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Natural strategies for photosynthetic light harvesting

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Photosynthetic organisms are crucial for life on Earth as they provide food and oxygen and are at the basis of most energy resources. They have a large variety of light-harvesting strategies that allow them to live nearly everywhere where sunlight can penetrate. They have adapted their pigmentation to the spectral composition of light in their habitat, they acclimate to slowly varying light intensities and they rapidly respond to fast changes in light quality and quantity. This is particularly important for oxygen-producing organisms because an overdose of light in combination with oxygen can be lethal. Rapid progress is being made in understanding how different organisms maximize light harvesting and minimize deleterious effects. Here we summarize the latest findings and explain the main design principles used in nature. The available knowledge can be used for optimizing light harvesting in both natural and artificial photosynthesis to improve light-driven production processes.

The amount of sunlight reaching the surface of Earth during one day is in principle sufficient to support human activities for many years[1]. At the moment solar thermal collectors and solar panels use a small fraction of this light to produce heat and electricity. A much larger fraction is used by photosynthetic organisms such as plants and algae to produce energy-rich chemical bonds that enable the production of biomass, including all the food and feed on this planet. However, the contribution of photosynthesis to energy production is limited largely to its past performance that has led to our fossil fuels. At the moment large research efforts are ongoing with the goal to improve photosynthetic efficiency in living organisms and to obtain efficient artificial systems that mimic photosynthesis for energy production. In both cases a decisive part is played by light harvesting and its regulation, and they form the topic of this Perspective.

In all organisms performing oxygenic photosynthesis, the transformation of sunlight into chemical energy occurs in and around the thylakoid membranes, in a series of steps collectively called the 'light reactions' (**Fig. 1**). The key players, photosystems I and II (PSI and PSII), are large protein assemblies (0.6–1.2 kDa in plants) containing hundreds of pigments. These pigments (**Figs. 2** and **3**) are excited upon light absorption and transfer excitation energy to the reaction centers of PSI and PSII, where these excitations initiate charge separation. This leads to net linear electron flow from PSII to PSI via the cytochrome b_6f complex ($cytb_6f$), followed by the reduction of $NADP^+$ to NADPH. The electrochemical gradient that is concomitantly generated across the thylakoid membrane is used for the synthesis of ATP by the ATP synthase. Together, NADPH and ATP lie at the basis of all further chemical reactions of the organisms.

The reaction centers (RCs) where charge separation takes place are highly specialized and 'expensive' (low pigment density leading to little light absorption) pigment-protein complexes that set the chemistry in motion. The RC complexes of PSI and PSII contain several cofactors, including the pigments that function as primary electron donors. The RCs differ from each other[2,3] but, together with their core antenna system, they are extremely well conserved in all oxygen-evolving organisms[4]. The expensive RCs are surrounded by far cheaper (high pigment density) light-harvesting complexes, also called antennae, typically containing a few hundred pigments per RC[5,6]. The antennae can absorb solar photons of many different colors, substantially increasing the effective absorption cross-section of the RC. These light-harvesting complexes are crucial for the success of photosynthesis because light is dilute and even on a sunny day, a chlorophyll (Chl) (**Fig. 2a**,**b**) will absorb no more than one photon every 0.1 s (ref. 7). This means that without an antenna the RC would be inactive most of the time, which in the case of multielectron redox processes could also lead to loss of excitation energy. The capacity of light harvesting is thus crucial, especially in light-limited conditions, when the organism needs to harvest every available photon. In addition, as light quantity and quality vary substantially in different natural habitats, the antenna system represents a modular unit that can be designed ad hoc. Indeed, in contrast to the RC complexes, the light-harvesting complexes show a remarkable variability in pigment composition, pigment organization and antenna size in different natural environments[4,5,8]. Finally, the capture and storage of light is a delicate and hazardous business. Changes in light quantity and quality occur daily on timescales as short as seconds and can easily lead to overexcitation of the photosynthetic machinery, inducing photodamage or even leading to the death of the organism. Photosynthetic organisms are able to deal with most of these situations. How do they manage?

Here we will illustrate how both chemical variation and varying pigment-protein interactions lead to coverage of different parts of the solar spectrum, enabling adaptation to different light conditions. Despite their diversity, all antennae must perform within the thermodynamic and kinetics constraints dictated by the RCs, and we discuss how this is done in an efficient way, elaborating also on the process of excitation energy transfer (EET) and on the role of the proteins. The need for regulation and adaptation will be addressed together with recently discovered examples thereof. From the analysis of light harvesting in nature we extract basic design principles for efficient light harvesting, which can be used as guidelines for the construction of artificial antennae. Finally, we will briefly discuss how organisms might be modified in the future for improving light harvesting.

