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## PERSPECTIVES

# Redesigning the photosynthetic light reactions to enhance photosynthesis – the *PhotoRedesign* consortium

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## SUMMARY

In this Perspective article, we describe the visions of the *PhotoRedesign* consortium funded by the European Research Council of how to enhance photosynthesis. The light reactions of photosynthesis in individual phototrophic species use only a fraction of the solar spectrum, and high light intensities can impair and even damage the process. In consequence, expanding the solar spectrum and enhancing the overall energy capacity of the process, while developing resilience to stresses imposed by high light intensities, could have a strong positive impact on food and energy production. So far, the complexity of the photosynthetic machinery has largely prevented improvements by conventional approaches. Therefore, there is an urgent need to develop concepts to redesign the light-harvesting and photochemical capacity of photosynthesis, as well as to establish new model systems and toolkits for the next generation of photosynthesis researchers. The overall objective of *PhotoRedesign* is to reconfigure the photosynthetic light reactions so they can harvest and safely convert energy from an expanded solar spectrum. To this end, a variety of synthetic biology approaches, including *de novo* design, will combine the attributes of photosystems from different photoautotrophic model organisms, namely the purple bacterium *Rhodobacter sphaeroides*, the cyanobacterium *Synechocystis* sp. PCC 6803 and the plant *Arabidopsis thaliana*. In parallel, adaptive laboratory evolution will be applied to improve the capacity of reimagined organisms to cope with enhanced input of solar energy, particularly in high and fluctuating light.

**Keywords:** adaptive laboratory evolution, *Arabidopsis*, assembly, evolution, genetic engineering, photosynthesis, photosystem, *Rhodobacter*, *Synechocystis*, synthetic biology.

## INTRODUCTION

In this Perspective article, we outline the visions of the *PhotoRedesign* (“Redesigning the Photosynthetic Light Reactions”) consortium, which has been funded for 6 years by the award of a Synergy Grant from the European Research Council (ERC). The *PhotoRedesign* consortium, launched in April 2020, comprises research groups based at LMU Munich, the Centre Algatech in Třeboň and the University of Sheffield. The research team uses different model species to investigate variants of photosynthesis, namely the oxygenic process in plants and cyanobacteria, as well as anoxygenic photosynthesis in purple bacteria. The *PhotoRedesign* groups now combine their expertise in the genetics, biochemistry and biophysics of photosynthetic

organisms and, by employing synthetic biology and adaptive laboratory evolution (ALE) approaches, aim to enhance the light reactions of photosynthesis. Due to the complexity of this topic, we will first introduce the basic principles and diversity of photosynthesis found in three model photosynthetic species, *Rhodobacter sphaeroides* (hereafter *Rhodobacter*), *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) and *Arabidopsis thaliana* (hereafter *Arabidopsis*), and highlight the current approaches used to enhance photosynthesis. We then provide an outline of the *PhotoRedesign* project, the overall objective of which is to reconfigure the light reactions of photosynthesis so they can harvest and safely convert energy from an expanded solar spectrum.

## PHOTOSYNTHESIS IS FUNDAMENTAL BUT IMPERFECT

In plants, algae and cyanobacteria, oxygenic photosynthesis uses water as the electron donor for chemical reduction of CO<sub>2</sub>, and as a consequence it releases oxygen for respiration (Amunts and Nelson, 2009; Barber, 2008; Nelson, 2013; Sanchez-Baracaldo and Cardona, 2020; Williamson et al., 2011). The photochemical light reactions in oxygenic photosynthesis are performed by photosystems I and II (PSI, PSII) that absorb photons and use their energy to generate a proton gradient and reducing power, subsequently used to fix CO<sub>2</sub> in the Calvin cycle (for review, see Blankenship, 2014). Phototrophs evolved diverse light-harvesting systems (antennas) that absorb solar energy and transfer it to the photosystem reaction centres (RCs). Here, the energy is trapped and used to drive downstream metabolism.

Plants and green algae utilise only a part of the sunlight because their pigments absorb only within the 400–700-nm range. Considering various thermodynamic limits, the overall maximum efficiency of the light reactions is theoretically only 26% (Zhu et al., 2008, 2010; Figure 1). Taking also the energy losses associated with carbohydrate biosynthesis and (photo)respiration into account, the theoretical maximum photosynthetic energy conversion efficiencies in land plants decrease to 4.6% (C<sub>3</sub> plants) and 6% (C<sub>4</sub> plants; Zhu et al., 2008, 2010), and to between 4.5 and 9% in microalgae (Walker, 2009; Wijffels and Barbosa, 2010). However, this efficiency does normally not exceed ~1% in crop plants under field conditions over the full growing season (Walker, 2009; Figure 1a) and ~3% in microalgae in bioreactors (Cotton et al., 2015; Wijffels and Barbosa, 2010).

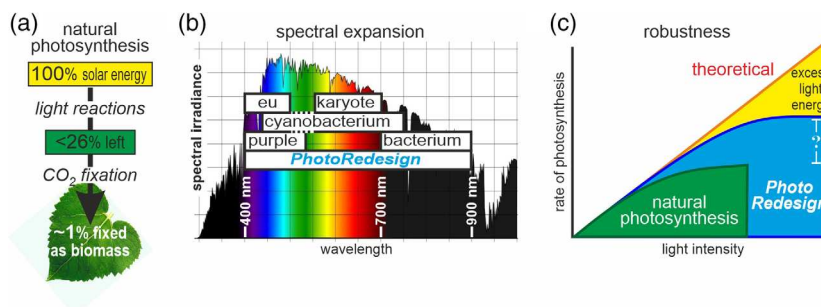
Given the aforementioned losses in energy conversion, the light reactions of photosynthesis provide ample scope for improvement. In particular, this includes: (i) expanding the solar spectrum for the light reactions, especially by using infrared (IR) light with wavelengths > 700 nm

(Blankenship et al., 2011; Figure 1b); and (ii) reducing the energy loss associated with photoinhibition or photodamage of photosystems due to oxidative stress caused by light saturation or fluctuating light conditions (Zhu et al., 2010; Figure 1c). In principle, expanding the solar spectrum for the light reactions should be possible, because different photosynthetic species have already evolved distinct antenna systems with different spectral coverage that could be combined in a single organism (Figure 1b). Moreover, certain cyanobacterial and algal species flourish under extremely high light intensities, and photoprotective mechanisms can be altered to improve photosynthetic efficiency under fluctuating light (Garcia-Molina and Leister, 2020; Kromdijk et al., 2016), suggesting that it is feasible to effectively enhance photosynthesis under high or fluctuating light levels.

However, photosystem complexes comprise tightly interacting ensembles of subunits and co-factors ('frozen metabolic accidents'), which limits the scope for modifying their composition and structure (Leister 2019b; Shi et al., 2005). So far, this problem has prevented improvement of the light reactions of photosynthesis through conventional breeding and genetic engineering approaches.

## COMMONALITIES AND DIFFERENCES OF PHOTOSYNTHESIS IN PROKARYOTES AND EUKARYOTES

The overall organisation of the oxygenic photosynthetic machinery was retained during the evolutionary transition from cyanobacteria to chloroplasts. Nonetheless, there were significant changes in the composition of peripheral subunits for light-harvesting and photosystem complexes (Figure 2). Apart from oxygenic phototrophs, a group of prokaryotes performs anoxygenic photosynthesis using a single type of RC. Of these, the purple phototrophic bacterium *Rhodospirillum rubrum* has been included in the *PhotoRedesign* project as model system.

