cells). All else being equal, one half of the harvested light energy is converted to heat when NPQ is active in these cells. Author's unpublished data.*

In parallel, a lumenal enzyme violaxanthin de-epoxidase (VDE) is activated by the low pH. VDE catalyzes removal of the two epoxide groups from violaxanthin in a two-step process, producing zeaxanthin.[46] The nature and the mechanism of the quenching state are not known [278]. Zeaxanthin and the protonated PsbS are likely not the protective species themselves but rather produce a quenching state of an unknown nature. It is speculated that the quenching state involves a conformational change in one or several light-harvesting proteins and also a different aggregation state of the membrane proteins. Presumed primary quenching targets include PSII monomeric light-harvesting proteins CP24 and CP29 and the major antenna LHCII [279–282].

Violaxanthin deepoxidase, the key enzyme of the xanthophyll cycle, is a 43 kDa protein which belongs to the lipocalin family.[47] VDE is monomeric and water-soluble at neutral pH but becomes dimeric and attached to the thylakoid membrane at low pH [286, 287]. The protein is rich in cysteine and contains six disulphide bonds [288].[48] VDE requires ascorbate as a source of electrons. The enzyme catalyzing the reverse reaction, epoxidation of zeaxanthin, is zeaxanthin epoxidase. Zeaxanthin epoxidase is located in the chloroplast stroma and it also has a lipocalin structure [283, 284]. While the deepoxidation reaction

[46]The full deepoxidation process is violaxanthin (with two epoxy groups) → antheraxanthin (one epoxy group) → zeaxanthin. The epoxy groups are on the opposite sides of the violaxanthin molecule, thus the carotenoid has to exit the VDE enzyme, rotate 180° and enter the enzyme again if the second epoxy group is required to be removed as fast as possible. Some eukaryotic algae use a different version of the cycle based on the carotenoid diadinoxanthin with a single epoxy group, resulting in a single-step deepoxidation reaction, forming the carotenoid diatoxanthin [277].

[47]Lipocalins are widely distributed and ubiquitous proteins known predominantly from animal studies and mostly involved in binding nonpolar molecules. Lipocalin functions are known e.g. in embryo development, nutrition (major bovine milk allergen β-lactoglobulin is a lipocalin) or the nervous system [283, 284]. Crustacyanin, the blue lobster protein binding the carotenoid astaxanthin is also a lipocalin [285].

[48]The reducing agent dithiothreitol (DTT), which is capable of breaking the disulphide bonds in VDE, is routinely used to disrupt the xanthophyll cycle mechanism in plant and algal experiments [289–291].

can be quite fast at high illumination intensities, running in tens of seconds or minutes, the back reaction is about 10× slower [292, 293].[49]

An excited chlorophyll molecule has a certain probability of intersystem crossing from a singlet state to a triplet state.[50] Chl *a* in a triplet state has a long lifetime (about five to six orders of magnitude longer than the lifetime of the singlet state) and can transfer its energy to molecular oxygen, quenching the Chl *a* back to the ground state and creating singlet oxygen, a highly reactive species [300]. The quantum yield of the triplet state formation in a free Chl *a* in solution is higher than 60 % [256, 301, 302]. A well-tuned photosynthetic membrane lowers the yield of the Chl *a* triplet formation to about 2–6 % by shortening the lifetime of the excited Chls *a* (either by energy transfer or by the quenching mechanisms indicated above) [268]. A second major Chl *a* triplet-forming mechanism is a charge recombination in the PSII RC which is of importance especially in conditions of high illumination intensities [298, 303]. During the recombination process, the charges separated by the primary donor do not necessarily proceed on the path described in the section 1.2.1 but come back together and recombine, yielding Chl *a* triplet state with a probability of about 20 % [304].

In spite of all the quenching power of the NPQ mechanisms, some Chl triplet states (and therefore singlet oxygen) are still produced in the antenna and also in the photosystem RCs. The protection against damage caused by these species is mostly carried out by carotenoids, which are able to accept the energy of the triplet Chls via a close-range electron exchange mechanism [305–308]. The result of the energy transfer is the Chl in the ground state (singlet) and an excited carotenoid in a triplet state. A carotenoid in a triplet state has sufficiently low energy so that it can not excite molecular oxygen and the excess energy is safely converted to heat.[51] An important condition for the energy transfer from the triplet Chl to a carotenoid is that the two molecules are in close contact. In LHCII, the Chl triplet states are quenched by two luteins located in the very centre of the protein [310–312]. Chl triplet states are quenched by carotenoids of the core antenna proteins as well, in this case by β-carotene [313–315]. In contrast to the light-harvesting proteins, the recombination triplet of the PSII RC is not quenched by carotenoids [316]. The β-carotenes present in the PSII RC have to be placed at a considerable distance from the primary donor lest they be oxidized due to its tremendous oxidative power. As a result, singlet oxygen is inevitably produced in the RC when all the other protective mechanisms fail. The β-carotenes of the RC likely do quench part of the produced singlet oxygen [303,

[49] It appears that in some conditions the photoprotective mechanisms are unnecessarily conservative, resulting in lost crop productivity when too much excitation energy is quenched. Under highly controlled conditions of modern agriculture, enhancing the rate of the zeaxanthin epoxidation could thus result in higher crop yields [294–296].

[50] The terms 'singlet' and 'triplet' are related to the spin of electrons and spectroscopy features associated with the spin configuration. An atom with electrons with paired spins in its orbitals has the total spin of zero. Such an atom is said to be in a singlet state (because there is only one energy level to be observed). Transitions between orbitals during the excitation and relaxation processes conserve the spin orientations. After excitation, the excited electron can, under some circumstances, obtain an opposite spin orientation (the process is called intersystem crossing) and thus reach a state which prevents it from relaxing back to the ground state (by doing so it would violate the Pauli exclusion principle). Such a state is called a triplet state because three spectral lines (a triplet, corresponding to three energy levels) are visible in the spectrum of such species after an application of an external magnetic field [297]. Most organic molecules in the ground state have paired electrons and are therefore in a singlet state. Molecular oxygen has a triplet ground state which constrains the rate at which it can react with organic molecules [298, 299].

[51] Carotenoids have extremely low fluorescence yields but very high rates of thermal deexcitation pathways [309].

317] but the PSII RC remains the most sensitive part of the electron transport chain [318–320].

1.3 Photosynthesis in the tree of life

Life is presently classified into three major branches or domains of organisms—Bacteria, Archaea and Eukarya [321, 322]. The name Bacteria is perhaps self-explanatory and includes a tremendous diversity of organisms both free-living and parasitic but also photosynthetic cyanobacteria, non-oxygenic phototrophs or ancestors of eukaryotic mitochondria. Archaea are the newest member of the club, previously pooled with Bacteria but their cellular features are so much different that establishment of a new group was deemed justified. Archaea are not as well known as bacteria but their importance is difficult to overstate as they are basically omnipresent and the group contains for example the methanogenic organisms present in bovine guts or many nitrogen-fixers and ammonia-oxidizers [323]. Eukarya differ from the other two domains by having complex cells with genetic information in nucleus, organelles (mitochondria and chloroplasts) and many other differences as well. The domain Eukarya contains basically all individual organisms one can see with bare eyes—plants, animals, mushrooms, lichens—and also a lot of microscopic organisms including e.g. unicellular algae.[52]

Photosynthesis can be found today only in Bacteria and Eukarya. Sadly, there is no known archaean organism using the photosynthetic machinery of the light reactions. Archaeans of course do not ignore light energy completely. Many archaeans are known to use proteins of the rhodopsin family, which are relatively simple light-driven proton pumps [324, 325]. Interestingly, many members of Archaea also possess genes for Rubisco, the key enzyme of the carbon reactions of photosynthesis [326, 327].

1.3.1 Prokaryotic photosynthesis

Bacteria as a group provide a very broad diversity of metabolic pathways, including photosynthesis. Besides cyanobacteria with complete oxygenic photosynthesis machinery, there are anoxygenic phototrophic bacteria which only use type-I or type-II reaction centers. Moreover, the diversity of bacterial photosystem reaction centers is much greater than that of eukaryotic phototrophs [328].

Cyanobacteria are at present by far the most important class of photosynthetic prokaryotes. Besides producing oxygen, they are also responsible for most of the oceanic nitrogen fixation[53] [329, 331]. It is presently not clear whether oxygenic photosynthesis in cyanobacteria is ancient and the anoxygenic bacterial photosynthesis is more modern or *vice versa* [20]. Early Earth lacked molecular oxygen in its atmosphere and an appearance of oxygen and accompanied geochemistry, as well as remains of photosynthetic organisms, can be followed in the geological record [332, 333]. The hard limit on the evolution of oxygenic photosynthesis and also cyanobacteria-like organisms is the Great Oxidation Event—a sudden increase of molecular oxygen concentration in the atmosphere from barely detectable to single digit percent of present level—dated to approximately 2.3 billion years before today [334–336]. Fossil signs of presumed cyanobacterial life are

[52] It is possible that we, as members of Eukarya, belong *sensu stricto* to a branch of Archaea but the functional differences justify the classification sufficiently, especially in the context of this text.

[53] It may be surprising to the reader that about half of the global nitrogen fixation is now provided by human-related activities. We truly live in an anthropocene [329, 330].

much older though. One of the first known fossilized remains of life are well-preserved 3.4 billion year old stromatolites, sedimentary structures even today occationally formed by cyanobacterial mats [337]. The assignment of these fossils to cyanobacteria is however disputed[54] and, although there is some evidence of prior transient oxygen content increases [333], in the end the only reliable date for emergence of oxygenic photosynthesis is the abovementioned Great Oxidation Event [335, 338, 339].

It is assumed that cyanobacteria-like organisms were the first to introduce complete oxygenic photosynthesis to the tree of life [21, 338, 339]. However, known extant cyanobacteria groups mostly evolved after the Great Oxidation Event [340] and current cyanobacterial marine phytoplankton groups are much younger, dating to the late Proterozoic [341]. Thus, it is not clear at all whether the appearance of oxygenic photosynthesis coincided with cyanobacteria-like organisms or whether some as yet undiscovered or extinct group developed modern photosynthetic apparatus. It is also not clear whether the ancient cyanobacteria combined pre-existing type-I and type-II photosystems into oxygenic photosynthesis or whether some other evolutionary pathway played out. There is actually a well-argued proposition that the very first photosynthetic reaction centers were oxygen-evolving and only later specialized into the present type-I and type-II structures [342]. Understanding the origin of photosynthesis is hampered by the vast time gap between the invention of oxygen-evolving enzymes and present time. Luckily, it seems that the age of discovery is not over as novel bacterial phototrophs are still being discovered [343–345]. Perhaps serendipity will help us by providing a 'missing link' organism with yet unknown type of photosystem reaction center. Coupled with structural biology insight this could lead to dramatic changes to the present theories of origin of photosynthesis.

Cyanobacteria possess both types of reaction centers, chlorophyll *a* as a major pigment[55] and simple carotenoid composition with β-carotene and zeaxanthin.[56] Besides chlorophyll *a* as the major light-harvesting tetrapyrrole, most cyanobacteria also use linear tetrapyrrole pigments, phycobilins, which are covalently bound to specific water-soluble light-harvesting proteins called phycobiliproteins [349]. While the above-mentioned features are present also in at least some eukaryotic organisms, there are a few photosynthetic features which are unique to cyanobacteria. Many cyanobacteria contain membrane-bound light harvesting protein IsiA which is not related to the eukaryotic light-harvesting proteins [350]. Some cyanobacteria are also known to use unique chlorophyll-type pigments—chlorophyll *d* and chlorophyll *f* [351, 352].

Other photosynthetic bacteria differ from cyanobacteria[57] by lack of oxygen production, lack of chlorophyll *a* pigmentation, lack of phycobilins and presence of only one type of reaction center in their photosynthetic membranes. Anoxygenic photosynthesis is known from six[58] bacterial groups [328]: Chlorobi (green sulfur bacteria), Chloroflexi (filamentous anoxygenic bacteria) [354], Proteobacteria (purple sulfur and non-sulfur bacteria), Heliobacteria [355], Acidobacteria [343] and Gemmatimonadetes [344]. Although the metabolic features present in anoxygenic bacteria are likely ancient, it is not clear how old are the extant groups and whether they correspond to the organisms which dominated

[54] It is not clear whether the organisms can be interpreted as cyanobacteria or as some other, potentially non-oxygenic, phototrophs.

[55] There are a few cyanobacteria which contain chlorophyll *b* in an accessory role [346].

[56] Other carotenoids like echinenone, canthaxanthin or synechoxanthin may be present in supporting roles [347, 348].

[57] From the point of view of photosynthesis.

[58] There seems to be another newly discovered group called *Candidatus* Eremiobacterota [345, 353]. Thus the present count is 7 groups of photosynthetic anoxygenic bacteria.

Earth before the Great Oxidation Event [20, 356].

Anoxygenic bacteria as a group present a remarkable demonstration of a modular approach to assembling the photosynthetic apparatus. Diverse combinations of reaction centers, light-harvesting antennas and metabolism in general are presented in the known phototrophic bacteria groups. Type-II reaction centers are used by Proteobacteria, Chloroflexi and Gemmatimonadetes. Type-I reaction centers in the form of homodimer are found in Chlorobi, Heliobacteria and Acidobacteria. Huge non-protein light-harvesting chlorosomes are found in Chlorobi, Chloroflexi and Acidobacteria but only Chlorobi and Acidobacteria use specialized FMO protein for connection between the chlorosome and the photosystem [357]. Some Proteobacteria, Acidobacteria and Gemmatimonadetes are capable of using photosynthesis in the presence of oxygen while the other groups need anoxic conditions to be able to use light energy. Heliobacteria are the only phototrophs which do not use any light-harvesting system at all. Bacterial photosystems are generally based on bacteriochlorophyll and bacteriopheophytin pigmentation but the type-I reaction centers of Chlorobi, Heliobacteria and Acidobacteria use chlorophyll *a*-derivatives as a primary acceptor [358–360].

1.3.2 Eukaryotic photosynthesis

Eukaryotic diversity

The incorporation of a (proto-)cyanobacterial cell into another cell in the process of endosymbiosis started the long path towards ferns, trees and flowers but also green, red and brown algae and a vast diversity of other interesting creatures. Eukaryotic cells developed complex multicellularity with specialized cells and tissues—a feature of obvious interest to us. Multicellularity evolved independently in five eukaryote groups: animals, fungi, green algae, red algae and brown algae, three of which are photosynthetic [361]. The entirety of eukaryotic life can be classified into a small number of relatively well-supported monophyletic supergroups[59] and a bunch of stragglers for which the evolutionary relationships are not clear [362, 363]. A simplified tree of eukaryotic life is presented in Fig. 1.10. Humans and other animals, as well as e.g. fungi, belong into a branch called Opisthokonta[60]. Opisthokonta, together with another closely related group Amoebozoa, are the only major branch of eukaryotic life which does not contain any photosynthetic organisms[61].

The descendants of the pioneering proto-alga form a branch called Archaeplastida [365]. Archaeplastida contain three lineages: red algae (Rhodophyta), green algae and plants (Viridiplantae) and a lesser-known group Glaucophyta. Archaeplastida are the source of photosynthesis for many other branches of eukaryotic life, which acquired it via secondary or even tertiary endosymbiosis [26]. Possibly closely related to Archaeplastida are Cryptophytes (Cryptista), a group of algae with photosynthetic features very similar

[59] Limited and simplified coverage of the tree of life is presented here. It is otherwise a very complex biological issue. An interested reader should consult Burki *et* al. [362] for very readable and probably the most up-to-date information.

[60] The name literally means flagellum at the rear [of the cell]. Other organisms often have flagella at the front or on the side of the cell.

[61] Organisms like corals, sea anemones and other cnidarians might be pushing the distinction a little because they need symbiotic algae to survive. Likewise lichens (which are a type of fungi) need symbiotic algae for survival. Nevertheless, these are examples of two organisms coexisting together rather than one organism having integrated in one cell all necessary components of the photosynthetic apparatus. Some amoebozoan organisms also contain green algae cells and likely can be also swept under the rug as exceptions [364].

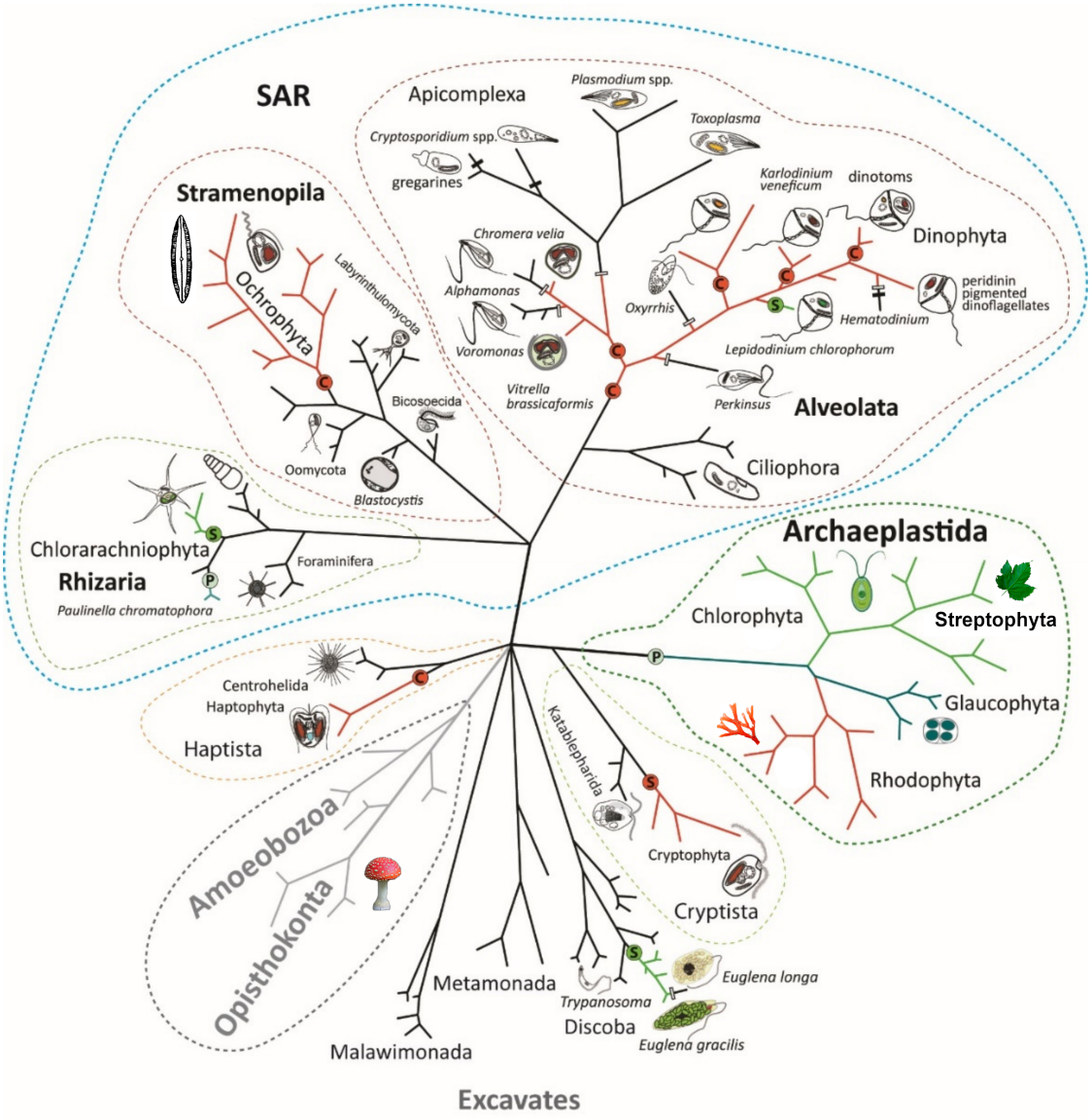

Figure 1.10: *A simplified tree of eukaryotic life. Major branches are enclosed in dotted lines. The SAR group (Stramenopila, Alveolata and Rhizaria) is enclosed in blue dotted line. Branches with organisms with chloroplasts of green algal origin are in green, of red algal origin are in red. Reused from Oborník (2019) [26] and modified, Creative Commons Attribution (CC BY) licence (http://creativecommons.org/licenses/by/4.0/.).*

to red algae and a chloroplast of clearly red algal origin [366]. Haptophytes (Haptista) are another separate branch. Haptophytes include many extremely abundant marine algae which are also prominent in the geological record. The photosynthetic features of Haptophytes are complex and their evolutionary history is not clear at the moment [367, 368]. A number of branches of unclear evolutionary position can be pooled together under the name Excavata[62]. This likely non-monophyletic group includes the well-known *Euglena* 'alga' which contains a chloroplast of green algal origin. Euglenas are not related to any other major algae group. Among the relatives of photosynthetic euglenas are the parasitic trypanosomas which cause diseases like sleeping sickness or Chagas disease but there are also *Phytomonas* organisms infecting plants [363, 369, 370].

A major part of the eukaryotic family is the SAR supergroup (enclosed by blue line in Fig. 1.10) [371]. SAR stands for Stramenopila, Apicomplexa and Rhizaria, the three branches which form the supergroup. SAR supergroup encompasses a multitude of life strategies and includes diverse algae, parasites of animals, agricultural pests, deep ocean protists with huge cells and many other organisms. Most photosynthetic members of SAR contain what appears to be a modified chloroplast of red algal origin but there are also some organisms with apparently green algal chloroplasts. Even the non-photosynthetic organisms in SAR often contain chloroplast remnant structures [372]. All of the work presented in subsequent chapters of this text was carried out on SAR member organisms, specifically stramenopiles. The major algal groups and some of their distinguishing characteristics are listed in Table 1.1.

Table 1.1: *Overview of major eukaryotic phototrophic groups. See text for details and references.*

Algae group	No. of species	oldest fossil [Mya]*	note
Red algae	7 500	1 000	phycobilins, Lhcr
Chlorophyta	8 000	700	Chl *b*, lutein
Streptophyta	300 000	420	Chl *b*, lutein, land plants
Cryptophyta	200		phycobilins, Chl *c*, alloxanthin
Euglenida	1 000		Chl *b*, diadinoxanthin
Stramenopila	20 000	130	Chl *c*, fucoxanthin
Dinoflagellata	4 000	240	Chl *c*, peridinin, PCP
Haptophyta	1 500	205	Chl *c*, modified pigments

* Mya = Millions of years before present

Archaeplastida

The oldest eukaryote fossils are sparse and often with inconclusive interpretation [373–375]. There appears to be sufficient evidence for the presence of eukaryotes around 1.5 billion years ago [374, 376] though due to the small size of the early eukaryotes and difficult preservation conditions there is always a danger of misinterpretation of the geologic evidence[63] or contamination from younger strata in the case of chemical fossils [375].

[62] Here I take the liberty of oversimplifying the complexity of these lineages as they're not that much involved in the photosynthetic story at hand.

[63] As a good example, one can quote Knoll et al. here: *"Among these, coiled fossils assigned to Grypania spiralis are most confidently interpreted as eukaryotic. (Most other forms could be fortuitously shaped fragments of microbial mats.)"* [374]

As concerns the evolution of photosynthetic organisms, the most ancient eukaryotic algae with fossil record appear to be **red algae** (Rhodophyta). The earliest eukaryotic fossil with generally accepted phylogenetic position is a ~1000 million years old red algal form *Bangiomorpha* from rocks in what is now arctic Canada [377, 378].[64] Red algae are predominantly marine and multicellular organisms [361, 379]. From the photosynthetic vantage point, red algae represent an intermediary step between the photosynthetic apparatus of cyanobacteria and that of green algae and land plants. Like cyanobacteria, red algae use chlorophyll *a* as the sole chlorophyll-type pigment[65], accompanied by β-carotene and zeaxanthin. Moreover, phycobiliproteins are used as peripheral light-harvesting antennas. An innovation of the red algae or, more probably, of the common ancestor of red and green algae are the membrane-intrinsic light-harvesting proteins (in red algae called Lhcr for Light Harvesting Complex Red) [383–387]. In red algae, the Lhcr proteins form an antenna of photosystem I whereas photosystem II uses membrane-extrinsic phycobiliproteins [385]. Despite the obvious importance of red algae for understanding evolution of plant photosynthesis and the number of species, about 7 500[66], comparable with green algae, research into red algae has been of relatively low intensity. A large part of the key work in photosynthesis was carried out on extremophilic *Cyanidium*, *Cyanidioschyzon* and *Galdieria* [385, 388–390]. This is unfortunate as these organisms are from a basal branch of red algae radiation and are known to possess reduced genomes so it is not clear how much they are representative of a broader sampling of red algae [391–393].

A minor and most likely basal group of Archaeplastida are glaucophytes. **Glaucophytes** are a small, exclusively freshwater, group of only about 15 species [394]. The photosynthetic apparatus of glaucophytes is similar to that of cyanobacteria [395], using phycobiliproteins for light harvesting and only chlorophyll *a* [396]. Glaucophyte chloroplasts—sometimes called cyanelles—are enveloped in a peptidoglycan layer which is often considered a typical bacterial feature and mostly missing in eukaryotes.[67] Glaucophytes are the only eukaryotic photosynthetic organisms which lack the Lhc–type light harvesting antenna proteins but they do contain two–helix SEPx proteins which could be the predecessors of the Lhc proteins [398].

The third group of Archaeplastida are **Viridiplantae**. Viridiplantae are formed by two major branches: marine and freshwater Chlorophyta and mostly freshwater Streptophyta [399, 400]. Chlorophytes contain a diverse assembly of green algae while streptophytes include land plants and green algae related to them. Multicellularity evolved independently in both branches of Viridiplantae [401]. The origin of Viridiplantae clearly lies in the proterozoic but producing a reliable date is difficult [399, 402]. The earliest identifiable green algae fossils come from the Cryogenian era of the late Proterozoic (~700 million years old *Palaeastrum* and *Proterocladus* [403, 404]) but most of the diversity is only found

[64] Interpretation of microfossils and chemofossils is often controversial and molecular dating is an evolving field with large error margins. Thus, only traditional fossils will be considered in the text. It should be understood that the organism groups must be sometimes much older than the given age.

[65] To this day the myth of presence of chlorophyll *d* in red algae [380] is perpetuated. It has been clearly shown that presence of Chl *d* in pigment extracts of red algal thalli comes from epiphytic cyanobacteria of the *Acaryochoris* affinity [381, 382]. Besides, there is no known Chl *d*-containing protein from red algae. The contamination by Chl *d* is apparently very common, it was also detected by the author in a sample of red algae from the Adriatic Sea (not published).

[66] Guiry, M.D. & Guiry, G.M. 2022. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. https://www.algaebase.org; searched on January 13, 2022.

[67] Peptidoglycan is missing in red algae and chlorophyte algae but present in streptophyte algae, mosses (bryophytes) and lycophytes. It is therefore not such a unique oddity of glaucophytes as sometimes presented [397].

after the Cambrian explosion (~530 million years ago) [405]. Streptophytes colonized dry land starting likely in the late Silurian (~420 million years before present) [406]. From photosynthetic point of view, all Viridiplantae are characterized by the presence of a large number of Lhc proteins with much larger functional diversity than in the red algae, and by a universal use of chlorophyll *b* as an accessory pigment. In Viridiplantae, Lhc proteins form light–harvesting antenna systems of both photosystems. The photoprotective four–helix PsbS protein is also exclusive for Viridiplantae [271, 398]. While the cannonical green plant of the Streptophyta branch uses neoxanthin, violaxanthin, lutein and β-carotene as well as the abovementioned Chl *b* in photosynthetic roles, some exceptions do exist in the chlorophytes.

Chlorophytes can be broadly split to four groups—basal and paraphyletic Prasinophyta and three 'crown' chlorophyte branches of Trebouxiophyceae, Chlorophyceae and Ulvophyceae [399, 407]. In total, chlorophytes include about 8 000 species,[66] with about 900 in Trebouxiophyceae, 4 000 in Chlorophyceae and 2 700 in Ulvophyceae. Ulvophyceae are mostly marine, some of them, e.g. *Acetabularia* or *Codium*, with unique macroscopic 'multicellular' bodies, which are in fact formed by one huge cell with thousands of nuclei [399]. One of the branches of Ulvophyceae, Bryopsidales, possesses a unique carotenoid profile with keto–carotenoids siphonaxanthin and siphonein largely replacing lutein and also a presence of Chl *c*, otherwise characteristic for the 'brown' algae [408–410]. Chlorophyceae include many model species well-known in the photosynthetic community (e.g. *Chlamydomonas*, *Dunaliella*, *Scenedesmus* or *Volvox*) [399]. Another staple of photosynthesis research, *Chlorella*, does not belong into Chlorophyceae as its name could suggest but is a member of the third crown group, Trebouxiophyceae.[68] Trebouxiophyceae are perhaps most interesting as a source of symbiont organisms, 'zooxanthellae' of lichens, anemones [411], various amoebae [364], ciliates (of the Alveolata group) [412–414] and even a *Ginkgo* tree [415, 416]. There are however also many examples of chlorophyte algae involved in relationships ranging in their nature from endophytism to outright parasitism, including in humans [417, 418].

The paraphyletic lineage called Prasinophyta is an assortment of basal chlorophyte groups which phylogeny is currently not very well understood [399, 401, 419]. Some prasinophyte organisms present common green algal pigmentation but there are at least two other pigmentation types. Some species[69] use siphonein/siphonaxanthin as major carotenoids [420]. There is also a large number of species, including relatively well studied *Ostreococcus* and *Mantoniella*, known to use an unusual mixture of multiple unique carotenoids—prasinoxanthin, uriolide, micromonal and dihydrolutein—as well as chlorophyll *c*-like pigment in addition to Chl *b* [421–424]. The prasinophyte diversity is likely not fully covered yet, for example the recently described *Picocystis*, which has a mixed carotenoid profile with alloxanthin, monadoxanthin and diatoxanthin (thus presenting in

[68] When we were about to start work which culminated in results presented in the following chapters, we have obtained a strain of *Nannochloropsis* and started cultivation. One of the first obtained data was an absorption spectrum of the culture. The spectrum showed a prominent shoulder at 650 nm, typically indicating presence of chlorophyll *b*. Suspected of contamination by a random 'wildtype' green algae, the culture was checked under the microscope but it appeared to be homogeneous. An analytical HPLC was carried out and showed abundant Chl *b*. Upon further investigation it was discovered that the person ordering the culture made a mistake and ordered *Nannochloris*, a member of the Trebouxiophyceae. We have since used the result as a demonstration to students that even a simple absorption spectrum can be very informative. No further work was carried out on this organism in our lab.

[69] Or groups, it is difficult to ascertain from current literature what is a general characteristics of a phylogenetic group and what is an idiosyncracy of specific species or even just a single cultivated strain of a species.

one cell the characteristic pigments of the green lineage, cryptophytes and stramenopiles), is likely a representative of a separate branch within prasinophytes [425]. Of the nine described prasinophyte branches, two are only known from environmental DNA sequences [426]. Available data suggest that prasinophytes lack proteins forming the cannonical plant LHCII complexes though 'crown' chlorophytes do have these [424, 427].

Streptophytes are the most well-known photosynthetic organisms as the group includes all land plants (Embryophyta, more than 300 000 species [428]) and most of the knowledge of photosynthesis comes from the study of this group.[70] Streptophytes include six algal groups, the most numerous of which are Charophyceae (1 000 species) and Zygnematophyceae (4 000 species).[66] The latter group is now believed to be the closest relative of land plants [429]. Land plants themselves can be split into bryophytes (mosses and liverworts) and tracheophytes (all other groups: lycophytes, ferns, gymnosperms and angiosperms) [430–432]. The earliest known tracheophytes come from the Silurian (~420 million years before present) [433, 434]. Ferns (Pteridophyta) appeared in the Middle Devonian (~385 million years before present) [435], gymnosperms in the Carboniferous (~315 million years before present)[436] and finally angiosperms in the Early Cretaceous (~115 million years before present) [437].

Eukaryotes with secondary plastids

Apart from Archaeplastida, one other case of primary endosymbiosis is known—the amoeba *Paulinella* acquired a cyanobacterial endosymbiont about 100 million years ago [438, 439].[71] All other known photosynthetic eukaryotes: cryptophytes, excavates, all three branches of the SAR supergroup, and haptophytes, acquired photosynthesis from organisms of the Archaeplastida affinity, mostly from red algae.

Cryptophyta is a small (about 200 species[66]) group of unicellular organisms with unique photosynthetic features inherited likely from a red algal endosymbiont. Cryptophyte chloroplasts are enveloped by four membranes and include a remnant of the endosymbiont nucleus called nucleomorph [440]. The organisms therefore contain four genomes—in the nucleus, mitochondrion, chloroplast and nucleomorph—and likely represent an intermediate stage of the secondary endosymbiotic process in which the endosymbiont is not yet fully controlled by the host nucleus. Cryptophyte photosynthetic light harvesting is based on a combination of water-soluble phycobiliproteins and membrane-intrinsic Lhc-type proteins. Cryptophyte phycobiliproteins do not form phycobilisomes like in cyanobacteria and red algae but rather smaller assemblies which are actually present on the opposite—lumenal—side of the thylakoid membrane than in the other organisms [441, 442]. The Lhc proteins of cryptophytes contain Chl c_2 as an accessory pigment and alkyne carotenoids alloxanthin, crocoxanthin and monadoxanthin [443–446]. Most of the photosynthesis research was done on *Rhodomonas*, *Chroomonas* and *Guillardia* species [447–450].