Primary electron donors dictate light-harvesting limits

The arrival of an excitation in the RC, typically within 100 ps after initial photon capture by the antenna, leads to very efficient electron transfer from primary donor to primary acceptor. The primary donor (P) in PSII is called P680, referring to the absorption

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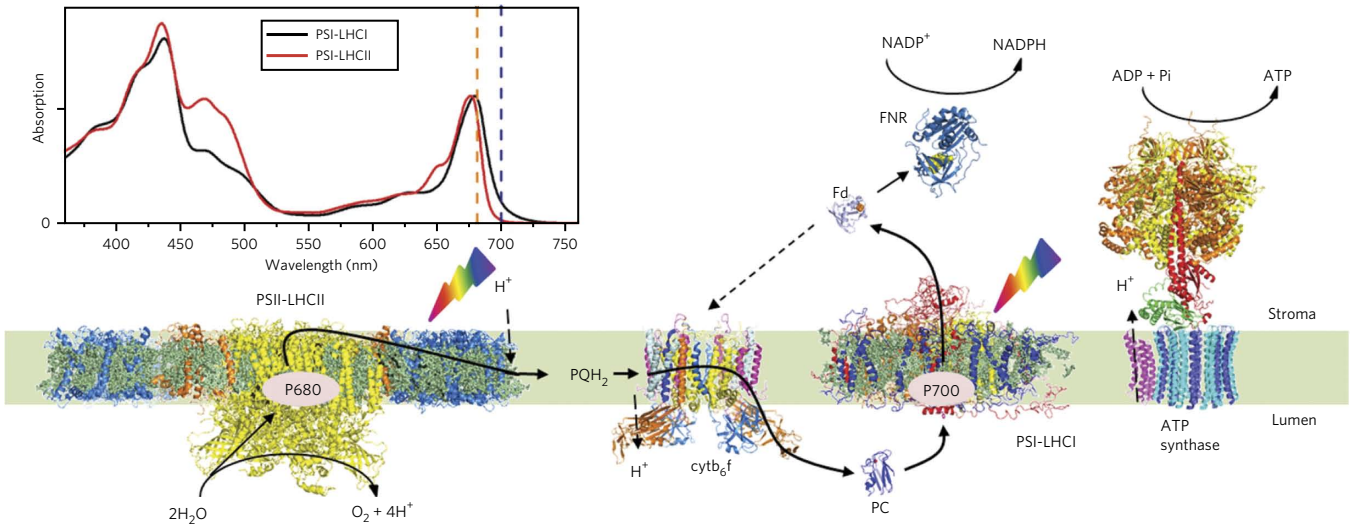
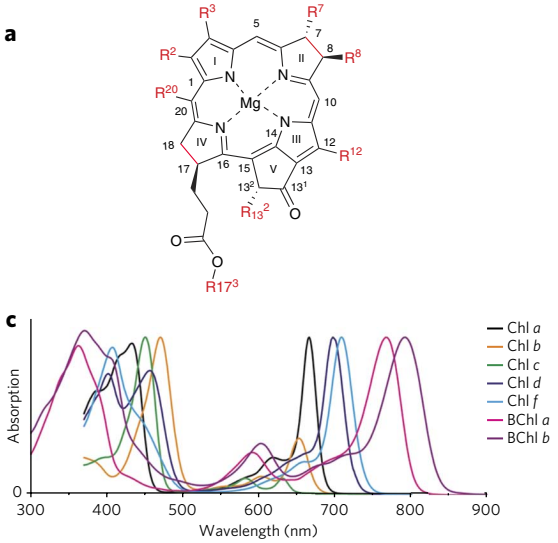


Figure 1 | A model of the photosynthetic membrane of higher plants with the four major multi-protein complexes that participate in the light reactions. Shown are PSII, the water-splitting system (here represented in its PSII-LHCII form); PSI (PSI-LHCI), the electrons of which are used to reduce $NADP^+$ to NADPH via ferredoxin (Fd) and ferredoxin- NADP- reductase (FNR); $cytb_6f$, plastoquinole (PQH_2)-plastocyanin (PC) oxido-reductase, which functionally connects the two photosystems, contributing to the generation of the electrochemical potential across the membrane; and the ATP synthase that uses the electrochemical gradient across the membrane to generate ATP. The dashed arrow from Fd to $cytb_6f$ indicates the cyclic electron transfer pathway, in which the electron cycles between PSI and $cytb_6f$, leading only to the production of ATP. Upon excitation by light P680 in PSII releases an electron, initiating the linear electron transfer pathway (solid arrows) and eventually leading to reduction of the primary donor P700 of PSI, which is oxidized after it has donated an electron to Fd after light excitation. The electron released from P680 is ultimately replaced by an electron extracted from water. Inset, the absorption spectra of PSI and PSII[56]. Dashed lines indicate the absorption wavelengths of the primary donors of PSII (680 nm, orange) and PSI (700 nm, blue).

maximum (680 nm, 175.9 kJ/mol) of the corresponding Chl *a* molecule. Although primary charge separation is more complex than originally thought, involving more pigments than only one donor and one acceptor, the primary donor is still considered to be a Chl *a* molecule[9]. To a first approximation, the excited-state energies of the antenna pigments should be the same or higher than that of P to energetically allow EET (**Box 1**, **Fig. 7**). This means that in the case of PSII the absorption maximum of most pigments should preferably lie below 680 nm. The energy required for charge separation in PSI is somewhat lower, with P700 (also a Chl *a* molecule) being the primary donor (700 nm, 170.9 kJ/mol), but with a different pigment-protein environment from that of P680. Antenna complexes feed both P680 and P700 with light-induced excitations created by photosynthetically active radiation (PAR) ranging from 400 to 700 nm. EET is often pictured as taking place in a funnel, following a downhill energy gradient with the RC at the bottom. However, it is important to realize that this picture is correct for various organisms but not for plants and green