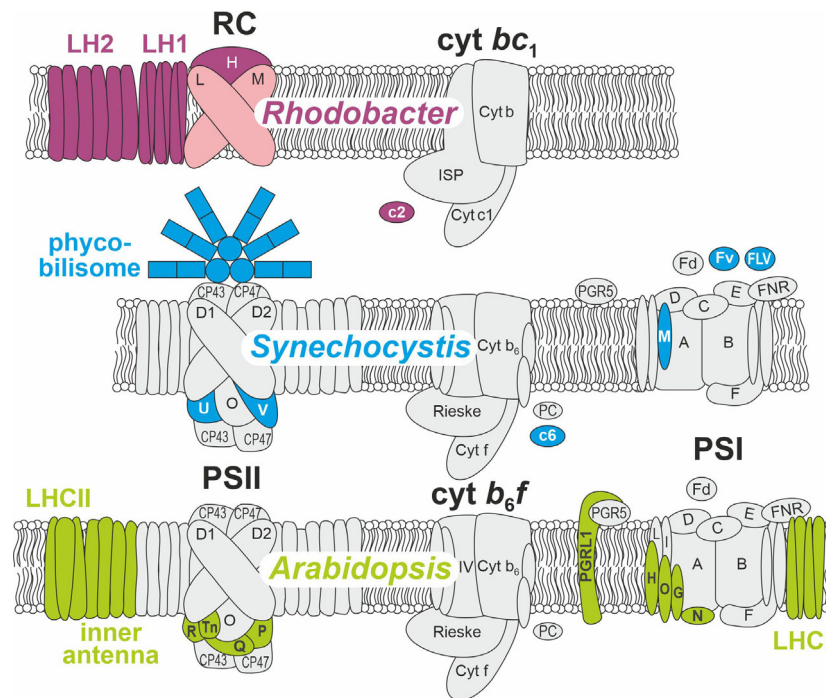


**Figure 1.** How *PhotoRedesign* will address limitations of photosynthesis and attempt to enhance the process.

(a) The overall maximal efficiency of the light reactions (in terms of the fraction of incident solar energy converted into chemical energy) is theoretically only 26% (Zhu et al., 2010). Taking into account carbon fixation, this value does normally not exceed ~1% in crop plants under field conditions and ~3% in microalgae grown in bioreactors (see main text).

(b) Natural photosynthesis in eukaryotes, cyanobacteria and purple bacteria uses different spectral regions of solar output, indicated in the white boxes. *PhotoRedesign* will expand the wavelength range of solar energy that can be used by a single organism.

(c) Natural photosynthesis becomes inhibited by excess light (photoinhibition). Increased HL tolerance will allow organisms to survive at higher light intensities, increase photosynthetic efficiency at HL, and provide a chassis that can cope with increased light harvesting due to expansion of the wavelength range (see b).



**Figure 2.** Composition of the photosynthetic machineries in purple bacteria, cyanobacteria and green algae/plants.

Both oxygenic and anoxygenic phototrophs employ similar principles for light harvesting and photochemistry, which provides opportunities for recombining native, engineered and newly developed photosystem modules to generate new, valuable traits. The cyanobacterial/plant photosystem II (PSII) is based on the photochemistry of chlorophyll (Chl) molecules, whereas the reaction centre (RC) of *Rhodobacter* contains bacteriochlorophylls (BChls), but both have a common ancestor and all are light-powered photo-oxidoreductases. PSII is able to oxidise water, whereas the *Rhodobacter* RC can be considered as a primordial version of PSII without its water-splitting capacity (Cardona et al., 2015). Instead, the RC oxidises soluble cytochrome (cyt)  $c_2$  (c2). Both PSII and the bacterial RC reduce quinones, plastoquinone in case of PSII and ubiquinone in case of RC, which carry electrons to the  $cyt\ b_6f$  (oxygenic photosynthesis) or  $cyt\ bc_1$  complex (*Rhodobacter*). The  $cyt\ bc_1$  reduces  $cyt\ c_2$ , establishing cyclic electron flow (CEF) to drive ATP synthesis (Sener et al., 2016). In oxygenic phototrophs, electrons are passed from  $cyt\ b_6f$  to photosystem I (PSI), employing soluble electron transporters [cyt  $c_6$  (c6) or plastocyanin (PC)]. Following an additional light absorption step, electrons are then transferred to soluble carriers [ferredoxin (Fd) or flavodoxin (Fv)] utilised for the generation of NADPH. Species-specific subunits are indicated by respective colours, with conserved subunits in light grey. LH1, LH2, phycobilisomes, light-harvesting complex (LHC) and LHCI are antenna complexes that harvest solar energy. Note that the photosynthetic machinery of plants like *Arabidopsis* and green algae like *Chlamydomonas* is very similar. For clarity, electron transport, the NDH complex and the ATP synthase are not shown.

### Antenna systems

The most striking differences between the three major variants of photosynthesis discussed here are their light-harvesting systems (antennas; Croce and van Amerongen, 2014). Cyanobacteria such as *Synechocystis* employ phycobilisomes, giant extrinsic complexes associated with the membrane surface (Figure 2) that absorb light in the 500–750 nm range (for review, see Adir et al., 2020; Bryant et al., 2020; Watanabe and Ikeuchi, 2013; Figure 1b). Green algae and plants such as *Arabidopsis* contain membrane-embedded antennas, the light-harvesting complexes I and II (LHCI/II; for review, see Cao et al., 2018; Caspy and Nelson, 2018) that absorb light mostly in the 400–510-nm and 620–700-nm regions (Figure 1b). The light-harvesting systems of *Rhodobacter* also consist of intrinsic membrane complexes, the light-harvesting complex 1 (LH1) and 2 (LH2) antenna, which harvest light in the 400–550-nm and 800–900-nm regions of the solar spectrum (for review, see Gardiner et al., 2020; Niederman, 2016; Saer and Blankenship, 2017; Figure 1b).

### Cofactors and lipids

The PSI and PSII cores in *Synechocystis* and *Arabidopsis* bind chlorophyll (Chl) *a* and  $\beta$ -carotene, whereas the *Rhodobacter* RC binds bacteriochlorophyll (BChl) *a* and spheroidene/spheroidenone. Phycobilisomes contain different types of linear tetrapyrroles called bilins, whereas purple bacterial LH1 and LH2 antennas contain near-infrared (NIR)-absorbing BChls and also bind a variety of carotenoids that provide the main pigmentation in the visible region of the spectrum (for review, see Gardiner et al., 2020; Leupold et al., 2000). In plant and algal LHCs, the spectrum of cofactors, and in particular carotenoids, is rather diverse. LHCs contain Chl *a* and *b*, as well as xanthophylls (Barros and Kühlbrandt, 2009; Schmid, 2008). Many other algal species, however, contain different carotenoids in LHCs (e.g. fucoxanthin, diadinoxanthin, zeaxanthin, alloxanthin) and possess Chl *c* instead of Chl *b*.

### Assembly factors

Photosystems are assembled from a number of protein subunits, pigments, lipids and other cofactors, and various

auxiliary (assembly) protein factors are required for their biogenesis. A plethora of assembly factors have been identified, but not all are conserved between photosynthetic organisms. Therefore, the exchange of thylakoid complexes between eukaryotes and cyanobacteria might require swapping of some of these auxiliary factors in addition to changing the structural proteins and enzymes to produce the required pigments. The complete set of assembly factors needs to be transferred between distantly related organisms; for instance, heterologous production of the plant ribulose-1,5-bisphosphat-carboxylase/oxygenase (RuBisCO) multiprotein complex in *Escherichia coli* requires all five assembly factors (Aigner et al., 2017). Nevertheless, there are clear common features between the biogenesis of photosystems and purple bacterial RCs, for instance between early steps of their assembly and (B) Chl biosynthesis in *Synechocystis* and *Rhodobacter* (Chidgey et al., 2014; Mothersole et al., 2016).

#### STRATEGIES FOR ENHANCING PHOTOSYNTHESIS AND THEIR LIMITATIONS

A substantial redesign of the light reactions of photosynthesis has not been attempted yet, although reconstruction of some photosynthetic modules in prokaryotes, such as biosynthesis of carotenoids and Chl, as well as RuBisCO (Aigner et al., 2017; Chen et al., 2018; Sandmann et al., 1999), provide a good basis for further progress. The tailoring of the light reactions of plant photosynthesis has been limited to a few instances of trial-and-error experiments, overproducing components of the light reactions or auxiliary proteins associated with the photosynthetic apparatus (for review, see Leister 2019a).

In contrast to plants, microorganisms are being employed as versatile cell factories for various metabolites, ranging from rare valuable pharmaceuticals to biofuels. The application of genetic engineering and synthetic biology to prokaryotic or unicellular eukaryotic cell factories involves the introduction of entire pathways and optimisation/streamlining by ALE (for review, see Cotton et al., 2015; Dragosits and Mattanovich, 2013). Compared with crop plants it is clear that microbial cell factory systems are better suited for synthetic biology. Therefore, there is a clear potential to improve plant productivity by applying innovative strategies that exploit a microbial chassis.

#### From single-protein approaches to entire pathways

Overproduction of single photosynthetic proteins has been reported to enhance plant photosynthesis (for review, see Leister 2019a), although it is unclear whether the reported enhancements hold true under field conditions. More recently, nuclear overproduction of the plastid-encoded D1 protein was reported to enhance both stress resistance and crop yield under field conditions (Chen et al., 2020).