All photosynthetic excavates are members of the **Euglenida** group [370]. Euglenids can be autotrophic or heterotrophic and the autotrophic members can be found in both freshwater and marine environments. Euglenids possess a secondary endosymbiotic green plastid, with Chl *b*, originating in one of the prasinophyte lineages [451, 452]. There are about 1 000 named species within the mostly autotrophic Euglenophyceae class.[66] The

[70] Land plant photosynthetic apparatus has been described sufficiently in chapter 1.2 and will not be covered in more detail here.

[71] Because *Paulinella* is a rhizarian, member of the SAR supergroup, it'll be briefly mentioned later.

light harvesting proteins are likely very similar to standard 'green' systems though the PSI antennas are derived from the Lhcb branch of proteins instead of the cannonic Lhca [427]. Not much is known about the biophysics of light harvesting in euglenids but the carotenoid complement of Lhcs is unique, especially in the context of 'green' plastids and very interesting as the major carotenoid is diadinoxanthin supplemented with a minor content of neoxanthin [453, 454].

The **SAR supergroup** contains what is often in photosynthetic literature pooled together as 'brown' algae or algae with chlorophyll *c*. A simplistic view suggests that SAR includes mainly diatoms (perhaps the most important example of stramenopile algae), dinoflagellates (of coral symbiont and red tide fame) and an assortment of 'others' which leaves out a significant part of the diversity. While all three branches of SAR—Stramenopila, Alveolata and Rhizaria—contain photosynthetic organisms, the relative share of photosynthetic species within these three assemblages is very different and none of the groups can be viewed as (almost) exclusively photosynthetic as is the case for Archaeplastida. While a major branch of stramenopiles is composed almost exclusively by phototrophs, only a fraction of alveolates carry out photosynthesis and just a few photosynthetic organisms can be found within Rhizaria.

Stramenopiles[72] are presently classified into dominantly photosynthetic Ochrophyta and two heterotrophic groups of Pseudofungi and Bigyra [456]. To provide a little insight into the obscure names, Bigyra includes *Opalina* [457], parasite of frogs (described and named by Czech physiologist Jan Evangelista Purkyně in the 19th century [458]) and *Blastocystis*, a parasite of human intestine [459]. Pseudofungi most importantly include a group called Oomycetes (water moulds in English). Oomycetes are important fungus-like[73] parasites of crops, a cannonical example being *Phytophthora infestans*, the cause of potato blight disease [460].

Ochrophytes[74] represent the dominantly photosynthetic branch of stramenopiles. Their (and SAR in general) chloroplast is most likely of red algal origin [461, 462]. Ochrophyte photosynthetic membrane apparatus differs from the assumed red algal ancestor predominantly by the lack of phycobiliproteins, great diversification of the Lhc proteins and very much altered pigmentation. Typical features of ochrophyte photosynthetic apparatus are Lhc proteins derived from the red algal Lhcr, diversified into Lhcf and photoprotective Lhcx protein classes, and the presence of Chl *c*, fucoxanthin as a major carotenoid and diadinoxanthin-based xanthophyll cycle. Exceptions are common, e.g. eustigmatophytes lack all of the mentioned pigments.

In current understanding, ochrophytes count about 20 classes [463–465], many of them with only a few known members. As a whole, ochrophytes are the second most species-rich group of photosynthetic organisms (after Viridiplantae) with the bulk of the species[75] being diatoms [467–469]. The other species-rich classes are phaeophytes (macroscopic mul-

[72] The term *Heterokonta* seems to be a synonym which has come out of fashion [455].

[73] Interestingly, despite being closely related to photosynthetic protists like diatoms and as far as possible distanced from fungi, the Czech popular name of these organisms translates into "true molds" ("pravé plísně"). This has consequences in pesticide use as common anti-fungal agents do not affect these 'odd brown algae' and *vice versa*.

[74] To make life of a non-specialist more difficult, also Ochrophyta have an older synonym—Chromophyta.

[75] The concept of species is rather complex, in unicellular organisms even more so, and therefore all species counts must be taken as very rough estimates. Moreover, morphologically distinct groups like diatoms are much more conducive to provide human eye-detected diversity than 'little brown balls' of some other groups so present knowledge must be understood such, the species counts are observer-dependent [466].

ticellular brown algae), chrysophytes and xanthophytes [468]. Publicly available genome data can be used here as a proxy for general research intensity in the individual ochrophyte classes. A recent review lists 48 nuclear genome assemblies of which 18 are for eustigmatophytes, 15 are for diatoms, six for phaeophytes and the remaining nine cover additional four classes [470]. Some of the ochrophyte classes will be briefly introduced in the following text without an ambition for exhaustive coverage. Work presented in subsequent chapters of this text was carried out on members of diatom and eustigmatophyte classes.

Diatoms (Bacillariophyceae), which possess the three abovementioned pigments (Chl *c*, fucoxanthin and diadinoxanthin), are the most well-known group of ochrophytes in terms of species richness (more than 15 000 species[66]), research depth, environmental significance, fossil record and arguably also economic significance. Diatoms are distinguished by characteristic cell walls made of amorphous hydrated silica ($SiO_2 \cdot nH_2O$)[76] and formed by two opposing halves fitting together to provide a sort of a box, called the frustule, in which the cell itself resides. Diatom frustules have very diverse species-specific shapes, can be radially or bilaterally symmetrical and typically contain a lot of perforations. Due to their chemical composition the frustules are very durable, accumulate in the sediment and survive in the geological record. Vast deposits of diatom frustules can form a material called diatomite (or Kieselguhr).[77] The oldest verifiable diatom frustule fossils come from early terrestrial Cretaceous of Korea (~130 million years old[78]) with a bit younger examples from marine Cretaceous Antarctica, Australia and Germany (~120–110 million years old) [476–479]. Diatom biomass was also the source material for some of petroleum deposits, starting about 100 million years ago [480]. Today, diatoms are responsible for a large share of the global primary production—up to 40 % of oceanic production or about 20 % of total world production [466, 481].[79] Diatoms are well-known even in the plant-focused photosynthetic community, organisms like *Phaeodactylum*, *Cyclotella* or *Chaetoceros* are among the most studied [483–488].

In terms of effort dedicated to obtaining genomic information, the most important class of Ochrophyta are eustigmatophytes. This class contains only about 100 named species[66] but manages to attract high attention due to its potential for use in biofuel production [489, 490]. Eustigmatophytes differ from most other ochrophytes by a lack of both Chl *c* and fucoxanthin which changes the cell color from typical ochrophyte brown to green. Violaxanthin is used as major light-harvesting carotenoid but also as the basis of the xanthophyll cycle, supplemented with allene-containing vaucheriaxanthin [491–493]. Eustig-

[76] The silica is just one component of an ultimately composite structure of organic and anorganic components [471].

[77] Diatomite of older neogenian origin (~20 million years old) is being mined about 15 km south-east from České Budějovice. The whole area from České Budějovice to the south-east was a shallow brackish and freshwater lake in late Cretaceous, Paleogene and Neogene as the area was being uplifted during the Alpine orogeny (actually the orogeny is still happening at present day). The deposits come from massive diatom growth in these environments. Due to the chemical stability and porosity of diatomite, it has found many uses in modern world. Diatomite was famously used as a key component of dynamite by Alfred Nobel. It is also used for water filtration purposes or as a pest control agent in agriculture [472].

[78] About the same age as early angiosperm plants, early iguanodont dinosaurs and very close to presumed divergence of marsupial and placental mammals [473–475].

[79] Despite the long and intensive research of the global carbon cycle, finding good information on major contributors to the global carbon fixation seems very difficult. The mentioned number of "40 %" comes from an introduction in a paper by D.G.Mann and it is clearly just an informed guess: *"Overall, therefore, it might not be unreasonable to estimate that diatoms could account for between 40 and 45% of oceanic production..."* [466]. Other values are even older and not much better linked to reliable data [482].

matophytes are likely not key global ecosystem players though they can become locally dominant [494]. Most of the research activity has been focused on species of the *Nannochloropsis* genus [495–499].

Phaeophyceae is a group of brown macroscopic algae with about 2 000 named species (second highest count within ochrophytes after diatoms).[66] Phaeophyceae contain well-known seaweeds and kelps, famously contributing to Christopher Columbus' trouble in passing through the Sargasso Sea but also forming the beautiful kelp forest ecosystems on the west coast of North America [500, 501]. Phaeophytes combine typical 'brown' pigmentation of Chl *c* and fucoxanthin with 'green' xanthophyll cycle based on violaxanthin, their light-harvesting proteins are closely related to those of the diatoms [290, 502]. Phaeophytes are not household names in the photosynthetic community, the most studied are perhaps the giant kelp *Macrocystis* [290, 502] and filamentous *Ectocarpus* [503, 504].

Chrysophyceae is a large group (more than 1 000 species[66]) of predominantly freshwater algae often from nutrient poor environments [505, 506].[80] Chrysophytes are one of the algae classes with a long taxonomic history.[81] In the past taxonomy treatments the group contained a lot of organisms now put in different places of the phylogenetic tree, e.g. Haptophytes or Pelagophyceae [506]. Chrysophytes as presently understood have not been favourites of photosynthetic research and not much is known besides the pigment composition [453, 508, 509]. Chrysophyte pigmentation is similar to Phaeophyceae: light harvesting pigments include Chl *c* and fucoxanthin and their xanthophyll cycle is based on violaxanthin [453].

Xanthophyceae are another long-known group with a lot of species (more than 700[66]) but relatively modest overall impact. Xanthophytes are mostly freshwater or even land (rock, soil etc.) algae but species of the *Vaucheria* genus are found also in marine environments [510]. Relatively large amount of information exists on the photosynthetic features of xanthophytes, with most of the work carried out on *Pleurochloris* and *Xanthonema* [511–516]. Typical xanthophyte pigmentation includes carotenoids vaucheriaxanthin, diadinoxanthin and heteroxanthin. Chl *c* is often reported but it is not present in all species and fucoxanthin is always missing, resulting in a greenish color of the cells. Macroscopic *Vaucheria* species are consumed by an interesting sea slug *Elysia*, which stores and maintains the ingested chloroplasts for an extended period of time, leading to (likely erroneous) suggestions that the animal is temporarily capable of autotrophy [517–520].

Other classes of ochrophytes include Dictyochophyceae (also called silicoflagellates[82], ~200 species), Raphidophyceae (~40 species), Phaeothamniophyceae (~30 species) or Pelagophyceae (~30 species).[66] They and the other even smaller groups will not be covered here though they often appear to be quite interesting creatures.

The 'A' in SAR stands for **Alveolata**, another important group of eukaryotic organisms. Alveolata themselves are a supergroup of multiple related lineages, the most numberous of which are Ciliata, Apicomplexa and Dinoflagellata.

Ciliates are ubiquitous unicellular protists, found both free-living and commensals or parasites of other organisms. Aproximately 8000 ciliate species have been described [522]. Ciliates are distinguished by presence of cilia—short flagella-type organelles covering

[80] There are recent indications that the class may also be important in the oceanic ecosystems [507].

[81] Long taxonomic history is well indicated by the existence of Czech names of the groups as modern science generates new taxonomy treatments faster than the language can reasonably keep up with. Thus chrysophytes are "zlativky"—an excellent translation of English "golden algae", xanthophytes are "různobrvky" (and "yellow-green algae" in English) and diatoms are "rozsivky".

[82] Silicoflagellates have considerable fossil record, starting in mid-Cretaceous Albian period [521]. Silicoflagellate cells are enclosed in an interesting spiked cage made of amorphous silica.

most of the cell and enabling fast locomotion. Free-living ciliates are mostly heterotrophic. It is not clear at the moment whether some truly autotrophic ciliate, which could be called an "alga", exists. However, ciliates are known to occasionally retain plastids from their prey for considerable time and could be construed as being in a transitory stage of an endosymbiotic event or having an acquired phototrophy characteristic [523]. Chloroplasts of multiple origins have been found in ciliates. Green chloroplasts from the trebouxiophyte algae are commonly found in ciliates feeding on these green algae [412, 413, 524]. Marine ciliate *Myrionecta* hunts cryptophyte algae and then sustains their chloroplasts for a considerable time [525–527]. Recently, a ciliate organism with two different 'endosymbionts' has been analyzed. Surprisingly, besides the expected green algal chloroplasts it contains also purple bacteria, and thus has rather stunning green+purple cells [414].

The second major alveolate group, Apicomplexa, contains almost exclusively parasites of animals.[83] The group's members include for example *Plasmodium* (the cause of malaria), *Babesia* (babesiosis), *Toxoplasma* (toxoplasmosis) or *Cryptosporidium* (coccidiosis) [529]. Many apicomplexans possess a special organelle, called apicoplast, which has been shown to be a highly reduced chloroplast of their ancestors [530, 531]. Photosynthetic organisms basal to the apicomplexans—*Chromera* and *Vitrella* (chromerids)—have been recently found in Australia [532–535]. These 'chromerid' algae have chloroplasts of most likely stramenopile origin [509], contain violaxanthin-based xanthophyll cycle, and lack Chl *c*, similar to eustigmatophytes. However, while *Vitrella* has very much eustigmatophyte pigmentation, *Chromera* uses a modified fucoxanthin-like pigment as the major carotenoid [536–539]. Moreover, chromerids use form II Rubisco like dinoflagellates (see below[86]) [529, 533]. Chromerids thus appear to be an important intermediate state between stramenopiles, dinoflagellates, and apicomplexans (in terms of photosynthesis, not evolution of the organisms themselves).

The most important alveolate phototrophs and arguably one of the most important group of algae in general are Dinoflagellata. Dinoflagellates include almost 4 000 living[66] and about 2 500 fossil species [540] from diverse habitats. Approximately half of the known species are photosynthetic. Dinoflagellates show a lot of unique features, starting with their nucleus, called dinokaryon, which contains much more DNA than in most other organisms, complicating molecular analyses [541]. Dinoflagellate cells are often covered in a sort of armor built from complex organic material called dinosporin [542]. The dinosporin shells are quite durable and are well preserved in the fossil record [543]. Dinoflagellates are presently effectively ubiquitous in marine and freshwater habitats [544]. They occasionally create toxic blooms in coastal seas [545, 546], are known as one of the major sources of marine bioluminiscence [547] and, perhaps most importantly, some dinoflagellates are symbionts of modern corals [541], helping them to form major parts of very impressive tropical coral reefs. The earliest generally accepted fossil dinoflagellates come from mid Triassic (about 240 million years before present), which coincides with an appearance and spread of modern corals [548, 549].[84] Dinoflagellate phytoplankton also significantly contributed to crude oil deposits [552–554].[85]

[83] There are approximately 6 000 named species in Apicomplexa but it is expected that there could be two orders of magnitude more species which are not known [528].

[84] Prior to the Mesosoic, the tropical limestone reefs were variously formed by microbial reef builders, red algae, bryozoans, exctinct corals and other groups [549]. The well-known Devonian reef in Koněprusy, about 30 km southwest of Prague, was built predominantly by red algae but also corals, crinoids, bryozoans and stromatopores (extinct calcareous sea sponges) [550, 551].

[85] Specific information seems to be hard to come by but e.g. the Minagish oil field in Kuwait contains oil sourced from a dinoflagellate-rich deposit accumulated in a part of the Tethys sea in the early Cretaceous

Dinoflagellate idiosyncrasies include several photosynthetic features. First of all, dinoflagellates (and chromerids) are the only photosynthetic eukaryotes to use a bacterial form II Rubisco.[86] In terms of pigmentation, Chl c_2 is a major accessory chlorophyll. Unique carbonyl carotenoid peridinin is the major dinoflagellate carotenoid, supplemented by diadinoxanthin as the basis of the xanthophyll cycle [560–562]. Another unique dinoflagellate feature is the presence of water-soluble light harvesting protein PCP (peridinin-chlorophyll protein) [563, 564]. The PCP light-harvesting antenna has no known analogs anywhere else in photosynthetic life.[87] Dinoflagellate thylakoid membrane also contains many isoforms of the common eukaryote Lhc-type light harvesting proteins, denoted acpPC for Chl *a*-Chl *c*-peridinin protein complex and otherwise likely related to the stramenopile Lhcf [565, 566]. Photosynthetic features of dinoflagellates have been mostly studied in *Amphidinium* and *Symbiodinium* [564, 566]. Besides the canonical photosynthetic dinoflagellates with peridinin, chloroplasts sourced from other photosynthetic organisms are known in some dinoflagellates. Marine dinoflagellate *Lepidodinium* contains chloroplasts obtained from chlorophyte green algae [451, 452, 567, 568]. A number of species related to *Durinskia* contain chloroplasts derived from diatoms [569–571]. Another group of species, including the toxic *Karenia brevis*, contains chloroplasts of haptophyte origin [572–574]. Finally, another toxic dinoflagellate *Dinophysis* is known to hunt ciliate *Myrionecta* and extract from it its own prey, which are chloroplasts of cryptophyte algae, likely an example of kleptoplasty and not true autotrophy [575–577]. Dinoflagellates are therefore prime targets for potential studies on assembly of photosynthetic supercomplexes with components of hybrid origin.

The last group of the SAR trio and the one with the smallest number of photosynthetic organisms is **Rhizaria**. Rhizaria encompass a very diverse and ubiquitous set of heterotrophic organisms with presumed distant origin in a photosynthetic lineage [578]. Most importantly, rhizarians include the very well-known and very abundant Foraminifera and Radiolaria [579–581]. Foraminiferans and radiolarians are unicellular or colonial heterotrophic planktonous or benthic organisms with durable cell walls ('tests') so abundant in the fossil record, and so unique, that they can be used for rock dating purposes.[88] Most of the global ocean floor is covered by so-called '*Globigerina* ooze' – a layer of foraminiferan tests [587]. Foraminiferans are also one of the largest unicellular organisms, their tests can reach more than 15 cm in size. The tests contain calcium carbonate or, in radiolarians, silica. The group is very old, the earliest foraminiferan and radiolarian fossil tests are found in early Cambrian (~530 million years ago[89]) [589, 590]. Of interest for this text, some

[555]. One prominent chemical marker present in crude oils but also elsewhere are dinosteranes, molecules presumably coming from dinoflagellate cells. Dinosteranes are known also from very old rocks, dating back perhaps even to Precambrium [556, 557].

[86]Four Rubisco forms have been discovered so far. Cyanobacteria and all eukaryotic algae, except dinoflagellates, use form I Rubisco, which is assembled from eight large subunits and eight small subunits (L_8S_8). The small subunit is only found in form I. Other Rubisco forms are mostly found in L_2 assembly. Some bacteria and dinoflagellates use form II. Form III Rubisco is present in many archaea and is presumably the most ancient type. Finally, form IV Rubisco is found in a diverse assortment of bacteria and archaea [326, 558, 559].

[87]Based on an ncbi database search at the time of writing.

[88]The Eocene (Lutetian stage, about 45 million years before present) limestone of the Giza pyramids includes tests of the foraminiferan *Nummulites* [582, 583]. Foraminifera tests also form major Carboniferous limestone formations of eastern Europe and Asia [584, 585]. About 40 000 species of extinct Foraminifera and about 10 000 living species have been described [586].

[89]The stated age is for Foraminifera, which appeared before the first trilobites. Radiolarian fossil record starts just a few million years later [588].

radiolarians are known to associate with haptophyte or dinoflagellate algae [591]. But most importantly, two minor rhizarian groups are photosynthetic. First, the *Paulinella* organism incorporated a cyanobacterium about 100 million years ago to create a sort of glaucophyte analog [439] which is the only known endosymbiotic event not related to the origin of Archaeplastida. The *Paulinella* symbiont likely retains most of the features of its cyanobacterial ancestor of the *Synechococcus* family, including phycobilisomes, and is apparently otherwise well established in the host. On the other hand, the host organism is known to grow very slowly in laboratory conditions, which could be adaptive or a result of the relatively recent endosymbiotic event and poor coordination of the two partners [592–594].

Second, a small rhizarian group Chlorarachniophyceae includes about 15 photosynthetic species with worldwide marine distribution. Most of the research so far has been carried out on the species *Bigelowiella natans* [440, 595–597]. Chlorarachniophyte chloroplast is apparently of green algae origin, with chlorophylls *a* and *b*, a violaxathin-based xanthophyll cycle, and with genes mostly similar to the crown chlorophytes [597–599]. On the other hand, there is evidence that chlorarachniophytes contain a lot of SAR or red algal genetic information and it is likely that their evolutionary history is very complex [600]. The light-harvesting protein complement is also predominantly green algal-like with the exception of a few Lhcz-type proteins, which likely come from the SAR lineage [597].

The last eukaryotic group to be covered here are **Haptophyta**. Haptophytes are a relatively small group with a global impact which is difficult to overstate. The group contains about 1500 species[66] of mostly marine unicellular algae. A major group of haptophytes, coccolithophorids, is characterised by cells covered in calcareous and uniquely shaped scales called coccoliths. These coccoliths are responsible for a big part of the global impact of the group. The coccoliths are continuously created and lost by the cells. The lost coccoliths first contribute to sea albedo and later, after sedimentation, form deposits of fine limestone, trapping significant amounts of carbon and calcium. The oldest known fossil coccoliths come from late Triassic (~205 million years ago, just before the Triassic-Jurassic extinction event) [601–604]. Presently, coccolithophorid blooms can be so extensive that they can be seen from space [605–607]. This phenomenon is not a recent occurrence, the coccolith deposits form massive chalk formations for example in the North Sea and surrounding areas [608].[90] The most studied coccolithophorid and haptophyte as well is probably *Emiliania huxleyi* [614]. Eight pigmentation types have been described in haptophytes [615]. The pigmentation types differ in presence of accessory Chl c_1 and Chl c_3, unusually modified Chl c_2-MGDG and modified fucoxanthin carotenoid 19'-hexanoyloxyfucoxanthin. The light-harvesting proteins of haptophytes are similar to those of stramenopiles with Lhcf, Lhcr and Lhcx proteins [616] but the overall genetic pattern of the organisms indicates a complex origin partly also from the green lineage [617]. The unique pigmentation makes haptophytes important for the study of the function and evolution of the photosynthetic machinery [618–620] but so far the research of the photosynthetic aspects of haptophyte metabolism is significantly lagging behind other algal

[90]The White Cliffs of Dover as well as the eastern coast of Denmark are parts of the chalk deposit. Another place where one can see the chalk is in Maastricht, The Netherlands. The relevant geologic period itself is called the Maastrichtian [609]. The deposit formed during the late Cretaceous until the very end of the period. The famous K-T boundary lies on top of this chalk formation [610]. The formation is up to 2 000 m thick and the parts of it which formed in the deeper parts of the Cretaceous ocean are now bearing large and economically important oil and natural gas deposits, drilled in the North Sea [611, 612]. It has been estimated that approximately half of the current calcite deposits in the global ocean are formed by haptophyte coccoliths (Foraminifera are responsible for the other half) [613].

groups.

1.4 Light harvesting in 'brown' algae

Having briefly covered the eukaryotic autotroph diversity, one has to ask the question, whether the photosynthetic apparatus of other eukaryotes differs from that of the green plants (covered in section 1.2) in minor details or whether it rather represents a significant deviation, showing us different ways of achieving the same goal. It is obvious that, principally, the oxygenic photosynthesis process is present in known life in only one form with perhaps just minor modifications here and there. On the other hand, innovations like Lhc proteins, xanthophyll cycle, PsbS and Lhcx photoprotective proteins, carbonyl carotenoids, or a single-step xanthophyll cycle suggest that the evolutionary process is (or at least has been) successfully experimenting with photosynthesis and producing alternatives where suitable (or available).

The following chapters of this text showcase several studies analyzing the specialization and function of Lhc proteins, including photoprotection, in eukaryotic algae, namely species from two groups of stramenopiles: diatoms and eustigmatophytes. There are two major outstanding questions in this field. First, the Lhc proteins of stramenopile algae are not direct descendants of plant LHCI (Lhca) and LHCII (Lhcb) subunits but are likely derived from red algal Lhcr proteins. The proteins are quite diverse across stramenopiles, therefore, can one expect that the overall assembly of photosystems, particularly PSII with its monomeric and trimeric Lhc forms, holds in this group (presumably because it is the best arrangement or the only one accessible)? Second, the pigment cofactor composition differs wildly in these proteins, potentially affecting their function as well. Experimenting with carefully composed Lhc proteins should be quite dangerous as it can easily lead to intense photodamage and death of the organism. Under these circumstances, why do these organisms experiment with the pigments? Can an evolutionarily relevant reason for the pigment diversity be figured out?

The wider Lhc protein family, as defined by the structure of the transmembrane α-helices, contains a variety of proteins [398].[91] The cannonical example and the topic of the following chapters, the three-transmembrane-helix Lhc proteins (cf. Fig. 1.7), are found fully developed in both red algae and the green lineage, whose common ancestor is unknown. Standard bioinformatical approaches do not provide much information on the Lhc evolution due to the short length of the relevant proteins and therefore limited information available in the polypeptide sequence. It is assumed that the three-helix Lhcs evolved from smaller one- or two-helix proteins associated with photosystem and chlorophyll synthesis processes. It has been hypothesized that the light-harvesting function evolved by switching off the photoprotective features of the originally cyanobacterial one- and two-helix proteins, perhaps of the SEP/LIL affinity[91], while maintaining the capability to bind Chls and carotenoids [270, 398].

In many algae, a clear-cut distinction between PSI and PSII antenna proteins on the basis of the polypeptide sequence, like in the case of plant Lhca and Lhcb [427], is difficult

[91] The full list of the Lhc-like family proteins is quite long. The one-helix proteins include cyanobacterial and eukaryote HLIP (high light-induced protein) [621] and OHP (one-helix protein) proteins [622, 623]. The two-helix proteins are known as SEP (stress-enhanced proteins) or LIL (light-harvesting-like) [624, 625]. Three-helix proteins, besides the Lhcs themselves, include ELIPs (early light-induced proteins) [626] and the RedCAP (red lineage chlorophyll a/b-binding (CAB)-like protein) proteins [627]. The four-helix PsbS photoprotective protein of the green lineage also belongs to the list.

or impossible to achieve. The algae also present considerable diversity in the antenna pigmentation. As a result, there is a lot of antenna protein names used in algal photosynthesis studies, which present a steep learning curve for an uninitiated researcher. Use of labels like LHCII and LHCI is (rightly) discouraged in algal studies as this practice does lead to serious misunderstanding of the function of these systems. Various labels such as FCP or Lhca/c were introduced in order to simplify writing about the studied antenna samples but these have the disadvantage of not being informative in the relationships between various systems. The attempts at algal Lhc classification started with the Lhcf and Lhcr labels for the FCP antennas of diatoms (with fucoxanthin) and PSI antenna of red algae, respectively [384, 628]. A comparatively reasonable distinction can be also argued for the photoprotective Lhcx proteins (called LhcSR[92] in green algae) [630, 631]. Besides the Lhcr, Lhcf and Lhcx trio, many other Lhc types have been introduced. Some of these additional labels are motivated by different pigmentation (e.g. Lhcv for violaxanthin chlorophyll proteins (VCP) of eustigmatophytes). Yet other types, as is the case of Lhcz, Lhcy and Lhcq, were denoted on the basis of phylogenetic analyses, with uncertain utility.[93] Recently used Lhc type labels from algae are summarized in table 1.2.[94]

Table 1.2: *Selected Lhc proteins of algae with chloroplasts of secondary endosymbiotic origin. Listed in alphabetical order, lesser-known Lhc types are in the bottom half of the table. Abbreviations: diadino, diadinoxanthin; fuco, fucoxanthin; hetero, heteroxanthin; vauch, vaucheriaxanthin; viola, violaxanthin;*

	Other label	Algae group	Pigments	Ref.
Lhcf	FCP	diatoms & others	Chl *c*, fuco	[384]
Lhcr	–	all groups	diverse	[384]
Lhcx	–	all groups, green algae	?	[630]
Lhcq	–	diatoms, haptophytes	diverse	[633]
Lhcv	VCP	eustigmatophytes	viola, vauch	[634]
Lhcy	–	chlorarachniophytes	?	[597]
Lhcz	–	cryptophytes, haptophytes	?	[427]
–	acpPC	dinoflagellates	Chl *c*, peridinin	[635]
–	CLH	chromerids	isofucoxanthin	[536]
–	XLH	xanthophytes	diadino, hetero, vauch	[515]

Regarding the supramolecular assembly of the algal Lhcs, only indirect indications were available until very recently. In contrast to plant studies, only PSII core complexes, lacking outer antenna, were observed in most biochemical studies of stramenopile algae. Diatom FCP complexes ('free' antenna) were believed to differ between the two most studied groups of diatoms, pennate and centric, in the degree of oligomerization. In pennate

[92] LhcSR was originally described as LI818 [629].

[93] It should not be implied here that the author does not believe in the utility of these labels within the definition scopes. Rather, it is not clear at the time of writing whether they are sufficiently robust and will be reliably recovered in different phylogenetic analyses. Indeed, even the Lhcr/Lhcf separation is not very robust [632].

[94] Some labels proposed by [384], like Lhcc (cryptophyte Cac) [445] or Lhcd (dinoflagellate Lhcs with peridinin often called acpPC) [566], were apparently not adopted by the community. It is unfortunate that many published genomes haphazardly use labels implying the presence of Chl *b* in gene and protein annotations even in cases where Chl *b* is obviously not present.

diatoms, trimeric FCP complexes built from Lhcf proteins were described in biochemical studies [636–638]. In contrast, analyses of the centric diatom antennas described also oligomeric assemblies [636, 638, 639]. It has also been noted that the centric diatom trimeric complex is more active in NPQ than the oligomeric form [640, 641]. The single-particle electron microscopy study presented in Chapter 2 revealed striking differences in the organization of oligomeric pennate and centric FCP complexes **[P1]**. The pennate diatom FCP oligomer, previously not observed in biochemical studies, showed an overall assembly similar to that of other stramenopile algae [513, 536]. In contrast, the centric diatom FCP presented a highly variable oligomer assembly unlike any described before. Assuming that the observed structures are formed by the Lhcf trimers, they must be an assembly of multiple trimers forming a kind of flexible belt, which in some of the presented projections curved to form a ring. Even at time of publication, it was not assumed that these structures were native to the membrane but rather represented a distorted fragment of part of the thylakoid membrane protein content. Similar, perhaps more understandable, results were also obtained by another group working on the same organism [642, 643].

The current view of the diatom FCP supramolecular assembly is still not very clear. The only published crystal structure of pennate diatom FCP represents a dimeric form of Lhcf4 protein [484]. The recent cryo-EM structures of centric diatom PSII present the supercomplex with two Lhcf tetramers and three monomeric antennas [487, 644]. These data suggest that diatom FCP assemble in dimeric or tetrameric form. However, the dimeric FCP crystal shows different relative position of the monomers than inside the tetramers of the cryo-EM structures. Further, other studies still suggest trimeric FCP assemblies similar to the LHCII of plants [645]. Regarding the monomeric antenna subunits of diatom PSII, the specific positions are not conserved upon comparison with plant PSII, as expected. Recently published analysis of diatom PSII supercomplex identifies specific polypeptides and a detailed analysis of homology within the stramenopiles as well as the identification of functional differences should be possible [646]. Diversity of structural approaches to spupercomplex assembly within the diatoms themselves remains an attractive option. The long-held simplified classification of diatoms into pennate and centric groups does not reflect the current understanding of the class. The most often studied ‘pennate’ and ‘centric’ diatoms are presently understood to be sister groups designated as pennates and multipolar centrics, respectively. On the base of these two groups are rarely studied radial centric diatoms, presumably representing an ancestral form [476, 647, 648].[95] These groups separated from each other likely in the late Jurassic and are thus older than the angiosperm plants [479]. With short generation times of the unicellular organisms, a diversification at the level of antenna oligomerization is surely possible. A broader sampling of the available diversity shall provide a better conclusion in the future.