b

Pigment	R2	R3	R7	R8	7–8 bond	R12	$R13^2$	17–18 bond	R20	$R17^3$
Chl *a*	CH_3	$CH{=}CH_2$	CH_3	C_2H_5	=	CH_3	$COOCH_3$	-	H	Phytol
Chl *b*	CH_3	$CH{=}CH_2$	CHO	C_2H_5	=	CH_3	$COOCH_3$	-	H	Phytol
Chl *c*1	CH_3	$CH{=}CH_2$	CH_3	C_2H_5	=	CH_3	$COOCH_3$	=	H	See note
Chl *c*2	CH_3	$CH{=}CH_2$	CH_3	$CH{=}CH_2$	=	CH_3	$COOCH_3$	=	H	See note
Chl *c*3	CH_3	$CH{=}CH_2$	$COOCH_3$	$CH{=}CH_2$	=	CH_3	$COOCH_3$	=	H	See note
Chl *d*	CH_3	CHO	CH_3	C_2H_5	=	CH_3	$COOCH_3$	-	H	Phytol
Chl *f*	CHO	C_2H_5	CH_3	C_2H_5	=	CH_3	$COOCH_3$	-	H	Phytol
BChl *a*	CH_3	$COCH_3$	CH_3	C_2H_5	-	CH_3	$COOCH_3$	-	H	Phytol
BChl *b*	CH_3	$COCH_3$	CH_3	$=CH{-}CH_3$	-	CH_3	$COOCH_3$	-	H	Phytol
BChl *c*	CH_3	$CHOH{-}CH_3$	CH_3	Various	=	Various	H	-	CH_3	Various
BChl *d*	CH_3	$CHOH{-}CH_3$	CH_3	Various	=	Various	H	-	H	Various
BChl *e*	CH_3	$CHOH{-}CH_3$	CHO	Various	=	C_2H_5	H	-	CH_3	Various

Figure 2 | Photosynthetic pigments: chlorophylls and bacteriochlorophylls. The main pigments used in natural light harvesting are substituted tetrapyrroles, including chlorophylls and bacteriochlorophylls. The absorption properties of these pigments vary owing to the extent of the conjugation and the number and nature of the substitutions, creating a 'palette' that covers the visible and infrared regions of the solar spectrum. (**a**) General structure of Chl. (**b**) The table illustrates the substitutions to the macrocycles that lead to the altered spectra[17,31] (Chls *c* have a –CH = CH-COOH attached to the ring at R17). (**c**) Absorption spectra of photosynthetic pigments in various solvents. The spectra of Chl *d* and *f* are from ref. 27; the spectra of BChl *a* and *b* are from ref. 103. The spectra are normalized to their absorption maxima.

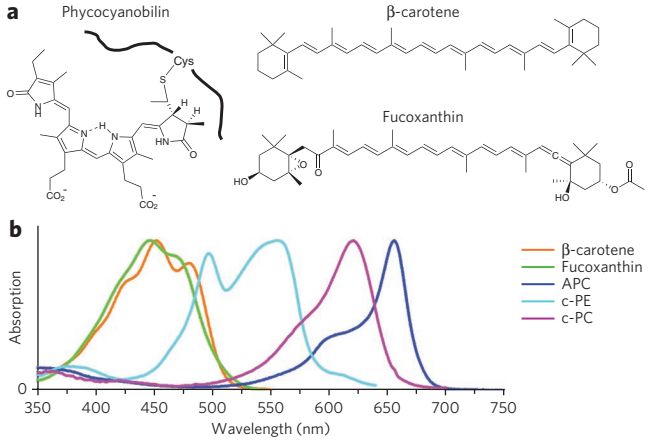


Figure 3 | Photosynthetic pigments: phycobilins and carotenoids. (**a**) Structure of phycocyanobilin, β-carotene and fucoxanthin. (**b**) Representative spectra of phycobilins and carotenoids, with spectra normalized to their absorption maxima. APC, allophycocyanin; c-PC, C-phycoyanin; c-PE, C-phycoerythrin.

algae, where most of the pigments are isoenergetic[10,11], as will be discussed below.

There is also a kinetic restriction for efficient light harvesting. After excitation, pigments remain in the excited state for a certain amount of time before losing the corresponding energy as heat, radiation or in other ways. A typical decay rate (k_d) for Chl *a* in solution is 0.2 ns^{-1}, and an average rate of $k_d = 0.5$ ns^{-1} was recently reported for the PSII antenna in plants[12]. Therefore, EET from the antenna to the RC and subsequent charge separation in the RC should occur with a much faster rate (rate of trapping or rate of photosynthesis, k_P) to guarantee a high quantum efficiency or yield (F_q) of charge separation. A useful approximation is $F_q = k_P/(k_P + k_d)$. Factors determining efficient transfer are discussed below and in **Box 1**. **Figure 4** shows the PSII organization in a 'supercomplex'[13], which is the form in which PSII is mainly organized in plants. In this complex, 300 Chls serve two RCs. The k_P value of this supercomplex is around 7 ns^{-1}; thus, the quantum efficiency is 0.93 (ref. 14). It should be realized that an increase of antenna size corresponds to more photons absorbed but also to a smaller trapping rate. This leads to an optimal antenna size, which in the case of plant PSII is on the order of several hundred isoenergetic pigments per RC in the absence of other restricting conditions[15].