A more complex approach was chosen to enhance plant photosynthesis under fluctuating light conditions by parallel overproduction of three thylakoid proteins, violaxanthin de-epoxidase, PsbS and zeaxanthin epoxidase. The resulting so-called 'VPZ' lines showed accelerated relaxation of non-photochemical quenching and exhibited enhanced biomass production in tobacco (Kromdijk et al., 2016), but not in *Arabidopsis* (Garcia-Molina and Leister, 2020). Similarly, in the GCGT approach, the four proteins glycolate oxidase, catalase, glyoxylate carboligase and tartronic semialdehyde reductase were targeted to chloroplasts, increasing the concentration of CO<sub>2</sub> and boosting photosynthesis, biomass production and grain yield, albeit at the cost of reduced seed setting rate (Wang et al., 2020). True synthetic biology approaches in regard to transferring entire pathways include the optimised production of multiple carotenoid and other plant colorant pathways (Yang et al., 2021), the aforementioned rebuilding of RuBisCO and the Chl biosynthesis pathway in *E. coli* (Aigner et al., 2017; Chen et al., 2018), and the screening and identification of highly active RuBisCO enzymes (Davidi et al., 2020). In addition, a Calvin-cycle like CO<sub>2</sub> fixation cycle was rebuilt in *E. coli* (Antonovsky et al., 2016; Flamholz et al., 2020; Herz et al., 2017). However, none of the approaches applied so far qualifies as fundamental redesign of the photosynthetic light reactions.

#### Employing the power of evolution: natural and laboratory-induced diversity

In principle, genetic factors involved in photosynthesis-relevant processes can be identified by comparing the genomes of closely related organisms that differ in the process of interest. Such an approach was used to determine the genetic basis of the rapid growth of the *Synechococcus elongatus* strain UTEX 2973 (relative to *S. elongatus* PCC 7942; Ungerer et al., 2018).

The ALE is a completely different concept because it generates a desired trait in a strain that initially lacks this attribute. Performing ALE in microbes makes large populations of rapidly dividing cells available for creating the genetic diversity required for selecting beneficial mutations (for review, see Sandberg et al., 2019). Such mutations can be identified by high-throughput sequencing in combination with bioinformatics, and their subsequent reintroduction to the non-adapted starting strain can confirm their effect. ALE has been applied to several photosynthetic organisms (for review, see Leister, 2018), and our recent study offered proof of practicability by showing that ALE enhances the robustness of photosynthesis (Dann et al., 2021). Here, ALE was used to generate *Synechocystis* strains that can grow under extremely high light intensities that even exceed naturally occurring terrestrial irradiation with sunlight. HL tolerance was found to be associated with more than 100 alterations in proteins, which were

involved in various cellular functions, including gene expression, photosynthesis and metabolism. Two of these mutations were introduced into wild-type cells, where they conferred an enhanced tolerance to HL, verifying the original effects (Dann et al., 2021).

### Chassis for studying and enhancing photosynthesis

Studying *Arabidopsis* and *Chlamydomonas reinhardtii* (hereafter: *Chlamydomonas*) led to tremendous progress in understanding eukaryotic photosynthesis, but the lack of efficient large-scale genetic engineering for these organisms prevents a deep structural and functional rebuilding of the photosynthetic machinery (Table 1). The moss *Physcomitrella patens* is accessible to homologous recombination, but like *Arabidopsis* it is not a suitable candidate for ALE (Table 1). Moreover, all photosynthetic eukaryotes display complex genomes distributed over three genetic compartments, complicating genetic engineering. *Synechocystis* and *Rhodobacter* are both prokaryotic species that are accessible to fast and efficient gene replacement and genetic complementation, as well as to ALE (Table 1). More recently, *E. coli* has been used as a host for expressing central components of photosynthesis, like Chl biosynthesis and carbon fixation (Table 1). One common and useful trait of these prokaryotic species is their capacity to grow heterotrophically, which makes them ideally suited for manipulating key components of photosynthesis that might involve intermediate steps of very low photosynthetic activity. In this regard, *Rhodobacter* is particularly useful because it can switch easily between phototrophic and heterotrophic growth modes.

Taken together, model plant species like *Arabidopsis* and *Physcomitrella* display clear technical limitations with respect to synthetic biology and ALE approaches, whereas *Rhodobacter* and *Synechocystis*, which are technically ideally suited for this endeavour, cannot easily serve as proxies for crop plants. The green alga *Chlamydomonas* occupies an intermediate position as it performs eukaryotic photosynthesis and can be used for ALE experiments.

## THE PHOTOREDESIGN PROJECT

### *Synechocystis* as a model system to study, modify and recombine three variants of photosynthesis

Enhancing photosynthesis is a promising approach for increasing biomass in microorganisms and crop yields. To this end, an organism is required that allows both the application of the technology required for deep rebuilding of photosynthesis, and the subsequent transfer of rebuilt parts to plants to enhance photosynthesis. As outlined above, none of the current model species for photosynthesis fulfils both requirements at the same time.

While rebuilding and studying photosynthesis in *E. coli* may lead to breakthroughs in understanding how photosynthetic complexes are assembled, this system has clear limitations with respect to developing concepts to enhance photosynthesis in multicellular plants because it simply cannot provide the necessary complex environment. Nevertheless, the *E. coli* system might be the system of choice to tackle fundamental flaws of photosynthesis, for example by enhancing or replacing the intrinsically inefficient enzyme RuBisCO, rebuilding CO<sub>2</sub> concentrating mechanisms (Davidi et al., 2020; Turmo et al., 2017), and developing fundamentally different photosystems.

*Chlamydomonas* is a popular algal model system for photosynthesis and other cellular processes (Dent et al., 2001; Grossman, 2000; Hanikenne, 2003; Hippler et al., 1998; Merchant et al., 2012; Rochaix, 1995; Salomé and Merchant 2019) and it is accessible to ALE, but one needs to be aware of some limitations. In addition to the aforementioned difficulties with large-scale genetic manipulation shared with *Arabidopsis*, photosynthesis in *Chlamydomonas* and land plants differs in several important respects. For instance, cyclic electron flow (CEF) in *Chlamydomonas* lacks the canonical NDH complex and the negative regulator PGRL2 (Rühle et al., 2021), yet it forms supercomplexes (Iwai et al., 2010) that do not exist in flowering plants. Moreover, some picoeukaryotic green algae like *Ostreococcus tauri* have much smaller genomes than *Chlamydomonas* and might have potential uses as

**Table 1** Characteristics of current model systems for photosynthesis

| Organism                | Type of photosynthesis | Genetic engineering | ALE | Heterotrophic propagation |
|-------------------------|------------------------|---------------------|-----|---------------------------|
| <i>Escherichia coli</i> | none                   | +++                 | +++ | Yes                       |
| <i>Rhodobacter</i>      | anoxygenic             | ++                  | ++  | Yes                       |
| <i>Synechocystis</i>    | oxygenic               | ++                  | ++  | Yes                       |
| <i>Chlamydomonas</i>    | oxygenic               | +                   | ++  | Yes                       |
| <i>Chlorella</i>        | oxygenic               | (+)                 | ++  | Yes                       |
| <i>Physcomitrella</i>   | oxygenic               | +                   | –   | Restricted                |
| <i>Arabidopsis</i>      | oxygenic               | +                   | –   | Restricted                |

+++ , highly efficient; ++ , efficient; + , possible; (+) , just possible; – , impossible.  
ALE, adaptive laboratory evolution.