The last two decades have seen a dramatic increase in the availability of genomic data from various organisms as well as huge improvements in proteomic methods. Analyses in Chapters 3 and 4 relied heavily on these resources in order to gain better understanding of the Lhc protein complements of eustigmatophyte algae and the differences between plant

[95] Pennate diatoms include for example *Phaeodactylum* or *Navicula*. Typical multipolar centrics are *Chaetoceros*, *Thalassiosira* and *Cyclotella*. *Coscinodiscus* is an example of the more basal radial centrics. Of note to the topic of this text, the cells from a group of pennate diatoms called raphid diatoms, of which *Phaeodactylum* is a member, contain only a limited number of chloroplasts (one to four) whereas the rest of the diatom species usually contain many tens of chloroplasts. It has been proposed that the species which can orient themselves in the environment, raphid pennates, have small number of chloroplasts. Species mostly characterized as planktonic (i.e. most of the centrics) and unable to actively orient themselves have a large number of chloroplasts [476].

and stramenopile systems **[P2, P3]**. The genome of *Nannochloropsis oceanica* contains 17 Lhc sequences and most of them were detected in the protein analysis. However, only a few could be reliably allocated to PSI and none at all to PSII. This contrasts with the typical plant complement of numerous Lhcs in PSII (monomeric and trimeric) and four Lhcs that are quite strongly attached to PSI. It also contrasted with the result of the single particle analysis, which revealed several types of PSI+Lhc complexes **[P3]**. While the major 'free' antenna (likely mostly attached to PSII *in vivo*) contained the carotenoids violaxanthin and vaucheriaxanthin in approximately 2:1 ratio [649], the PSI antenna complement was depleted of vaucheriaxanthin. This mirrors the situation in diatoms, which have major antenna with fucoxanthin but the PSI is enriched in diadinoxanthin. The largest PSI particle in **[P3]** was estimated to contain between seven and nine Lhc subunits, a smaller number than found in the recent huge diatom PSI complex structure [488, 650]. On the other hand, this observation would agree with the smaller number of Lhc sequences in eustigmatophytes in comparison to many diatoms. The functional differences of these features are not clear at the moment. The habitats of *Nannochloropsis* and planktonic diatoms however appear to be quite similar [494, 651].

The PSI particles from the eustigmatophyte *Nannochloropsis oceanica* contained a unique Lhc-family antenna protein denoted RedCAP **[P2]**. RedCAP has been first noted in an *in silico* study [398, 627] and only later observed experimentally in diatoms [638, 652]. The RedCAP seems to be present in red algae and most algae with chloroplasts derived from red algae. Interestingly, the RedCAP is only found in one of the two published cryo-EM diatom PSI structures [488]. The cited cryo-EM study identifies the RedCAP as FCPI-1, in a position occupied by 'Lhcr1*' in a PSI structure from a red alga *Cyanidioschyzon* [390]. If there is a specific function of this protein and whether it's presence is related to some acclimation process is not known at the moment.

Beyond structural considerations, one of the most exciting examples of the adaptability of the basic Lhc structure to environmental conditions are red-shifted Lhc antennas. It has been known since early 20th century that cyanobacteria respond to ambient light quality by synthesis of specific phycobiliproteins. This process, called chromatic acclimation [653], has also been extended to include acclimation to shaded environments [654, 655]. While the cyanobacterial chromatic acclimation is sometimes observable with a naked eye, processes of similar magnitude are unheard of in eukaryotic organisms. However, reports dating back to the 1960s occasionally mentioned diatom cells exhibiting significantly red-shifted fluorescence emission spectra when grown under low light intensity. These observations often correctly concluded that the observed fluorescence is coming from a light-harvesting system associated with PSII [656, 657].

Initiated by a coincidental discovery of a red-shifted antenna in chromerid *Chromera velia* [658, 659] and the resulting insight into old data published about diatoms, we have studied the chromatic acclimation mechanism of the model diatom *Phaeodactylum tricornutum* (Chapter 5) **[P4]**. By a combination of careful biochemical work and tandem mass spectroscopy, informed by genomic data, it was possible to identify the Lhc-type protein Lhcf15 as the necessary actor responsible for a remarkable 30 nm shift of fluorescence maximum and a corresponding increase of absorbance around 700 nm. This analysis was recently confirmed by targeted mutagenesis of the same organism [660]. The cyanobacterial acclimation to shaded environment involves the synthesis of novel pigments like Chl *d* and Chl *f*. In contrast, diatoms and other algae rely on pigment-protein and pigment-pigment interactions which modify the absorption properties of Chl *a* in analogy to known effect of the protein on fucoxanthin (Fig. 1.11) and to the very similar, yet much smaller

in magnitude, process known from plant LHCI antenna [661].

Figure 1.11: *The effect of protein environment on pigment properties. A comparison of the color of haptophyte cells (Emiliania huxleyi, left, in water-based cultivation medium), a pigment extract prepared from these cells (center, in methanol) and a pigment extract from a green plant leaf (Hibiscus rosa-sinensis, right, in methanol). Upon removal of the pigments from the protein environment, the absorption maxima shift which results in a color change. In the case of haptophyte cells, the brown color is caused mostly by the presence of fucoxanthin and its derivatives. Human eye is highly sensitive to various hues of green and the differences in absorption spectra in the green spectral region are therefore easily recognizable. Samples and photo prepared for this text by František Matoušek and the author.*

The diatom chromatic acclimation does not stop at synthesis of a specific antenna protein. As shown in Chapter 6, the cells grown on red-enhanced light[96] show greatly expanded thylakoid membrane system, which contains large areas ('rafts') occupied exclusively by PSI particles (See Fig. 1.12) **[P5]**. This work was one of the first to indicate some degree of lateral homogeneity in diatom thylakoid membranes. Unlike plants with their grana and stromal lamellae, diatoms and other algae usually possess a comparatively large thylakoid membrane system spreading across the chloroplast and organized usually into bands of three thylakoids, i.e. six membranes close together. Until recently, it was not clear whether the diatom photosystems were separated into domains or not [632, 662]. Presently, it appears that, within the three banded thylakoids, PSII is predominantly localized in the central thylakoid and PSI in the outer thylakoids [663, 664]. Presumably, the large membrane system of our red-acclimated *Phaeodactylum* made it easier to extract the patches of PSI-only membranes which are also present in the day-acclimated cells.

The diatom red acclimation story is (for now) concluded in Chapter 7 **[P6]**, which describes the properties of the red-shifted antenna oligomer and the changes to cell shape of our model *Phaeodactylum*. *Phaeodactylum tricornutum* has been observed in various coastal environment, often with an estuary nearby, in the north Atlantic but also in the

[96]Many laboratories use 'monochromatic' LEDs for illumination in the far-red region. Our work on red-shifted Lhcs used traditional incandescent light bulbs, i.e. what one could call 'white' light. These light sources produce very little blue light and induce the red-acclimation syndrome well but their sale was all but banned recently due to their low energy efficiency. The replacement halogen lamps also work but the response of the organisms is often slower or not so perfect. We prefer these broad-spectrum light sources as, besides photosynthesis, there are other processes in the cells which might need light of some other wavelength. The natural environment will probably only very rarely be strictly monochromatic.

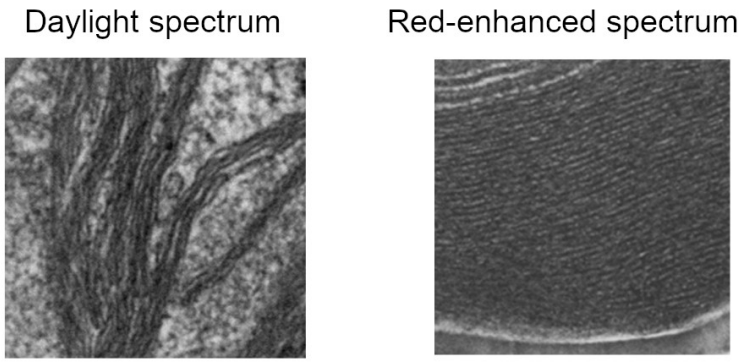

Figure 1.12: *Comparison of the thylakoid membrane systems of day light-acclimated (left) and red light-acclimated (right) cells of the diatom Phaeodactylum tricornutum. In this aspect alone, there is little difference between low light and red light response. Most other diatom species however cannot produce the red-shifted Lhc. Transmission electron microscopy images adopted from Chapter 6* ***[P5]****.*

Pacific ocean [665]. The life cycle presumably includes a planktonic as well as a benthic form. The coastal waters and estuaries are frequently murky, presenting a low-light environment. Winter storms will also contribute by presenting very low light intensity environments, at least in the northern parts of the species' distribution. We believe that the planktonic form, with cells in 'fusiform' or 'triradiate' shapes, is present when contitions are favourable and provide sufficient light intensity. The benthic form with cells of 'oval' shape then resides on the bottom of the home waters and requires high light interception capability to drive photosynthesis in order to survive until another favourable period appears. Needless to say, our red-acclimated cells display oval forms and thus our findings corroborate the model of benthic oval cells, with greatly expanded thylakoid membrane system and the red-shifted antenna to help survive low-light conditions.

The red-antennas not only of diatoms but also of green algae [666, 667], eustigmatophytes (see below), and *Chromera* [658, 659] have characteristic absorption bands around 700 nm. It is often our first instinct to realize that there is no red light underwater, due to the absorption properties of the water itself. The niche for the red-shifted Lhcs is however limited to the top layers of the water environment.[97] One can get an appreciation of the light spectrum niches available under a thin layer of water in Fig. 1.13. Similar illumination profiles are more rare in terrestrial habitats. Blue sky provides almost the same illumination spectrum as direct sunshine but habitats fully shaded from the sky, in rock or tree bark crevices, and with indirect light filtered or reflected off canopies do offer very similar illumination quality. Among the algal groups which are able to use such light in terrestrial habitats are eustigmatophytes.

One of the results of the work on red-shifted antenna in *Chromera velia* ('redCLH') [659] was that it shares sequence similarity to eustigmatophyte 'VCP' (Lhcv) antennas. We have subsequently analyzed a marine species of this group with negative results concerning chromatic acclimation (Chapter 3 **[P2]**). However, the freshwater or, perhaps better, terrestric members of this group often do show presence of red-shifted Lhc [668, P7, 669]. Chapter 8 **[P7]** describes an investigation of several red-shifted Lhcs (rVCPs) found in the eustigmatophyte *Trachydiscus*. The rVCP of *Trachydiscus* is much more stable upon purifi-

[97] The cyanobacterial Chl *f* was discovered in isolates from stromatolites, growing just below water surface [352].

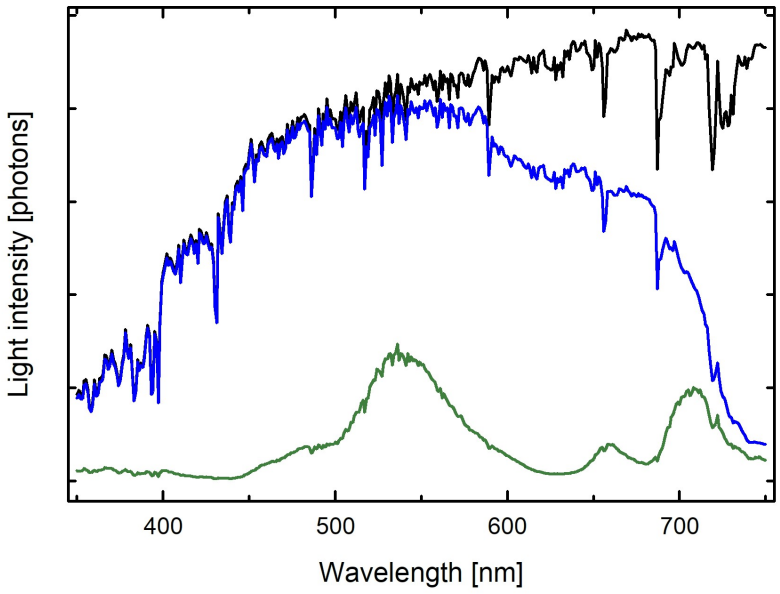

Figure 1.13: *The niche for the red-shifted light-harvesting systems. Solar spectrum available at the surface (black) is filtered by 1 m of water column (blue) and then by a very dilute population of cyanobacteria (green, Synnechocystis, $OD_{670\ nm}$=1.0). The remaining light spectrum is dominated by green light around 540 nm, which can be harvested by keto-carotenoids fucoxanthin or peridinin (diatoms or dinoflagellates, respectively). Note side bands at ~650 nm and ~470 nm, characteristic for Chl b of the green lineage. The last significant band is at ~700 nm, utilized by cyanobacterial pigments Chl d and Chl f as well as by eukaryotic red-shifted Lhc. Adopted from Chapter 5* ***[P4]****.*

cation than the diatom red antenna[98] and, very interestingly, a whole array of these antennas with different degree of oligomerization was found in this organism. These oligomers also differ in the amount of red Chl *a* forms. The number of different oligomeric VCP forms in *Trachydiscus* is especially striking in comparison to the situation in diatoms [639]. The cited diatom FCP analysis shows that diatom species differ in the degree of oligomerization of their FCP complexes. The largest FCP complexes were found in *Chaetoceros*, which was later used as the source of large supercomplexes for structural cryo-electron microscopy studies [487, 488, 644, 650]. However, no diatom species has as high number or variety in FCP organization as the eustigmatophyte *Trachydiscus*. It is not clear how much of the observed diversity in Lhc complex oligomerization is native and how much is a result of the used experimental approaches, which may be optimal only for a few species. The nature of the red-shifted chromophore in *Trachydiscus* rVCP is not clear either and could well be complex [670], analyses of the system are ongoing at present time.

Eustigmatophyte algae use violaxanthin for both light harvesting and in the photoprotective xanthophyll cycle. It is thus of interest to take a look at the photoprotective non-photochemical quenching of these organisms. One of the outstanding questions in NPQ research is the location of the deepoxidized carotenoid. Violaxanthin is present in the core of the plant LHCII complex [216] and presumably also in an analogous position in the eustigmatophyte VCP [671]. Evidence from diatoms however points to a significant amount of xanthophyll cycle pigment (diadinoxanthin) localized in the lipid phase, only weakly associated with the Lhc proteins [672]. Some data suggest violaxanthin deepoxidation in the membrane phase also in plants [673].

[98] The red antenna of *Pheodactylum* is almost completely lost after cell disruption or even just when the cells are frozen and later thawed **[P4]**.

Assuming that diatoms are good representatives of the NPQ mechanisms in the stramenopile algae in general, the following three theses are expected to be also valid for eustigmatophytes. First, it is generally accepted that a proton gradient (ΔpH) on the thylakoid membrane induced by excessive illumination intensity is the key trigger for NPQ. Second, the ΔpH activates deepoxidation of diadinoxanthin to diatoxanthin, which is the major NPQ actor, not the ΔpH itself. Third, the Lhcx protein is necessary for NPQ and it has been suggested that the Lhcx protein with bound diatoxanthin is either the quencher itself or a direct actor in the process [674, 675].[99] A peculiarity of many diatoms is that the epoxidation reaction is very slow or nonexistent in darkness, resulting in a lack of NPQ recovery [676]. The xanthophyll cycle back reaction (epoxidation) and therefore NPQ recovery speeds up significantly in low light intensity. It has been suggested that the slow or nonexistent xanthophyll cycle recovery is caused by the lack of NADPH as the necessary cofactor of diatoxanthin epoxidase, presumably due to the competition of the Calvin cycle reactions [674].

We have analyzed four species of eustigmatophyte algae to assess whether their violaxanthin xanthophyll cycle-based NPQ can be viewed through the diatom glasses (Chapter 9 **[P8]**).[100] At first glance, our results confirmed the central theses of diatom NPQ: the observed NPQ correlates linearly with zeaxanthin concentration and the dark recovery is often nonexistent but NPQ recovers nicely at low illumination intensity. There is of course a significant xanthophyll cycle activity in eustigmatophytes. However, at closer look there is quite a more complex story. First, as is obvious from the increase of fluorescence yield after releasing the actinic illumination, there is surely an 'energetic' component, which requires ΔpH. Second, while the share of violaxanthin pool converted to zeaxanthin is comparatively low (about 8 % of violaxanthin converted to zeaxanthin, Chapter 9), it is obviously not a sign of a weak xanthophyll cycle but rather of violaxanthin use in the light harvesting protein VCP, where it is likely not accessible to the violaxanthin deepoxidase. Third, and most importantly, something is 'wrong' with the response of (not only) eustigmatophytes to saturating light pulses.

In theory, a saturating light pulse is so strong that it achieves closure of all PSII in the membrane and thus it blocks electron transport and allows one to quantify nonphotochemical quenching pathways. Many PAM instruments[101] can export calculated NPQ values, read automatically by the software. This is very convenient for the user but it can hide some important data features. Due to the technology used, we were able to analyze fluorescence yield during the saturating pulses. Despite using an intensity of ~10 000 μmol photons.m^{-2}.s^{-1}, the cells were able to respond to the light pulse and managed to quench it completely within its 800 ms duration (as if the membrane could use all of the supplied energy, Figure 1.14). The stated light pulse intensity is 5$\times$ higher than full sunlight and the cells were of course grown on fractions of that intensity. The quenching during the pulse takes some time to relax after the pulse ends, resulting in a characteristic feature of

[99] PsbS protein of plants is present in the genome often in just one copy. In contrast, Lhcx proteins often form extended families of more than 10 isoforms of unclear functional differences. A good example is the phaeophyte *Ectocarpus* with 11 Lhcx genes [503].

[100] Some reports indicated that eustigmatophytes have limited or nonexistent protective NPQ [677] or that their xanthophyll cycle is effectively a single step mechanism (violaxanthin $\rightleftharpoons$ antheraxanthin) [497]. Analyses of photoprotection and indeed physiology in algae in general are hampered by much higher sensitivity of the organism to cultivation conditions in comparison to plant models. It is entirely possible, and quite likely, that two scientists working on the same organism in different laboratories will obtain conflicting data.

[101] PAM stands for Pulse Amplitude Modulation and identifies a standard method of analysis of chlorophyll fluorescence quenching and NPQ [16, 678].

the PAM record, which has been sometimes called 'low wave' [679] and which is also visible in some other published PAM data but not commented upon [676]. This 'fast' NPQ, observable within the saturating pulses, depends on the zeaxanthin concentration and can be eliminated by an uncoupler, i.e. by removing the ΔpH on the membrane, even in the presence of zeaxanthin. The molecular mechanism of this phenomenon is not understood.

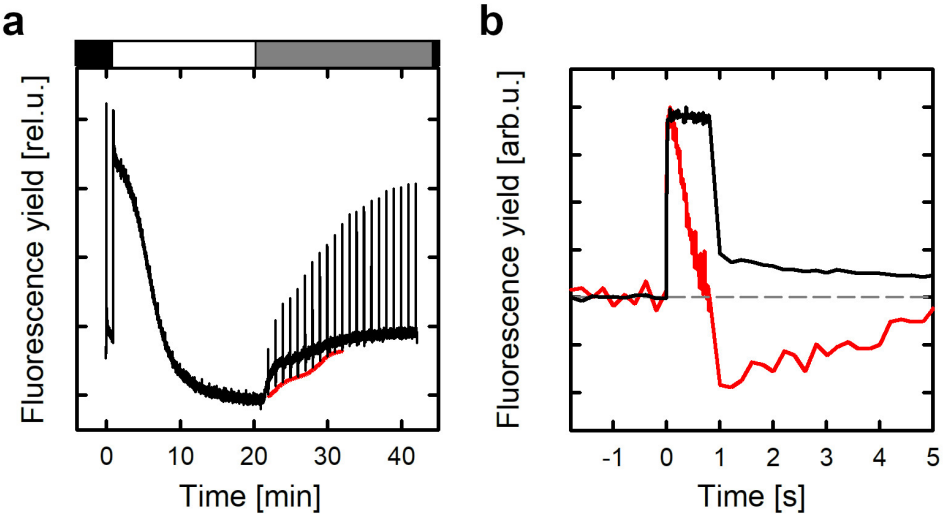

Figure 1.14: *An example of PAM fluorescence record showing the low-wave phenomenon in cells of Nannochloropsis oceanica.* ***a)*** *Kautsky curve with saturating pulse analysis. Cells were illuminated by actinic light for 20 minutes, starting at 1 minute time point. Afterwards, weak light was applied to speed up the relaxation. Note good recovery after the actinic light was turned off (after 21 minute) and the low-wave phenomenon, outlined by a red line.* ***b)*** *Zoom into two selected saturating pulse responses. Black line is the very first saturating pulse, applied after dark acclimation of the cells. Red line is the profile of the saturating pulse response after the actinic light treatment (the 23 min pulse from panel a). Image prepared from one of the datasets obtained when working on Chapter 9* ***[P8]****.*

Finally, we were also able to calculate the reaction rates of the two deepoxidation steps. The conversion of antheraxanthin to zeaxanthin was faster than the violaxanthin-antheraxanthin step by a factor of two. This is similar to the behavior reported for plant violaxanthin deepoxidase, where the rates are however an order of magnitude faster [680, 681]. Equal rates were also reported in the literature [289]. Having a wider sampling of species and growth condition-specific (de-)epoxidation rates linked to NPQ rates and capacities could be very informative on understanding the regulation of the xanthophyll cycle but obtaining such data is quite a labour-intensive endeavor.

1.5 Conclusion and outlook

In comparison to the situation just a few years ago, there are now several published structures of diatom photosystem supercomplexes. However, these come from just one organism and it remains to be determined how representative these will be for the diatoms or even stramenopile algae at large. The study of the function of the individual Lhc protein forms in brown algae is still in its infancy. For example, the specifics of the RedCAP protein function are wholly unknown. Can RedCAP be swept under the rug as an example of convergent evolution? Or is it a relict of the wild days of Precambrium, when Lhcs as we

know them today evolved? Very little is also known about the PSI antenna in algae. Even the information on red algal Lhcs is sparse [682, 683].

In terms of the role of different pigmentation, we do have compelling hypotheses at least in a few cases. It is generally accepted that fucoxanthin and peridinin, supplemented with Chl *c* forms, developed in order to survive in a world dominated by cyanobacteria. The aforementioned pigments also turned out to be quite suitable for planktonic oceanic existence in deeper waters entirely depleted of red and orange light. The red-shifted Lhcs are of obvious use for survival in an environment highly shaded by other Chl *a*-containing organisms. The cost of having a significant portion of the light-harvesting antenna lying energetically below the photosystem RC has not been quantified yet. The penalty is however quite likely not very drastic. Introduction of a red-shifted Lhc into biotechnologically relevant algae strains should open the options in using diverse light profiles or multi-layer cultivation approaches. The significance of several NPQ features of secondary endosymbiotic algae, like using one or the other xanthophyll cycle (violaxanthin or diadinoxanthin), limited rates of the recovery reactions of the xanthophyll cycle, multiple Lhcx isoforms or the 'fast' NPQ, is generally not understood.

The evolutionary pressures and conditions of the time when some of the innovations of secondary endosymbiotic algae evolved are only known from indirect indications. Although, in principle, experiments with pigment composition should be costly or even lethal, it is obvious that life has found a way. There are at least two factors suggesting a favourable outcome of such attempts. First, the Lhc protein scaffold and the resulting structure are quite robust and can more or less successfully accomodate significant disturbances to the norm [684]. Second, the algae groups with divergent pigmentation evolved most likely in the Precambrium, when atmospheric oxygen concentration was a fraction of today's values [404, 685]. The oxygen radical risk was therefore likely much lower than today. We will probably never learn whether the original niches, which opened with the invention of the new pigments, were the same that we observe now.

Perhaps the last item to mention here is the biggest question. What is the long game? Why should we care about the study of the minutiae of photosynthetic light harvesting? After all, crops were domesticated without the knowledge of detailed molecular mechanisms of plant life and it is quite likely that a brute force approach to algae domestication will be quite successful in the near future. One can of course write about all the traditional suggestions of targeted evolution, informed crop breeding and nature inspiring artificial structures. These notions are mostly potentially useful but also not particularly enticing to me. I'd like to provide something of a different nature. Foremost, I do believe in the inherent value of knowledge and do find great inspiration and awe in the natural phenomena we observe. This is probably my primary motivation in science but it isn't, maybe even shouldn't be, the society's motive to fund science.

Our world is very much driven by efficiency nowadays. We often ask, or are asked, questions like "Is the cost/benefit ratio of approach A lower or higher than that of approach B?" or "What is the expected yield of your approach in comparison to this other approach?" While these economy- and utility-driven perspectives have their place, they are often short-sighted and can be very costly in the long term [686]. We should not be interested in a maximal yield in a short time period but rather in stability, robustness and resilience against (yet) unknown external forces [687]. While organisms also optimize to a specific set of conditions in the short term, there is a catch. The natural conditions are often quite unstable and an organism has to be able to survive this instability in order for life to continue. Optimization to a pseudo-stable set of conditions can lead to an inabil-

ity of long-term survival [688–690]. In a world with uncertain variance, organisms able to 'expect the unexpected' can survive longer than highly optimized specialists. It is one of the reasons that we see many metabolic pathways with complex regulatory networks. The mechanism and regulation of circadian clocks [691], nonphotochemical quenching, or light harvesting is not optimal—or the most efficient—in the eyes of a human engineer. We would never design such systems. Yet they did survive eons and quite a few cataclysms as well. Apparently, there is sufficient value in not having a simple and highly efficient system and the pressure to optimize was not strong enough to eliminate the complexities. A generalist approach to research with a wide interest scope can provide open avenues which are often only obvious in retrospect. In human culture, robustness requires diversity of thought, which should not be limited by immediate profit considerations [692]. This does not necessarily mean that the study of algal photosynthesis is a good thing to do. But I've found that, in its combination of methods and the necessary theory, it is certainly a unique field, distinct—one could say orthogonal—from other fields of study.

Regarding the long game, I can also offer the following 'long' idea. There is a silly sci-fi TV show called Farscape, created in the late 90s. In Farscape, the characters travel distant universe in a biomechanoid spacecraft. What is interesting, the spacecraft can repair itself and even grow new capability when needed. I dream of a future where it would be possible to grow your house, other infrastructure, or technology, and have it nicely tuned and maintained due to it being alive and therefore capable of self-repair. The big advantage of living matter is that it manufactures its components from simple materials, does not require large infrastructure or massive energy sources, and that it manufactures the components with atomic precision. The advantage of human technology is, of course, its adaptation to our needs and desires as well as a comparatively fast evolution rate not dependent on offspring and random events. The examples of photosynthesis and manufactured solar cells provide sufficient illustration of the comparative advantages and disadvantages of the two approaches. By joining them, we could hope for getting the best of both worlds. One can visualize living technology powered by light and grown from air and water (and a pinch of fertilizer). Perhaps it is a Frankensteinian vision but I hope that this sort of biopunk [693] does provide an interesting perspective to close this text.

References

(1) Stokes, G. G. II. On the supposed identity of biliverdin with chlorophyll, with remarks on the constitution of chlorophyll. *Proceedings of the Royal Society of London* **1864**, *13*, 144–145.

(2) Nitecki, M. H.; Lemke, J. L.; Pullman, H. W.; Johnson, M. E. Acceptance of plate tectonic theory by geologists. *Geology* **1978**, *6*, 661–664.

(3) Klemas, V. Remote Sensing of Algal Blooms: An Overview with Case Studies. *Journal of Coastal Research* **2012**, *28*, 34–43.

(4) Christner, B. C. et al. A microbial ecosystem beneath the West Antarctic ice sheet. *Nature* **2014**, *512*, 310–313.

(5) Cloud, P. Paleoecological Significance of the Banded Iron-Formation. *Economic Geology* **1973**, *68*, 1135–1143.

(6) Procházka, S.; Macháčková, I.; Krekule, J.; Šebánek, J., et al., *Fyziologie rostlin*; Academia Praha: 1998.

(7) Ke, B., *Photosynthesis photobiochemistry and photobiophysics*; Springer Science & Business Media: 2001; Vol. 10.

(8) Blankenship, R. E., *Molecular Mechanisms of Photosynthesis*; Blackwell Science Ltd: 2002.

(9) Renger, G. In *Primary Processes of Photosynthesis, Part 1: Principles and Apparatus*; The Royal Society of Chemistry: 2008; Vol. 8, pp 5–35.

(10) Blackman, F. F. Optima and limiting factors. *Annals of Botany* **1905**, *19*, 281–295.

(11) Trebst, A. V.; Tsujimoto, H. Y.; Arnon, D. I. Separation of Light and Dark Phases in the Photosynthesis of Isolated Chloroplasts. *Nature* **1958**, *182*, 351–355.

(12) Buchanan, B. B. The carbon (formerly dark) reactions of photosynthesis. *Photosynthesis Research* **2016**, *128*, 215–217.

(13) Duysens, L. N. M.; Amesz, J.; Kamp, B. M. Two Photochemical Systems in Photosynthesis. *Nature* **1961**, *190*, 510–511.

(14) Ruben, S.; Randall, M.; Kamen, M.; Hyde, J. L. Heavy oxygen (O^{18}) as a tracer in the study of photosynthesis. *Journal of the American Chemical Society* **1941**, *63*, 877–879.

(15) In *Plant Energetics*, Ksenzhek, O. S., Volkov, A. G., Eds.; Academic Press: San Diego, 1998, pp 31–54.

(16) Schreiber, U.; Schliwa, U.; Bilger, W. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis Research* **1986**, *10*, 51–62.

(17) Müller, P.; Li, X.-P.; Niyogi, K. K. Non-Photochemical Quenching. A Response to Excess Light Energy. *Plant Physiology* **2001**, *125*, 1558–1566.

(18) Govindjee; Seufferheld, M. J. Non-photochemical quenching of chlorophyll *a* fluorescence: early history and characterization of two xanthophyll-cycle mutants of *Chlamydomonas reinhardtii*. *Functional Plant Biology* **2002**, *29*, 1141–1155.

(19) Mulkidjanian, A. Y.; Koonin, E. V.; Makarova, K. S.; Mekhedov, S. L.; Sorokin, A.; Wolf, Y. I.; Dufresne, A.; Partensky, F.; Burd, H.; Kaznadzey, D., et al. The cyanobacterial genome core and the origin of photosynthesis. *Proceedings of the National Academy of Sciences* **2006**, *103*, 13126–13131.

(20) Cardona, T. Thinking twice about the evolution of photosynthesis. *Open Biology* **2019**, *9*, 180246.

(21) Sánchez-Baracaldo, P.; Cardona, T. On the origin of oxygenic photosynthesis and Cyanobacteria. *New Phytologist* **2020**, *225*, 1440–1446.

(22) Dorrell, R. G.; Howe, C. J. What makes a chloroplast? Reconstructing the establishment of photosynthetic symbioses. *Journal of Cell Science* **2012**, *125*, 1865–1875.

(23) Sagan, L. On the origin of mitosing cells. *Journal of Theoretical Biology* **1967**, *14*, 225–274.

(24) Archibald, J. M. Endosymbiosis and Eukaryotic Cell Evolution. *Current Biology* **2015**, *25*, R911–R921.

(25) Martin, W.; Herrmann, R. G. Gene Transfer from Organelles to the Nucleus: How Much, What Happens, and Why? *Plant Physiology* **1998**, *118*, 9–17.

(26) Oborník, M. Endosymbiotic Evolution of Algae, Secondary Heterotrophy and Parasitism. *Biomolecules* **2019**, *9*, 266.

(27) Menke, W. Structure and Chemistry of Plastids. *Annual Review of Plant Physiology* **1962**, *13*, 27–44.

(28) Menke, W. Über die Chloroplasten von *Anthoceros punctatus*: (5. Mitteilung zur Entwicklungsgeschichte der Plastiden). *Zeitschrift für Naturforschung B* **1961**, *16*, 334–336.

(29) Allen, J. F. Cyclic, pseudocyclic and noncyclic photophosphorylation: new links in the chain. *Trends in Plant Science* **2003**, *8*, 15–19.

(30) Khatter, H.; Myasnikov, A. G.; Natchiar, S. K.; Klaholz, B. P. Structure of the human 80S ribosome. *Nature* **2015**, *520*, 640–645.

(31) Wang, X.; Zhu, L.; Dang, M.; Hu, Z.; Gao, Q.; Yuan, S.; Sun, Y.; Zhang, B.; Ren, J.; Kotecha, A.; Walter, T. S.; Wang, J.; Fry, E. E.; Stuart, D. I.; Rao, Z. Potent neutralization of hepatitis A virus reveals a receptor mimic mechanism and the receptor recognition site. *Proceedings of the National Academy of Sciences* **2017**, *114*, 770–775.

(32) Wei, X.; Su, X.; Cao, P.; Liu, X.; Chang, W.; Li, M.; Zhang, X.; Liu, Z. Structure of spinach photosystem II-LHCII supercomplex at 3.2Å resolution. *Nature* **2016**, *534*, 69.

(33) Su, X.; Ma, J.; Wei, X.; Cao, P.; Zhu, D.; Chang, W.; Liu, Z.; Zhang, X.; Li, M. Structure and assembly mechanism of plant $C_2S_2M_2$-type PSII-LHCII supercomplex. *Science* **2017**, *357*, 815–820.

(34) Graça, A. T.; Hall, M.; Persson, K.; Schröder, W. P. High-resolution model of *Arabidopsis* Photosystem II reveals the structural consequences of digitonin-extraction. *Scientific Reports* **2021**, *11*, 15534.

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

(36) Tang, X.-S.; Satoh, K. The oxygen-evolving photosystem II core complex. *FEBS Letters* **1985**, *179*, 60–64.

(37) Chua, N. H.; Gillham, N. W. The sites of synthesis of the principal thylakoid membrane polypeptides in *Chlamydomonas reinhardtii*. *Journal of Cell Biology* **1977**, *74*, 441–452.

(38) Green, B. R. The chlorophyll-protein complexes of higher plant photosynthetic membranes or Just what green band is that? *Photosynthesis Research* **1988**, *15*, 3–32.