Antenna building blocks: the pigments

Harvesting visible light. In order to absorb light, nature has chosen chlorophylls, substituted porphyrins containing a large array of conjugated double bonds, which assure absorption in the visible and near-infrared regions of the solar spectrum[16,17] (**Fig. 2b**). The Chl absorption properties are highly tunable, as the saturation of chemical bonds and the use of different substituents on the pyrrole rings strongly influence the energy levels[18–20]. Chl *a* seems to be present in all RCs in oxygenic photosynthesis and also to constitute a significant part of most antenna complexes. Chl *a* is well suited for light harvesting, having a relatively long excited-state lifetime (a low k_d value, which corresponds to a large F_q value, i.e., high efficiency) and the transition to the lowest excited state (S_1) is characterized by a strong dipole strength that scales linearly with the absorption coefficient and is extremely important for efficient EET. EET between two pigments is typically governed by dipole-dipole interaction, and the rate of transfer often scales with the product of their dipole strengths (**Box 1**). In addition to Chl *a*, with its lowest energy absorption maximum around 670 nm, a series of chlorophylls that harvest light in different regions of the solar spectrum (**Fig. 2b**) exist, providing the flexibility needed for adaptation to different natural habitats where light quality can differ substantially[8] (**Fig. 5**). In the light-harvesting complexes of plants and green algae (LHCs) (**Fig. 6a**), which constitute a widespread LHC family of many homologous proteins[21], Chl *a* is complemented by Chl *b*, whose lowest excited-state energy level (650 nm) lies above that of Chl *a* (**Box 1**). Chl *c* can be found in the antennae of other organisms, including diatoms and dinoflagellates[22]. It absorbs mainly around 450 nm, which is particularly advantageous in underwater conditions (see below). Chls *b* and *c* transfer their excitation energy typically within 1 ps to Chl *a*, and EET proceeds via Chl *a* (refs. 22,23). This is the recurring design principle in the antenna systems of plants and green algae: pigments covering a large part of the solar spectrum rapidly (within 1 ps) transfer their excitations to the lowest excited state of a nearby Chl *a* molecule, together forming a local funnel. EET then occurs among Chl *a* molecules, along a network of excitation pathways to the RC. These pathways are usually not interrupted by high-energy pigments which would substantially slow down EET[24]. The absorption bandwidths of the Chl *a* molecules constituting the excitation transfer pathway is typically in the order of k_bT (200 cm^{-1}), which is required for optimal efficiency and robustness, provided that the energetic coupling between the pigments is of a similar size[25].

The organism *Acaryochloris marina*, living in an ecological niche depleted in visible light by Chl *a*–containing organisms living above it and thereby relatively enriched in near-infrared light[26], mainly contains Chl *d* (95%) instead of Chl *a* (ref. 27). The low-energy absorption band of Chl *d* is also characterized by a large dipole strength, but its maximum is shifted 30 nm toward longer wavelengths (red-shifted) (**Fig. 2b**), which would make it virtually impossible to excite the primary electron donor P680 efficiently. However, the primary donor also absorbs at longer wavelengths, and most Chls in the PSII RC are replaced by Chls *d*, although there might still be one Chl *a* involved in charge separation[28]. It is apparently possible to split water efficiently with the excited-state energy of the primary donor corresponding to approximately 725 nm[28,29], which may be related to an altered midpoint potential of the primary acceptor[30]. Recently a new chlorophyll, Chl *f*, absorbing up to 750 nm *in vivo*, was discovered in a cyanobacterium hosted by stromatolites[31], and thus also living in a near-infrared-rich environment. However, the proportion of Chl *f* is only 10–15%[27], probably because higher percentages would significantly lower the excitation trapping efficiency, in particular for PSII with its high-energy primary donor P680. Indeed, EET can also occur from a low-energy pigment to a high-energy pigment, but the corresponding rate is lower than for the reverse process (**Box 1**). If k_P of a system is very high, then it can 'tolerate' several low-energy pigments to extend the absorption spectrum, although this slows down the trapping process, as happens, for instance, in PSI of plants[32]. If k_P is already small, the addition of many long-wavelength pigments may not be advantageous.

Harvesting near-infrared light. Organisms that perform oxygenic photosynthesis are mostly stuck with the limits imposed by P680 and P700, but there are very different organisms with different types of RCs, which do not split water and utilize light of much longer wavelengths. They use different light-harvesting systems, and perhaps the most remarkable of all are the chlorosomes of green bacteria, which are probably an early invention in biology[33] and can live in deep water where little light penetrates. They contain up to many hundreds of thousands of BacterioChl (BChl) *c*, BChl *d* and/or BChl *e* molecules[34] (**Fig. 2a**), which are self-organized into layered structures[33] and represent a primary source of inspiration for the building of artificial antennae[35]. The BChl *c*, *d* and *e* pigments, individually absorbing around 670 nm, are responsible for an intense broad absorption band around 740 nm, the red-shift being the result of strong pigment-pigment interactions[36]. However, 740-nm photons

Box 1 | Excitation energy transfer

How does excitation energy transfer (EET) take place?