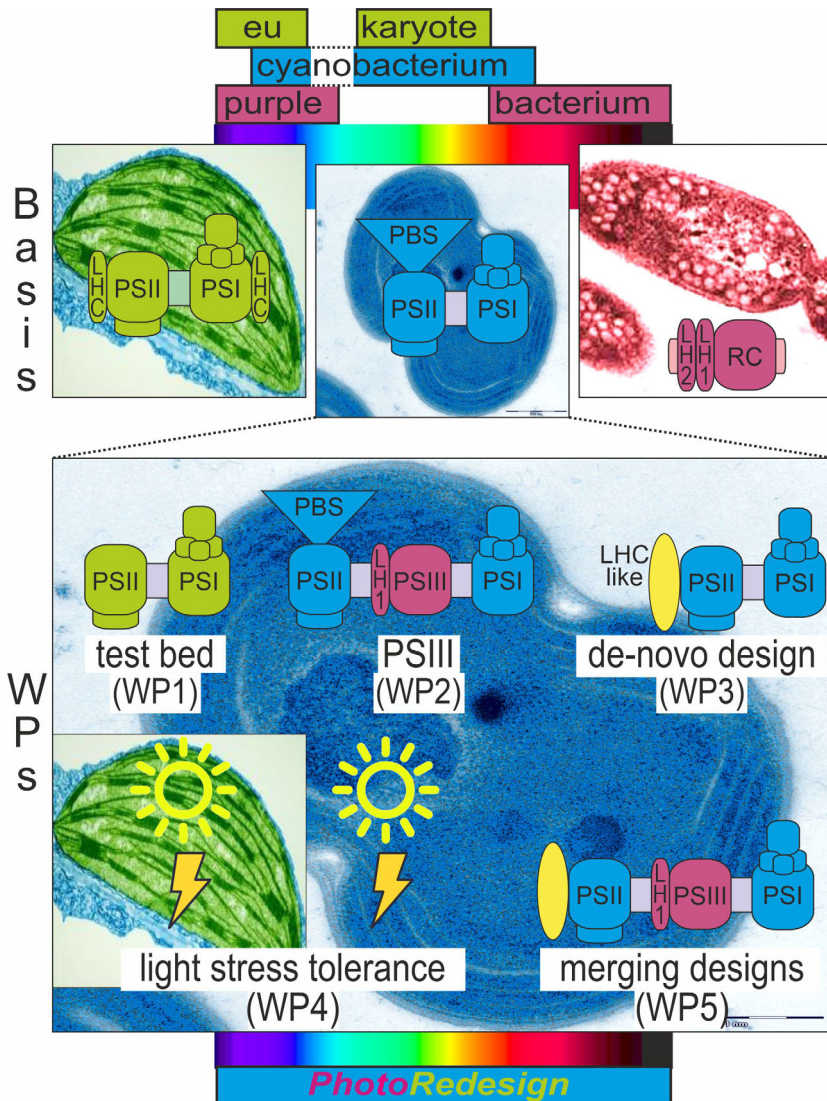
versions of a 'green *E. coli*' (Cock and Coelho, 2011; Hicks et al., 2001).

Cyanobacteria are the only prokaryotes capable of oxygenic photosynthesis. Although their auxiliary photosynthetic electron pathways differ even more from the ones in flowering plants than those in *Chlamydomonas*, cyanobacterial photosynthesis acts as a blueprint for the eukaryotic process. In fact, despite the evolutionary distance between cyanobacteria and flowering plants, photosynthetic modules for CEF from flowering plants can be functionally rebuilt in *Synechocystis* (Dann and Leister, 2019; Rühle et al., 2021), demonstrating the compatibility of plant and cyanobacterial photosynthesis. Therefore, we chose *Synechocystis* as our workhorse for combining modules of photosynthesis derived from very distantly related organisms. Details of this concept are outlined in the next sections.

### Implementation and organisation of the *PhotoRedesign* project

*PhotoRedesign* has two major objectives with a number of work packages (WPs) that are briefly outlined below and illustrated in Figure 3.

Objective 1 comprises the generation of hybrid light reactions in *Synechocystis* that can utilise a broader bandwidth of the solar spectrum. *Synechocystis* is the ideal test bed (WP1: 'Test bed') for enhancing the light reactions of oxygenic photosynthesis and can serve as an experimentally accessible proxy for plant photosynthesis. To this end we intend to rebuild the two plant photosystems, together with all auxiliary components necessary for their function, in *Synechocystis*. While it is not planned to directly generate plants with enhanced light reactions during the project, *PhotoRedesign* will provide the basis for such work. We will



**Figure 3.** Work packages (WPs) of *PhotoRedesign*. Plant photosystems (WP1), a novel photosystem 'PSIII' (WP2) and *de novo*-designed antennas (WP3) will be (re)built. Increasing tolerance to light stress (WP4), and combining the outcomes of the different tasks (WP5) will result in light reactions with enhanced utilisation of solar energy. See text for further details.

generate variants of photosynthesis in *Synechocystis* that can utilise a wider range of the light spectrum. This includes, as a first step, production of BChl in *Synechocystis*, followed by assembly of a novel photosystem based on the modified *Rhodobacter* RC complex (WP2: 'PSIII'). The NIR-powered photochemistry in this third RC has the potential to augment the energy input for oxygenic photosynthesis. In WP3: 'de novo design', we will design Chl-based (LHC-like) and BChl-based antenna modules in *Synechocystis*. As described earlier, our ambitions are to augment the cyanobacterial PSII with an LHC-type antenna and to couple an LH1-type antenna to PSIII.

Objective 2 aims to enhance light stress tolerance and to merge redesigned modules. We expect that improving the harvesting and conversion of light energy in WP2 and WP3 will initially produce rather inefficient systems, which could generate harmful side-effects for the cell, particularly in fluctuating light. Therefore, we will use ALE to make oxygenic photosynthesis more resilient towards light stress, using continuous and fluctuating light as stressors. To this end, we will continue our work on HL-adapted *Synechocystis* strains (Dann et al., 2021) and extend it to eukaryotic systems, namely using two green algal species, which are also accessible to ALE. We will identify and verify the causative mutations in the adapted strains and characterise the discovered factors in detail (WP4: 'increased light stress tolerance').

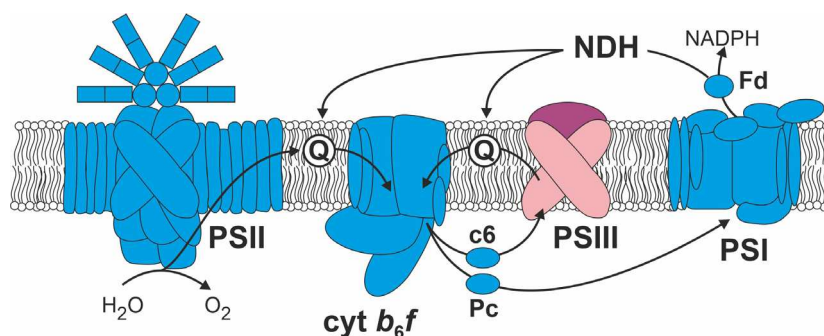
At later stages of the project, we will also employ ALE to optimise the hybrid systems generated during WP1, WP2 and WP3, and we will recombine key plant components with PSIII and the *de novo*-designed antenna (WP5: 'merging designs').

#### A new photosystem: 'PSIII'

Why has natural evolution not led to organisms that can use a broader bandwidth of light or higher light intensities? The answer might be simple: the naturally occurring

variants of photosynthesis have been, until now, sufficient for their evolved roles. However, rapid growth and biomass production is a desirable trait for crops grown under man-made and controlled conditions, and would benefit from enhanced energy capture.

How can *PhotoRedesign* create novel photosynthetic variants with increased light usage that nature failed to implement? Nature has already developed pigments capable of absorbing far-red (FR) or NIR irradiance, such as the Chls *d* and *f* found in some cyanobacteria (Chen et al., 2010; Elias et al., 2021; Mascoli et al., 2020). Although the gain in spectral expansion by Chl *d* and *f* compared with Chl *a* and *b* is rather small (up to 750 nm compared with 700 nm), there are deep-shade environments where Chl *f* confers significant benefits (Mascoli et al., 2020). However, the requirements for the biosynthesis and incorporation of these pigments into photosystems are partially (for Chl *f*; Ho et al., 2016; Trinugroho et al., 2020) or completely (for Chl *d*) unknown. Because its biosynthesis is known and its absorption ranges up to 900 nm, *PhotoRedesign* will focus on BChl *a* found in anoxygenic photosynthetic prokaryotes as a means to markedly expand the spectral range of oxygenic phototrophs, as suggested previously (Blankenship et al., 2011; Ort et al., 2015; Figure 1). To utilise FR/NIR photons, BChl *a* heterologously synthesised in *Synechocystis* and incorporated in the corresponding antennas (see below) will be attached to a modified *Rhodobacter* RC that can be assembled in *Synechocystis*. Together with the two native cyanobacterial photosystems, 'PSIII' will form a three-photosystem hybrid array that covers visible, red, FR and NIR regions of the solar spectrum (Figure 4). ALE might support the establishment of PSIII in *Synechocystis* by optimising functional links to the cytochrome (cyt) *b<sub>6</sub>f* complex. The necessary selection pressure could be achieved by suppressing native CEF through mutating the NDH complex and/or providing NIR-enriched light for photosynthesis. Potentially, the introduced PSIII might