(39) Camm, E. L.; Green, B. R. Fractionation of Thylakoid Membranes with the Nonionic Detergent Octyl-β-d-glucopyranoside: Resolution Of Chlorophyll-protein Complex II Into Two Chlorophyll-protein Complexes. *Plant Physiology* **1980**, *66*, 428–432.

(40) Tanaka, A.; Fukushima, Y.; Kamiya, N. Two Different Structures of the Oxygen-Evolving Complex in the Same Polypeptide Frameworks of Photosystem II. *Journal of the American Chemical Society* **2017**, *139*, 1718–1721.

(41) Kühlbrandt, W. Three-dimensional structure of the light-harvesting chlorophyll a/b–protein complex. *Nature* **1984**, *307*, 478–480.

(42) Butler, P. J. G.; Kühlbrandt, W. Determination of the aggregate size in detergent solution of the light-harvesting chlorophyll a/b-protein complex from chloroplast membranes. *Proceedings of the National Academy of Sciences* **1988**, *85*, 3797–3801.

(43) Boekema, E. J.; van Roon, H.; Dekker, J. P. Specific association of photosystem II and light-harvesting complex II in partially solubilized photosystem II membranes. *FEBS Letters* **1998**, *424*, 95–99.

(44) Boekema, E. J.; van Roon, H.; Calkoen, F.; Bassi, R.; Dekker, J. P. Multiple Types of Association of Photosystem II and Its Light-Harvesting Antenna in Partially Solubilized Photosystem II Membranes. *Biochemistry* **1999**, *38*, 2233–2239.

(45) Ballottari, M.; Dall'Osto, L.; Morosinotto, T.; Bassi, R. Contrasting behavior of higher plant photosystem I and II antenna systems during acclimation. *Journal of Biological Chemistry* **2007**, *282*, 8947–8958.

(46) Rappaport, F.; Guergova-Kuras, M.; Nixon, P. J.; Diner, B. A.; Lavergne, J. Kinetics and Pathways of Charge Recombination in Photosystem II. *Biochemistry* **2002**, *41*, 8518–8527.

(47) Döring, G.; Stiehl, H. H.; Witt, H. T. A Second Chlorophyll Reaction in the Electron Chain of Photosynthesis — Registration by the Repetitive Excitation Technique. *Zeitschrift für Naturforschung B* **1967**, *22*, 639–644.

(48) Floyd, R. A.; Chance, B.; Devault, D. Low temperature photo-induced reactions in green leaves and chloroplasts. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1971**, *226*, 103–112.

(49) Döring, G.; Witt, H. T. In *Photosynthesis, two centuries after its discovery by Joseph Priestley*, ed. by Forti, G.; Avron, M.; Melandri, A., Dordrecht, 1972, pp 39–45.

(50) Barber, J.; Archer, M. P680, the primary electron donor of photosystem II. *Journal of Photochemistry and Photobiology A: Chemistry* **2001**, *142*, 97–106.

(51) Xiong, L.; Seibert, M.; Gusev, A. V.; Wasielewski, M. R.; Hemann, C.; Hille, C. R.; Sayre, R. T. Substitution of a Chlorophyll into the Inactive Branch Pheophytin-Binding Site Impairs Charge Separation in Photosystem II. *The Journal of Physical Chemistry B* **2004**, *108*, 16904–16911.

(52) Cardona, T.; Sedoud, A.; Cox, N.; Rutherford, A. W. Charge separation in Photosystem II: A comparative and evolutionary overview. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2012**, *1817*, 26–43.

(53) Cardona, T. A fresh look at the evolution and diversification of photochemical reaction centers. *Photosynthesis Research* **2015**, *126*, 111–134.

(54) Rappaport, F.; Blanchard-Desce, M.; Lavergne, J. Kinetics of electron transfer and electrochromic change during the redox transitions of the photosynthetic oxygen-evolving complex. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1994**, *1184*, 178–192.

(55) de Wijn, R.; van Gorkom, H. J. Kinetics of Electron Transfer from Q_A to Q_B in Photosystem II. *Biochemistry* **2001**, *40*, 11912–11922.

(56) Joliot, P.; Barbieri, G.; Chabaud, R. Un nouveau modele des centres photochimiques du systeme II. *Photochemistry and Photobiology* **1969**, *10*, 309–329.

(57) Kok, B.; Forbush, B.; Mcgloin, M. Cooperation of charges in photosynthetic O_2 evolution–I. A linear four step mechanism. *Photochemistry and Photobiology* **1970**, *11*, 457–475.

(58) Klauss, A.; Haumann, M.; Dau, H. Alternating electron and proton transfer steps in photosynthetic water oxidation. *Proceedings of the National Academy of Sciences* **2012**, *109*, 16035–16040.

(59) Lubitz, W.; Chrysina, M.; Cox, N. Water oxidation in photosystem II. *Photosynthesis Research* **2019**, *142*, 105–125.

(60) Nelson, N.; Neumann, J. Isolation of a Cytochrome b_6-f Particle from Chloroplasts. *Journal of Biological Chemistry* **1972**, *247*, 1817–1824.

(61) Malkin, R.; Aparicio, P. J. Identification of a g = 1.90 high-potential iron-sulfur protein in chloroplasts. *Biochemical and Biophysical Research Communications* **1975**, *63*, 1157–1160.

(62) Ke, B.; Sugahara, K.; Shaw, E. Further purification of "Triton subchloroplast fraction I" (TSF-I particles). Isolation of a cytochrome-free High-P-700 particle and a complex containing cytochromes f and b_6, plastocyanin and iron-sulfur protein(s). *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1975**, *408*, 12–25.

(63) Cramer, W. A. Structure–function of the cytochrome b_6f lipoprotein complex: a scientific odyssey and personal perspective. *Photosynthesis Research* **2019**, *139*, 53–65.

(64) Huang, D.; Everly, R. M.; Cheng, R. H.; Heymann, J. B.; Schaegger, H.; Sled, V.; Ohnishi, T.; Baker, T. S.; Cramer, W. A. Characterization of the Chloroplast Cytochrome b_6f Complex as a Structural and Functional Dimer. *Biochemistry* **1994**, *33*, 4401–4409.

(65) Kurisu, G.; Zhang, H.; Smith, J. L.; Cramer, W. A. Structure of the Cytochrome b_6f Complex of Oxygenic Photosynthesis: Tuning the Cavity. *Science* **2003**, *302*, 1009–1014.

(66) Stroebel, D.; Choquet, Y.; Popot, J.-L.; Picot, D. An atypical haem in the cytochrome b_6f complex. *Nature* **2003**, *426*, 413–418.

(67) Rieske, J. S.; Hansen, R. E.; Zaugg, W. S. Studies on the Electron Transfer System: LVIII. Properties of a new oxidation-reduction component of the respiratory chain as studied by electron paramagnetic resonance spectroscopy. *Journal of Biological Chemistry* **1964**, *239*, 3017–3022.

(68) Malone, L. A.; Qian, P.; Mayneord, G. E.; Hitchcock, A.; Farmer, D. A.; Thompson, R. F.; Swainsbury, D. J. K.; Ranson, N. A.; Hunter, C. N.; Johnson, M. P. Cryo-EM structure of the spinach cytochrome b_6f complex at 3.6 Å resolution. *Nature* **2019**, *575*, 535–539.

(69) Allen, J. F. Cytochrome b_6f: structure for signalling and vectorial metabolism. *Trends in Plant Science* **2004**, *9*, 130–137.

(70) Dumas, L.; Chazaux, M.; Peltier, G.; Johnson, X.; Alric, J. Cytochrome b_6f function and localization, phosphorylation state of thylakoid membrane proteins and consequences on cyclic electron flow. *Photosynthesis Research* **2016**, *129*, 307–320.

(71) Mitchell, P. Possible molecular mechanisms of the protonmotive function of cytochrome systems. *Journal of Theoretical Biology* **1976**, *62*, 327–367.

(72) Caughey, W.; Smythe, G.; O'Keeffe, D.; Maskasky, J.; Smith, M. Heme A of cytochrome c oxicase. Structure and properties: comparisons with hemes B, C, and S and derivatives. *Journal of Biological Chemistry* **1975**, *250*, 7602–7622.

(73) Kim, H. J.; Khalimonchuk, O.; Smith, P. M.; Winge, D. R. Structure, function, and assembly of heme centers in mitochondrial respiratory complexes. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **2012**, *1823*, 1604–1616.

(74) Kleingardner, J. G.; Bren, K. L. Biological Significance and Applications of Heme c Proteins and Peptides. *Accounts of Chemical Research* **2015**, *48*, 1845–1852.

(75) Schoepp, B.; Chabaud, E.; Breyton, C.; Verméglio, A.; Popot, J.-L. On the Spatial Organization of Hemes and Chlorophyll in Cytochrome b_6f: A linear and circular dichroism study. *Journal of Biological Chemistry* **2000**, *275*, 5275–5283.

(76) Yan, J.; Cramer, W. A. Functional Insensitivity of the Cytochrome b_6f Complex to Structure Changes in the Hinge Region of the Rieske Iron-Sulfur Protein. *Journal of Biological Chemistry* **2003**, *278*, 20925–20933.

(77) Malone, L. A.; Proctor, M. S.; Hitchcock, A.; Hunter, C. N.; Johnson, M. P. Cytochrome b_6f – Orchestrator of photosynthetic electron transfer. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2021**, *1862*, 148380.

(78) Alric, J.; Pierre, Y.; Picot, D.; Lavergne, J.; Rappaport, F. Spectral and redox characterization of the heme c_i of the cytochrome b_6f complex. *Proceedings of the National Academy of Sciences* **2005**, *102*, 15860–15865.

(79) Mitchell, P. The protonmotive Q cycle: A general formulation. *FEBS Letters* **1975**, *59*, 137–139.

(80) Baniulis, D.; Yamashita, E.; Zhang, H.; Hasan, S. S.; Cramer, W. A. Structure–Function of the Cytochrome b_6f Complex. *Photochemistry and Photobiology* **2008**, *84*, 1349–1358.

(81) Cramer, W. A.; Hasan, S. S.; Yamashita, E. The Q cycle of cytochrome bc complexes: A structure perspective. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2011**, *1807*, 788–802.

(82) Haehnel, W. Photosynthetic Electron Transport in Higher Plants. *Annual Review of Plant Physiology* **1984**, *35*, 659–693.

(83) Stiehl, H. H.; Witt, H. T. Quantitative Treatment of the Function of Plastoquinone in Photosynthesis. *Zeitschrift für Naturforschung B* **1969**, *24*, 1588–1598.

(84) Zhang, H.; Huang, D.; Cramer, W. A. Stoichiometrically Bound β-Carotene in the Cytochrome b_6f Complex of Oxygenic Photosynthesis Protects against Oxygen Damage. *Journal of Biological Chemistry* **1999**, *274*, 1581–1587.

(85) Kim, H.; Dashdorj, N.; Zhang, H.; Yan, J.; Cramer, W. A.; Savikhin, S. An Anomalous Distance Dependence of Intraprotein Chlorophyll-Carotenoid Triplet Energy Transfer. *Biophysical Journal* **2005**, *89*, L28–L30.

(86) Zuo, P.; Li, B.-X.; Zhao, X.-H.; Wu, Y.-S.; Ai, X.-C.; Zhang, J.-P.; Li, L.-B.; Kuang, T.-Y. Ultrafast Carotenoid-to-Chlorophyll Singlet Energy Transfer in the Cytochrome b_6f Complex from *Bryopsis corticulans*. *Biophysical Journal* **2006**, *90*, 4145–4154.

(87) Li, B.-X.; Zuo, P.; Chen, X.-B.; Li, L.-B.; Zhang, J.-P.; Kuang, T.-Y. Study on energy transfer between carotenoid and chlorophyll ain cytochrome b6f complex from *Bryopsis corticulans*. *Photosynthesis Research* **2006**, *88*, 43–50.

(88) Ma, F.; Chen, X.-B.; Sang, M.; Wang, P.; Zhang, J.-P.; Li, L.-B.; Kuang, T.-Y. Singlet oxygen formation and chlorophyll a triplet excited state deactivation in the cytochrome b_6f complex from *Bryopsis corticulans*. *Photosynthesis Research* **2009**, *100*, 19–28.

(89) Katoh, S. A New Copper Protein from *Chlorella ellipsoidea*. *Nature* **1960**, *186*, 533–534.

(90) Katoh, S.; Takamiya, A. A New Leaf Copper Protein 'Plastocyanin', a Natural Hill Oxidant. *Nature* **1961**, *189*, 665–666.

(91) Colman, P. M.; Freeman, H. C.; Guss, J. M.; Murata, M.; Norris, V. A.; Ramshaw, J. A. M.; Venkatappa, M. P. X-ray crystal structure analysis of plastocyanin at 2.7 Å resolution. *Nature* **1978**, *272*, 319–324.

(92) Holwerda, R. A.; Wherland, S.; Gray, H. B. Electron transfer reactions of copper proteins. *Annual Review of Biophysics and Bioengineering* **1976**, *5*, 363–396.

(93) Battistuzzi, G.; Borsari, M.; Loschi, L.; Righi, F.; Sola, M. Redox Thermodynamics of Blue Copper Proteins. *Journal of the American Chemical Society* **1999**, *121*, 501–506.

(94) Sigfridsson, K. Plastocyanin, an electron-transfer protein. *Photosynthesis Research* **1998**, *57*, 1–28.

(95) Hope, A. Electron transfers amongst cytochrome f, plastocyanin and photosystem I: kinetics and mechanisms. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2000**, *1456*, 5–26.

(96) Hippler, M.; Drepper, F. In *Photosystem I: The Light-Driven Plastocyanin:Ferredoxin Oxidoreductase*, Golbeck, J. H., Ed.; Springer Netherlands: Dordrecht, 2006, pp 499–513.

(97) Meyer, T. E.; Zhao, Z. G.; Cusanovich, M. A.; Tollin, G. Transient kinetics of electron transfer from a variety of c-type cytochromes to plastocyanin. *Biochemistry* **1993**, *32*, 4552–4559.

(98) Haehnel, W.; Ratajczak, R.; Robenek, H. Lateral distribution and diffusion of plastocyanin in chloroplast thylakoids. *Journal of Cell Biology* **1989**, *108*, 1397–1405.

(99) Haehnel, W.; Jansen, T.; Gause, K.; Klösgen, R.; Stahl, B.; Michl, D.; Huvermann, B.; Karas, M.; Herrmann, R. Electron transfer from plastocyanin to photosystem I. *The EMBO Journal* **1994**, *13*, 1028–1038.

(100) Takano, M.; Takahashi, M.-A.; Asada, K. Reduction of photosystem I reaction center, P-700, by plastocyanin in stroma thylakoids from spinach: Lateral diffusion of plastocyanin. *Archives of Biochemistry and Biophysics* **1982**, *218*, 369–375.

(101) Hoehner, R.; Pribil, M.; Herbstová, M.; Lopez, L. S.; Kunz, H.; Li, M.; Wood, M.; Svoboda, V.; Puthiyaveetil, S.; Leister, D.; Kirchhoff, H. Plastocyanin is the long-range electron carrier between photosystem II and photosystem I in plants. *Proceedings of the National Academy of Sciences of the United States of America* **2020**, *117*, 15354–15362.

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

(103) Castell, C.; Rodríguez-Lumbreras, L. A.; Hervás, M.; Fernández-Recio, J.; Navarro, J. A. New Insights into the Evolution of the Electron Transfer from Cytochrome f to Photosystem I in the Green and Red Branches of Photosynthetic Eukaryotes. *Plant and Cell Physiology* **2021**, *62*, 1082–1093.

(104) Kerfeld, C. A.; Anwar, H. P.; Interrante, R.; Merchant, S.; Yeates, T. O. The Structure of Chloroplast Cytochrome c_6 at 1.9 Å Resolution: Evidence for Functional Oligomerization. *Journal of Molecular Biology* **1995**, *250*, 627–647.

(105) Sandmann, G.; Böger, P. Copper-induced exchange of plastocyanin and cytochrome c-533 in cultures of *Anabaena variabilis* and *Plectonema boryanum*. *Plant Science Letters* **1980**, *17*, 417–424.

(106) De la Rosa, M. A.; Navarro, J. A.; Hervás, M. In *Bioenergetic Processes of Cyanobacteria: From Evolutionary Singularity to Ecological Diversity*, Peschek, G. A., Obinger, C., Renger, G., Eds.; Springer Netherlands: Dordrecht, 2011, pp 607–630.

(107) Ben-Shem, A.; Frolow, F.; Nelson, N. Crystal structure of plant photosystem I. *Nature* **2003**, *426*, 630–635.

(108) Amunts, A.; Drory, O.; Nelson, N. The structure of a plant photosystem I supercomplex at 3.4 Å resolution. *Nature* **2007**, *447*, 58–63.

(109) Qin, X.; Suga, M.; Kuang, T.; Shen, J.-R. Structural basis for energy transfer pathways in the plant PSI-LHCI supercomplex. *Science* **2015**, *348*, 989–995.

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

(111) Ivanov, A. G.; Krol, M.; Sveshnikov, D.; Selstam, E.; Sandström, S.; Koochek, M.; Park, Y.-I.; Vasil'ev, S.; Bruce, D.; Öquist, G.; Huner, N. P. Iron Deficiency in Cyanobacteria Causes Monomerization of Photosystem I Trimers and Reduces the Capacity for State Transitions and the Effective Absorption Cross Section of Photosystem I in Vivo. *Plant Physiology* **2006**, *141*, 1436–1445.

(112) Watanabe, M.; Kubota, H.; Wada, H.; Narikawa, R.; Ikeuchi, M. Novel Supercomplex Organization of Photosystem I in *Anabaena* and *Cyanophora paradoxa*. *Plant and Cell Physiology* **2010**, *52*, 162–168.

(113) Li, M.; Semchonok, D. A.; Boekema, E. J.; Bruce, B. D. Characterization and Evolution of Tetrameric Photosystem I from the Thermophilic Cyanobacterium *Chroococcidiopsis* sp TS-821. *Plant Cell* **2014**, *26*, 1230–1245.

(114) Chen, M.; Liu, X.; He, Y.; Li, N.; He, J.; Zhang, Y. Diversity Among Cyanobacterial Photosystem I Oligomers. *Frontiers in Microbiology* **2022**, *12*, 781826.

(115) Schubert, W.-D.; Klukas, O.; Saenger, W.; Witt, H. T.; Fromme, P.; Krauß, N. A common ancestor for oxygenic and anoxygenic photosynthetic systems: A comparison based on the structural model of photosystem I. *Journal of Molecular Biology* **1998**, *280*, 297–314.

(116) Sadekar, S.; Raymond, J.; Blankenship, R. E. Conservation of Distantly Related Membrane Proteins: Photosynthetic Reaction Centers Share a Common Structural Core. *Molecular Biology and Evolution* **2006**, *23*, 2001–2007.

(117) Hippler, M.; Reichert, J.; Sutter, M.; Zak, E.; Altschmied, L.; Schröer, U.; Herrmann, R. G.; Haehnel, W. The plastocyanin binding domain of photosystem I. *The EMBO Journal* **1996**, *15*, 6374–6384.

(118) Chitnis, P. R. Photosystem I: Function and Physiology. *Annual Review of Plant Physiology and Plant Molecular Biology* **2001**, *52*, 593–626.

(119) Galka, P.; Santabarbara, S.; Khuong, T. T. H.; Degand, H.; Morsomme, P.; Jennings, R. C.; Boekema, E. J.; Caffarri, S. Functional analyses of the plant photosystem I-light-harvesting complex II supercomplex reveal that light-harvesting complex II loosely bound to photosystem II is a very efficient antenna for photosystem I in state II. *Plant Cell* **2012**, *24*, 2963–2978.

(120) Pan, X.; Ma, J.; Su, X.; Cao, P.; Chang, W.; Liu, Z.; Zhang, X.; Li, M. Structure of the maize photosystem I supercomplex with light-harvesting complexes I and II. *Science* **2018**, *360*, 1109–1113.

(121) Mazor, Y.; Borovikova, A.; Caspy, I.; Nelson, N. Structure of the plant photosystem I supercomplex at 2.6 Å resolution. *Nature Plants* **2017**, *3*, 17014.

(122) Dam, H. The Antihæmorrhagic Vitamin of the Chick: Occurrence And Chemical Nature. *Nature* **1935**, *135*, 652–653.

(123) Binkley, S.; MacCorquodale, D.; Thayer, S. A.; Doisy, E. A. The isolation of Vitamin K_1. *Journal of Biological Chemistry* **1939**, *130*, 219–234.

(124) Shearer, M. J.; Newman, P. Metabolism and cell biology of vitamin K. *Thromb Haemost* **2008**, *100*, 530–547.

(125) Ferland, G. The Discovery of Vitamin K and Its Clinical Applications. *Annals of Nutrition and Metabolism* **2012**, *61*, 213–218.

(126) Kok, B.; Gott, W. Activation Spectra of 700 mμ Absorption Change in Photosynthesis. *Plant Physiology* **1960**, *35*, 802–808.

(127) Kok, B. Partial purification and determination of oxidation reduction potential of the photosynthetic chlorophyll complex absorbing at 700 mμ. *Biochimica et Biophysica Acta* **1961**, *48*, 527–533.

(128) Watanabe, T.; Nakazato, M.; Mazaki, H.; Hongu, A.; Konno, M.; Saitoh, S.; Honda, K. Chlorophyll *a* epimer and pheophytin *a* in green leaves. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1985**, *807*, 110–117.

(129) Busch, A.; Hippler, M. The structure and function of eukaryotic photosystem I. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2011**, *1807*, 864–877.

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

(131) Brettel, K.; Leibl, W. Electron transfer in photosystem I. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2001**, *1507*, 100–114.

(132) Shelaev, I. V.; Gostev, F. E.; Mamedov, M. D.; Sarkisov, O. M.; Nadtochenko, V. A.; Shuvalov, V. A.; Semenov, A. Y. Femtosecond primary charge separation in *Synechocystis* sp. PCC 6803 photosystem I. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2010**, *1797*, 1410–1420.

(133) Poluektov, O. G.; Paschenko, S. V.; Utschig, L. M.; Lakshmi, K. V.; Thurnauer, M. C. Bidirectional Electron Transfer in Photosystem I: Direct Evidence from High-Frequency Time-Resolved EPR Spectroscopy. *Journal of the American Chemical Society* **2005**, *127*, 11910–11911.

(134) Müller, M. G.; Slavov, C.; Luthra, R.; Redding, K. E.; Holzwarth, A. R. Independent initiation of primary electron transfer in the two branches of the photosystem I reaction center. *Proceedings of the National Academy of Sciences* **2010**, *107*, 4123–4128.

(135) Santabarbara, S.; Galuppini, L.; Casazza, A. P. Bidirectional Electron Transfer in the Reaction Centre of Photosystem I. *Journal of Integrative Plant Biology* **2010**, *52*, 735–749.

(136) Tagawa, K.; Arnon, D. I. Ferredoxins as Electron Carriers in Photosynthesis and in the Biological Production and Consumption of Hydrogen Gas. *Nature* **1962**, *195*, 537–543.

(137) Tsukihara, T.; Fukuyama, K.; Nakamura, M.; Katsube, Y.; Tanaka, N.; Kakudo, M.; Wada, K.; Hase, T.; Matsubara, H. X-Ray Analysis of a [2Fe-2S] Ferredoxin from *Spirulina platensis*. Main Chain Fold and Location of Side Chains at 2.5 Å Resolution. *The Journal of Biochemistry* **1981**, *90*, 1763–1773.

(138) Hanke, G.; Mulo, P. Plant type ferredoxins and ferredoxin-dependent metabolism. *Plant, Cell & Environment* **2013**, *36*, 1071–1084.

(139) Nawrocki, W.; Bailleul, B.; Picot, D.; Cardol, P.; Rappaport, F.; Wollman, F.-A.; Joliot, P. The mechanism of cyclic electron flow. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2019**, *1860*, 433–438.

(140) Reinbothe, C.; Bartsch, S.; Eggink, L. L.; Hoober, J. K.; Brusslan, J.; Andrade-Paz, R.; Monnet, J.; Reinbothe, S. A role for chlorophyllide *a* oxygenase in the regulated import and stabilization of light-harvesting chlorophyll *a/b* proteins. *Proceedings of the National Academy of Sciences* **2006**, *103*, 4777–4782.

(141) Muramoto, T.; Tsurui, N.; Terry, M. J.; Yokota, A.; Kohchi, T. Expression and Biochemical Properties of a Ferredoxin-Dependent Heme Oxygenase Required for Phytochrome Chromophore Synthesis. *Plant Physiology* **2002**, *130*, 1958–1966.

(142) Kohchi, T.; Mukougawa, K.; Frankenberg, N.; Masuda, M.; Yokota, A.; Lagarias, J. C. The Arabidopsis HY2 Gene Encodes Phytochromobilin Synthase, a Ferredoxin-Dependent Biliverdin Reductase. *The Plant Cell* **2001**, *13*, 425–436.

(143) Schmidt, H.; Heinz, E. Involvement of Ferredoxin in Desaturation of Lipid-Bound Oleate in Chloroplasts. *Plant Physiology* **1990**, *94*, 214–220.

(144) Pierella Karlusich, J. J.; Carrillo, N. Evolution of the acceptor side of photosystem I: ferredoxin, flavodoxin, and ferredoxin-NADP$^+$ oxidoreductase. *Photosynthesis Research* **2017**, *134*, 235–250.

(145) Smillie, R. M. Isolation of two proteins with chloroplast ferredoxin activity from a blue-green alga. *Biochemical and Biophysical Research Communications* **1965**, *20*, 621–629.

(146) Sancho, J. Flavodoxins: sequence, folding, binding, function and beyond. *Cellular and Molecular Life Sciences CMLS* **2006**, *63*, 855–864.

(147) Pierella Karlusich, J. J.; Lodeyro, A. F.; Carrillo, N. The long goodbye: the rise and fall of flavodoxin during plant evolution. *Journal of Experimental Botany* **2014**, *65*, 5161–5178.

(148) Fitzgerald, M. P.; Sykes, G. A.; Rogers, L. J. Apoflavodoxin aggregation following dissociation of flavin. *Biochimica et Biophysica Acta (BBA) - Protein Structure* **1980**, *625*, 127–132.

(149) Fukuyama, K.; Wakabayashi, S.; Matsubara, H.; Rogers, L. Tertiary structure of oxidized flavodoxin from an eukaryotic red alga *Chondrus crispus* at 2.35-Å resolution. Localization of charged residues and implication for interaction with electron transfer partners. *Journal of Biological Chemistry* **1990**, *265*, 15804–15812.

(150) Eck, R. V.; Dayhoff, M. O. Evolution of the Structure of Ferredoxin Based on Living Relics of Primitive Amino Acid Sequences. *Science* **1966**, *152*, 363–366.

(151) Hall, D. O.; Cammack, R.; Rao, K. K. Role for Ferredoxins in the Origin of Life and Biological Evolution. *Nature* **1971**, *233*, 136–138.

(152) Wang, M.; Boca, S. M.; Kalelkar, R.; Mittenthal, J. E.; Caetano-Anollés, G. A phylogenomic reconstruction of the protein world based on a genomic census of protein fold architecture. *Complexity* **2006**, *12*, 27–40.

(153) Camprubi, E.; Jordan, S. F.; Vasiliadou, R.; Lane, N. Iron catalysis at the origin of life. *IUBMB Life* **2017**, *69*, 373–381.

(154) Arakaki, A. K.; Ceccarelli, E. A.; Carrillo, N. Plant-type ferredoxin-NADP$^+$ reductases: a basal structural framework and a multiplicity of functions. *The FASEB Journal* **1997**, *11*, 133–140.

(155) Hermoso, J. A.; Mayoral, T.; Faro, M.; Gómez-Moreno, C.; Sanz-Aparicio, J.; Medina, M. Mechanism of Coenzyme Recognition and Binding Revealed by Crystal Structure Analysis of Ferredoxin–NADP$^+$ Reductase Complexed with NADP$^+$. *Journal of Molecular Biology* **2002**, *319*, 1133–1142.

(156) Mulo, P. Chloroplast-targeted ferredoxin-NADP$^+$ oxidoreductase (FNR): Structure, function and location. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2011**, *1807*, 927–934.

(157) Medina, M.; Gómez-Moreno, C. Interaction of Ferredoxin–NADP$^+$ Reductase with its Substrates: Optimal Interaction for Efficient Electron Transfer. *Photosynthesis Research* **2004**, *79*, 113–131.

(158) Mulo, P.; Medina, M. Interaction and electron transfer between ferredoxin–NADP$^+$ oxidoreductase and its partners: structural, functional, and physiological implications. *Photosynthesis Research* **2017**, *134*, 265–280.

(159) Arnon, D. I.; Whatley, F. R.; Allen, M. B. Assimilatory Power in Photosynthesis. *Science* **1958**, *127*, 1026–1034.

(160) Endo, T.; Mil, H.; Shikanai, T.; Asada, K. Donation of Electrons to Plastoquinone by NAD(P)H Dehydrogenase and by Ferredoxin-Quinone Reductase in Spinach Chloroplasts. *Plant and Cell Physiology* **1997**, *38*, 1272–1277.

(161) Peng, L.; Shikanai, T. Supercomplex Formation with Photosystem I Is Required for the Stabilization of the Chloroplast NADH Dehydrogenase-Like Complex in *Arabidopsis*. *Plant Physiology* **2011**, *155*, 1629–1639.

(162) Ifuku, K.; Endo, T.; Shikanai, T.; Aro, E.-M. Structure of the Chloroplast NADH Dehydrogenase-Like Complex: Nomenclature for Nuclear-Encoded Subunits. *Plant and Cell Physiology* **2011**, *52*, 1560–1568.

(163) Laughlin, T. G.; Bayne, A. N.; Trempe, J.-F.; Savage, D. F.; Davies, K. M. Structure of the complex I-like molecule NDH of oxygenic photosynthesis. *Nature* **2019**, *566*, 411–414.

(164) Munekage, Y.; Hojo, M.; Meurer, J.; Endo, T.; Tasaka, M.; Shikanai, T. PGR5 Is Involved in Cyclic Electron Flow around Photosystem I and Is Essential for Photoprotection in *Arabidopsis*. *Cell* **2002**, *110*, 361–371.

(165) DalCorso, G.; Pesaresi, P.; Masiero, S.; Aseeva, E.; Schünemann, D.; Finazzi, G.; Joliot, P.; Barbato, R.; Leister, D. A Complex Containing PGRL1 and PGR5 Is Involved in the Switch between Linear and Cyclic Electron Flow in *Arabidopsis*. *Cell* **2008**, *132*, 273–285.

(166) Hertle, A. P.; Blunder, T.; Wunder, T.; Pesaresi, P.; Pribil, M.; Armbruster, U.; Leister, D. PGRL1 Is the Elusive Ferredoxin-Plastoquinone Reductase in Photosynthetic Cyclic Electron Flow. *Molecular Cell* **2013**, *49*, 511–523.

(167) Strand, D. D.; Fisher, N.; Kramer, D. M. The higher plant plastid NAD(P)H dehydrogenase-like complex (NDH) is a high efficiency proton pump that increases ATP production by cyclic electron flow. *Journal of Biological Chemistry* **2017**, *292*, 11850–11860.

(168) Suorsa, M.; Rossi, F.; Tadini, L.; Labs, M.; Colombo, M.; Jahns, P.; Kater, M. M.; Leister, D.; Finazzi, G.; Aro, E.-M.; Barbato, R.; Pesaresi, P. PGR5-PGRL1-Dependent Cyclic Electron Transport Modulates Linear Electron Transport Rate in *Arabidopsis thaliana*. *Molecular Plant* **2016**, *9*, 271–288.

(169) Racker, E. Studies of factors involved in oxidative phosphorylation. *Proceedings of the National Academy of Sciences* **1962**, *48*, 1659–1663.

(170) Kagawa, Y.; Racker, E. Partial Resolution of the Enzymes Catalyzing Oxidative Phosphorylation: VIII. Properties of a factor conferring oligomycin sensitivity on mitochondrial adenosine triphosphatase. *Journal of Biological Chemistry* **1966**, *241*, 2461–2466.

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

(172) Junge, W.; Sielaff, H.; Engelbrecht, S. Torque generation and elastic power transmission in the rotary F_OF_1-ATPase. *Nature* **2009**, *459*, 364–370.

(173) Kühlbrandt, W.; Davies, K. M. Rotary ATPases: A New Twist to an Ancient Machine. *Trends in Biochemical Sciences* **2016**, *41*, 106–116.

(174) Guo, H.; Rubinstein, J. L. Structure of ATP synthase under strain during catalysis. *Nature Communications* **2022**, *13*, 2232.

(175) Pogoryelov, D.; Reichen, C.; Klyszejko, A. L.; Brunisholz, R.; Muller, D. J.; Dimroth, P.; Meier, T. The Oligomeric State of c Rings from Cyanobacterial F-ATP Synthases Varies from 13 to 15. *Journal of Bacteriology* **2007**, *189*, 5895–5902.

(176) Watt, I. N.; Montgomery, M. G.; Runswick, M. J.; Leslie, A. G. W.; Walker, J. E. Bioenergetic cost of making an adenosine triphosphate molecule in animal mitochondria. *Proceedings of the National Academy of Sciences* **2010**, *107*, 16823–16827.