When electrostatic interactions between pigments are relatively weak, i.e., their absorption spectra are unaltered, EET can be described by Förster resonance energy transfer (FRET), which is based on electric dipole-dipole interactions between chromophores: excitations hop from donor to acceptor pigments with a rate given by the Förster equation[94]. This Förster rate scales with R^{-6}, where R is the center-to-center distance between the interacting chromophores, but it also depends on the relative orientations of the pigments and the overlap of energy levels. Particularly important is the product of the dipole strengths of the corresponding electronic transitions of donor and acceptor. For practical reasons, the acceptor dipole strength is expressed in terms of the molar extinction coefficient for absorption and in terms of its radiative rate for the donor (the fluorescence yield divided by the excited-state lifetime in the absence of an acceptor). The average rate of transfer between two isoenergetic Chl *a* molecules at R = 1.5 nm with random orientations is 0.7–0.8 ps^{-1} in an environment with a refractive index of 1.5 (ref. 6). For transfer from a Chl *b* molecule with an absorption maximum at 650 nm (corresponding energy 3.060×10^{-19} J) to a Chl *a* molecule with an absorption maximum at 675 nm (energy 2.947×10^{-19} J) the rate k_{ba} is ~0.2 ps^{-1} (**Fig. 7**). The reverse rate k_{ab} follows from $k_{ba}/k_{ab} = \exp(-\Delta E/k_B T)$, where $\Delta E = 3.060 \times 10^{-19} - 2.947 \times 10^{-19}$ J. At room temperature (T = 293 K) the reverse rate k_{ab} is then a factor ~16 smaller. So uphill energy transfer is possible but can be considerably slower.

However, a large part of EET in photosynthesis proceeds on a timescale below 1 ps, where FRET theory is not applicable. Pigment interactions are far stronger, and energy levels and absorption spectra change. The interactions lead to new excitonic energy levels[6], shared between the strongly interacting molecules, which can even behave as one big super-molecule (**Fig. 7b**). The dipole strengths for the corresponding transitions depend on the relative orientations of the pigments. For EET it is in general favorable that the low-energy states correspond to high dipole strengths[6]. Well-known examples for such a distribution of dipole strengths are the LH1 and LH2 complexes in purple bacteria and the chlorosomes in green bacteria, where the main absorption bands lie substantially lower in energy than those of the noninteracting pigments. Excitations do not simply hop around, but they can coherently oscillate for a short amount of time between pigments, depending on excitation conditions. Excitation dynamics can be described by Redfield theory[95], which also takes into account the interactions with the environment, particularly its vibrations. These interactions are responsible for transitions between exciton levels and can be described quantitatively. Such transitions can usually lead to net movement of excitations.

Most photosynthetic complexes operate in the regime where excitations are only partly delocalized[6]. In that case the theoretical description of EET becomes far more difficult[96]. Advanced femtosecond techniques[97,98] have been developed and used, particularly during the last decade, to study EET in light-harvesting complexes with special interest for the time that excitations remain coherent. It appears that coherence can persist for 0.1–1.0 ps in various pigment-protein complexes[97–99] for temperatures ranging from 77 K to room temperature, although several conclusions in these studies have also been disputed[100,101]. Coherence has been suggested to be responsible for light-harvesting efficiencies close to 100% (see, for example, ref. 97). Remarkably, in chlorosomes where the pigment-pigment couplings are very large, rapid loss of excitonic coherence was reported together with incoherent diffusion on a sub-100-fs timescale[102]. Still these excitations can travel over many thousands of pigments within several tens of picoseconds[34], suggesting that coherence might not always be required to reach very high efficiencies and that strong interactions might be sufficient.

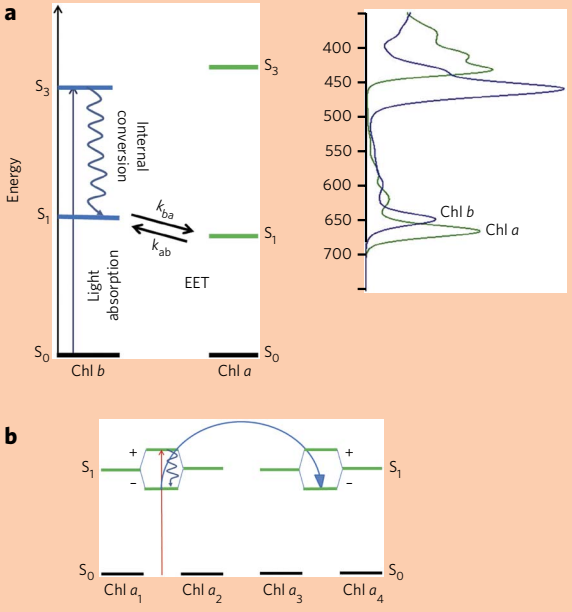


Figure 7 | Mechanisms of EET. (**a**) Simplified energy-level diagrams for Chls *a* and *b*, with the main levels corresponding to absorption peaks (right). In this case, after Chl *b* is excited by 'blue' light that promotes the molecule from the ground state (S_0) to the third excited state (S_3), it rapidly relaxes to its first excited state (S_1) from which EET to Chl *a* can take place with rate k_{ba}. The reverse rate k_{ab} is slower because the transfer occurs energetically uphill. (**b**) Energy-level scheme for two pairs of strongly coupled Chl *a* molecules (so close together that they behave almost as one molecule) that have only weak coupling with each other. The strong coupling leads to two new exciton states (+ and −) that are shared by the molecules. In case of weak coupling between the two pairs, EET between them can be described with generalized Förster theory[11,104]. For efficient energy transfer the lowest exciton level should correspond to the highest dipole strength.