**Figure 4.** Near-infrared (NIR)-enhanced, oxygenic photosynthesis, adapted from Ort et al. (2015). The native cyanobacterial photosystem II (PSII; using light from 400 to 680 nm) delivers protons for ATP synthesis and electrons from water oxidation for plastoquinone reduction. The novel bacteriochlorophyll (BChl)-containing photosystem III (PSIII) derived from a modified *Rhodobacter* reaction centre (RC; operating out to 900 nm) and cytochrome (cyt) *b<sub>6</sub>f* collaborate in a cyclic electron flow (CEF) loop. For NADPH production we will rely on linear electron flow involving native cyt *b<sub>6</sub>f* and photosystem I (PSI). Selection pressure for successful establishment of PSIII by adaptive laboratory evolution (ALE) could be achieved by propagating cells in NIR-enriched light and (partially) inactivating NDH-dependent CEF. NDH, NDH complex; c6, cyt *c<sub>6</sub>*; Pc, plastocyanin; Fd, ferredoxin; Q, plastoquinone.

compete with PSI for a source of electrons, i.e. cytochrome  $c_6$  or plastocyanin, although this competition could be controlled by applying light that preferentially excites either Chl (PSII and PSI) or BChl (PSIII). Alternatively, two populations of *cyt b<sub>6</sub>f* complexes could be designed, with one of them providing electrons for PSI and another one with an engineered reduction site that reduces an electron donor targeted to an engineered binding site on PSIII.

### New and recombined antenna

We further plan to extend the capacity of the *Synechocystis* PSII to collect photons in the blue, yellow and red regions using plant LHC(-like) antennas. As the *in vivo* synthesis of Chl *b* is toxic for *Synechocystis* (Xu et al., 2002), we do not plan to utilise antenna proteins from plants or green algae. Instead, we will produce LHC proteins containing a simple set of cofactors (Chl *a* and a single carotenoid) from *Nanochloropsis* and *Vitrella* algae. *Synechocystis* strains producing various eukaryotic carotenoids have already been developed (Cao et al., 2020; Lehmann et al., 2021). An alternative, more synthetic, strategy is the construction of completely new, LHC-like antennas, using cyanobacterial single helix high-light-inducible proteins (Hlips), ancestors of the whole LHC superfamily (Komenda and Sobotka, 2016). These proteins bind to PSII via the CP47 antenna and adjacent small subunits (Promnares et al., 2006); we aim to create three-helix LHC-like proteins by linking two Hlip helices with another TM helix. Such an antenna can also be fused to other PSII accessory proteins bound to CP47 such as Psb35 (Pascual Aznar et al., 2021) in order to increase the PSII binding stability, while cofactor-binding sites in these synthetic proteins will be mutated to achieve efficient light-harvesting. Energy to the introduced PSIII in *Synechocystis* will be delivered by a native cyanobacterial antenna, or by heterologously assembled BChl-based antenna complexes related to *Rhodobacter* LH1, which have the flexibility to bind a range of carotenoids (Chi et al., 2015).

### Improving the capacity to safely convert increased doses of light energy

Cyanobacteria and eukaryotic phototrophs have evolved distinct mechanisms to dissipate excess incident light energy (Erickson et al., 2015; Kirilovsky and Kerfeld, 2012, 2016; Pinnola and Bassi, 2018; Ruban, 2016) in order to avoid the production of dangerous reactive oxygen species and photodamage (Nath et al., 2013). The provision of more light-harvesting capacity could result in increased light stress, in particular under natural conditions with fluctuating light intensities. Therefore, *PhotoRedesign* will use ALE to increase the light tolerance of native and configured organisms containing PSIII.

Because variants of *Synechocystis* tolerant to HL have already been generated by ALE (Dann et al., 2021), it should also be possible to evolve green algae with

similarly enhanced HL tolerance. In fact, the existence of the desert green alga *Chlorella ohadii*, which can flourish under extremely high intensities of light (Treves et al., 2013, 2016, 2017), strongly supports the notion that genera of green algae such as *Chlorella* or *Chlamydomonas* can be made HL tolerant by ALE in a straightforward way.

In conclusion, using ALE, *PhotoRedesign* will identify light stress tolerance factors in cyanobacteria and green algae to compensate for negative side-effects of increasing the spectral range of photosynthesis.

### Facilitating effective biogenesis of reconfigured photosynthetic complexes

What factors are required for biogenesis of fully functional photosystems and their antennas? How is stepwise assembly of photosystems achieved? What intermediates are involved? In what sequence do the assembly factors act? The assembly of all photosynthetic complexes, in particular of PSII, has been studied in detail (see above); however, even in *Synechocystis* the full complement of assembly factors is not yet known, and indeed some PSII assembly factors were initially discovered in *Arabidopsis* and then characterised in *Synechocystis* (Armbruster et al., 2010; Bučinská et al., 2018; Meurer et al., 1998). For plant PSI, the situation is similar, whereas in *Rhodobacter* factors for LH1 and LH2 assembly have been identified (Mothersole et al., 2016), although no details on the mechanism of their action are available. During rebuilding PSIII and other key modules of photosynthesis in *Synechocystis*, we will include known components required for their assembly and in parallel attempt to identify as yet unknown ones. These unknown assembly factors pose a tentative threat to our approach of rebuilding heterologous multi-protein complexes in *Synechocystis*, but previous results indicate that lack of assembly factors can be compensated by the activation of other processes, for instance Chl biosynthesis (Bučinská et al., 2018; Yu et al., 2018). Furthermore, in general, *Synechocystis* appears to tolerate the loss of assembly factors better than plants (Nixon et al., 2010).

### CONCLUDING REMARKS

The light reactions of photosynthesis are one of the most complex systems in nature and so far they have largely resisted man-made improvements. Significant progress has been made in the last years in the areas of synthetic biology and in tuning of cellular processes by ALE, which can now be combined with the wealth of information on the genetics, biochemistry and structure of the light reactions of photosynthesis. Thus, photosynthesis research can take a next step, improving photosynthesis in a rational way by establishing a genetic model system in the cyanobacterium *Synechocystis*. The proposed research is ultra-high risk: a number of pitfalls and challenges are obvious and have been listed in Table 2 together with