(177) Seelert, H.; Poetsch, A.; Dencher, N. A.; Engel, A.; Stahlberg, H.; Müller, D. J. Proton-powered turbine of a plant motor. *Nature* **2000**, *405*, 418–419.

(178) Meyer, A., *Das Chlorophyllkorn in chemischer, morphologischer und biologischer Beziehung*; A. Felix: 1883.

(179) Staehelin, L. A.; Paolillo, D. J. A brief history of how microscopic studies led to the elucidation of the 3D architecture and macromolecular organization of higher plant thylakoids. *Photosynthesis Research* **2020**, *145*, 237–258.

(180) Austin Jotham R., I.; Staehelin, L. A. Three-Dimensional Architecture of Grana and Stroma Thylakoids of Higher Plants as Determined by Electron Tomography. *Plant Physiology* **2011**, *155*, 1601–1611.

(181) Bussi, Y.; Shimoni, E.; Weiner, A.; Kapon, R.; Charuvi, D.; Nevo, R.; Efrati, E.; Reich, Z. Fundamental helical geometry consolidates the plant photosynthetic membrane. *Proceedings of the National Academy of Sciences* **2019**, *116*, 22366–22375.

(182) Dekker, J. P.; Boekema, E. J. Supramolecular organization of thylakoid membrane proteins in green plants. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2005**, *1706*, 12–39.

(183) Kirchhoff, H. Diffusion of molecules and macromolecules in thylakoid membranes. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2014**, *1837*, 495–502.

(184) Kirchhoff, H. Chloroplast ultrastructure in plants. *New Phytologist* **2019**, *223*, 565–574.

(185) Lichtenthaler, H. K.; Kuhn, G.; Prenzel, U.; Buschmann, C.; Meier, D. Adaptation of Chloroplast-Ultrastructure and of Chlorophyll- Protein Levels to High-Light and Low-Light Growth Conditions. *Zeitschrift für Naturforschung C* **1982**, *37*, 464–475.

(186) Fristedt, R.; Willig, A.; Granath, P.; Crèvecoeur, M.; Rochaix, J.-D.; Vener, A. V. Phosphorylation of Photosystem II Controls Functional Macroscopic Folding of Photosynthetic Membranes in *Arabidopsis*. *The Plant Cell* **2009**, *21*, 3950–3964.

(187) Herbstová, M.; Tietz, S.; Kinzel, C.; Turkina, M. V.; Kirchhoff, H. Architectural switch in plant photosynthetic membranes induced by light stress. *Proceedings of the National Academy of Sciences* **2012**, *109*, 20130–20135.

(188) Garab, G. Hierarchical organization and structural flexibility of thylakoid membranes. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2014**, *1837*, 481–494.

(189) Kirchhoff, H.; Hall, C.; Wood, M.; Herbstová, M.; Tsabari, O.; Nevo, R.; Charuvi, D.; Shimoni, E.; Reich, Z. Dynamic control of protein diffusion within the granal thylakoid lumen. *Proceedings of the National Academy of Sciences* **2011**, *108*, 20248–20253.

(190) Pribil, M.; Labs, M.; Leister, D. Structure and dynamics of thylakoids in land plants. *Journal of Experimental Botany* **2014**, *65*, 1955–1972.

(191) Wood, W. H. J.; MacGregor-Chatwin, C.; Barnett, S. F. H.; Mayneord, G. E.; Huang, X.; Hobbs, J. K.; Hunter, C. N.; Johnson, M. P. Dynamic thylakoid stacking regulates the balance between linear and cyclic photosynthetic electron transfer. *Nature Plants* **2018**, *4*, 116–127.

(192) Li, M.; Mukhopadhyay, R.; Svoboda, V.; Oung, H. M. O.; Mullendore, D. L.; Kirchhoff, H. Measuring the dynamic response of the thylakoid architecture in plant leaves by electron microscopy. *Plant Direct* **2020**, *4*, e00280.

(193) Andersson, B.; Anderson, J. M. Lateral heterogeneity in the distribution of chlorophyll-protein complexes of the thylakoid membranes of spinach chloroplasts. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1980**, *593*, 427–440.

(194) Nevo, R.; Charuvi, D.; Tsabari, O.; Reich, Z. Composition, architecture and dynamics of the photosynthetic apparatus in higher plants. *The Plant Journal* **2012**, *70*, 157–176.

(195) Boekema, E. J.; van Breemen, J. F.; van Roon, H.; Dekker, J. P. Arrangement of photosystem II supercomplexes in crystalline macrodomains within the thylakoid membrane of green plant chloroplasts. *J Mol Biol* **2000**, *301*, 1123–1133.

(196) Kouril, R.; Wientjes, E.; Bultema, J. B.; Croce, R.; Boekema, E. J. High-light vs. low-light: Effect of light acclimation on photosystem II composition and organization in *Arabidopsis thaliana*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2013**, *1827*, 411–419.

(197) Tietz, S.; Puthiyaveetil, S.; Enlow, H. M.; Yarbrough, R.; Wood, M.; Semchonok, D. A.; Lowry, T.; Li, Z.; Jahns, P.; Boekema, E. J.; Lenhert, S.; Niyogi, K. K.; Kirchhoff, H. Functional Implications of Photosystem II Crystal Formation in Photosynthetic Membranes. *Journal of Biological Chemistry* **2015**, *290*, 14091–14106.

(198) Yadav, K. N. S.; Semchonok, D. A.; Nosek, L.; Kouřil, R.; Fucile, G.; Boekema, E. J.; Eichacker, L. A. Supercomplexes of plant photosystem I with cytochrome b6f, light-harvesting complex II and NDH. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2017**, *1858*, 12–20.

(199) Steinbeck, J.; Ross, I. L.; Rothnagel, R.; Gäbelein, P.; Schulze, S.; Giles, N.; Ali, R.; Drysdale, R.; Sierecki, E.; Gambin, Y.; Stahlberg, H.; Takahashi, Y.; Hippler, M.; Hankamer, B. Structure of a PSI-LHCI-cyt b_6f supercomplex in *Chlamydomonas reinhardtii* promoting cyclic electron flow under anaerobic conditions. *Proceedings of the National Academy of Sciences* **2018**, *115*, 10517–10522.

(200) Kouřil, R.; Strouhal, O.; Nosek, L.; Lenobel, R.; Chamrád, I.; Boekema, E. J.; Sebela, M.; Ilík, P. Structural characterization of a plant photosystem I and NAD(P)H dehydrogenase supercomplex. *The Plant Journal* **2013**, *77*, 568–576.

(201) Shen, L.; Tang, K.; Wang, W.; Wang, C.; Wu, H.; Mao, Z.; An, S.; Chang, S.; Kuang, T.; Shen, J.-R.; Han, G.; Zhang, X. Architecture of the chloroplast PSI-NDH supercomplex in *Hordeum vulgare*. *Nature* **2022**, *601*, 649–654.

(202) Su, X.; Cao, D.; Pan, X.; Shi, L.; Liu, Z.; Dall'Osto, L.; Bassi, R.; Zhang, X.; Li, M. Supramolecular assembly of chloroplast NADH dehydrogenase-like complex with photosystem I from *Arabidopsis thaliana*. *Molecular Plant* **2022**, *15*, 454–467.

(203) Järvi, S.; Suorsa, M.; Paakkarinen, V.; Aro, E.-M. Optimized native gel systems for separation of thylakoid protein complexes: novel super- and mega-complexes. *Biochemical Journal* **2011**, *439*, 207–214.

(204) Yokono, M.; Takabayashi, A.; Akimoto, S.; Tanaka, A. A megacomplex composed of both photosystem reaction centres in higher plants. *Nature Communications* **2015**, *6*, 6675.

(205) Rantala, M.; Tikkanen, M.; Aro, E.-M. Proteomic characterization of hierarchical megacomplex formation in *Arabidopsis* thylakoid membrane. *The Plant Journal* **2017**, *92*, 951–962.

(206) Yokono, M.; Takabayashi, A.; Kishimoto, J.; Fujita, T.; Iwai, M.; Murakami, A.; Akimoto, S.; Tanaka, A. The PSI–PSII Megacomplex in Green Plants. *Plant and Cell Physiology* **2019**, *60*, 1098–1108.

(207) Trissl, H.-W.; Wilhelm, C. Why do thylakoid membranes from higher plants form grana stacks? *Trends in Biochemical Sciences* **1993**, *18*, 415–419.

(208) Anderson, J. M.; Chow, W. S.; De Las Rivas, J. Dynamic flexibility in the structure and function of photosystem II in higher plant thylakoid membranes: the grana enigma. *Photosynthesis Research* **2008**, *98*, 575–587.

(209) Santanoo, S.; Vongcharoen, K.; Banterng, P.; Vorasoot, N.; Jogloy, S.; Roytrakul, S.; Theerakulpisut, P. Canopy Structure and Photosynthetic Performance of Irrigated Cassava Genotypes Growing in Different Seasons in a Tropical Savanna Climate. *Agronomy* **2020**, *10*, 2018.

(210) Shannon, E. E.; Brezonik, P. L. Limnological characteristics of north and central Florida lakes. *Limnology and Oceanography* **1972**, *17*, 97–110.

(211) Porra, R.; Thompson, W.; Kriedemann, P. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1989**, *975*, 384–394.

(212) Oostergetel, G. T.; van Amerongen, H.; Boekema, E. J. The chlorosome: a prototype for efficient light harvesting in photosynthesis. *Photosynthesis Research* **2010**, *104*, 245–255.

(213) Van Amerongen, H.; Croce, R. Light harvesting in photosystem II. *Photosynthesis Research* **2013**, *116*, 251–263.

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

(215) Kühlbrandt, W.; Wang, D. N. Three-dimensional structure of plant light-harvesting complex determined by electron crystallography. *Nature* **1991**, *350*, 130–134.

(216) Standfuss, J.; Terwisscha van Scheltinga, A. C.; Lamborghini, M.; Kühlbrandt, W. Mechanisms of photoprotection and nonphotochemical quenching in pea light-harvesting complex at 2.5 Å resolution. *The EMBO Journal* **2005**, *24*, 919–928.

(217) Croce, R.; van Amerongen, H. Light-harvesting in photosystem I. *Photosynthesis Research* **2013**, *116*, 153–166.

(218) Plumley, F. G.; Schmidt, G. W. Reconstitution of chlorophyll a/b light-harvesting complexes: Xanthophyll-dependent assembly and energy transfer. *Proceedings of the National Academy of Sciences* **1987**, *84*, 146–150.

(219) Ros, F.; Bassi, R.; Paulsen, H. Pigment-binding properties of the recombinant photosystem II subunit CP26 reconstituted in vitro. *European Journal of Biochemistry* **1998**, *253*, 653–658.

(220) Forster, T. Energiewanderung und Fluoreszenz. *Die Naturwissenschaften* **1946**, *33*, 166–175.

(221) Förster, T. Energy migration and fluorescence. *Journal of Biomedical Optics* **2012**, *17*, 011002.

(222) Scholes, G. D.; Mirkovic, T.; Turner, D. B.; Fassioli, F.; Buchleitner, A. Solar light harvesting by energy transfer: from ecology to coherence. *Energy & Environmental Science* **2012**, *5*, 9374–9393.

(223) Wilhelm, C.; Jakob, T. Uphill energy transfer from long-wavelength absorbing chlorophylls to PS II in *Ostreobium* sp. is functional in carbon assimilation. *Photosynthesis Research* **2006**, *87*, 323–329.

(224) Schlodder, E.; Lendzian, F.; Meyer, J.; Cetin, M.; Brecht, M.; Renger, T.; Karapetyan, N. V. Long-wavelength limit of photochemical energy conversion in photosystem I. *Journal of the American Chemical Society* **2014**, *136*, 3904–3918.

(225) Van Amerongen, H.; van Grondelle, R. Understanding the Energy Transfer Function of LHCII, the Major Light-Harvesting Complex of Green Plants. *The Journal of Physical Chemistry B* **2001**, *105*, 604–617.

(226) Schubert, A.; Beenken, W. J.; Stiel, H.; Voigt, B.; Leupold, D.; Lokstein, H. Excitonic Coupling of Chlorophylls in the Plant Light-Harvesting Complex LHC-II. *Biophysical Journal* **2002**, *82*, 1030–1039.

(227) Niedzwiedzki, D. M.; Blankenship, R. E. Singlet and triplet excited state properties of natural chlorophylls and bacteriochlorophylls. *Photosynthesis Research* **2010**, *106*, 227–238.

(228) Peterman, E. J.; Monshouwer, R.; van Stokkum, I. H.; van Grondelle, R.; van Amerongen, H. Ultrafast singlet excitation transfer from carotenoids to chlorophylls via different pathways in light-harvesting complex II of higher plants. *Chemical Physics Letters* **1997**, *264*, 279–284.

(229) Agarwal, R.; Krueger, B. P.; Scholes, G. D.; Yang, M.; Yom, J.; Mets, L.; Fleming, G. R. Ultrafast Energy Transfer in LHC-II Revealed by Three-Pulse Photon Echo Peak Shift Measurements. *The Journal of Physical Chemistry B* **2000**, *104*, 2908–2918.

(230) Croce, R.; Müller, M. G.; Bassi, R.; Holzwarth, A. R. Carotenoid-to-chlorophyll energy transfer in recombinant major light-harvesting complex (LHCII) of higher plants. I. Femtosecond transient absorption measurements. *Biophysical Journal* **2001**, *80*, 901–915.

(231) Melkozernov, A. N.; Barber, J.; Blankenship, R. E. Light harvesting in photosystem I supercomplexes. *Biochemistry* **2006**, *45*, 331–345.

(232) Caffarri, S.; Broess, K.; Croce, R.; van Amerongen, H. Excitation Energy Transfer and Trapping in Higher Plant Photosystem II Complexes with Different Antenna Sizes. *Biophysical Journal* **2011**, *100*, 2094–2103.

(233) Le Quiniou, C.; van Oort, B.; Drop, B.; van Stokkum, I. H. M.; Croce, R. The high efficiency of Photosystem I in the green alga *Chlamydomonas reinhardtii* is maintained after the antenna size is substantially increased by the association of Light Harvesting Complex II. *Journal of Biological Chemistry* **2015**, *290*, 30587–30595.

(234) Zhu, X.-G.; Long, S. P.; Ort, D. R. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Current Opinion in Biotechnology* **2008**, *19*, 153–159.

(235) Fu, Q. A.; Ehleringer, J. R. Modification of paraheliotropic leaf movement in *Phaseolus vulgaris* by photon flux density. *Plant, Cell & Environment* **1991**, *14*, 339–343.

(236) Arena, C.; Vitale, L.; De Santo, A. V. Paraheliotropism in *Robinia pseudoacacia* L.: an efficient strategy to optimise photosynthetic performance under natural environmental conditions. *Plant Biology* **2008**, *10*, 194–201.

(237) Thomas, D. A.; Barber, H. N. Studies on Leaf Characteristics of a Cline of *Eucalyptus urnigera* From Mount Wellington, Tasmania. II. Reflection, Transmission and Absorption of Radiation. *Australian Journal of Botany* **1974**, *22*, 701–707.

(238) Robinson, S. A.; Lovelock, C. E.; Osmond, C. B. Wax as a Mechanism for Protection against Photoinhibition — A Study of *Cotyledon orbiculata*. *Botanica Acta* **1993**, *106*, 307–312.

(239) Morales, F.; Abadía, A.; Abadía, J.; Montserrat, G.; Gil-Pelegrín, E. Trichomes and photosynthetic pigment composition changes: responses of *Quercus ilex subsp. ballota* (Desf.) Samp. and *Quercus coccifera* L. to Mediterranean stress conditions. *Trees* **2002**, *16*, 504–510.

(240) Inoue, Y.; Shibata, K. Comparative examination of terrestrial plant leaves in terms of light-induced absorption changes due to chloroplast rearrangements. *Plant and Cell Physiology* **1974**, *15*, 717–721.

(241) Davis, P. A.; Hangarter, R. P. Chloroplast movement provides photoprotection to plants by redistributing PSII damage within leaves. *Photosynthesis Research* **2012**, *112*, 153–161.

(242) Zubik-Duda, M.; Luchowski, R.; Maksim, M.; Nosalewicz, A.; Zgłobicki, P.; Banaś, A. K.; Grudzinski, W.; Gruszecki, W. I. The photoprotective dilemma of a chloroplast: to avoid high light or to quench the fire? *The Plant Journal* **2023**, *115*, 7–17.

(243) Takahashi, T.; Watanabe, M. Photosynthesis modulates the sign of phototaxis of wild-type *Chlamydomonas reinhardtii*. *FEBS Letters* **1993**, *336*, 516–520.

(244) Kaňa, R.; Kotabová, E.; Šedivá, B.; Kuthanová Trsková, E. Photoprotective strategies in the motile cryptophyte alga *Rhodomonas salina*—role of non-photochemical quenching, ions, photoinhibition, and cell motility. *Folia Microbiologica* **2019**, *64*, 691–703.

(245) Park II, Y.; Chow, W. S.; Anderson, J. M. Antenna Size Dependency of Photoinactivation of Photosystem II in Light-Acclimated Pea Leaves. *Plant Physiology* **1997**, *115*, 151–157.

(246) Ruban, A. V.; Johnson, M. P. Dynamics of higher plant photosystem cross-section associated with state transitions. *Photosynthesis Research* **2009**, *99*, 173–183.

(247) Goldschmidt-Clermont, M.; Bassi, R. Sharing light between two photosystems: mechanism of state transitions. *Curr Opin Plant Biol* **2015**, *25*, 71–78.

(248) Wood, W. H.; Johnson, M. P. Modeling the Role of LHCII-LHCII, PSII-LHCII, and PSI-LHCII Interactions in State Transitions. *Biophysical Journal* **2020**, *119*, 287–299.

(249) Vener, A. V.; Van Kan, P. J. M.; Rich, P. R.; Ohad, I.; Andersson, B. Plastoquinol at the quinol oxidation site of reduced cytochrome bf mediates signal transduction between light and protein phosphorylation: Thylakoid protein kinase deactivation by a single-turnover flash. *Proceedings of the National Academy of Sciences of the United States of America* **1997**, *94*, 1585–1590.

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

(251) Shapiguzov, A.; Ingelsson, B.; Samol, I.; Andres, C.; Kessler, F.; Rochaix, J.-D.; Vener, A. V.; Goldschmidt-Clermont, M. The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in *Arabidopsis*. *Proceedings of the National Academy of Sciences* **2010**, *107*, 4782–4787.

(252) Pribil, M.; Pesaresi, P.; Hertle, A.; Barbato, R.; Leister, D. Role of Plastid Protein Phosphatase TAP38 in LHCII Dephosphorylation and Thylakoid Electron Flow. *PLOS Biology* **2010**, *8*, 1–12.

(253) Murata, N. Control of excitation transfer in photosynthesis I. Light-induced change of chlorophyll a fluoresence in *Porphyridium cruentum*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1969**, *172*, 242–251.

(254) Bonaventura, C.; Myers, J. Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1969**, *189*, 366–383.

(255) Asada, K. The water-water cycle in chloroplasts: Scavenging of Active Oxygens and Dissipation of Excess Photons. *Annual Review of Plant Physiology and Plant Molecular Biology* **1999**, *50*, 601–639.

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

(257) Mastroianni, A. M.; Gilbert, D. T. The illusion of moral decline. *Nature* **2023**, *618*, 782–789.

(258) McAlister, E. D.; Myers, J. The time course of photosynthesis and fluorescence observed simultaneously. *Smithsonian Miscellaneous Collections* **1940**, *99*, 1–37.

(259) Krause, G. H.; Weis, E. Chlorophyll fluorescence as a tool in plant physiology. *Photosynthesis Research* **1984**, *5*, 139–157.

(260) Lichtenthaler, H. The Kautsky effect: 60 years of chlorophyll fluorescence. *Photosynthetica* **1992**, *27*, 45–55.

(261) Sweetser, P.; Todd, C.; Hersh, R. Effect of photosynthesis inhibitors on light re-emission in photosynthesis. *Biochimica et Biophysica Acta* **1961**, *51*, 509–518.

(262) Murata, N.; Nishimura, M.; Takamiya, A. Fluorescence of chlorophyll in photosynthetic systems II. Induction of fluorescence in isolated spinach chloroplasts. *Biochimica et Biophysica Acta (BBA) – Biophysics including Photosynthesis* **1966**, *120*, 23–33.

(263) Horton, P.; Ruban, A. V.; Walters, R. G. Regulation of Light Harvesting in Green Plants (Indication by Nonphotochemical Quenching of Chlorophyll Fluorescence). *Plant Physiology* **1994**, *106*, 415–420.

(264) Ruban, A. V.; Wilson, S. The Mechanism of Non-Photochemical Quenching in Plants: Localization and Driving Forces. *Plant and Cell Physiology* **2020**, *62*, 1063–1072.

(265) Bethmann, S.; Haas, A.-K.; Melzer, M.; Jahns, P. The impact of long-term acclimation to different growth light intensities on the regulation of zeaxanthin epoxidase in different plant species. *Physiologia Plantarum* **2023**, *175*, e13998.

(266) Gilmore, A. M.; Hazlett, T. L.; Govindjee Xanthophyll cycle-dependent quenching of photosystem II chlorophyll a fluorescence: formation of a quenching complex with a short fluorescence lifetime. *Proceedings of the National Academy of Sciences* **1995**, *92*, 2273–2277.

(267) Kasajima, I.; Takahara, K.; Kawai-Yamada, M.; Uchimiya, H. Estimation of the Relative Sizes of Rate Constants for Chlorophyll De-excitation Processes Through Comparison of Inverse Fluorescence Intensities. *Plant and Cell Physiology* **2009**, *50*, 1600–1616.

(268) Ostroumov, E. E.; Khan, Y. R.; Scholes, G. D.; Govindjee In *Non-Photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria*, Demmig-Adams, B., Garab, G., Adams III, W., Govindjee, Eds.; Springer Netherlands: Dordrecht, 2014, pp 97–128.

(269) Demmig-Adams, B.; Koh, S.-C.; Cohu, C. M.; Muller, O.; Stewart, J. J.; Adams, W. W. In *Non-Photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria*, Demmig-Adams, B., Garab, G., Adams III, W., Govindjee, Eds.; Springer Netherlands: Dordrecht, 2014, pp 531–552.

(270) Green, R.; Pichersky, E. Hypothesis for the evolution of three-helix Chl a/b and Chl a/c light-harvesting antenna proteins from two-helix and four-helix ancestors. *Photosynthesis Research* **1994**, *39*, 149–162.

(271) Li, X. P.; Björkman, O.; Shih, C.; Grossman, A. R.; Rosenquist, M.; Jansson, S.; Niyogi, K. K. A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature* **2000**, *403*, 391–395.

(272) Yamamoto, H.; Nakayama, T.; Chichester, C. Studies on the light and dark interconversions of leaf xanthophylls. *Archives of Biochemistry and Biophysics* **1962**, *97*, 168–173.

(273) Demmig, B.; Winter, K.; Krüger, A.; Czygan, F.-C. Photoinhibition and Zeaxanthin Formation in Intact Leaves: A Possible Role of the Xanthophyll Cycle in the Dissipation of Excess Light Energy. *Plant Physiology* **1987**, *84*, 218–224.

(274) Li, X.-P.; Müller-Moulé, P.; Gilmore, A. M.; Niyogi, K. K. PsbS-dependent enhancement of feedback de-excitation protects photosystem II from photoinhibition. *Proceedings of the National Academy of Sciences* **2002**, *99*, 15222–15227.

(275) Fan, M.; Li, M.; Liu, Z.; Cao, P.; Pan, X.; Zhang, H.; Zhao, X.; Zhang, J.; Chang, W. Crystal structures of the PsbS protein essential for photoprotection in plants. *Nature Structural and Molecular Biology* **2015**, *22*, 729–735.

(276) Bergantino, E.; Segalla, A.; Brunetta, A.; Teardo, E.; Rigoni, F.; Giacometti, G. M.; Szabò, I. Light- and pH-dependent structural changes in the PsbS subunit of photosystem II. *Proceedings of the National Academy of Sciences* **2003**, *100*, 15265–15270.

(277) Latowski, D.; Kuczyńska, P.; Strzałka, K. Xanthophyll cycle – a mechanism protecting plants against oxidative stress. *Redox Report* **2011**, *16*, 78–90.

(278) Gray, C.; Wei, T.; Polívka, T.; Daskalakis, V.; Duffy, C. D. P. Trivial Excitation Energy Transfer to Carotenoids Is an Unlikely Mechanism for Non-photochemical Quenching in LHCII. *Frontiers in Plant Science* **2022**, *12*, 797373.

(279) Betterle, N.; Ballottari, M.; Zorzan, S.; de Bianchi, S.; Cazzaniga, S.; Dall'Osto, L.; Morosinotto, T.; Bassi, R. Light-induced Dissociation of an Antenna Hetero-oligomer Is Needed for Non-photochemical Quenching Induction. *Journal of Biological Chemistry* **2009**, *284*, 15255–15266.

(280) Pinnola, A.; Bassi, R. Molecular mechanisms involved in plant photoprotection. *Biochemical Society Transactions* **2018**, *46*, 467–482.

(281) Nicol, L.; Croce, R. The PsbS protein and low pH are necessary and sufficient to induce quenching in the light-harvesting complex of plants LHCII. *Scientific Reports* **2021**, *11*, 7415.

(282) Bassi, R.; Dall'Osto, L. Dissipation of Light Energy Absorbed in Excess: The Molecular Mechanisms. *Annual Review of Plant Biology* **2021**, *72*, 47–76.

(283) Hieber, A. D.; Bugos, R. C.; Yamamoto, H. Y. Plant lipocalins: violaxanthin de-epoxidase and zeaxanthin epoxidase. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2000**, *1482*, 84–91.

(284) Grzyb, J.; Latowski, D.; Strzałka, K. Lipocalins – a family portrait. *Journal of Plant Physiology* **2006**, *163*, 895–915.

(285) Cianci, M.; Rizkallah, P. J.; Olczak, A.; Raftery, J.; Chayen, N. E.; Zagalsky, P. F.; Helliwell, J. R. The molecular basis of the coloration mechanism in lobster shell: β-Crustacyanin at 3.2-Å resolution. *Proceedings of the National Academy of Sciences* **2002**, *99*, 9795–9800.

(286) Eskling, M.; Arvidsson, P.-O.; Åkerlund, H.-E. The xanthophyll cycle, its regulation and components. *Physiologia Plantarum* **1997**, *100*, 806–816.

(287) Arnoux, P.; Morosinotto, T.; Saga, G.; Bassi, R.; Pignol, D. A structural basis for the pH-dependent xanthophyll cycle in *Arabidopsis thaliana*. *Plant Cell* **2009**, *21*, 2036–2044.

(288) Hallin, E.; Guo, K.; Åkerlund, H.-E. Violaxanthin de-epoxidase disulphides and their role in activity and thermal stability. *Photosynthesis Research* **2015**, *124*, 191–198.

(289) Havir, E. A.; Tausta, S.; Peterson, R. B. Purification and properties of violaxanthin de-epoxidase from spinach. *Plant Science* **1997**, *123*, 57–66.

(290) García-Mendoza, E.; Colombo-Pallotta, M. F. The giant kelp *Macrocystis pyrifera* presents a different nonphotochemical quenching control than higher plants. *New Phytologist* **2007**, *173*, 526–536.

(291) Proctor, M. C. F.; Smirnoff, N. Photoprotection in bryophytes: rate and extent of dark relaxation of non-photochemical quenching of chlorophyll fluorescence. *Journal of Bryology* **2015**, *37*, 171–177.

(292) Bilger, W.; Björkman, O.; Thayer, S. S. Light-Induced Spectral Absorbance Changes in Relation to Photosynthesis and the Epoxidation State of Xanthophyll Cycle Components in Cotton Leaves. *Plant Physiology* **1989**, *91*, 542–551.

(293) Jahns, P.; Latowski, D.; Strzalka, K. Mechanism and regulation of the violaxanthin cycle: The role of antenna proteins and membrane lipids. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2009**, *1787*, 3–14.

(294) Kromdijk, J.; Głowacka, K.; Leonelli, L.; Gabilly, S. T.; Iwai, M.; Niyogi, K. K.; Long, S. P. Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science* **2016**, *354*, 857–861.

(295) Murchie, E. H.; Ruban, A. V. Dynamic non-photochemical quenching in plants: from molecular mechanism to productivity. *The Plant Journal* **2020**, *101*, 885–896.

(296) Souza, A. P. D.; Burgess, S. J.; Doran, L.; Hansen, J.; Manukyan, L.; Maryn, N.; Gotarkar, D.; Leonelli, L.; Niyogi, K. K.; Long, S. P. Soybean photosynthesis and crop yield are improved by accelerating recovery from photoprotection. *Science* **2022**, *377*, 851–854.

(297) Zeeman, P. VII. Doublets and triplets in the spectrum produced by external magnetic forces. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science* **1897**, *44*, 55–60.

(298) Krieger-Liszkay, A.; Fufezan, C.; Trebst, A. Singlet oxygen production in photosystem II and related protection mechanism. *Photosynthesis Research* **2008**, *98*, 551–564.

(299) Blankenship, R. E., *Molecular Mechanisms of Photosynthesis*; Wiley & Sons, Limited, John: 2021, p 352.

(300) Krieger-Liszkay, A. Singlet oxygen production in photosynthesis. *Journal of Experimental Botany* **2004**, *56*, 337–346.

(301) Bowers, P. G.; Porter, G. Quantum yields of triplet formation in solutions of chlorophyll. *Proceedings of the Royal Society of London. Series A. Mathematical and Physical Sciences* **1967**, *296*, 435–441.

(302) Tanielian, C.; Wolff, C. Porphyrin-Sensitized Generation of Singlet Molecular Oxygen: Comparison of Steady-State and Time-Resolved Methods. *The Journal of Physical Chemistry* **1995**, *99*, 9825–9830.

(303) Telfer, A. Singlet oxygen production by photosystem II under light stress: mechanism, detection and the protective role of β-carotene. *Plant and Cell Physiology* **2014**, *55*, 1216–1223.

(304) Takahashi, Y.; Hansson, Ö.; Mathis, P.; Satoh, K. Primary radical pair in the Photosystem II reaction centre. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1987**, *893*, 49–59.

(305) Griffiths, M.; Sistrom, W. R.; Cohen-Bazire, G.; Stanier, R. Y. Function of Carotenoids in Photosynthesis. *Nature* **1955**, *176*, 1211–1214.

(306) Van der Vos, R.; Carbonera, D.; Hoff, A. J. Microwave and optical spectroscopy of carotenoid triplets in light-harvesting complex LHC II of spinach by absorbance-detected magnetic resonance. *Applied Magnetic Resonance* **1991**, *2*, 179–202.

(307) Peterman, E.; Dukker, F.; van Grondelle, R.; van Amerongen, H. Chlorophyll a and carotenoid triplet states in light-harvesting complex II of higher plants. *Biophysical Journal* **1995**, *69*, 2670–2678.

(308) Di Valentin, M.; Carbonera, D. The fine tuning of carotenoid–chlorophyll interactions in light-harvesting complexes: an important requisite to guarantee efficient photoprotection via triplet–triplet energy transfer in the complex balance of the energy transfer processes. *Journal of Physics B: Atomic, Molecular and Optical Physics* **2017**, *50*, 162001.

(309) Gillbro, T.; Cogdell, R. J. Carotenoid fluorescence. *Chemical Physics Letters* **1989**, *158*, 312–316.

(310) Mozzo, M.; Dall'Osto, L.; Hienerwadel, R.; Bassi, R.; Croce, R. Photoprotection in the antenna complexes of photosystem II: role of individual xanthophylls in chlorophyll triplet quenching. *Journal of Biological Chemistry* **2008**, *283*, 6184–6192.

(311) Di Valentin, M.; Biasibetti, F.; Ceola, S.; Carbonera, D. Identification of the Sites of Chlorophyll Triplet Quenching in Relation to the Structure of LHC-II from Higher Plants. Evidence from EPR Spectroscopy. *The Journal of Physical Chemistry B* **2009**, *113*, 13071–13078.

(312) Jahns, P.; Holzwarth, A. R. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2012**, *1817*, 182–193.

(313) Carbonera, D.; Giacometti, G.; Agostini, G.; Angerhofer, A.; Aust, V. ODMR of carotenoid and chlorophyll triplets in CP43 and CP47 complexes of spinach. *Chemical Physics Letters* **1992**, *194*, 275–281.

(314) Groot, M. L.; Peterman, E. J.; van Stokkum, I. H.; Dekker, J. P.; van Grondelle, R. Triplet and fluorescing states of the CP47 antenna complex of photosystem II studied as a function of temperature. *Biophysical Journal* **1995**, *68*, 281–290.

(315) Herbstová, M.; **Litvín**, R.; Gardian, Z.; Komenda, J.; Vácha, F. Localization of Pcb antenna complexes in the photosynthetic prokaryote *Prochlorothrix hollandica*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2010**, *1797*, 89–97.