cannot penetrate the water very deeply, and the organisms must then rely on the strong carotenoid and BChl absorption bands at shorter wavelengths. Excitations rapidly end up in the lowest excited states of the BChl *c*, *d* and *e* pigments, and although these do not necessarily contribute to light absorption, they are responsible for EET. This large pigment pool is not directly connected to the RCs because this would create a thermodynamics problem[34]: simply speaking, an excitation would spend only a very small fraction of its time on a pigment that is close enough to the RC to allow efficient energy transfer. Therefore, green bacteria use a pool of BChl *a* molecules as intermediates in a funnel-like design: these pigments are lower in energy (absorption maxima 795–825 nm), and they are present in much lower numbers and concentrate the excitations near the RCs. BChl *a* is also the pigment of choice in many purple bacteria and, like Chl *a*, also has very favorable EET properties. The best-studied BChl *a*–containing light-harvesting system is the LH2 complex (**Fig. 6b**). It contains two broad absorption bands in the near infrared, at 800 and 850 nm. The LH2 complexes transfer excitation energy to

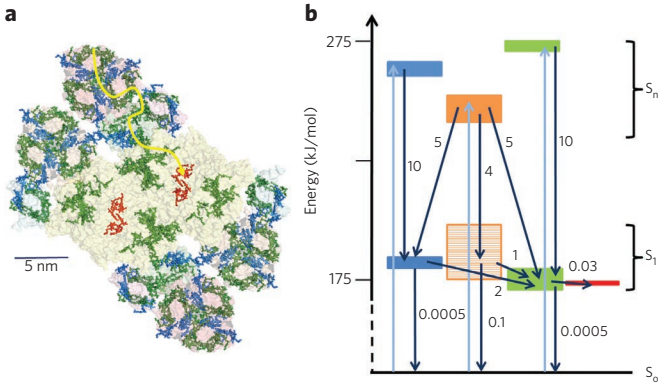


Figure 4 | Role of the antenna complexes. (**a**) Schematic representation of a PSII supercomplex in plants[13]; proteins of the core complex are shown in light yellow and the LHCs in light pink, green and blue. Chls *a* are in green and Chls *b* in blue. Carotenoids are omitted. The light harvested by the carotenoids and Chl *b* is rapidly transferred to Chl *a* molecules (local funnel), typically within 1 ps, after which excitation transfer proceeds mainly via Chls *a* (yellow arrow) (**Box 1**) to the primary donor P680 (red) in a more or less random way, where it is used for charge separation. On average, charge separation occurs 150 ps after a photon is absorbed somewhere in this supercomplex[23]. (**b**) Simplified energy-level diagram of the pigments in the PSII supercomplex. The color coding is as in **a** and carotenoids are shown in orange. The light blue arrows indicate excitation by a photon. The numbers near the arrows are the approximate rate constants of the processes (in ps^{-1}). The dashed orange box represents the energy range of the carotenoid S_1 state, which is not known precisely and varies for different carotenoids.

BChl *a*–containing LH1 complexes, peaking around 875 nm, which surround and feed the RCs with excitations[37,38], constituting another example of an overall funnel in which excitations are concentrated near the RC. It is important to note that the bands at 800, 850 and 875 nm originate from BChl *a*, showing that the absorption can be tuned extensively by modulating pigment-protein and pigment-pigment interactions (see below). Finally, it is worth mentioning that some purple bacteria contain BChl *b* instead of BChl *a*, which can lead to absorption even beyond 1,000 nm[39].

Filling the green gap. Chlorophylls (like modified porphyrins)[19,20] show little absorption in the region of 500–600 nm, leading to the 'green gap', which is responsible for the green color of most leaves and green algae. In water, at depths of 10 m the amount of transmitted light above 600 nm is almost negligible, meaning that red Chl absorption cannot be used and the presence of a green gap would allow absorption only of photons with wavelengths below 500 nm. Organisms that live underwater use special carotenoids and phycobilins (**Fig. 3a**,**b**) to fill the gap[7]. Diatoms, for instance, contain high amounts of fucoxanthin[40] (**Fig. 3a**). But carotenoids have a strong limitation: their excited-state lifetime is very short, typically on the order of 10 ps, which means that excitations are almost immediately lost if left unchecked. However, they are nearly always in close contact with Chls, thereby allowing EET to Chl within 1 ps[41], after which excitations are stabilized for several nanoseconds. An interesting example is the peridinin-chlorophyll protein (PCP), present in dinoflagellates (**Fig. 6c**). It contains eight peridinin carotenoids surrounding only two Chl molecules[42], which is an efficient way of buying (excitation) time: whereas the peridinins are responsible for most of the light absorption, the Chls subsequently transport the excitation energy to the RCs. At the same time carotenoids also provide photoprotection: Chl excitations can lead to dangerous triplets, causing formation of reactive oxygen species (ROS), which can be lethal for the organism. Carotenoids are highly efficient quenchers of Chl triplets when they are in direct contact with the Chls, and the overlap of their wavefunctions allows exchange of electrons and quenching of triplets[43]. Indeed most Chls *a* in the antenna are in direct contact with a carotenoid.