**Table 2** Pitfalls and challenges in the *PhotoRedesign* project and how to address them

| Pitfalls and challenges                                                                                                                                                                                                                               | Tentative solutions                                                                                                                                                                                                                                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Technical level</b>                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                      |
| Several unknown assembly factors might be required for rebuilding entire photosystems in other organisms.                                                                                                                                             | Comparison of the effects of lack of conserved assembly factors in <i>Synechocystis</i> and <i>Arabidopsis</i> shows that they are less essential in <i>Synechocystis</i> (Nixon et al., 2010), and lack of assembly factors in <i>Synechocystis</i> can be overcome by activation of other processes (suppressor mutations; Bućinská et al., 2018).                                                 |
| Regulation of photosynthetic gene expression in <i>Synechocystis</i> is tightly controlled and this impedes rebuilding of entire photosystems.                                                                                                        | Original regulatory sequences of photosynthetic genes will be used as much as possible. ALE should enable fine-tuning of gene expression.                                                                                                                                                                                                                                                            |
| ALE generates strains with various mutations that are often epistatically connected. This makes it difficult to combine ALE with synthetic biology approaches.                                                                                        | This is not an issue if ALE is applied after the synthetic biology approaches, e.g. for optimising hybrid systems. If ALE is used before synthetic biology, e.g. for generating a stress-tolerant chassis for hybrid photosynthesis, ALE-based mutations need to be identified, characterised and suitably combined.                                                                                 |
| <b>Compatibility and functionality</b>                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                      |
| Lowered levels of pigments lead to higher yields of biomass in photobioreactors (Melis, 2009). Therefore, a 'black cyanobacterium' that absorbs more wavelengths might not display enhanced photosynthesis and biomass production.                    | This is true for cultivation of photosynthetic microbes in photobioreactors, in which cells at the surface effectively absorb light, which then does not penetrate into the deeper layers of the culture. For plants, this problem might be less relevant, if leaf size, canopy height and density can be manipulated.                                                                               |
| Without careful redesign and regulation of the electron flow (including modification of ATP synthase and NDH activity), as well as a balanced stoichiometry between photosystems, addition of a 'PSIII' will harm rather than enhance photosynthesis. | <i>PhotoRedesign</i> does not aim to couple PSIII directly to any of the other photosystems for bioenergetic reasons. The aim is to introduce PSIII and link it then functionally to other components to create a selection pressure that allows fine-tuning by ALE.                                                                                                                                 |
| BChl-driven charge separation might be unfavourable <i>per se</i> for <i>Synechocystis</i> photosynthesis with respect to its bioenergetics. BChl might be less effective than employing Chl <i>d</i> or Chl <i>f</i> .                               |                                                                                                                                                                                                                                                                                                                                                                                                      |
| ALE might enhance tolerance of the applied stress, but the generated mutations might come with a trade-off such as decreased photosynthetic efficiency under non-stress conditions.                                                                   | Conceptually, increasing HL tolerance should counterbalance potential harmful effects of increased light usage. Actually, proof-of-concept for HL-ALE of <i>Synechocystis</i> resulted in only limited trade-offs (Dann et al., 2021).                                                                                                                                                               |
| Cyanobacterial ALE might enhance tolerance to oxidative stress by mutations in gene expression and this is not transferable to plants.                                                                                                                | Previous results from ALE for HL tolerance in <i>Synechocystis</i> also identified mutations in metabolism, including photosynthesis and respiration, as well as in proteins of unknown function (Dann et al., 2021).                                                                                                                                                                                |
| <b>Conceptual issues</b>                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                      |
| Enhanced cyanobacterial photosynthesis is not transferable to plants because photosynthesis in plants is spatially separated from other processes (for instance respiration) in contrast to prokaryotic systems.                                      | Modifications in basic components of the light reactions might be transferable between cyanobacteria and plants. Enhancements based on ALE of green algae are expected to be transferable to plants.                                                                                                                                                                                                 |
| Enhancements in cyanobacterial and green algal photosynthesis are not transferable to plants, because plant photosynthesis involves different cellular compartments and different organs.                                                             | Enhancing photosynthesis in cyanobacteria and green algae is limited to unicellular organisms. Nevertheless, sink-source relationships also exist in these organisms to a certain extent.                                                                                                                                                                                                            |
| For crop yield the efficiency of the light reactions is not limiting. Enhancing only the light reactions will not enhance biomass and yield.                                                                                                          | A number of experiments show that enhancing the light reactions can increase biomass production; e.g. by increasing the amounts of plastocyanin (Pesaresi et al., 2009), the Rieske Fe-S protein (Simkin et al., 2017), the D1 protein (Chen et al., 2020) or a set of three photoprotective proteins (Kromdijk et al., 2016), implying a certain level of source limitation in current crop plants. |
| Natural evolution should have already created any possible enhancement of photosynthesis. Therefore, photosynthesis is already as perfect as it can be.                                                                                               | Natural evolution does not involve the exchange of gene sets between very distantly related organisms like cyanobacteria, purple bacteria and plants. Crop species are not the result of natural evolution but of man-made breeding.                                                                                                                                                                 |
| Employing natural variation might be more powerful than ALE for identifying components contributing to desired photosynthetic traits like HL tolerance.                                                                                               | Identifying the genetic basis of natural HL tolerance as in the green alga <i>Chlorella ohadii</i> or certain cyanobacterial species or diatoms is complementary to our approach of generating stress tolerance.                                                                                                                                                                                     |

ALE, adaptive laboratory evolution; BChl, bacteriochlorophyll; Chl, chlorophyll; HL, XXX; PSIII, photosystem III.

potential solutions. Notably, our work in progress has already removed some tentative obstacles for the project.

The simpler cyanobacterial model system will act as a valuable stepping-stone for the far-reaching aim of *PhotoRedesign*, to improve the light reactions of photosynthesis in higher plants, which will undoubtedly be a far more complicated task. As a consortium we acknowledge that there are rapidly escalating risks in engineering a PSIII into a cyanobacterium, then increasing photosynthetic yield and finally with making such a strategy work for plants. Nonetheless, *en route* we expect to learn various hitherto unknown aspects of how the photosynthetic machinery is assembled and regulated, allowing us to define a set of photosynthetic modules that can be shuttled between species.

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## AUTHOR CONTRIBUTIONS

All authors contributed to the writing of this manuscript. Writing was coordinated by DL.

## CONFLICT OF INTEREST

The authors of this manuscript claim no conflict of interest.

## REFERENCES

- Adir, N., Bar-Zvi, S. & Harris, D. (2020) The amazing phycobilisome. *Biochim Biophys Acta Bioenerg*, **1861**, 148047.
- Aigner, H., Wilson, R.H., Bracher, A., Calisse, L., Bhat, J.Y., Hartl, F.U. *et al.* (2017) Plant RuBisCo assembly in *E. coli* with five chloroplast chaperones including BSD2. *Science*, **358**, 1272–1278.
- Amunts, A. & Nelson, N. (2009) Plant photosystem I design in the light of evolution. *Structure*, **17**, 637–650.
- Antonovsky, N., Gleizer, S., Noor, E., Zohar, Y., Herz, E., Barenholz, U. *et al.* (2016) Sugar synthesis from CO<sub>2</sub> in *Escherichia coli*. *Cell*, **166**, 115–125.
- Armbruster, U., Zuhlke, J., Rengstl, B., Kreller, R., Makarenko, E., Rühle, T. *et al.* (2010) The Arabidopsis thylakoid protein PAM68 is required for efficient D1 biogenesis and photosystem II assembly. *The Plant Cell*, **22**, 3439–3460.
- Barber, J. (2008) Photosynthetic generation of oxygen. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **363**, 2665–2674.
- Barros, T. & Kühlbrandt, W. (2009) Crystallisation, structure and function of plant light-harvesting complex II. *Biochimica Et Biophysica Acta*, **1787**, 753–772.
- Blankenship, R.E. (2014) *Molecular Mechanisms of Photosynthesis*, 2nd edition. Hoboken, NJ: Wiley Blackwell.
- Blankenship, R.E., Tiede, D.M., Barber, J., Brudvig, G.W., Fleming, G., Ghirardi, M. *et al.* (2011) Comparing photosynthetic and photovoltaic efficiencies and recognizing the potential for improvement. *Science*, **332**, 805–809.
- Bryant, D.A., Hunter, C.N. & Warren, M.J. (2020) Biosynthesis of the modified tetrapyrroles—the pigments of life. *Journal of Biological Chemistry*, **295**, 6888–6925.
- Bučinská, L., Kiss, E., Konik, P., Knoppová, J., Komenda, J. & Sobotka, R. (2018) The ribosome-bound protein Pam68 promotes insertion of chlorophyll into the CP47 subunit of photosystem II. *Plant Physiology*, **176**, 2931–2942.
- Cao, P., Su, X., Pan, X., Liu, Z., Chang, W. & Li, M. (2018) Structure, assembly and energy transfer of plant photosystem II supercomplex. *Biochim Biophys Acta Bioenerg*, **1859**, 633–644.
- Cao, T.J., Wang, L.J., Huang, X.Q., Deng, Y.Y., Yang, L.E. & Lu, S. (2020) Manipulation of *Synechocystis* sp. PCC 6803 as a platform for functional identification of genes involved in carotenoid metabolism. *Plant Biotechnology Journal*, **18**, 605–607.
- Cardona, T., Murray, J.W. & Rutherford, A.W. (2015) Origin and evolution of water oxidation before the last common ancestor of the cyanobacteria. *Molecular Biology and Evolution*, **32**, 1310–1328.
- Caspy, I. & Nelson, N. (2018) Structure of the plant photosystem I. *Biochemical Society Transactions*, **46**, 285–294.
- Chen, G.E., Canniffe, D.P., Barnett, S.F.H., Hollingshead, S., Brindley, A.A., Vasilev, C. *et al.* (2018) Complete enzyme set for chlorophyll biosynthesis in *Escherichia coli*. *Science Advances*, **4**, eaq1407.
- Chen, J.H., Chen, S.T., He, N.Y., Wang, Q.L., Zhao, Y., Gao, W. *et al.* (2020) Nuclear-encoded synthesis of the D1 subunit of photosystem II increases photosynthetic efficiency and crop yield. *Nature Plants*, **6**, 570–580.
- Chen, M., Schliep, M., Willows, R.D., Cai, Z.L., Neilan, B.A. & Scheer, H. (2010) A red-shifted chlorophyll. *Science*, **329**, 1318–1319.
- Chi, S.C., Mothersole, D.J., Dilbeck, P., Niedzwiedzki, D.M., Zhang, H., Qian, P. *et al.* (2015) Assembly of functional photosystem complexes in *Rhodobacter sphaeroides* incorporating carotenoids from the spirilloxanthin pathway. *Biochimica Et Biophysica Acta*, **1847**, 189–201.
- Chidgey, J.W., Linhartová, M., Komenda, J., Jackson, P.J., Dickman, M.J., Canniffe, D.P. *et al.* (2014) A cyanobacterial chlorophyll synthase-HliD complex associates with the Ycf39 protein and the YidC/Alb3 insertase. *The Plant Cell*, **26**, 1267–1279.
- Cock, J.M. & Coelho, S.M. (2011) Algal models in plant biology. *Journal of Experimental Botany*, **62**, 2425–2430.
- Cotton, C.A., Douglass, J.S., De Causmaecker, S., Brinkert, K., Cardona, T., Fantuzzi, A. *et al.* (2015) Photosynthetic constraints on fuel from microbes. *Front Bioeng Biotechnol*, **3**, 36.
- Croce, R. & van Amerongen, H. (2014) Natural strategies for photosynthetic light harvesting. *Nature Chemical Biology*, **10**, 492–501.
- Dann, M. & Leister, D. (2019) Evidence that cyanobacterial Sll1217 functions analogously to PGRL1 in enhancing PGR5-dependent cyclic electron flow. *Nature Communications*, **10**, 5299.
- Dann, M., Ortiz, E.M., Thomas, M., Guljamow, A., Lehmann, M., Schaefer, H. *et al.* (2021) Enhancing photosynthesis at high light levels by adaptive laboratory evolution. *Nature Plants*, **7**, 681–695.
- Davidi, D., Shamshoum, M., Guo, Z., Bar-On, Y.M., Prywes, N., Oz, A. *et al.* (2020) Highly active rubiscos discovered by systematic interrogation of natural sequence diversity. *EMBO Journal*, **39**, e104081.
- Dent, R.M., Han, M. & Niyogi, K.K. (2001) Functional genomics of plant photosynthesis in the fast lane using *Chlamydomonas reinhardtii*. *Trends in Plant Science*, **6**, 364–371.
- Dragosits, M. & Mattanovich, D. (2013) Adaptive laboratory evolution – principles and applications for biotechnology. *Microbial Cell Factories*, **12**, 64.
- Elias, E., Liguori, N., Saga, Y., Schäfers, J. & Croce, R. (2021) Harvesting far-red light with plant antenna complexes incorporating chlorophyll d. *Biomacromolecules*, **22**, 3313–3322.
- Erickson, E., Wakao, S. & Niyogi, K.K. (2015) Light stress and photoprotection in *Chlamydomonas reinhardtii*. *The Plant Journal*, **82**, 449–465.
- Flamholz, A.I., Dugan, E., Blikstad, C., Gleizer, S., Ben-Nissan, R., Amram, S. *et al.* (2020) Functional reconstitution of a bacterial CO<sub>2</sub> concentrating mechanism in *Escherichia coli*. *Elife*, **9**, e59882.
- García-Molina, A. & Leister, D. (2020) Accelerated relaxation of photoprotection impairs biomass accumulation in Arabidopsis. *Nature Plants*, **6**, 9–12.
- Gardiner, A.T., Nguyen-Phan, T.C. & Cogdell, R.J. (2020) A comparative look at structural variation among RC-LH1 ‘Core’ complexes present in anoxygenic phototrophic bacteria. *Photosynthesis Research*, **145**, 83–96.
- Grossman, A.R. (2000) *Chlamydomonas reinhardtii* and photosynthesis: genetics to genomics. *Current Opinion in Plant Biology*, **3**, 132–137.