(316) Noguchi, T. Dual Role of Triplet Localization on the Accessory Chlorophyll in the Photosystem II Reaction Center: Photoprotection and Photodamage of the D1 Protein. *Plant and Cell Physiology* **2002**, *43*, 1112–1116.

(317) Telfer, A. What is β–carotene doing in the photosystem II reaction centre? *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **2002**, *357*, 1431–1440.

(318) Nixon, P. J.; Michoux, F.; Yu, J.; Boehm, M.; Komenda, J. Recent advances in understanding the assembly and repair of photosystem II. *Annals of Botany* **2010**, *106*, 1–16.

(319) Tyystjärvi, E. In *International Review of Cell and Molecular Biology*, Jeon, K. W., Ed.; International Review of Cell and Molecular Biology, Vol. 300; Academic Press: 2013, pp 243–303.

(320) Järvi, S.; Suorsa, M.; Aro, E.-M. Photosystem II repair in plant chloroplasts — Regulation, assisting proteins and shared components with photosystem II biogenesis. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2015**, *1847*, 900–909.

(321) Woese, C. R.; Kandler, O.; Wheelis, M. L. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences* **1990**, *87*, 4576–4579.

(322) Castelle, C. J.; Banfield, J. F. Major New Microbial Groups Expand Diversity and Alter our Understanding of the Tree of Life. *Cell* **2018**, *172*, 1181–1197.

(323) Baker, B. J.; De Anda, V.; Seitz, K. W.; Dombrowski, N.; Santoro, A. E.; Lloyd, K. G. Diversity, ecology and evolution of Archaea. *Nature Microbiology* **2020**, *5*, 887–900.

(324) Oesterhelt, D.; Stoeckenius, W. Rhodopsin-like Protein from the Purple Membrane of *Halobacterium halobium*. *Nature New Biology* **1971**, *233*, 149–152.

(325) Ernst, O. P.; Lodowski, D. T.; Elstner, M.; Hegemann, P.; Brown, L. S.; Kandori, H. Microbial and Animal Rhodopsins: Structures, Functions, and Molecular Mechanisms. *Chemical Reviews* **2014**, *114*, 126–163.

(326) Tabita, F. R.; Satagopan, S.; Hanson, T. E.; Kreel, N. E.; Scott, S. S. Distinct form I, II, III, and IV Rubisco proteins from the three kingdoms of life provide clues about Rubisco evolution and structure/function relationships. *Journal of Experimental Botany* **2008**, *59*, 1515–1524.

(327) Kono, T.; Mehrotra, S.; Endo, C.; Kizu, N.; Matusda, M.; Kimura, H.; Mizohata, E.; Inoue, T.; Hasunuma, T.; Yokota, A.; Matsumura, H.; Ashida, H. A RuBisCO-mediated carbon metabolic pathway in methanogenic archaea. *Nature Communications* **2017**, *8*, 14007.

(328) Bryant, D. A.; Frigaard, N.-U. Prokaryotic photosynthesis and phototrophy illuminated. *Trends in Microbiology* **2006**, *14*, 488–496.

(329) Fowler, D. et al. The global nitrogen cycle in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences* **2013**, *368*, 20130164.

(330) Crutzen, P. J. In *Earth system science in the anthropocene*, Eckart, E., Thomas, K., Eds.; Springer: 2006, pp 13–18.

(331) Voss, M.; Bange, H. W.; Dippner, J. W.; Middelburg, J. J.; Montoya, J. P.; Ward, B. The marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences* **2013**, *368*, 20130121.

(332) Fischer, W. W.; Hemp, J.; Johnson, J. E. Evolution of Oxygenic Photosynthesis. *Annual Review of Earth and Planetary Sciences* **2016**, *44*, 647–683.

(333) Young, G. M. Aspects of the Archean-Proterozoic transition: How the great Huronian Glacial Event was initiated by rift-related uplift and terminated at the rift-drift transition during break-up of Lauroscandia. *Earth-Science Reviews* **2019**, *190*, 171–189.

(334) Bekker, A.; Holland, H. D.; Wang, P.-L.; Rumble, D.; Stein, H. J.; Hannah, J. L.; Coetzee, L. L.; Beukes, N. J. Dating the rise of atmospheric oxygen. *Nature* **2004**, *427*, 117–120.

(335) Farquhar, J.; Zerkle, A.; Bekker, A. Geological constraints on the origin of oxygenic photosynthesis. *Photosynthesis Research* **2011**, *107*, 11–36.

(336) Luo, G.; Ono, S.; Beukes, N. J.; Wang, D. T.; Xie, S.; Summons, R. E. Rapid oxygenation of Earth's atmosphere 2.33 billion years ago. *Sci Adv* **2016**, *2*, e1600134.

(337) Allwood, A. C.; Walter, M. R.; Kamber, B. S.; Marshall, C. P.; Burch, I. W. Stromatolite reef from the Early Archaean era of Australia. *Nature* **2006**, *441*, 714–718.

(338) Soo, R. M.; Hemp, J.; Parks, D. H.; Fischer, W. W.; Hugenholtz, P. On the origins of oxygenic photosynthesis and aerobic respiration in Cyanobacteria. *Science* **2017**, *355*, 1436–1440.

(339) Demoulin, C. F.; Lara, Y. J.; Cornet, L.; François, C.; Baurain, D.; Wilmotte, A.; Javaux, E. J. Cyanobacteria evolution: Insight from the fossil record. *Free Radical Biology and Medicine* **2019**, *140*, 206–223.

(340) Sánchez-Baracaldo, P. Origin of marine planktonic cyanobacteria. *Scientific Reports* **2015**, *5*, 17418.

(341) Sánchez-Baracaldo, P.; Ridgwell, A.; Raven, J. A. A Neoproterozoic Transition in the Marine Nitrogen Cycle. *Current Biology* **2014**, *24*, 652–657.

(342) Cardona, T.; Rutherford, A. W. Evolution of Photochemical Reaction Centres: More Twists? *Trends in Plant Science* **2019**, *24*, 1008–1021.

(343) Bryant, D. A.; Costas, A. M. G.; Maresca, J. A.; Chew, A. G. M.; Klatt, C. G.; Bateson, M. M.; Tallon, L. J.; Hostetler, J.; Nelson, W. C.; Heidelberg, J. F.; Ward, D. M. *Candidatus* Chloracidobacterium thermophilum: An Aerobic Phototrophic Acidobacterium. *Science* **2007**, *317*, 523–526.

(344) Zeng, Y.; Feng, F.; Medová, H.; Dean, J.; Koblížek, M. Functional type 2 photosynthetic reaction centers found in the rare bacterial phylum Gemmatimonadetes. *Proceedings of the National Academy of Sciences* **2014**, *111*, 7795–7800.

(345) Ward, L. M.; Cardona, T.; Holland-Moritz, H. Evolutionary Implications of Anoxygenic Phototrophy in the Bacterial Phylum *Candidatus* Eremiobacterota (WPS-2). *Frontiers in Microbiology* **2019**, *10*, 1658.

(346) Urbach, E.; Robertson, D. L.; Chisholm, S. W. Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. *Nature* **1992**, *355*, 267–270.

(347) Takaichi, S.; Mochimaru, M. Carotenoids and carotenogenesis in cyanobacteria: unique ketocarotenoids and carotenoid glycosides. *Cellular and Molecular Life Sciences* **2007**, *64*, 2607.

(348) Graham, J. E.; Lecomte, J. T. J.; Bryant, D. A. Synechoxanthin, an aromatic C40 xanthophyll that is a major carotenoid in the cyanobacterium *Synechococcus* sp. PCC 7002. *J Nat Prod* **2008**, *71*, 1647–1650.

(349) Ting, C. S.; Rocap, G.; King, J.; Chisholm, S. W. Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light-harvesting strategies. *Trends in Microbiology* **2002**, *10*, 134–142.

(350) Chen, H.-Y. S.; Bandyopadhyay, A.; Pakrasi, H. B. Function, regulation and distribution of IsiA, a membrane-bound chlorophyll *a*-antenna protein in cyanobacteria. *Photosynthetica* **2018**, *56*, 322–333.

(351) Miyashita, H.; Ikemoto, H.; Kurano, N.; Adachi, K.; Chihara, M.; Miyachi, S. Chlorophyll d as a major pigment. *Nature* **1996**, *383*, 402–402.

(352) Chen, M.; Schliep, M.; Willows, R. D.; Cai, Z.-L.; Neilan, B. A.; Scheer, H. A red-shifted chlorophyll. *Science* **2010**, *329*, 1318–1319.

(353) Ji, M.; Williams, T. J.; Montgomery, K.; Wong, H. L.; Zaugg, J.; Berengut, J. F.; Bissett, A.; Chuvochina, M.; Hugenholtz, P.; Ferrari, B. C. *Candidatus* Eremiobacterota, a metabolically and phylogenetically diverse terrestrial phylum with acid-tolerant adaptations. *The ISME Journal* **2021**, *15*, 2692–2707.

(354) Pierson, B. K.; Castenholz, R. W. A phototrophic gliding filamentous bacterium of hot springs, *Chloroflexus aurantiacus*, gen. and sp. nov. *Archives of Microbiology* **1974**, *100*, 5–24.

(355) Gest, H.; Favinger, J. L. *Heliobacterium chlorum*, an anoxygenic brownish-green photosynthetic bacterium containing a "new" form of bacteriochlorophyll. *Archives of Microbiology* **1983**, *136*, 11–16.

(356) Cardona, T. Origin of Bacteriochlorophyll *a* and the Early Diversification of Photosynthesis. *PLoS ONE* **2016**, *11*, 1–9.

(357) Saer, R. G.; Blankenship, R. E. Light harvesting in phototrophic bacteria: structure and function. *Biochemical Journal* **2017**, *474*, 2107–2131.

(358) Kobayashi, M.; Oh-oka, H.; Akutsu, S.; Akiyama, M.; Tominaga, K.; Kise, H.; Nishida, F.; Watanabe, T.; Amesz, J.; Koizumi, M.; Ishida, N.; Kano, H. The primary electron acceptor of green sulfur bacteria, bacteriochlorophyll 663, is chlorophyll a esterified with Δ 2,6-phytadienol. *Photosynthesis Research* **2000**, *63*, 269–280.

(359) Gisriel, C.; Sarrou, I.; Ferlez, B.; Golbeck, J. H.; Redding, K. E.; Fromme, R. Structure of a symmetric photosynthetic reaction center–photosystem. *Science* **2017**, *357*, 1021–1025.

(360) He, Z.; Ferlez, B.; Kurashov, V.; Tank, M.; Golbeck, J. H.; Bryant, D. A. Reaction centers of the thermophilic microaerophile, *Chloracidobacterium thermophilum* (*Acidobacteria*) I: biochemical and biophysical characterization. *Photosynthesis Research* **2019**, *142*, 87–103.

(361) Knoll, A. H. The Multiple Origins of Complex Multicellularity. *Annual Review of Earth and Planetary Sciences* **2011**, *39*, 217–239.

(362) Burki, F.; Roger, A. J.; Brown, M. W.; Simpson, A. G. The New Tree of Eukaryotes. *Trends in Ecology & Evolution* **2020**, *35*, 43–55.

(363) Hampl, V.; Hug, L.; Leigh, J. W.; Dacks, J. B.; Lang, B. F.; Simpson, A. G. B.; Roger, A. J. Phylogenomic analyses support the monophyly of Excavata and resolve relationships among eukaryotic "supergroups". *Proc Natl Acad Sci U S A* **2009**, *106*, 3859–3864.

(364) Gomaa, F.; Kosakyan, A.; Heger, T. J.; Corsaro, D.; Mitchell, E. A.; Lara, E. One Alga to Rule them All: Unrelated Mixotrophic Testate Amoebae (Amoebozoa, Rhizaria and Stramenopiles) Share the Same Symbiont (Trebouxiophyceae). *Protist* **2014**, *165*, 161–176.

(365) Jackson, C. J.; Reyes-Prieto, A. The Mitochondrial Genomes of the Glaucophytes *Gloeochaete wittrockiana* and *Cyanoptyche gloeocystis*: Multilocus Phylogenetics Suggests a Monophyletic Archaeplastida. *Genome Biology and Evolution* **2014**, *6*, 2774–2785.

(366) Kim, J. I.; Moore, C. E.; Archibald, J. M.; Bhattacharya, D.; Yi, G.; Yoon, H. S.; Shin, W. Evolutionary Dynamics of Cryptophyte Plastid Genomes. *Genome Biology and Evolution* **2017**, *9*, 1859–1872.

(367) Burki, F.; Kaplan, M.; Tikhonenkov, D. V.; Zlatogursky, V.; Minh, B. Q.; Radaykina, L. V.; Smirnov, A.; Mylnikov, A. P.; Keeling, P. J. Untangling the early diversification of eukaryotes: a phylogenomic study of the evolutionary origins of Centrohelida, Haptophyta and Cryptista. *Proceedings of the Royal Society of London B: Biological Sciences* **2016**, *283*, 20152802.

(368) Eikrem, W.; Medlin, L. K.; Henderiks, J.; Rokitta, S.; Rost, B.; Probert, I.; Throndsen, J.; Edvardsen, B. In *Handbook of the Protists*, Archibald, J. M., Simpson, A. G., Slamovits, C. H., Margulis, L., Melkonian, M., Chapman, D. J., Corliss, J. O., Eds.; Springer International Publishing: Cham, 2016, pp 1–61.

(369) Jaskowska, E.; Butler, C.; Preston, G.; Kelly, S. *Phytomonas*: Trypanosomatids Adapted to Plant Environments. *PLOS Pathogens* **2015**, *11*, 1–17.

(370) Zakryś, B.; Milanowski, R.; Karnkowska, A. In *Euglena: Biochemistry, Cell and Molecular Biology*, Schwartzbach, S. D., Shigeoka, S., Eds.; Springer International Publishing: Cham, 2017, pp 3–17.

(371) Burki, F.; Shalchian-Tabrizi, K.; Minge, M.; Skjæveland, Å.; Nikolaev, S. I.; Jakobsen, K. S.; Pawlowski, J. Phylogenomics Reshuffles the Eukaryotic Supergroups. *PLOS ONE* **2007**, *2*, 1–6.

(372) Grattepanche, J.-D.; Walker, L. M.; Ott, B. M.; Paim Pinto, D. L.; Delwiche, C. F.; Lane, C. E.; Katz, L. A. Microbial Diversity in the Eukaryotic SAR Clade: Illuminating the Darkness Between Morphology and Molecular Data. *BioEssays* **2018**, *40*, 1700198.

(373) Brocks, J. J.; Logan, G. A.; Buick, R.; Summons, R. E. Archean Molecular Fossils and the Early Rise of Eukaryotes. *Science* **1999**, *285*, 1033–1036.

(374) Knoll, A.; Javaux, E.; Hewitt, D.; Cohen, P. Eukaryotic organisms in Proterozoic oceans. *Philosophical Transactions of the Royal Society B: Biological Sciences* **2006**, *361*, 1023–1038.

(375) Rasmussen, B.; Fletcher, I. R.; Brocks, J. J.; Kilburn, M. R. Reassessing the first appearance of eukaryotes and cyanobacteria. *Nature* **2008**, *455*, 1101–1104.

(376) Javaux, E. J.; Knoll, A. H. Micropaleontology of the lower Mesoproterozoic Roper Group, Australia, and implications for early eukaryotic evolution. *Journal of Paleontology* **2017**, *91*, 199–229.

(377) Butterfield, N. J. *Bangiomorpha pubescens* n. gen., n. sp.: implications for the evolution of sex, multicellularity, and the Mesoproterozoic/Neoproterozoic radiation of eukaryotes. *Paleobiology* **2000**, *26*, 386–404.

(378) Gibson, T. M.; Shih, P. M.; Cumming, V. M.; Fischer, W. W.; Crockford, P. W.; Hodgskiss, M. S.; Wörndle, S.; Creaser, R. A.; Rainbird, R. H.; Skulski, T. M.; Halverson, G. P. Precise age of *Bangiomorpha pubescens* dates the origin of eukaryotic photosynthesis. *Geology* **2018**, *46*, 135–138.

(379) Sheath, R. G. In *Freshwater Algae of North America*, Wehr, J. D., Sheath, R. G., Eds.; Aquatic Ecology; Academic Press: Burlington, 2003, pp 197–224.

(380) Manning, W. M.; Strain, H. H. Chlorophyll *d*, a green pigment of red algae. *Journal of Biological Chemistry* **1943**, *151*, 1–19.

(381) Murakami, A.; Miyashita, H.; Iseki, M.; Adachi, K.; Mimuro, M. Chlorophyll *d* in an Epiphytic Cyanobacterium of Red Algae. *Science* **2004**, *303*, 1633–1633.

(382) Larkum, A. W. D.; Kühl, M. Chlorophyll *d*: the puzzle resolved. *Trends Plant Sci* **2005**, *10*, 355–357.

(383) Wolfe, G. R.; Jr., F. X. C.; Grabowski, B.; Gantt, E. Isolation and characterization of Photosystems I and II from the red alga *Porphyridium cruentum*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1994**, *1188*, 357–366.

(384) Jansson, S.; Green, B.; Grossman, A. R.; Hiller, R. A Proposal for Extending the Nomenclature of Light-Harvesting Proteins of the Three Transmembrane Helix Type. *Plant Molecular Biology Reporter* **1999**, *17*, 221–224.

(385) Gardian, Z.; Bumba, L.; Schrofel, A.; Herbstova, M.; Nebesarova, J.; Vacha, F. Organisation of Photosystem I and Photosystem II in red alga *Cyanidium caldarium*: encounter of cyanobacterial and higher plant concepts. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2007**, *1767*, 725–731.

(386) Muñoz-Gómez, S. A.; Mejía-Franco, F. G.; Durnin, K.; Colp, M.; Grisdale, C. J.; Archibald, J. M.; Slamovits, C. H. The New Red Algal Subphylum Proteorhodophytina Comprises the Largest and Most Divergent Plastid Genomes Known. *Current Biology* **2017**, *27*, 1677–1684.e4.

(387) Haniewicz, P.; Abram, M.; Nosek, L.; Kirkpatrick, J.; El-Mohsnawy, E.; Olmos, J. D. J.; Kouřil, R.; Kargul, J. M. Molecular Mechanisms of Photoadaptation of Photosystem I Supercomplex from an Evolutionary Cyanobacterial/Algal Intermediate. *Plant Physiology* **2018**, *176*, 1433–1451.

(388) Thangaraj, B.; Jolley, C. C.; Sarrou, I.; Bultema, J. B.; Greyslak, J.; Whitelegge, J. P.; Lin, S.; Kouřil, R.; Subramanyam, R.; Boekema, E. J.; Fromme, P. Efficient light harvesting in a dark, hot, acidic environment: the structure and function of PSI-LHCI from Galdieria sulphuraria. *Biophysical Journal* **2011**, *100*, 135–143.

(389) Ago, H.; Adachi, H.; Umena, Y.; Tashiro, T.; Kawakami, K.; Kamiya, N.; Tian, L.; Han, G.; Kuang, T.; Liu, Z.; Wang, F.; Zou, H.; Enami, I.; Miyano, M.; Shen, J.-R. Novel features of eukaryotic photosystem II revealed by its crystal structure analysis from a red alga. *J. Biol. Chem.* **2016**, *291*, 5676–5687.

(390) Pi, X.; Tian, L.; Dai, H.-E.; Qin, X.; Cheng, L.; Kuang, T.; Sui, S.-F.; Shen, J.-R. Unique organization of photosystem I–light-harvesting supercomplex revealed by cryo-EM from a red alga. *Proceedings of the National Academy of Sciences* **2018**, *115*, 4423–4428.

(391) Yoon, H. S.; Müller, K. M.; Sheath, R. G.; Ott, F. D.; Bhattacharya, D. Defining the major lineages of red algae (Rhodophyta). *Journal of Phycology* **2006**, *42*, 482–492.

(392) Qiu, H.; Price, D. C.; Yang, E. C.; Yoon, H. S.; Bhattacharya, D. Evidence of ancient genome reduction in red algae (Rhodophyta). *Journal of Phycology* **2015**, *51*, 624–636.

(393) Yang, E. C.; Boo, S. M.; Bhattacharya, D.; Saunders, G. W.; Knoll, A. H.; Fredericq, S.; Graf, L.; Yoon, H. S. Divergence time estimates and the evolution of major lineages in the florideophyte red algae. *Scientific Reports* **2016**, *6*, 21361.

(394) Figueroa-Martinez, F.; Jackson, C.; Reyes-Prieto, A. Plastid Genomes from Diverse Glaucophyte Genera Reveal a Largely Conserved Gene Content and Limited Architectural Diversity. *Genome Biology and Evolution* **2018**, *11*, 174–188.

(395) Jaynes, J. M.; Vernon, L. P. The cyanelle of *Cyanophora paradoxa*: almost a cyanobacterial chloroplast. *Trends in Biochemical Sciences* **1982**, *7*, 22–24.

(396) Steiner, J. M.; Löffelhardt, W. In *Bioenergetic Processes of Cyanobacteria*, Peschek, G. A., Obinger, C., Renger, G., Eds.; Springer Netherlands: 2011, pp 71–87.

(397) Björn, L. O. Peptidoglycan in eukaryotes: Unanswered questions. *Phytochemistry* **2020**, *175*, 112370.

(398) Engelken, J.; Brinkmann, H.; Adamska, I. Taxonomic distribution and origins of the extended LHC (light-harvesting complex) antenna protein superfamily. *BMC Evolutionary Biology* **2010**, *10*, 233.

(399) Leliaert, F.; Smith, D. R.; Moreau, H.; Herron, M. D.; Verbruggen, H.; Delwiche, C. F.; De Clerck, O. Phylogeny and Molecular Evolution of the Green Algae. *Critical Reviews in Plant Sciences* **2012**, *31*, 1–46.

(400) Ruhfel, B. R.; Gitzendanner, M. A.; Soltis, P. S.; Soltis, D. E.; Burleigh, J. G. From algae to angiosperms–inferring the phylogeny of green plants (Viridiplantae) from 360 plastid genomes. *BMC Evolutionary Biology* **2014**, *14*, 23.

(401) Leliaert, F.; Tronholm, A.; Lemieux, C.; Turmel, M.; DePriest, M. S.; Bhattacharya, D.; Karol, K. G.; Fredericq, S.; Zechman, F. W.; Lopez-Bautista, J. M. Chloroplast phylogenomic analyses reveal the deepest-branching lineage of the Chlorophyta, Palmophyllophyceae class. nov. *Scientific Reports* **2016**, *6*, 25367.

(402) Verbruggen, H.; Ashworth, M.; LoDuca, S. T.; Vlaeminck, C.; Cocquyt, E.; Sauvage, T.; Zechman, F. W.; Littler, D. S.; Littler, M. M.; Leliaert, F.; De Clerck, O. A multi-locus time-calibrated phylogeny of the siphonous green algae. *Molecular Phylogenetics and Evolution* **2009**, *50*, 642–653.

(403) Butterfield, N. J. Modes of pre-Ediacaran multicellularity. *Precambrian Research* **2009**, *173*, 201–211.

(404) Brocks, J. J.; Jarrett, A. J. M.; Sirantoine, E.; Hallmann, C.; Hoshino, Y.; Liyanage, T. The rise of algae in Cryogenian oceans and the emergence of animals. *Nature* **2017**, *548*, 578–581.

(405) Nowak, H.; Servais, T.; Monnet, C.; Molyneux, S. G.; Vandenbroucke, T. R. Phytoplankton dynamics from the Cambrian Explosion to the onset of the Great Ordovician Biodiversification Event: A review of Cambrian acritarch diversity. *Earth-Science Reviews* **2015**, *151*, 117–131.

(406) Kenrick, P.; Wellman, C. H.; Schneider, H.; Edgecombe, G. D. A timeline for terrestrialization: consequences for the carbon cycle in the Palaeozoic. *Philosophical Transactions of the Royal Society B: Biological Sciences* **2012**, *367*, 519–536.

(407) Lemieux, C.; Otis, C.; Turmel, M. Six newly sequenced chloroplast genomes from prasinophyte green algae provide insights into the relationships among prasinophyte lineages and the diversity of streamlined genome architecture in picoplanktonic species. *BMC Genomics* **2014**, *15*, 857.

(408) Ricketts, T. The structures of siphonein and siphonaxanthin from *Codium fragile*. *Phytochemistry* **1971**, *10*, 155–160.

(409) Nakayama, K.; Okada, M. Purification and Characterization of Light-Harvesting Chlorophyll a/b-Protein Complexes of Photosystem II from the Green alga, *Bryopsis maxima*. *Plant and Cell Physiology* **1990**, *31*, 253–260.

(410) Staleva-Musto, H.; Kuznetsova, V.; Bína, D.; **Litvín, R.**; Polívka, T. Intramolecular charge-transfer state of carotenoids siphonaxanthin and siphonein: function of non-conjugated acyl-oxy group. *Photosynthesis Research* **2020**, *144*, 127–135.

(411) Lewis, L. A.; Muller-Parker, G. Phylogenetic Placement of "Zoochlorellae" (Chlorophyta), Algal Symbiont of the Temperate Sea Anemone *Anthopleura elegantissima*. *The Biological Bulletin* **2004**, *207*, 87–92.

(412) Hoshina, R.; Imamura, N. Multiple Origins of the Symbioses in *Paramecium bursaria*. *Protist* **2008**, *159*, 53–63.

(413) Summerer, M.; Sonntag, B.; Sommaruga, R. Ciliate-symbiont specificity of freshwater endosymbiotic *Chlorella* (Trebouxiophyceae, Chlorophyta). *Journal of Phycology* **2008**, *44*, 77–84.

(414) Muñoz-Gómez, S. A.; Kreutz, M.; Hess, S. A microbial eukaryote with a unique combination of purple bacteria and green algae as endosymbionts. *Science Advances* **2021**, *7*, eabg4102.

(415) Trémouillaux-Guiller, J.; Rohr, T.; Rohr, R.; Huss, V. A. R. Discovery of an endophytic alga in *Ginkgo biloba*. *American Journal of Botany* **2002**, *89*, 727–733.

(416) Trémouillaux-Guiller, J.; Huss, V. A. R. A cryptic intracellular green alga in *Ginkgo biloba*: ribosomal DNA markers reveal worldwide distribution. *Planta* **2007**, *226*, 553–557.

(417) Kano, R. Emergence of Fungal-Like Organisms: *Prototheca*. *Mycopathologia* **2020**, *185*, 747–754.

(418) Barcytė, D.; Pusztai, M.; Škaloud, P.; Eliáš, M. When you Like Other Algae: *Adglutina synurophila* gen. et sp. nov. (Moewusinia, Chlorophyceae), a Clingy Green Microalga Associated with *Synura* Colonies. *Protist* **2022**, *173*, 125858.

(419) Fang, L.; Leliaert, F.; Zhang, Z.-H.; Penny, D.; Zhong, B.-J. Evolution of the Chlorophyta: Insights from chloroplast phylogenomic analyses. *Journal of Systematics and Evolution* **2017**, *55*, 322–332.

(420) Egeland, E. S.; Guillard, R. R.; Liaaen-Jensen, S. Additional carotenoid prototype representatives and a general chemosystematic evaluation of carotenoids in prasinophyceae (chlorophyta). *Phytochemistry* **1997**, *44*, 1087–1097.

(421) Egeland, E. S.; Eikrem, W.; Throndsen, J.; Wilhelm, C.; Zapata, M.; Liaaen-Jensen, S. Carotenoids from Further Prasinophytes. *Biochemical Systematics and Ecology* **1995**, *23*, 747–755.

(422) Wilhelm, C.; Kolz, S.; Meyer, M.; Schmitt, A.; Zuber, H.; Egeland, E. S.; Liaaen-Jensen, S. Refined carotenoid analysis of the major light-harvesting complex of *Mantoniella squamata*. *Photosynthetica* **1997**, *33*, 161.

(423) Rhiel, E.; Mörschel, E. The atypical chlorophyll a/b/c light-harvesting complex of *Mantoniella squamata*: molecular cloning and sequence analysis. *Molecular and General Genetics* **1993**, *240*, 403–413.

(424) Six, C.; Worden, A. Z.; Rodríguez, F.; Moreau, H.; Partensky, F. New insights into the nature and phylogeny of prasinophyte antenna proteins: *Ostreococcus tauri*, a case study. *Molecular Biology and Evolution* **2005**, *22*, 2217–2230.

(425) Lewin, R. A.; Krienitz, L.; Goericke, R.; Takeda, H.; Hepperle, D. *Picocystis salinarum* gen. et sp. nov. (Chlorophyta) – a new picoplanktonic green alga. *Phycologia* **2000**, *39*, 560–565.

(426) Viprey, M.; Guillou, L.; Ferréol, M.; Vaulot, D. Wide genetic diversity of picoplanktonic green algae (Chloroplastida) in the Mediterranean Sea uncovered by a phylum-biased PCR approach. *Environmental Microbiology* **2008**, *10*, 1804–1822.

(427) Koziol, A. G.; Borza, T.; Ishida, K.-I.; Keeling, P.; Lee, R. W.; Durnford, D. G. Tracing the evolution of the light-harvesting antennae in chlorophyll *a/b*-containing organisms. *Plant Physiology* **2007**, *143*, 1802–1816.

(428) Christenhusz, M. J.; Byng, J. W. The number of known plants species in the world and its annual increase. *Phytotaxa* **2016**, *261*, 201–217.

(429) Busch, A.; Hess, S. A diverse group of underappreciated zygnematophytes deserves in-depth exploration. *Applied Phycology* **2022**, *3*, 306–323.

(430) Gitzendanner, M. A.; Soltis, P. S.; Wong, G. K.-S.; Ruhfel, B. R.; Soltis, D. E. Plastid phylogenomic analysis of green plants: A billion years of evolutionary history. *American Journal of Botany* **2018**, *105*, 291–301.

(431) Puttick, M. N.; Morris, J. L.; Williams, T. A.; Cox, C. J.; Edwards, D.; Kenrick, P.; Pressel, S.; Wellman, C. H.; Schneider, H.; Pisani, D.; Donoghue, P. C. The Interrelationships of Land Plants and the Nature of the Ancestral Embryophyte. *Current Biology* **2018**, *28*, 733–745.e2.

(432) Li, H.-T. et al. Origin of angiosperms and the puzzle of the Jurassic gap. *Nature Plants* **2019**, *5*, 461–470.

(433) Garratt, M. J. New evidence for a Silurian (Ludlow) age for the earliest *Baragwanathia* flora. *Alcheringa: An Australasian Journal of Palaeontology* **1978**, *2*, 217–224.

(434) Edwards, D.; Feehan, J. Records of *Cooksonia*-type sporangia from late Wenlock strata in Ireland. *Nature* **1980**, *287*, 41–42.

(435) Skog, J. E.; Banks, H. P. *Ibyka amphikoma*, gen. et sp. n., a new protoarticulate precursor from the late middle Devonian of New York state. *American Journal of Botany* **1973**, *60*, 366–380.

(436) Beck, C. B. On the Origin of Gymnosperms. *TAXON* **1966**, *15*, 337–339.

(437) Mohr, B. A. R.; Bernardes-de-Oliveira, M. E. C. *Endressinia brasiliana*, a Magnolialean Angiosperm from the Lower Cretaceous Crato Formation (Brazil). *International Journal of Plant Sciences* **2004**, *165*, 1121–1133.

(438) Marin, B.; M. Nowack, E. C.; Melkonian, M. A Plastid in the Making: Evidence for a Second Primary Endosymbiosis. *Protist* **2005**, *156*, 425–432.

(439) Delaye, L.; Valadez-Cano, C.; Pérez-Zamorano, B. How Really Ancient Is *Paulinella chromatophora*? *PLoS Currents: Tree of Life* **2016**, *8*, 28515968[pmid].

(440) Curtis, B. A. et al. Algal genomes reveal evolutionary mosaicism and the fate of nucleomorphs. *Nature* **2012**, *492*, 59–65.

(441) Glazer, A. N.; Wedemayer, G. J. Cryptomonad biliproteins — an evolutionary perspective. *Photosynthesis Research* **1995**, *46*, 93–105.

(442) Hoef-Emden, K. Molecular Phylogeny Of Phycocyanin-containing Cryptophytes: Evolution Of Biliproteins And Geographical Distribution. *Journal of Phycology* **2008**, *44*, 985–993.

(443) Bathke, L.; Rhiel, E.; Krumbein, W. E.; Marquardt, J. Biochemical and Immunochemical Investigations on the Light-Harvesting System of the Cryptophyte *Rhodomonas* sp.: Evidence for a Photosystem I Specific Antenna. *Plant Biology* **1999**, *1*, 516–523.

(444) Kereïche, S.; Kouřil, R.; Oostergetel, G. T.; Fusetti, F.; Boekema, E. J.; Doust, A. B.; van der Weij-de Wit, C. D.; Dekker, J. P. Association of chlorophyll a/c2 complexes to photosystem I and photosystem II in the cryptophyte *Rhodomonas* CS24. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2008**, *1777*, 1122–1128.

(445) Kuthanová Trsková, E.; Bína, D.; Santabarbara, S.; Sobotka, R.; Kaňa, R.; Belgio, E. Isolation and characterization of CAC antenna proteins and photosystem I supercomplex from the cryptophytic alga *Rhodomonas salina*. *Physiologia Plantarum* **2019**, *166*, 309–319.