The other pigments used to cover the green gap are phycobilins, open tetrapyrroles that absorb between 550 and 680 nm (**Fig. 3**). They are the chromophores of light-harvesting phycobilisomes in cyanobacteria and red algae, which are large hemispherical antenna structures containing many hundreds of phycobilins[44] (a subunit of the phycobilisomes is shown in **Fig. 6d**). Phycobilisomes form a funnel with a decrease in excited-state energy toward their center, where several low-energy pigments deliver excitations to the RCs of either PSI or PSII[44].

Antenna building blocks: the protein

With the major exception of chlorosomes, most light-harvesting pigments are associated with proteins in the photosynthetic apparatus. The natural variation of the protein scaffold is very large (a gallery of different antenna complexes is presented in **Fig. 6**). In most cases antennae are integrated in the photosynthetic membranes (**Fig. 6a**–**c**), but there are also water-soluble antennae (**Fig. 6d**) that are associated with the membrane. The protein mass, structure and pigment/protein ratio differ substantially for different antenna families. Antennae should, ideally, have a high pigment/protein ratio, thus lowering the cost of protein synthesis and improving the rate of EET (**Box 1**). This is particularly well achieved by the LHC family of plants and green algae[23], where 25 kilodaltons (kDa) of protein coordinates 15 kDa of pigment (**Fig. 6a**).

The role of the protein is to organize the pigments close together and in the correct orientation to facilitate EET (**Box 1**) and photoprotection. This means that in general the protein is organizing the pigment to form a local funnel, avoiding energy gaps and especially avoiding concentration quenching: Chls in solution at concentrations similar to those present in pigment-protein complexes (up to 0.5 M) are heavily quenched[45], which would negatively influence the light-harvesting efficiency. In most antenna complexes the average center-to-center distance between neighboring Chls is 10 Å (see, for example, refs. 2 and 46). Proteins also provide vibrations of the right frequency that can help bridge energy gaps between excitonic states (**Box 1** and **Fig. 7b**), thus facilitating EET[25].

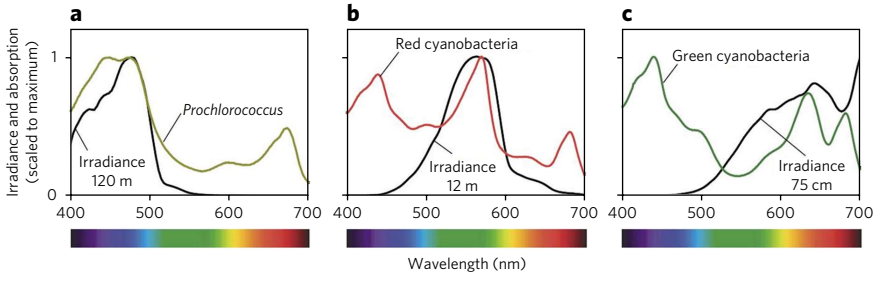


Figure 5 | Examples of adaptation. Absorption spectra of photosynthetic organisms (in color) and the available light spectrum in their specific habitats underwater (black) at the indicated depths[8]. (**a**) *Prochlorococcus*, which lives at a depth of 120 m in the subtropical Pacific Ocean absorbs the available blue light using divinyl-chlorophyll *a* and *b*. (**b**) *Synechococcus* living at 12 m depth in the Baltic Sea absorbs the available green light using phycoerythrin. (**c**) Green cyanobacteria living at 75 cm depth in Lake Groote Moost in The Netherlands absorb the available red light using phycocyanin (peak at 635 nm) and Chl *a* (peak at 680 nm).