- Hanikenne, M. (2003) *Chlamydomonas reinhardtii* as a eukaryotic photosynthetic model for studies of heavy metal homeostasis and tolerance. *New Phytologist*, **159**, 331–340.
- Herz, E., Antonovsky, N., Bar-On, Y., Davidi, D., Gleizer, S., Prywes, N. *et al.* (2017) The genetic basis for the adaptation of *E. coli* to sugar synthesis from CO<sub>2</sub>. *Nature Communications*, **8**, 1705.
- Hicks, G.R., Hironaka, C.M., Dauvillee, D., Funke, R.P., D'Hulst, C., Waffenschmidt, S. *et al.* (2001) When simpler is better. Unicellular green algae for discovering new genes and functions in carbohydrate metabolism. *Plant Physiology*, **127**, 1334–1338.
- Hippler, M., Redding, K. & Rochaix, J.D. (1998) *Chlamydomonas* genetics, a tool for the study of bioenergetic pathways. *Biochimica Et Biophysica Acta*, **1367**, 1–62.
- Ho, M.Y., Shen, G., Canniffe, D.P., Zhao, C. & Bryant, D.A. (2016) Light-dependent chlorophyll f synthase is a highly divergent paralog of PsbA of photosystem II. *Science*, **353**, aaf9178.
- Iwai, M., Takizawa, K., Tokutsu, R., Okamuro, A., Takahashi, Y. & Minagawa, J. (2010) Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. *Nature*, **464**, 1210–1213.
- Kirilovsky, D. & Kerfeld, C.A. (2012) The orange carotenoid protein in photoprotection of photosystem II in cyanobacteria. *Biochimica Et Biophysica Acta*, **1817**, 158–166.
- Kirilovsky, D. & Kerfeld, C.A. (2016) Cyanobacterial photoprotection by the orange carotenoid protein. *Nature Plants*, **2**, 16180.
- Komenda, J. & Sobotka, R. (2016) Cyanobacterial high-light-inducible proteins - protectors of chlorophyll-protein synthesis and assembly. *Biochimica Et Biophysica Acta*, **1857**, 288–295.
- Kromdijk, J., Glowacka, K., Leonelli, L., Gabilly, S.T., Iwai, M., Niyogi, K.K. *et al.* (2016) Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science*, **354**, 857–861.
- Lehmann, M., Vamvaka, E., Torrado, A., Jahns, P., Dann, M., Rosenhammer, L. *et al.* (2021) Introduction of the carotenoid biosynthesis  $\alpha$ -branch into *Synechocystis* sp. PCC 6803 for lutein production. *Frontiers in Plant Science*, **12**, 699424.
- Leister, D. (2018) Experimental evolution in photoautotrophic microorganisms as a means of enhancing chloroplast functions. *Essays in Biochemistry*, **62**, 77–84.
- Leister, D. (2019b) Genetic engineering, synthetic biology and the light reactions of photosynthesis. *Plant Physiology*, **179**, 778–793.
- Leister, D. (2019a) Thawing out frozen metabolic accidents. *BMC Biology*, **17**, 8.
- Leupold, D., Voigt, B., Beenken, W. & Stiel, H. (2000) Pigment-protein architecture in the light-harvesting antenna complexes of purple bacteria: does the crystal structure reflect the native pigment-protein arrangement? *FEBS Letters*, **480**, 73–78.
- Mascoli, V., Bersanini, L. & Croce, R. (2020) Far-red absorption and light-use efficiency trade-offs in chlorophyll f photosynthesis. *Nature Plants*, **6**, 1044–1053.
- Melis, A. (2009) Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. *Plant Science*, **177**, 272–280.
- Merchant, S.S., Kropat, J., Liu, B., Shaw, J. & Warakanont, J. (2012) TAG, you're it! *Chlamydomonas* as a reference organism for understanding algal triacylglycerol accumulation. *Current Opinion in Biotechnology*, **23**, 352–363.
- Meurer, J., Plucken, H., Kowallik, K.V. & Westhoff, P. (1998) A nuclear-encoded protein of prokaryotic origin is essential for the stability of photosystem II in *Arabidopsis thaliana*. *EMBO Journal*, **17**, 5286–5297.
- Mothersole, D.J., Jackson, P.J., Vasilev, C., Tucker, J.D., Brindley, A.A., Dickman, M.J. *et al.* (2016) PucC and LhaA direct efficient assembly of the light-harvesting complexes in *Rhodospirillum rubrum*. *Molecular Microbiology*, **99**, 307–327.
- Nath, K., Jajoo, A., Poudyal, R.S., Timilsina, R., Park, Y.S., Aro, E.M. *et al.* (2013) Towards a critical understanding of the photosystem II repair mechanism and its regulation during stress conditions. *FEBS Letters*, **587**, 3372–3381.
- Nelson, N. (2013) Evolution of photosystem I and the control of global enthalpy in an oxidizing world. *Photosynthesis Research*, **116**, 145–151.
- Niederman, R.A. (2016) Development and dynamics of the photosynthetic apparatus in purple phototrophic bacteria. *Biochimica Et Biophysica Acta*, **1857**, 232–246.
- Nixon, P.J., Michoux, F., Yu, J., Boehm, M. & Komenda, J. (2010) Recent advances in understanding the assembly and repair of photosystem II. *Annals of Botany*, **106**, 1–16.
- Ort, D.R., Merchant, S.S., Alric, J., Barkan, A., Blankenship, R.E., Bock, R. *et al.* (2015) Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proceedings of the National Academy of Sciences U S A*, **112**, 8529–8536.
- Pascual Aznar, G., Konert, G., Bečková, M., Kotabová, E., Gardian, Z., Knopová, J. *et al.* (2021) Psb35 protein stabilizes the CP47 assembly module and associated high-light inducible proteins during the biogenesis of Photosystem II in the cyanobacterium *Synechocystis* sp. PCC6803. *Plant and Cell Physiology*, **62**, 178–190.
- Pesaresi, P., Scharfenberg, M., Weigel, M., Granlund, I., Schroder, W.P., Finazzi, G. *et al.* (2009) Mutants, overexpressors, and interactors of *Arabidopsis* plastocyanin isoforms: revised roles of plastocyanin in photosynthetic electron flow and thylakoid redox state. *Molecular Plant*, **2**, 236–248.
- Pinnola, A. & Bassi, R. (2018) Molecular mechanisms involved in plant photoprotection. *Biochemical Society Transactions*, **46**, 467–482.
- Promnares, K., Komenda, J., Bumba, L., Nebesarova, J., Vacha, F. & Tichy, M. (2006) Cyanobacterial small chlorophyll binding protein ScpD (HliB) is located on the periphery of Photosystem II in the vicinity of PsbH and CP47 subunits. *Journal of Biological Chemistry*, **281**, 32705–32713.
- Rochaix, J.D. (1995) *Chlamydomonas reinhardtii* as the photosynthetic yeast. *Annual Review of Genetics*, **29**, 209–230.
- Ruban, A.V. (2016) Nonphotochemical chlorophyll fluorescence quenching: mechanism and effectiveness in protecting plants from photodamage. *Plant Physiology*, **170**, 1903–1916.
- Rühle, T., Dann, M., Reiter, B., Schünemann, D., Naranjo, B., Penzler, J.F. *et al.* (2021) PGRL2 triggers degradation of PGR5 in the absence of PGRL1. *Nature Communications*, **12**, 3941.
- Saer, R.G. & Blankenship, R.E. (2017) Light harvesting in phototrophic bacteria: structure and function. *The Biochemical Journal*, **474**, 2107–2131.
- Salomé, P.A. & Merchant, S.S. (2019) A series of fortunate events: introducing *Chlamydomonas* as a reference organism. *The Plant Cell*, **31**, 1682–1707.
- Sanchez-Baracaldo, P. & Cardona, T. (2020) On the origin of oxygenic photosynthesis and cyanobacteria. *New Phytologist*, **225**, 1440–1446.
- Sandberg, T.E., Salazar, M.J., Weng, L.L., Palsson, B.O. & Feist, A.M. (2019) The emergence of adaptive laboratory evolution as an efficient tool for biological discovery and industrial biotechnology. *Metabolic Engineering*, **56**, 1–16.
- Sandmann, G., Albrecht, M., Schnurr, G., Knörzer, O. & Böger, P. (1999) The biotechnological potential and design of novel carotenoids by gene combination in *Escherichia coli*. *Trends in Biotechnology*, **17**, 233–237.
- Schmid, V.H. (2008) Light-harvesting complexes of vascular plants. *Cellular and Molecular Life Sciences*, **65**, 3619–3639.
- Sener, M., Strumpfer, J., Singharoy, A., Hunter, C.N. & Schulten, K. (2016) Overall energy conversion efficiency of a photosynthetic vesicle. *Elife*, **5**, e09541.
- Shi, T., Bibby, T.S., Jiang, L., Irwin, A.J. & Falkowski, P.G. (2005) Protein interactions limit the rate of evolution of photosynthetic genes in cyanobacteria. *Molecular Biology and Evolution*, **22**, 2179–2189.
- Simkin, A.J., McAusland, L., Lawson, T. & Raines, C.A. (2017) Overexpression of the RieskeFeS protein increases electron transport rates and biomass yield. *Plant Physiology*, **175**, 134–145.
- Treves, H., Murik, O., Kedem, I., Eisenstadt, D., Meir, S., Rogachev, I. *et al.* (2017) Metabolic flexibility underpins growth capabilities of the fastest growing alga. *Current Biology*, **27**(2559–2567), e2553.
- Treves, H., Raanan, H., Finkel, O.M., Berkowicz, S.M., Keren, N., Shotland, Y. *et al.* (2013) A newly isolated *Chlorella* sp. from desert sand crusts exhibits a unique resistance to excess light intensity. *FEMS Microbiology Ecology*, **86**, 373–380.
- Treves, H., Raanan, H., Kedem, I., Murik, O., Keren, N., Zer, H. *et al.* (2016) The mechanisms whereby the green alga *Chlorella ohadii*, isolated from desert soil crust, exhibits unparalleled photodamage resistance. *New Phytologist*, **210**, 1229–1243.
- Tringroho, J.P., Bečková, M., Shao, S., Yu, J., Zhao, Z., Murray, J.W. *et al.* (2020) Chlorophyll f synthesis by a super-roque photosystem II complex. *Nature Plants*, **6**, 238–244.
- Turmo, A., Gonzalez-Esquer, C.R. & Kerfeld, C.A. (2017) Carboxysomes: metabolic modules for CO<sub>2</sub> fixation. *FEMS Microbiology Letters*, **364**, fnx176.