(446) Šebelík, V.; West, R.; Trsková, E. K.; Kaňa, R.; Polívka, T. Energy transfer pathways in the CAC light-harvesting complex of *Rhodomonas salina*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2020**, *1861*, 148280.

(447) Doust, A. B.; Wilk, K. E.; Curmi, P. M.; Scholes, G. D. The photophysics of cryptophyte light-harvesting. *Journal of Photochemistry and Photobiology A: Chemistry* **2006**, *184*, 1–17.

(448) Collini, E.; Wong, C. Y.; Wilk, K. E.; Curmi, P. M. G.; Brumer, P.; Scholes, G. D. Coherently wired light-harvesting in photosynthetic marine algae at ambient temperature. *Nature* **2010**, *463*, 644–647.

(449) Kaňa, R.; Eva, K.; Sobotka, R.; Prášil, O. Non-Photochemical Quenching in Cryptophyte Alga *Rhodomonas salina* Is Located in Chlorophyll a/c Antennae. *PLOS ONE* **2012**, *7*, 1–12.

(450) Kieselbach, T.; Cheregi, O.; Green, B. R.; Funk, C. Proteomic analysis of the phycobiliprotein antenna of the cryptophyte alga *Guillardia theta* cultured under different light intensities. *Photosynthesis Research* **2018**, *135*, 149–163.

(451) Kamikawa, R.; Tanifuji, G.; Kawachi, M.; Miyashita, H.; Hashimoto, T.; Inagaki, Y. Plastid genome-based phylogeny pinpointed the origin of the green-colored plastid in the dinoflagellate *Lepidodinium chlorophorum*. *Genome Biol Evol* **2015**, *7*, 1133–1140.

(452) Jackson, C.; Knoll, A. H.; Chan, C. X.; Verbruggen, H. Plastid phylogenomics with broad taxon sampling further elucidates the distinct evolutionary origins and timing of secondary green plastids. *Scientific Reports* **2018**, *8*, 1523.

(453) Casper-Lindley, C.; Björkman, O. Fluorescence quenching in four unicellular algae with different light-harvesting and xanthophyll-cycle pigments. *Photosynthesis Research* **1998**, *56*, 277–289.

(454) Nagao, R.; Yokono, M.; Kato, K.-H.; Ueno, Y.; Shen, J.-R.; Akimoto, S. High-light modification of excitation-energy-relaxation processes in the green flagellate *Euglena gracilis*. *Photosynthesis Research* **2021**, *149*, 303–311.

(455) Derelle, R.; López-García, P.; Timpano, H.; Moreira, D. A phylogenomic framework to study the diversity and evolution of stramenopiles (=heterokonts). *Molecular Biology and Evolution* **2016**, *33*, 2890–2898.

(456) Thakur, R.; Shiratori, T.; Ishida, K.-i. Taxon-rich Multigene Phylogenetic Analyses Resolve the Phylogenetic Relationship Among Deep-branching Stramenopiles. *Protist* **2019**, *170*, 125682.

(457) Yubuki, N.; Galindo, L. J.; Reboul, G.; López-García, P.; Brown, M. W.; Pollet, N.; Moreira, D. Ancient Adaptive Lateral Gene Transfers in the Symbiotic *Opalina–Blastocystis* Stramenopile Lineage. *Molecular Biology and Evolution* **2020**, *37*, 651–659.

(458) Purkinje, J.; Valentin, G., *De Phenomeno generali et Fundamentali in Membranis cum externis tum internis Animalium Plurimorum et Superiorum et Infertorium Ordinum Obvii*; Schulz Wratislaviae: Wratislaviae, 1835.

(459) Stensvold, C. R.; Clark, C. G. Current status of *Blastocystis*: A personal view. *Parasitology International* **2016**, *65*, 763–771.

(460) Haas, B. J. et al. Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature* **2009**, *461*, 393–398.

(461) Yoon, H. S.; Hackett, J. D.; Pinto, G.; Bhattacharya, D. The single, ancient origin of chromist plastids. *Proc Natl Acad Sci USA* **2002**, *99*, 15507–15512.

(462) Burki, F. In *Secondary Endosymbioses*, Hirakawa, Y., Ed.; Advances in Botanical Research, Vol. 84; Academic Press: 2017, pp 1–30.

(463) Yang, E. C.; Boo, G. H.; Kim, H. J.; Cho, S. M.; Boo, S. M.; Andersen, R. A.; Yoon, H. S. Supermatrix data highlight the phylogenetic relationships of photosynthetic stramenopiles. *Protist* **2012**, *163*, 217–231.

(464) Wetherbee, R.; Jackson, C. J.; Repetti, S. I.; Clementson, L. A.; Costa, J. F.; van de Meene, A.; Crawford, S.; Verbruggen, H. The golden paradox – a new heterokont lineage with chloroplasts surrounded by two membranes. *Journal of Phycology* **2019**, *55*, 257–278.

(465) Barcytė, D.; Eikrem, W.; Engesmo, A.; Seoane, S.; Wohlmann, J.; Horák, A.; Yurchenko, T.; Eliáš, M. *Olisthodiscus* represents a new class of Ochrophyta. *Journal of Phycology* **2021**, *57*, 1094–1118.

(466) Mann, D. G. The species concept in diatoms. *Phycologia* **1999**, *38*, 437–495.

(467) Bhattacharya, D.; Yoon, H. S.; Hackett, J. D. Photosynthetic eukaryotes unite: endosymbiosis connects the dots. *BioEssays* **2004**, *26*, 50–60.

(468) Yoon, H.; Andersen, R.; Boo, S.; Bhattacharya, D. In *Encyclopedia of Microbiology (Third Edition)*, Schaechter, M., Ed., Third Edition; Academic Press: Oxford, 2009, pp 721–731.

(469) Mann, D. G.; Vanormelingen, P. An Inordinate Fondness? The Number, Distributions, and Origins of Diatom Species. *Journal of Eukaryotic Microbiology* **2013**, *60*, 414–420.

(470) Hanschen, E. R.; Starkenburg, S. R. The state of algal genome quality and diversity. *Algal Research* **2020**, *50*, 101968.

(471) Kröger, N.; Poulsen, N. Diatoms–From Cell Wall Biogenesis to Nanotechnology. *Annual Review of Genetics* **2008**, *42*, 83–107.

(472) Zahradník, J.; Jirásek, J.; Zahradník, J.; Sivek, M. Development of the diatomite production, reserves and its processing in the Czech Republic in 1999–2018. *Gospodarka Surowcami Mineralnymi* **2019**, *35*, 31–48.

(473) Sun, G.; Dilcher, D. L.; Zheng, S.; Zhou, Z. In Search of the First Flower: A Jurassic Angiosperm, *Archaefructus*, from Northeast China. *Science* **1998**, *282*, 1692–1695.

(474) Dos Reis, M.; Inoue, J.; Hasegawa, M.; Asher, R. J.; Donoghue, P. C. J.; Yang, Z. Phylogenomic datasets provide both precision and accuracy in estimating the timescale of placental mammal phylogeny. *Proceedings of the Royal Society B: Biological Sciences* **2012**, *279*, 3491–3500.

(475) Mcdonald, A. T.; Espilez, E.; Mampel, L.; Kirkland, J. I.; Alcala, L. An unusual new basal iguanodont (Dinosauria: Ornithopoda) from the Lower Cretaceous of Teruel, Spain. *Zootaxa* **2012**, *3595*, 61–76.

(476) Sims, P. A.; Mann, D. G.; Medlin, L. K. Evolution of the diatoms: insights from fossil, biological and molecular data. *Phycologia* **2006**, *45*, 361–402.

(477) Harwood, D. M.; Nikolaev, V. A.; Winter, D. M. Cretaceous Records of Diatom Evolution, Radiation, and Expansion. *The Paleontological Society Papers* **2007**, *13*, 33–59.

(478) Kotrc, B.; Knoll, A. H. A morphospace of planktonic marine diatoms. I. Two views of disparity through time. *Paleobiology* **2015**, *41*, 45–67.

(479) Medlin, L. A timescale for diatom evolution based on four molecular markers: reassessment of ghost lineages and major steps defining diatom evolution. *Vie et Milieu Life and Environment* **2016**, *65*, 219–238.

(480) Damsté, J. S. S. et al. The rise of the rhizosolenid diatoms. *Science* **2004**, *304*, 584–587.

(481) Falkowski, P. G.; Katz, M. E.; Knoll, A. H.; Quigg, A.; Raven, J. A.; Schofield, O.; Taylor, F. J. R. The evolution of modern eukaryotic phytoplankton. *Science* **2004**, *305*, 354–360.

(482) Tréguer, P.; Nelson, D. M.; Bennekom, A. J. V.; DeMaster, D. J.; Leynaert, A.; Quéguiner, B. The Silica Balance in the World Ocean: A Reestimate. *Science* **1995**, *268*, 375–379.

(483) Nymark, M.; Sharma, A. K.; Sparstad, T.; Bones, A. M.; Winge, P. A CRISPR/Cas9 system adapted for gene editing in marine algae. *Scientific Reports* **2016**, *6*, 24951.

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

(485) Di Valentin, M.; Meneghin, E.; Orian, L.; Polimeno, A.; Büchel, C.; Salvadori, E.; Kay, C. W. M.; Carbonera, D. Triplet-triplet energy transfer in fucoxanthin-chlorophyll protein from diatom *Cyclotella meneghiniana*: insights into the structure of the complex. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2013**, *1827*, 1226–1234.

(486) Ramanan, C.; Berera, R.; Gundermann, K.; van Stokkum, I.; Büchel, C.; van Grondelle, R. Exploring the mechanism(s) of energy dissipation in the light harvesting complex of the photosynthetic algae *Cyclotella meneghiniana*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2014**, *1837*, 1507–1513.

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

(488) Xu, C.; Pi, X.; Huang, Y.; Han, G.; Chen, X.; Qin, X.; Huang, G.; Zhao, S.; Yang, Y.; Kuang, T.; Wang, W.; Sui, S.-F.; Shen, J.-R. Structural basis for energy transfer in a huge diatom PSI-FCPI supercomplex. *Nature Communications* **2020**, *11*, 5081.

(489) Hu, Q.; Sommerfeld, M.; Jarvis, E.; Ghirardi, M.; Posewitz, M.; Seibert, M.; Darzins, A. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *The Plant Journal* **2008**, *54*, 621–639.

(490) Mata, T. M.; Martins, A. A.; Caetano, N. S. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews* **2010**, *14*, 217–232.

(491) Nitsche, H. The Structure of Vaucheriaxanthin. *Zeitschrift für Naturforschung C* **1973**, *28*, 641–645.

(492) Owens, T. G.; Gallagher, J. C.; Alberte, R. S. Photosynthetic Light-Harvesting Function of Violaxanthin in *Nannochloropsis* spp. (Eustigmatophyceae). *Journal of Phycology* **1987**, *23*, 79–85.

(493) Figueroa, F. L.; Jiménez, C.; Lubián, L. M.; Montero, O.; Lebert, M.; Häder, D.-P. Effects of high irradiance and temperature on photosynthesis and photoinhibition in *Nannochloropsis gaditana* Lubián (Eustigmatophyceae). *Journal of Plant Physiology* **1997**, *151*, 6–15.

(494) Andreoli, C.; Bresciani, E.; Moro, I.; Scarabel, L.; La Rocca, N.; Dalla Valle, L.; Ghion, F. A Survey on a Persistent Greenish Bloom in the Comacchio Lagoons (Ferrara, Italy). *Botanica Marina* **1999**, *42*, 467–479.

(495) Sukenik, A.; Livne, A.; Neori, A.; Yacobi, Y. Z.; Katcoff, D. Purification and Characterization of a Light-Harvesting Chlorophyll-Protein Complex from the Marine Eustigmatophyte *Nannochloropsis* sp. *Plant and Cell Physiology* **1992**, *33*, 1041–1048.

(496) Alboresi, A.; Le Quiniou, C.; Yadav, S. K. N.; Scholz, M.; Meneghesso, A.; Gerotto, C.; Simionato, D.; Hippler, M.; Boekema, E. J.; Croce, R.; Morosinotto, T. Conservation of core complex subunits shaped the structure and function of photosystem I in the secondary endosymbiont alga *Nannochloropsis gaditana*. *New Phytologist* **2016**, *213*, 714–726.

(497) Chukhutsina, V.; Fristedt, R.; Morosinotto, T.; Croce, R. Photoprotection strategies of the alga *Nannochloropsis gaditana*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2017**, *1858*, 544–552.

(498) Røkke, G.; Melø, T. B.; Hohmann-Marriott, M. F. The plastoquinone pool of *Nannochloropsis oceanica* is not completely reduced during bright light pulses. *PLOS ONE* **2017**, *12*, 1–14.

(499) Yokono, M.; Umetani, I.; Takabayashi, A.; Akimoto, S.; Tanaka, A. Regulation of excitation energy in *Nannochloropsis* photosystem II. *Photosynthesis Research* **2018**, *139*, 155–161.

(500) Steneck, R. S.; Graham, M. H.; Bourque, B. J.; Corbett, D.; Erlandson, J. M.; Estes, J. A.; Tegner, M. J. Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation* **2002**, *29*, 436–459.

(501) Wang, M.; Hu, C.; Barnes, B. B.; Mitchum, G.; Lapointe, B.; Montoya, J. P. The great Atlantic *Sargassum* belt. *Science* **2019**, *365*, 83–87.

(502) Apt, K. E.; Clendennen, S. K.; Powers, D. A.; Grossman, A. R. The gene family encoding the fucoxanthin chlorophyll proteins from the brown alga *Macrocystis pyrifera*. *Molecular and General Genetics* **1995**, *246*, 455–464.

(503) Cock, J. M. et al. The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature* **2010**, *465*, 617–621.

(504) Mikami, K.; Hosokawa, M. Biosynthetic Pathway and Health Benefits of Fucoxanthin, an Algae-Specific Xanthophyll in Brown Seaweeds. *International Journal of Molecular Sciences* **2013**, *14*, 13763–13781.

(505) Andersen, R. A.; de Peer, Y. V.; Potter, D.; Sexton, J. P.; Kawachi, M.; LaJeunesse, T. Phylogenetic Analysis of the SSU rRNA from Members of the Chrysophyceae. *Protist* **1999**, *150*, 71–84.

(506) Andersen, R. A. In *Unravelling the algae the past, present, and future of algal systematics*, Brodie, J., Lewis, J., Eds.; CRC Press: 2007.

(507) Kirkham, A. R.; Lepère, C.; Jardillier, L. E.; Not, F.; Bouman, H.; Mead, A.; Scanlan, D. J. A global perspective on marine photosynthetic picoeukaryote community structure. *The ISME Journal* **2013**, *7*, 922–936.

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

(509) Ševčíková, T.; Horák, A.; Klimeš, V.; Zbránková, V.; Demir-Hilton, E.; Sudek, S.; Jenkins, J.; Schmutz, J.; Přibyl, P.; Fousek, J.; Vlček, Č.; Lang, B. F.; Oborník, M.; Worden, A. Z.; Eliáš, M. Updating algal evolutionary relationships through plastid genome sequencing: did alveolate plastids emerge through endosymbiosis of an ochrophyte? *Scientific Reports* **2015**, *5*, 10134.

(510) Ott, D. W.; Oldham-Ott, C. K.; Rybalka, N.; Friedl, T. In *Freshwater Algae of North America (Second Edition)*, Wehr, J. D., Sheath, R. G., Kociolek, J. P., Eds., Second Edition; Aquatic Ecology; Academic Press: Boston, 2015, pp 485–536.

(511) Wilhelm, C.; Büchel, C.; Rousseau, B. The molecular organization of chlorophyll-protein complexes in the Xanthophycean alga *Pleurochloris meiringensis*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1988**, *934*, 220–226.

(512) Büchel, C.; Wilhelm, C. Isolation and characterization of a photosystem I-associated antenna (LHC I) and a photosystem I-core complex from the chlorophyll c-containing alga *Pleurochloris meiringensis* (Xanthophyceae). *Journal of Photochemistry and Photobiology B: Biology* **1993**, *20*, 87–93.

(513) Gardian, Z.; Tichý, J.; Vácha, F. Structure of PSI, PSII and antennae complexes from yellow-green alga *Xanthonema debile*. *Photosynthesis Research* **2011**, *108*, 25–32.

(514) Durchan, M.; Tichý, J.; **Litvín**, R.; Šlouf, V.; Gardian, Z.; Hříbek, P.; Vácha, F.; Polívka, T. Role of carotenoids in light-harvesting processes in an antenna protein from the chromophyte *Xanthonema debile*. *Journal of Physical Chemistry B* **2012**, *116*, 8880–8889.

(515) Streckaite, S.; Gardian, Z.; Li, F.; Pascal, A. A.; **Litvín**, R.; Robert, B.; Llansola-Portoles, M. J. Pigment configuration in the light-harvesting protein of the xanthophyte alga *Xanthonema debile*. *Photosynthesis Research* **2018**, *138*, 139–148.

(516) Khoroshyy, P.; Bína, D.; Gardian, Z.; **Litvín**, R.; Alster, J.; Pšenčík, J. Quenching of chlorophyll triplet states by carotenoids in algal light-harvesting complexes related to fucoxanthin-chlorophyll protein. *Photosynthesis Research* **2018**, *135*, 213–225.

(517) Mujer, C. V.; Andrews, D. L.; Manhart, J. R.; Pierce, S. K.; Rumpho, M. E. Chloroplast genes are expressed during intracellular symbiotic association of *Vaucheria litorea* plastids with the sea slug *Elysia chlorotica*. *Proceedings of the National Academy of Sciences* **1996**, *93*, 12333–12338.

(518) Soule, K. M.; Rumpho, M. E. Light-regulated photosynthetic gene expression and phosphoribulokinase enzyme activity in the heterokont alga *Vaucheria litorea* (Xanthophyceae) and its symbiotic molluskan partner *Elysia chlorotica* (Gastropoda). *Journal of Phycology* **2012**, *48*, 373–383.

(519) Cruz, S.; Cartaxana, P.; Newcomer, R.; Dionísio, G.; Calado, R.; Serôdio, J.; Pelletreau, K. N.; Rumpho, M. E. Photoprotection in sequestered plastids of sea slugs and respective algal sources. *Scientific Reports* **2015**, *5*, 7904.

(520) Cruz, S.; Cartaxana, P. Kleptoplasty: Getting away with stolen chloroplasts. *PLOS Biology* **2022**, *20*, 1–14.

(521) McCartney, K. A review of past and recent research on Cretaceous silicoflagellates. *Phytotaxa* **2013**, *127*, 190.

(522) Foissner, W.; Chao, A.; Katz, L. A. In *Protist Diversity and Geographical Distribution*, Foissner, W., Hawksworth, D. L., Eds.; Springer Netherlands: Dordrecht, 2009, pp 111–129.

(523) Stoecker, D.; Johnson, M.; deVargas, C.; Not, F. Acquired phototrophy in aquatic protists. *Aquatic Microbial Ecology* **2009**, *57*, 279–310.

(524) Hines, H. N.; Onsbring, H.; Ettema, T. J.; Esteban, G. F. Molecular Investigation of the Ciliate *Spirostomum semivirescens*, with First Transcriptome and New Geographical Records. *Protist* **2018**, *169*, 875–886.

(525) Johnson, M.; Stoecker, D. Role of feeding in growth and photophysiology of *Myrionecta rubra*. *Aquatic Microbial Ecology* **2005**, *39*, 303–312.

(526) Johnson, M. D.; Tengs, T.; Oldach, D.; Stoecker, D. K. Sequestration, performance, and functional control of cryptophyte plastids in the ciliate *Myrionecta rubra* (Ciliophora). *Journal of Phycology* **2006**, *42*, 1235–1246.

(527) Johnson, M. D.; Oldach, D.; Delwiche, C. F.; Stoecker, D. K. Retention of transcriptionally active cryptophyte nuclei by the ciliate *Myrionecta rubra*. *Nature* **2007**, *445*, 426–428.

(528) Morrison, D. A. Evolution of the Apicomplexa: where are we now? *Trends in Parasitology* **2009**, *25*, 375–382.

(529) Janouškovec, J.; Paskerova, G. G.; Miroliubova, T. S.; Mikhailov, K. V.; Birley, T.; Aleoshin, V. V.; Simdyanov, T. G. Apicomplexan-like parasites are polyphyletic and widely but selectively dependent on cryptic plastid organelles. *eLife* **2019**, *8*, ed. by McCutcheon, J.; Weigel, D.; Howe, C.; McFadden, G., e49662.

(530) McFadden, G. I.; Reith, M. E.; Munholland, J.; Lang-Unnasch, N. Plastid in human parasites. *Nature* **1996**, *381*, 482.

(531) Kwong, W. K.; del Campo, J.; Mathur, V.; Vermeij, M. J. A.; Keeling, P. J. A widespread coral-infecting apicomplexan with chlorophyll biosynthesis genes. *Nature* **2019**, *568*, 103–107.

(532) Moore, R. B.; Oborník, M.; Janouškovec, J.; Chrudimský, T.; Vancová, M.; Green, D. H.; Wright, S. W.; Davies, N. W.; Bolch, C. J. S.; Heimann, K.; Šlapeta, J.; Hoegh-Guldberg, O.; Logsdon, J. M.; Carter, D. A. A photosynthetic alveolate closely related to apicomplexan parasites. *Nature* **2008**, *451*, 959–963.

(533) Janouskovec, J.; Horák, A.; Oborník, M.; Lukes, J.; Keeling, P. J. A common red algal origin of the apicomplexan, dinoflagellate, and heterokont plastids. *Proc Natl Acad Sci U S A* **2010**, *107*, 10949–10954.

(534) Oborník, M.; Modrý, D.; Lukeš, M.; Cernotíková-Stříbrná, E.; Cihlář, J.; Tesařová, M.; Kotabová, E.; Vancová, M.; Prášil, O.; Lukeš, J. Morphology, ultrastructure and life cycle of *Vitrella brassicaformis* n. sp., n. gen., a novel chromerid from the Great Barrier Reef. *Protist* **2012**, *163*, 306–323.

(535) Woo, Y. H. et al. Chromerid genomes reveal the evolutionary path from photosynthetic algae to obligate intracellular parasites. *Elife* **2015**, *4*, e06974.

(536) Tichý, J.; Gardian, Z.; Bína, D.; Koník, P.; **Litvín**, R.; Herbstová, M.; Pain, A.; Vácha, F. Light harvesting complexes of *Chromera velia*, photosynthetic relative of apicomplexan parasites. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2013**, *1827*, 723–729.

(537) Durchan, M.; Keşan, G.; Slouf, V.; Fuciman, M.; Staleva, H.; Tichý, J.; **Litvín**, R.; Bína, D.; Vácha, F.; Polívka, T. Highly efficient energy transfer from a carbonyl carotenoid to chlorophyll a in the main light harvesting complex of *Chromera velia*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2014**, *1837*, 1748–1755.

(538) Keşan, G.; Durchan, M.; Tichy, J.; Minofar, B.; Kuznetsova, V.; Fuciman, M.; Slouf, V.; Parlak, C.; Polivka, T. Different Response of Carbonyl Carotenoids to Solvent Proticity Helps to Estimate Structure of the Unknown Carotenoid From *Chromera velia*. *Journal of Physical Chemistry B* **2015**, *119*, 12653–12663.

(539) Llansola-Portoles, M. J.; Uragami, C.; Pascal, A. A.; Bína, D.; **Litvín**, R.; Robert, B. Pigment structure in the FCP-like light-harvesting complex from *Chromera velia*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2016**, *1857*, 1759–1765.

(540) Taylor, F. J. R.; Hoppenrath, M.; Saldarriaga, J. F. Dinoflagellate diversity and distribution. *Biodiversity and Conservation* **2008**, *17*, 407–418.

(541) LaJeunesse, T. C.; Lambert, G.; Andersen, R. A.; Coffroth, M. A.; Galbraith, D. W. *Symbiodinium* (Pyrrhophyta) genome sizes (dna content) are smallest among dinoflagellates. *Journal of Phycology* **2005**, *41*, 880–886.

(542) Bogus, K.; Mertens, K. N.; Lauwaert, J.; Harding, I. C.; Vrielinck, H.; Zonneveld, K. A. F.; Versteegh, G. J. M. Differences in the chemical composition of organic-walled dinoflagellate resting cysts from phototrophic and heterotrophic dinoflagellates. *Journal of Phycology* **2014**, *50*, 254–266.

(543) Penaud, A.; Hardy, W.; Lambert, C.; Marret, F.; Masure, E.; Servais, T.; Siano, R.; Wary, M.; Mertens, K. N. Dinoflagellate fossils: Geological and biological applications. *Revue de Micropaléontologie* **2018**, *61*, 235–254.

(544) Hackett, J. D.; Anderson, D. M.; Erdner, D. L.; Bhattacharya, D. Dinoflagellates: a remarkable evolutionary experiment. *American Journal of Botany* **2004**, *91*, 1523–1534.

(545) Berdalet, E.; Fleming, L. E.; Gowen, R.; Davidson, K.; Hess, P.; Backer, L. C.; Moore, S. K.; Hoagland, P.; Enevoldsen, H. Marine harmful algal blooms, human health and wellbeing: challenges and opportunities in the 21st century. *Journal of the Marine Biological Association of the United Kingdom* **2016**, *96*, 61–91.

(546) Gong, W.; Browne, J.; Hall, N.; Schruth, D.; Paerl, H.; Marchetti, A. Molecular insights into a dinoflagellate bloom. *The ISME Journal* **2017**, *11*, 439–452.

(547) Haddock, S. H.; Moline, M. A.; Case, J. F. Bioluminescence in the Sea. *Annual Review of Marine Science* **2010**, *2*, 443–493.

(548) Fensome, R. A.; MacRae, R. A.; Moldowan, J. M.; Taylor, F. J. R.; Williams, G. L. The early Mesozoic radiation of dinoflagellates. *Paleobiology* **1996**, *22*, 329–338.

(549) Kiessling, W. Geologic and Biologic Controls on the Evolution of Reefs. *Annual Review of Ecology, Evolution, and Systematics* **2009**, *40*, 173–192.

(550) Chlupáč, I. Devonský útes u Koněprus. *Vesmír* **1994**, *73*, 618.

(551) Janoušek, V.; Hladil, J.; Frýda, J.; Slavik, L. In *Journal of Conference Abstracts*, 2000; Vol. 5, p 552.

(552) Grantham, P.; Wakefield, L. Variations in the sterane carbon number distributions of marine source rock derived crude oils through geological time. *Organic Geochemistry* **1988**, *12*, 61–73.

(553) Aboglila, S.; Grice, K.; Trinajstic, K.; Snape, C.; Williford, K. The significance of 24-norcholestanes, 4-methylsteranes and dinosteranes in oils and source-rocks from East Sirte Basin (Libya). *Applied Geochemistry* **2011**, *26*, 1694–1705.

(554) Araújo, B. Q.; de Almeida Azevedo, D. Uncommon steranes in Brazilian marginal crude oils: Dinoflagellate molecular fossils in the Sergipe-Alagoas Basin, Brazil. *Organic Geochemistry* **2016**, *99*, 38–52.

(555) Abdullah, F.; Kinghorn, R. A preliminary evaluation of lower and middle Cretaceous source rocks in Kuwait. *Journal of Petroleum Geology* **1996**, *19*, 461–480.

(556) Summons, R. E.; Volkman, J. K.; Boreham, C. J. Dinosterane and other steroidal hydrocarbons of dinoflagellate origin in sediments and petroleum. *Geochimica et Cosmochimica Acta* **1987**, *51*, 3075–3082.

(557) Suzuki, N.; Oba, M. In *Marine Protists*, Ohtsuka, S., Suzaki, T., Horiguchi, T., Suzuki, N., Not, F., Eds.; Springer Japan: 2015, pp 359–394.

(558) Erb, T. J.; Zarzycki, J. A short history of RubisCO: the rise and fall (?) of Nature's predominant CO_2 fixing enzyme. *Current Opinion in Biotechnology* **2018**, *49*, 100–107.

(559) Rickaby, R. E.; Eason Hubbard, M. Upper ocean oxygenation, evolution of RuBisCO and the Phanerozoic succession of phytoplankton. *Free Radical Biology and Medicine* **2019**, *140*, 295–304.

(560) Johansen, J.; Svec, W.; Liaaen-Jensen, S.; Haxo, F. Carotenoids of the dinophyceae. *Phytochemistry* **1974**, *13*, 2261–2271.

(561) Jeffrey, S. W.; Sielicki, M.; Haxo, F. T. Chloroplast pigment patterns in dinoflagellates. *Journal of Phycology* **1975**, *11*, 374–384.

(562) Demers, S.; Roy, S.; Gagnon, R.; Vignault, C. Rapid light-induced changes in cell fluorescence and in xanthophyll-cycle pigments of *Alexandrium excavatum* (Dinophyceae) and *Thalassiosira pseudonana* (Bacillariophyceae): a photo-protection mechanism. *Marine Ecology Progress Series* **1991**, *76*, 185–193.

(563) Haidak, D. J.; Mathews, C. K.; Sweeney, B. M. Pigment Protein Complex from *Gonyaulax*. *Science* **1966**, *152*, 212–213.

(564) Hofmann, E.; Wrench, P. M.; Sharples, F. P.; Hiller, R. G.; Welte, W.; Diederichs, K. Structural basis of light harvesting by carotenoids: peridinin-chlorophyll-protein from *Amphidinium carterae*. *Science* **1996**, *272*, 1788–1791.

(565) Boldt, L.; Yellowlees, D.; Leggat, W. Hyperdiversity of genes encoding integral light-harvesting proteins in the dinoflagellate *Symbiodinium* sp. *PLoS One* **2012**, *7*, e47456.

(566) Niedzwiedzki, D. M.; Jiang, J.; Lo, C. S.; Blankenship, R. E. Spectroscopic properties of the Chlorophyll a—Chlorophyll c 2—Peridinin-Protein-Complex (acpPC) from the coral symbiotic dinoflagellate *Symbiodinium*. *Photosynthesis Research* **2014**, *120*, 125–139.

(567) Elbrächter, M.; Schnepf, E. *Gymnodinium chlorophorum*, a new, green, bloom-forming dinoflagellate (Gymnodiniales, Dinophyceae) with a vestigial prasinophyte endosymbiont. *Phycologia* **1996**, *35*, 381–393.

(568) Green-colored Plastids in the Dinoflagellate Genus *Lepidodinium* are of Core Chlorophyte Origin. *Protist* **2011**, *162*, 268–276.

(569) Yamada, N.; Sym, S. D.; Horiguchi, T. Identification of Highly Divergent Diatom-Derived Chloroplasts in Dinoflagellates, Including a Description of *Durinskia kwazulunatalensis* sp. nov. (Peridiniales, Dinophyceae). *Molecular Biology and Evolution* **2017**, *34*, 1335–1351.

(570) Kretschmann, J.; Žerdoner Čalasan, A.; Gottschling, M. Molecular phylogenetics of dinophytes harboring diatoms as endosymbionts (Kryptoperidiniaceae, Peridiniales), with evolutionary interpretations and a focus on the identity of *Durinskia oculata* from Prague. *Molecular Phylogenetics and Evolution* **2018**, *118*, 392–402.

(571) Mann, D. G.; Yamada, N.; Bolton, J. J.; Witkowski, A.; Trobajo, R. *Nitzschia captiva* sp. nov. (Bacillariophyta), the essential prey diatom of the kleptoplastic dinoflagellate *Durinskia capensis*, compared with *N. agnita*, *N. kuetzingioides* and other species. *Phycologia* **2023**, *62*, 136–151.

(572) Tengs, T.; Dahlberg, O. J.; Shalchian-Tabrizi, K.; Klaveness, D.; Rudi, K.; Delwiche, C. F.; Jakobsen, K. S. Phylogenetic Analyses Indicate that the 19'Hexanoyloxy-fucoxanthin-Containing Dinoflagellates Have Tertiary Plastids of Haptophyte Origin. *Molecular Biology and Evolution* **2000**, *17*, 718–729.

(573) Yoon, H. S.; Hackett, J. D.; Van Dolah, F. M.; Nosenko, T.; Lidie, K. L.; Bhattacharya, D. Tertiary Endosymbiosis Driven Genome Evolution in Dinoflagellate Algae. *Molecular Biology and Evolution* **2005**, *22*, 1299–1308.

(574) Nosenko, T.; Lidie, K. L.; Van Dolah, F. M.; Lindquist, E.; Cheng, J.-F.; of Energy–Joint Genome Institute, U. D.; Bhattacharya, D. Chimeric Plastid Proteome in the Florida "Red Tide" Dinoflagellate *Karenia brevis*. *Molecular Biology and Evolution* **2006**, *23*, 2026–2038.

(575) Takishita, K.; Kolke, K.; Maruyama, T.; Ogata, T. Molecular Evidence for Plastid Robbery (Kleptoplastidy) in *Dinophysis*, a Dinoflagellate causing Diarrhetic Shellfish Poisoning. *Protist* **2002**, *153*, 293–302.