- Ungerer, J., Wendt, K.E., Hendry, J.I., Maranas, C.D. & Pakrasi, H.B. (2018) Comparative genomics reveals the molecular determinants of rapid growth of the cyanobacterium *Synechococcus elongatus* UTEX 2973. *Proceedings of the National Academy of Sciences U S A*, **115**, E11761–E11770.
- Walker, D.A. (2009) Biofuels, facts, fantasy, and feasibility. *Journal of Applied Phycology*, **21**, 509–517.
- Wang, L.M., Shen, B.R., Li, B.D., Zhang, C.L., Lin, M., Tong, P.P. et al. (2020) A synthetic photorespiratory shortcut enhances photosynthesis to boost biomass and grain yield in rice. *Molecular Plant*, **13**, 1802–1815.
- Watanabe, M. & Ikeuchi, M. (2013) Phycobilisome: architecture of a light-harvesting supercomplex. *Photosynthesis Research*, **116**, 265–276.
- Wijffels, R.H. & Barbosa, M.J. (2010) An outlook on microalgal biofuels. *Science*, **329**, 796–799.
- Williamson, A., Conlan, B., Hillier, W. & Wydrzynski, T. (2011) The evolution of photosystem II: insights into the past and future. *Photosynthesis Research*, **107**, 71–86.
- Xu, H., Vavilin, D., Funk, C. & Vermaas, W. (2002) Small CAB-like proteins regulating tetrapyrrole biosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Molecular Biology*, **49**, 149–160.
- Yang, D., Park, S.Y. & Lee, S.Y. (2021) Production of rainbow colorants by metabolically engineered *Escherichia coli*. *Advanced Science*, **8**, 2100743.
- Yu, J.F., Knoppová, J., Michoux, F., Bialek, W., Cota, E., Shukla, M.K. et al. (2018) Ycf48 involved in the biogenesis of the oxygen-evolving photosystem II complex is a seven-bladed beta-propeller protein. *Proceedings of the National Academy of Sciences of the United States of America*, **115**, E7824–E7833.
- Zhu, X.G., Long, S.P. & Ort, D.R. (2008) What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Current Opinion in Biotechnology*, **19**, 153–159.
- Zhu, X.G., Long, S.P. & Ort, D.R. (2010) Improving photosynthetic efficiency for greater yield. *Annual Review of Plant Biology*, **61**, 235–261.