(576) Janson, S. Molecular evidence that plastids in the toxin-producing dinoflagellate genus *Dinophysis* originate from the free-living cryptophyte *Teleaulax amphioxeia*. *Environmental Microbiology* **2004**, *6*, 1102–1106.

(577) Park, M. G.; Kim, S.; Kim, H. S.; Myung, G.; Kang, Y. G.; Yih, W. First successful culture of the marine dinoflagellate *Dinophysis acuminata*. *Aquatic Microbial Ecology* **2006**, *45*, 101–106.

(578) Cavalier-Smith, T.; Chao, E. E.; Lewis, R. Multigene phylogeny and cell evolution of chromist infrakingdom Rhizaria: contrasting cell organisation of sister phyla Cercozoa and Retaria. *Protoplasma* **2018**, *255*, 1517–1574.

(579) Dennett, M. R.; Caron, D. A.; Michaels, A. F.; Gallager, S. M.; Davis, C. S. Video plankton recorder reveals high abundances of colonial Radiolaria in surface waters of the central North Pacific. *Journal of Plankton Research* **2002**, *24*, 797–805.

(580) Biard, T.; Stemmann, L.; Picheral, M.; Mayot, N.; Vandromme, P.; Hauss, H.; Gorsky, G.; Guidi, L.; Kiko, R.; Not, F. In situ imaging reveals the biomass of giant protists in the global ocean. *Nature* **2016**, *532*, 504–507.

(581) Biard, T. Diversity and ecology of Radiolaria in modern oceans. *Environmental Microbiology* **2022**, *24*, 2179–2200.

(582) Purton, L. M. A.; Brasier, M. D. Giant protist *Nummulites* and its Eocene environment: Life span and habitat insights from δ18O and δ13C data from *Nummulites* and *Venericardia*, Hampshire basin, UK. *Geology* **1999**, *27*, 711–714.

(583) Boukhary, M.; Hussein, A.; Kamal, D. Precursors of the *Nummulites gizehensis* and *N. partschi* group from Egypt. *Journal of African Earth Sciences* **2010**, *56*, 43–54.

(584) Izart, A.; Briand, C.; Vaslet, D.; Vachard, D.; Coquel, R.; Maslo, A. Stratigraphy and sequence stratigraphy of the Moscovian in the Donets basin. *Tectonophysics* **1996**, *268*, 189–209.

(585) Davydov, V. Warm water benthic foraminifera document the Pennsylvanian–Permian warming and cooling events — The record from the Western Pangea tropical shelves. *Palaeogeography, Palaeoclimatology, Palaeoecology* **2014**, *414*, 284–295.

(586) Hayward, B. W.; Coze, F. L.; Vandepitte, L.; Vanhoorne, B. Foraminifera in the World Register of Marine Species (Worms) Taxonomic Database. *Journal of Foraminiferal Research* **2020**, *50*, 291–300.

(587) Langer, M. R. Assessing the Contribution of Foraminiferan Protists to Global Ocean Carbonate Production. *Journal of Eukaryotic Microbiology* **2008**, *55*, 163–169.

(588) McIlroy, D.; Green, O. R.; Brasier, M. D. Palaeobiology and evolution of the earliest agglutinated Foraminifera: *Platysolenites*, *Spirosolenites* and related forms. *Lethaia* **2001**, *34*, 13–29.

(589) Pawlowski, J.; Holzmann, M.; Berney, C.; Fahrni, J.; Gooday, A. J.; Cedhagen, T.; Habura, A.; Bowser, S. S. The evolution of early Foraminifera. *Proceedings of the National Academy of Sciences* **2003**, *100*, 11494–11498.

(590) Maletz, J. The identification of putative Lower Cambrian Radiolaria. *Revue de Micropaléontologie* **2017**, *60*, 233–240.

(591) Decelle, J.; Veronesi, G.; LeKieffre, C.; Gallet, B.; Chevalier, F.; Stryhanyuk, H.; Marro, S.; Ravanel, S.; Tucoulou, R.; Schieber, N.; Finazzi, G.; Schwab, Y.; Musat, N. Subcellular architecture and metabolic connection in the planktonic photosymbiosis between *Collodaria* (radiolarians) and their microalgae. *Environmental Microbiology* **2021**, *23*, 6569–6586.

(592) Yoon, H. S.; Nakayama, T.; Reyes-Prieto, A.; Andersen, R. A.; Boo, S. M.; Ishida, K.-i.; Bhattacharya, D. A single origin of the photosynthetic organelle in different *Paulinella* lineages. *BMC Evolutionary Biology* **2009**, *9*, 98.

(593) Gabr, A.; Grossman, A. R.; Bhattacharya, D. *Paulinella*, a model for understanding plastid primary endosymbiosis. *Journal of Phycology* **2020**, *56*, 837–843.

(594) Gabr, A.; Zournas, A.; Stephens, T. G.; Dismukes, G. C.; Bhattacharya, D. Evidence for a robust photosystem II in the photosynthetic amoeba *Paulinella*. *New Phytologist* **2022**, *234*, 934–945.

(595) Archibald, J. M.; Rogers, M. B.; Toop, M.; Ishida, K.-i.; Keeling, P. J. Lateral gene transfer and the evolution of plastid-targeted proteins in the secondary plastid-containing alga *Bigelowiella natans*. *Proceedings of the National Academy of Sciences* **2003**, *100*, 7678–7683.

(596) Hopkins, J. F.; Spencer, D. F.; Laboissiere, S.; Neilson, J. A.; Eveleigh, R. J.; Durnford, D. G.; Gray, M. W.; Archibald, J. M. Proteomics Reveals Plastid- and Periplastid-Targeted Proteins in the Chlorarachniophyte Alga *Bigelowiella natans*. *Genome Biology and Evolution* **2012**, *4*, 1391–1406.

(597) Neilson, J. A.; Rangsrikitphoti, P.; Durnford, D. G. Evolution and regulation of *Bigelowiella natans* light-harvesting antenna system. *Journal of Plant Physiology* **2017**, *217*, 68–76.

(598) Ishida, K.-I.; Hara, Y. Taxonomic studies on the Chlorarachniophyta. I. *Chlorarachnion globosum* sp. nov. *Phycologia* **1994**, *33*, 351–358.

(599) Rogers, M. B.; Gilson, P. R.; Su, V.; McFadden, G. I.; Keeling, P. J. The Complete Chloroplast Genome of the Chlorarachniophyte *Bigelowiella natans*: Evidence for Independent Origins of Chlorarachniophyte and Euglenid Secondary Endosymbionts. *Molecular Biology and Evolution* **2006**, *24*, 54–62.

(600) Ponce-Toledo, R. I.; Moreira, D.; López-García, P.; Deschamps, P. Secondary Plastids of Euglenids and Chlorarachniophytes Function with a Mix of Genes of Red and Green Algal Ancestry. *Molecular Biology and Evolution* **2018**, *35*, 2198–2204.

(601) Gardin, S.; Krystyn, L.; Richoz, S.; Bartolini, A.; Galbrun, B. Where and when the earliest coccolithophores? *Lethaia* **2012**, *45*, 507–523.

(602) Suchéras-Marx, B.; Mattioli, E.; Allemand, P.; Giraud, F.; Pittet, B.; Plancq, J.; Escarguel, G. The colonization of the oceans by calcifying pelagic algae. *Biogeosciences* **2019**, *16*, 2501–2510.

(603) Wignall, P. B.; Atkinson, J. W. A two-phase end-Triassic mass extinction. *Earth-Science Reviews* **2020**, *208*, 103282.

(604) Rigo, M. et al. The Late Triassic Extinction at the Norian/Rhaetian boundary: Biotic evidence and geochemical signature. *Earth-Science Reviews* **2020**, *204*, 103180.

(605) Holligan, P. M.; Viollier, M.; Harbour, D. S.; Camus, P.; Champagne-Philippe, M. Satellite and ship studies of coccolithophore production along a continental shelf edge. *Nature* **1983**, *304*, 339–342.

(606) Brown, C. W.; Yoder, J. A. Coccolithophorid blooms in the global ocean. *Journal of Geophysical Research: Oceans* **1994**, *99*, 7467–7482.

(607) Jeffrey, S. W.; Anderson, J. M. *Emiliania huxleyi* (Haptophyta) holds promising insights for photosynthesis. *Journal of Phycology* **2000**, *36*, 449–452.

(608) Hancock, J. M. The petrology of the Chalk. *Proceedings of the Geologists' Association* **1975**, *86*, 499–535.

(609) Mai, H. Paleocene coccoliths and coccospheres in deposits of the Maastrichtian stage at the 'type locality' and type area in SE Limburg, The Netherlands. *Marine Micropaleontology* **1999**, *36*, 1–12.

(610) Alvarez, L. W.; Alvarez, W.; Asaro, F.; Michel, H. V. Extraterrestrial Cause for the Cretaceous-Tertiary Extinction. *Science* **1980**, *208*, 1095–1108.

(611) Childs, F.; Reed, P.; Andersen, K., *Geology of the Dan field and the Danish North Sea*; I kommission hos CA Reitzels Forlag: 1975.

(612) Hatton, I. R. Geometry of allochthonous Chalk Group members, Central Trough, North Sea. *Marine and Petroleum Geology* **1986**, *3*, 79–98.

(613) Broecker, W.; Clark, E. Ratio of coccolith CaCO3 to foraminifera CaCO3 in late Holocene deep sea sediments. *Paleoceanography* **2009**, *24*, PA3205.

(614) Read, B. A. et al. Pan genome of the phytoplankton *Emiliania* underpins its global distribution. *Nature* **2013**, *499*, 209–213.

(615) Zapata, M.; Jeffrey, S. W.; Wright, S. W.; Rodríguez, F.; Garrido, J. L.; Clementson, L. Photosynthetic pigments in 37 species (65 strains) of Haptophyta: implications for oceanography and chemotaxonomy. *Marine Ecology Progress Series* **2004**, *270*, 83–102.

(616) Hoffman, G. E.; Sanchez Puerta, M. V.; Delwiche, C. F. Evolution of light-harvesting complex proteins from Chl c-containing algae. *BMC Evolutionary Biology* **2011**, *11*, 101.

(617) Dorrell, R. G.; Gile, G.; McCallum, G.; Méheust, R.; Bapteste, E. P.; Klinger, C. M.; Brillet-Guéguen, L.; Freeman, K. D.; Richter, D. J.; Bowler, C. Chimeric origins of ochrophytes and haptophytes revealed through an ancient plastid proteome. *eLife* **2017**, *6*, ed. by Bhattacharya, D., e23717.

(618) Staleva-Musto, H.; Kuznetsova, V.; West, R. G.; Kesan, G.; Minofar, B.; Fuciman, M.; Bína, D.; **Litvín**, R.; Polívka, T. Non-Conjugated Acyloxy Group Deactivates the Intramolecular Charge Transfer State in the Carotenoid Fucoxanthin. *The Journal of Physical Chemistry B* **2018**, *122*, PMID: 29469573, 2922–2930.

(619) Staleva-Musto, H.; West, R.; Trathnigg, M.; Bína, D.; **Litvín**, R.; Polívka, T. Carotenoid-chlorophyll energy transfer in the fucoxanthin-chlorophyll complex binding a fucoxanthin acyloxy derivative. *Faraday Discussions* **2019**, *216*, 460–475.

(620) Agostini, A.; Bína, D.; Carbonera, D.; **Litvín**, R. Conservation of triplet-triplet energy transfer photoprotective pathways in fucoxanthin chlorophyll-binding proteins across algal lineages. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2023**, *1864*, 148935.

(621) Funk, C.; Vermaas, W. A Cyanobacterial Gene Family Coding for Single-Helix Proteins Resembling Part of the Light-Harvesting Proteins from Higher Plants. *Biochemistry* **1999**, *38*, 9397–9404.

(622) Komenda, J.; Sobotka, R. Cyanobacterial high-light-inducible proteins - protectors of chlorophyll-protein synthesis and assembly. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2016**, *1857*, 288–295.

(623) Psencik, J.; Hey, D.; Grimm, B.; Lokstein, H. Photoprotection of Photosynthetic Pigments in Plant One-Helix Protein 1/2 Heterodimers. *The Journal of Physical Chemistry Letters* **2020**, *11*, 9387–9392.

(624) Mork-Jansson, A. E.; Eichacker, L. A. Characterization of chlorophyll binding to LIL3. *PLOS ONE* **2018**, *13*, 1–14.

(625) Skotnicová, P.; Staleva-Musto, H.; Kuznetsova, V.; Bína, D.; Konert, M. M.; Lu, S.; Polívka, T.; Sobotka, R. Plant LHC-like proteins show robust folding and static non-photochemical quenching. *Nature Communications* **2021**, *12*, 6890.

(626) Beck, J.; Lohscheider, J. N.; Albert, S.; Andersson, U.; Mendgen, K. W.; Rojas-Stütz, M. C.; Adamska, I.; Funck, D. Small One-Helix Proteins Are Essential for Photosynthesis in Arabidopsis. *Frontiers in Plant Science* **2017**, *8*, 7.

(627) Sturm, S.; Engelken, J.; Gruber, A.; Vugrinec, S.; Kroth, P. G.; Adamska, I.; Lavaud, J. A novel type of light-harvesting antenna protein of red algal origin in algae with secondary plastids. *BMC Evolutionary Biology* **2013**, *13*, 159.

(628) Fawley, M. W.; Grossman, A. R. Polypeptides of a Light-Harvesting Complex of the Diatom *Phaeodactylum tricornutum* Are Synthesized in the Cytoplasm of the Cell as Precursors. *Plant Physiology* **1986**, *81*, 149–155.

(629) Gagné, G.; Guertin, M. The early genetic response to light in the green unicellular alga *Chlamydomonas eugametos* grown under light/dark cycles involves genes that represent direct responses to light and photosynthesis. *Plant Molecular Biology* **1992**, *18*, 429–445.

(630) Zhu, S.-H.; Green, B. R. In *Photosynthesis. Energy from the Sun*, ed. by Allen, J. F.; Gantt, E.; Golbeck, J. H.; Osmond, B., Springer Netherlands: Dordrecht, 2008, pp 261–264.

(631) Peers, G.; Truong, T. B.; Ostendorf, E.; Busch, A.; Elrad, D.; Grossman, A. R.; Hippler, M.; Niyogi, K. K. An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. *Nature* **2009**, *462*, 518–521.

(632) Büchel, C. Evolution and function of light harvesting proteins. *Journal of Plant Physiology* **2015**, *172*, 62–75.

(633) Kumazawa, M.; Nishide, H.; Nagao, R.; Inoue-Kashino, N.; Shen, J.-R.; Nakano, T.; Uchiyama, I.; Kashino, Y.; Ifuku, K. Molecular phylogeny of fucoxanthin-chlorophyll a/c proteins from *Chaetoceros gracilis* and Lhcq/Lhcf diversity. *Physiologia Plantarum* **2022**, *174*, e13598.

(634) Sukenik, A.; Livne, A.; Apt, K. E.; Grossman, A. R. Characterization of a gene encoding the light-harvesting violaxanthin-chlorophyll protein of *Nannochloropsis* sp. (Eustigmatophyceae). *Journal of Phycology* **2000**, *36*, 563–570.

(635) Hiller, R. G.; Wrench, P. M.; Gooley, A. P.; Shoebridge, G.; Breton, J. The major intrinsic light-harvesting protein of *Amphidinium*: characterization and relation to other light-harvesting proteins. *Photochemistry and Photobiology* **1993**, *57*, 125–131.

(636) Büchel, C. Fucoxanthin-chlorophyll proteins in diatoms: 18 and 19 kDa subunits assemble into different oligomeric states. *Biochemistry* **2003**, *42*, 13027–13034.

(637) Joshi-Deo, J.; Schmidt, M.; Gruber, A.; Weisheit, W.; Mittag, M.; Kroth, P. G.; Büchel, C. Characterization of a trimeric light-harvesting complex in the diatom *Phaeodactylum tricornutum* built of FcpA and FcpE proteins. *Journal of Experimental Botany* **2010**, *61*, 3079–3087.

(638) Grouneva, I.; Rokka, A.; Aro, E.-M. The thylakoid membrane proteome of two marine diatoms outlines both diatom-specific and species-specific features of the photosynthetic machinery. *Journal of Proteome Research* **2011**, *10*, 5338–5353.

(639) Nagao, R.; Takahashi, S.; Suzuki, T.; Dohmae, N.; Nakazato, K.; Tomo, T. Comparison of oligomeric states and polypeptide compositions of fucoxanthin chlorophyll a/c-binding protein complexes among various diatom species. *Photosynthesis Research* **2013**, *117*, 281–288.

(640) Gundermann, K.; Büchel, C. The fluorescence yield of the trimeric fucoxanthin-chlorophyll-protein FCPa in the diatom *Cyclotella meneghiniana* is dependent on the amount of bound diatoxanthin. *Photosynthesis Research* **2008**, *95*, 229–235.

(641) Elnour, H. M.; Dietzel, L.; Ramanan, C.; Büchel, C.; van Grondelle, R.; Krüger, T. P. Energy dissipation mechanisms in the FCPb light-harvesting complex of the diatom *Cyclotella meneghiniana*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2018**, *1859*, 1151–1160.

(642) Röding, A.; Büchel, C.; Sandmann, G., *Strukturelle und funktionelle Analyse von Lichtsammelkomplexen in Nicotiana tabacum und Cyclotella meneghiniana*; Univ.-Bibliothek Frankfurt am Main: 2013.

(643) Röding, A.; Boekema, E.; Büchel, C. The structure of FCPb, a light-harvesting complex in the diatom *Cyclotella meneghiniana*. *Photosynthesis Research* **2018**, 203–211.

(644) Pi, X.; Zhao, S.; Wang, W.; Liu, D.; Xu, C.; Han, G.; Kuang, T.; Sui, S.-F.; Shen, J.-R. The pigment-protein network of a diatom photosystem II-light-harvesting antenna supercomplex. *Science* **2019**, *365*, eaax4406.

(645) Arshad, R.; Calvaruso, C.; Boekema, E. J.; Büchel, C.; Kouřil, R. Revealing the architecture of the photosynthetic apparatus in the diatom *Thalassiosira pseudonana*. *Plant Physiology* **2021**, *186*, 2124–2136.

(646) Nagao, R.; Kato, K.; Kumazawa, M.; Ifuku, K.; Yokono, M.; Suzuki, T.; Dohmae, N.; Akita, F.; Akimoto, S.; Miyazaki, N.; Shen, J.-R. Structural basis for different types of hetero-tetrameric light-harvesting complexes in a diatom PSII-FCPII supercomplex. *Nature Communications* **2022**, *13*, 1764.

(647) Medlin, L. K.; Kaczmarska, I. Evolution of the diatoms: V. Morphological and cytological support for the major clades and a taxonomic revision. *Phycologia* **2004**, *43*, 245–270.

(648) Kooistra, W. H.; Gersonde, R.; Medlin, L. K.; Mann, D. G. In *Evolution of Primary Producers in the Sea*, Falkowski, P. G., Knoll, A. H., Eds.; Academic Press: Burlington, 2007, pp 207–249.

(649) Keşan, G.; **Litvín**, R.; Bína, D.; Durchan, M.; Šlouf, V.; Polívka, T. Efficient light-harvesting using non-carbonyl carotenoids: Energy transfer dynamics in the VCP complex from *Nannochloropsis oceanica*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2016**, *1857*, 370–379.

(650) Nagao, R.; Kato, K.; Ifuku, K.; Suzuki, T.; Kumazawa, M.; Uchiyama, I.; Kashino, Y.; Dohmae, N.; Akimoto, S.; Shen, J.-R.; Miyazaki, N.; Akita, F. Structural basis for assembly and function of a diatom photosystem I-light-harvesting supercomplex. *Nature Communications* **2020**, *11*, 2481.

(651) De Luca, D.; Kooistra, W. H.; Sarno, D.; Gaonkar, C. C.; Piredda, R. Global distribution and diversity of *Chaetoceros* (Bacillariophyta, Mediophyceae): integration of classical and novel strategies. *PeerJ* **2019**, *7*, e7410.

(652) Ikeda, Y.; Yamagishi, A.; Komura, M.; Suzuki, T.; Dohmae, N.; Shibata, Y.; Itoh, S.; Koike, H.; Satoh, K. Two types of fucoxanthin-chlorophyll-binding proteins I tightly bound to the photosystem I core complex in marine centric diatoms. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2013**, *1827*, 529–539.

(653) Sanfilippo, J. E.; Garczarek, L.; Partensky, F.; Kehoe, D. M. Chromatic Acclimation in Cyanobacteria: A Diverse and Widespread Process for Optimizing Photosynthesis. *Annual Review of Microbiology* **2019**, *73*, 407–433.

(654) Schiller, H.; Senger, H.; Miyashita, H.; Miyachi, S.; Dau, H. Light-harvesting in *Acaryochloris marina*–spectroscopic characterization of a chlorophyll *d*-dominated photosynthetic antenna system. *FEBS Lett* **1997**, *410*, 433–436.

(655) Chen, M.; Li, Y.; Birch, D.; Willows, R. D. A cyanobacterium that contains chlorophyll *f*–a red-absorbing photopigment. *FEBS Lett* **2012**, *586*, 3249–3254.

(656) Brown, J. S. Fluorometric evidence for the participation of chlorophyll *a*-695 in System 2 of photosynthesis. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1967**, *143*, 391–398.

(657) Fujita, Y.; Ohki, K. On the 710 nm fluorescence emitted by the diatom *Phaeodactylum tricornutum* at room temperature. *Plant Cell Physiol* **2004**, *45*, 392–397.

(658) Kotabová, E.; Jarešová, J.; Kaňa, R.; Sobotka, R.; Bína, D.; Prášil, O. Novel type of red-shifted chlorophyll *a* antenna complex from *Chromera velia*. I. Physiological relevance and functional connection to Photosystems. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2014**, *1837*, 734–743.

(659) Bína, D.; Gardian, Z.; Herbstová, M.; Kotabová, E.; Koník, P.; **Litvín**, R.; Prášil, O.; Tichý, J.; Vácha, F. Novel type of red-shifted chlorophyll a antenna complex from *Chromera velia* II. Biochemistry and spectroscopy. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2014**, *1837*, 802–810.

(660) Wang, L.; Xie, X.; Gu, W.; Zheng, Z.; Chen, M.; Wang, G. LHCF15 facilitates the absorption of longer wavelength light and promotes growth of *Phaeodactylum tricornutum* under red light. *Algal Research* **2023**, *75*, 103249.

(661) Wientjes, E.; Roest, G.; Croce, R. From red to blue to far-red in Lhca4: How does the protein modulate the spectral properties of the pigments? *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2012**, *1817*, 711–717.

(662) Pyszniak, A. M.; Gibbs, S. P. Immunocytochemical localization of photosystem I and the fucoxanthin-chlorophyll *a/c* light-harvesting complex in the diatom *Phaeodactylum tricornutum*. *Protoplasma* **1992**, *166*, 208–217.

(663) Flori, S.; Jouneau, P.-H.; Bailleul, B.; Gallet, B.; Estrozi, L. F.; Moriscot, C.; Bastien, O.; Eicke, S.; Schober, A.; Bártulos, C. R., et al. Plastid thylakoid architecture optimizes photosynthesis in diatoms. *Nature Communications* **2017**, *8*, 15885.

(664) Wietrzynski, W.; Schaffer, M.; Tegunov, D.; Albert, S.; Kanazawa, A.; Plitzko, J. M.; Baumeister, W.; Engel, B. D. Charting the native architecture of *Chlamydomonas* thylakoid membranes with single-molecule precision. *eLife* **2020**, *9*, e53740.

(665) De Martino, A.; Meichenin, A.; Shi, J.; Pan, K.; Bowler, C. Genetic and phenotypic characterization of *Phaeodactylum tricornutum* (Bacillariophyceae) accessions. *Journal of Phycology* **2007**, *43*, 992–1009.

(666) Fork, D.; Larkum, A. Light harvesting in the green alga *Ostreobium* sp., a coral symbiont adapted to extreme shade. *Marine Biology* **1989**, *103*, 381–385.

(667) Koehne, B.; Elli, G.; Jennings, R. C.; Wilhelm, C.; Trissl, H. Spectroscopic and molecular characterization of a long wavelength absorbing antenna of *Ostreobium* sp. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1999**, *1412*, 94–107.

(668) Chrystal, J.; Larkum, A. Preservation of long-wavelength fluorescence in the isolated thylakoids of two phytoplanktonic algae at 77 K. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1988**, *932*, 189–194.

(669) Wolf, B. M.; Niedzwiedzki, D. M.; Magdaong, N. C. M.; Roth, R.; Goodenough, U.; Blankenship, R. E. Characterization of a newly isolated freshwater Eustigmatophyte alga capable of utilizing far-red light as its sole light source. *Photosynthesis Research* **2018**, *135*, 177–189.

(670) Bína, D.; Durchan, M.; Kuznetsova, V.; Vácha, F.; **Litvín**, R.; Polívka, T. Energy transfer dynamics in a red-shifted violaxanthin-chlorophyll a light-harvesting complex. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2019**, *1860*, 111–120.

(671) Carbonera, D.; Agostini, A.; Valentin, M. D.; Gerotto, C.; Basso, S.; Giacometti, G. M.; Morosinotto, T. Photoprotective sites in the Violaxanthin-Chlorophyll a binding Protein (VCP) from *Nannochloropsis gaditana*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2014**, *1837*, 1235–1246.

(672) Lepetit, B.; Volke, D.; Gilbert, M.; Wilhelm, C.; Goss, R. Evidence for the existence of one antenna-associated, lipid-dissolved and two protein-bound pools of diadinoxanthin cycle pigments in diatoms. *Plant Physiology* **2010**, *154*, 1905–1920.

(673) Arvidsson, P.-O.; Carlsson, M.; Stefánsson, H.; Albertsson, P.; Åkerlund, H.-E. Violaxanthin accessibility and temperature dependency for de-epoxidation in spinach thylakoid membranes. *Photosynthesis Research* **1997**, *52*, 39–48.

(674) Goss, R.; Pinto, E. A.; Wilhelm, C.; Richter, M. The importance of a highly active and ΔpH-regulated diatoxanthin epoxidase for the regulation of the PSII antenna function in diadinoxanthin cycle containing algae. *Journal of Plant Physiology* **2006**, *163*, 1008–1021.

(675) Lepetit, B.; Sturm, S.; Rogato, A.; Gruber, A.; Sachse, M.; Falciatore, A.; Kroth, P. G.; Lavaud, J. High light acclimation in the secondary plastids containing diatom *Phaeodactylum tricornutum* is triggered by the redox state of the plastoquinone pool. *Plant Physiology* **2013**, *161*, 853–865.

(676) Grouneva, I.; Jakob, T.; Wilhelm, C.; Goss, R. The regulation of xanthophyll cycle activity and of non-photochemical fluorescence quenching by two alternative electron flows in the diatoms *Phaeodactylum tricornutum* and *Cyclotella meneghiniana*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2009**, *1787*, 929–938.

(677) Kotabová, E.; Kaňa, R.; Jarešová, J.; Prášil, O. Non-photochemical fluorescence quenching in *Chromera velia* is enabled by fast violaxanthin de-epoxidation. *FEBS Letters* **2011**, *585*, 1941–1945.

(678) Kalaji, H. M. et al. Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynthesis Research* **2017**, *132*, 13–66.

(679) Tsuyama, M.; Shibata, M.; Kawazu, T.; Kobayashi, Y. An Analysis of the Mechanism of the Low-wave Phenomenon of Chlorophyll Fluorescence. *Photosynthesis Research* **2004**, *81*, 67–76.

(680) Yamamoto, H.; Higashi, R. Violaxanthin de-epoxidase: Lipid composition and substrate specificity. *Archives of Biochemistry and Biophysics* **1978**, *190*, 514–522.

(681) Frommolt, R.; Goss, R.; Wilhelm, C. The de-epoxidase and epoxidase reactions of *Mantoniella squamata* (Prasinophyceae) exhibit different substrate-specific reaction kinetics compared to spinach. *Planta* **2001**, *213*, 446–456.

(682) Grabowski, B.; Tan, S.; Cunningham Jr, F.; Gantt, E. Characterization of the *Porphyridium cruentum* Chl *a* binding LHC by in vitro reconstitution: LHCaR1 binds 8 Chl a molecules and proportionately more carotenoids than CAB proteins. *Photosynthesis Research* **2000**, *63*, 85–96.

(683) Grabowski, B.; Cunningham, F. X.; Gantt, E. Chlorophyll and carotenoid binding in a simple red algal light-harvesting complex crosses phylogenetic lines. *Proceedings of the National Academy of Sciences* **2001**, *98*, 2911–2916.

(684) Liguori, N.; Xu, P.; van Stokkum, I.; van Oort, B.; Lu, Y.; Karcher, D.; Bock, R.; Croce, R. Different carotenoid conformations have distinct functions in light-harvesting regulation in plants. *Nature Communications* **2017**, *8*, 1994.

(685) Planavsky, N. J.; Reinhard, C. T.; Wang, X.; Thomson, D.; McGoldrick, P.; Rainbird, R. H.; Johnson, T.; Fischer, W. W.; Lyons, T. W. Low Mid-Proterozoic atmospheric oxygen levels and the delayed rise of animals. *Science* **2014**, *346*, 635–638.

(686) Shukla, A.; Agarwal Lalit, V.; Venkatasubramanian, V. Optimizing efficiency-robustness trade-offs in supply chain design under uncertainty due to disruptions. *International Journal of Physical Distribution & Logistics Management* **2011**, *41*, 623–647.

(687) Ugent, D. The Potato. *Science* **1970**, *170*, 1161–1166.

(688) Rankin, D. J.; Bargum, K.; Kokko, H. The tragedy of the commons in evolutionary biology. *Trends in Ecology & Evolution* **2007**, *22*, 643–651.

(689) Parvinen, K.; Dieckmann, U. Self-extinction through optimizing selection. *Journal of Theoretical Biology* **2013**, *333*, 1–9.

(690) Raia, P.; Carotenuto, F.; Mondanaro, A.; Castiglione, S.; Passaro, F.; Saggese, F.; Melchionna, M.; Serio, C.; Alessio, L.; Silvestro, D.; Fortelius, M. Progress to extinction: increased specialisation causes the demise of animal clades. *Scientific Reports* **2016**, *6*, 30965.

(691) Sanchez, S. E.; Kay, S. A. The Plant Circadian Clock: From a Simple Timekeeper to a Complex Developmental Manager. *Cold Spring Harbor Perspectives in Biology* **2016**, *8*, DOI: `10.1101/cshperspect.a027748`.

(692) Smaldino, P. E.; McElreath, R. The natural selection of bad science. *Royal Society Open Science* **2016**, *3*, 160384.

(693) Wilbanks, R. Engineering the Liquid Posthuman. *Science Fiction Studies* **2018**, *45*, 390–394.

List of publications included in this thesis

(P1) Gardian, Z.; **Litvín**, R.; Bína, D.; Vácha, F. Supramolecular organization of fucoxanthin–chlorophyll proteins in centric and pennate diatoms. *Photosynthesis Research* **2014**, *121*, 79–86.

(P2) **Litvín**, R.; Bína, D.; Herbstová, M.; Gardian, Z. Architecture of the light-harvesting apparatus of the eustigmatophyte alga *Nannochloropsis oceanica*. *Photosynthesis Research* **2016**, *130*, 137–150.

(P3) Bína, D.; Gardian, Z.; Herbstová, M.; **Litvín**, R. Modular antenna of photosystem I in secondary plastids of red algal origin: a *Nannochloropsis oceanica* case study. *Photosynthesis Research* **2017**, *131*, 255–266.

(P4) Herbstová, M.; Bína, D.; Koník, P.; Gardian, Z.; Vácha, F.; **Litvín**, R. Molecular basis of chromatic adaptation in pennate diatom *Phaeodactylum tricornutum*. *Biochimica et Biophysica Acta - Bioenergetics* **2015**, *1847*, 534–543.

(P5) Bína, D.; Herbstová, M.; Gardian, Z.; Vácha, F.; **Litvín**, R. Novel structural aspect of the diatom thylakoid membrane: lateral segregation of photosystem I under red-enhanced illumination. *Scientific Reports* **2016**, *6*, 25583.

(P6) Herbstová, M.; Bína, D.; Kaňa, R.; Vácha, F.; **Litvín**, R. Red-light phenotype in a marine diatom involves a specialized oligomeric red-shifted antenna and altered cell morphology. *Scientific Reports* **2017**, *7*, 11976.

(P7) **Litvín**, R.; Bína, D.; Herbstová, M.; Pazderník, M.; Kotabová, E.; Gardian, Z.; Trtílek, M.; Prášil, O.; Vácha, F. Red-shifted light-harvesting system of freshwater eukaryotic alga *Trachydiscus minutus* (Eustigmatophyta, Stramenopila). *Photosynthesis Research* **2019**, *142*, 137–151.

(P8) Bína, D.; Bouda, K.; **Litvín**, R. A two-component nonphotochemical fluorescence quenching in eustigmatophyte algae. *Photosynthesis Research* **2017**, *131*, 65–77.