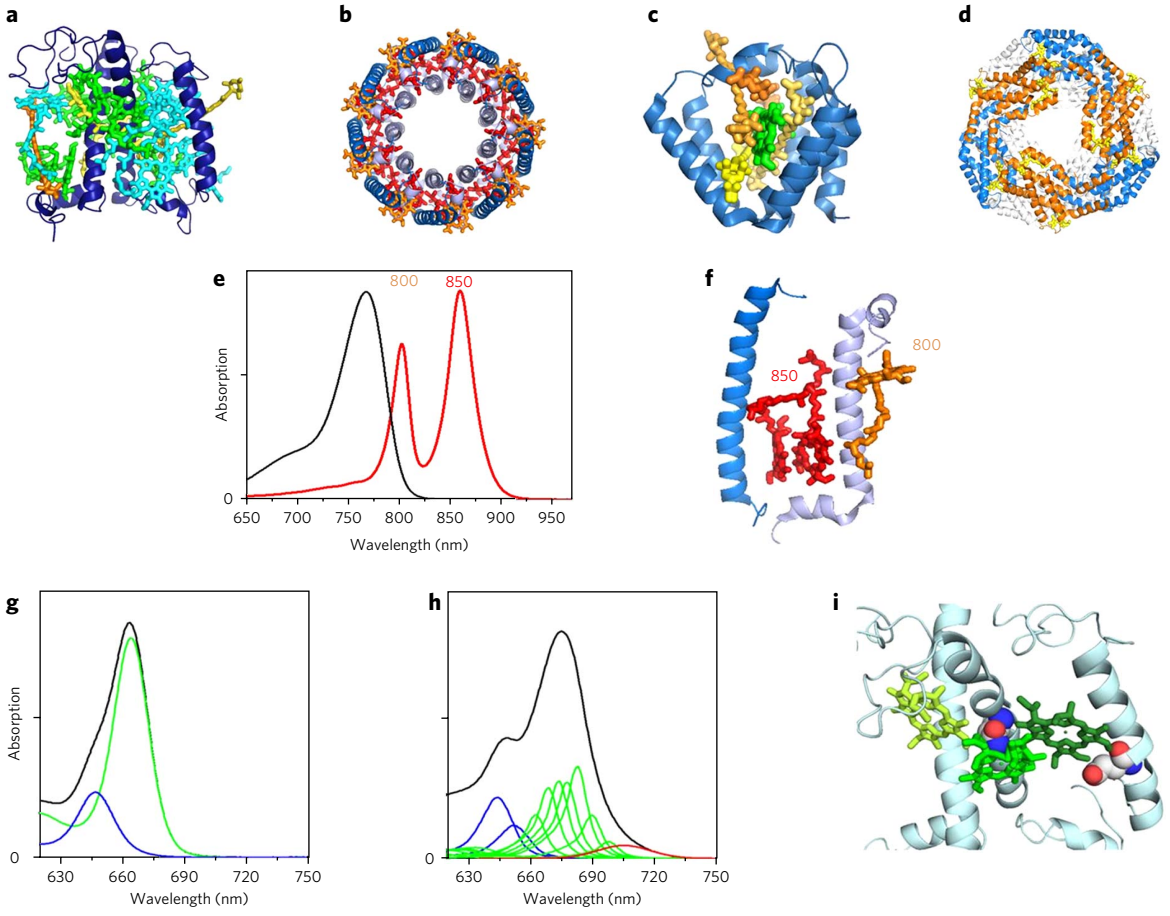


Figure 6 | Light-harvesting complexes and tuning of the pigment spectra. (**a**) LHCII of spinach (PDB 1RWT)[46]; blue, apoprotein; green, Chl *a*; cyan: Chl *b*; yellow and orange: carotenoids. (**b**) LH2 of *Rps. Acidophila*[37] (PDB 1NKZ) composed of nine times two subunits (α and β in blue and gray, respectively); BChl *a*-800, orange; BChl *a*-850, red. (**c**) Part of PCP[42] of *Amphidinium carterae* showing one Chl (green) in contact with four carotenoids (PDB 3IIS). (**d**) Hexamer (two trimers) of the C-phycocyanin component of the *S. elongatus* phycobilisome (PDB 4H0M)[105]; the top trimer is shown in color (blue, α-subunit; orange, β-subunit) and the top phycocyanobilins in yellow. (**e**) Absorption spectrum of BChl *a* in organic solvent (black) compared with that of BChl *a* in LH2 (red). (**f**) Organization of a LH2 subunit with three BChl *a* molecules[37]. The shift from 780 nm in solution to 800 nm in the protein is caused mainly by the protein environment of the orange BChl; the shift to 850 nm is also due largely to strong excitonic interactions (**Box 1**) between the red BChls. (**g**,**h**) Spectra of a mixture (black) of Chls *a* (green) and Chls *b* (blue) in organic solvent (**g**) and in the pigment-protein complex Lhca4 (**h**). The ratio of [Chl *a*] to [Chl *b*] is the same in both cases. (**i**) A model (based on the structure of LHCII) showing the three Chls responsible for the lowest-energy state of Lhca4 (the red Gaussian in **h**) and the two residues (represented as spheres with O in red, N in blue and C in gray) that strongly affect the absorption of this band[49,51].

BChls and Chls are associated with the proteins through the coordination of their central magnesium. The ligand is most often a histidine, although coordination via glutamine, asparagine, glutamic acid and even water molecules has been observed[46,47]. Efforts are ongoing to understand the design principle of the binding sites, also comparing the available crystal structures and constructing and studying mutated complexes[48,49]. The factors determining the association of the carotenoids with the protein are not fully understood: hydrophobic interactions in the transmembrane region can be responsible for their stable association with the protein, although the involvement of charged or polar residues close to their end rings has also been proposed[46]. At variance with the other pigments, phycobilins are covalently linked to the proteins, via a thioester bond to cysteine.

The protein also changes the absorption properties and can influence the excited-state lifetime of the pigments. This effect is extremely large in the case of the phycobilins, which are structurally very flexible. The binding to the protein strongly increases the extinction coefficient, the excited state lifetimes and the stability of the phycobilins, restricting their structural flexibility and thus transforming a bad chromophore into an excellent light harvester[16]. In general the protein strongly tunes the absorption wavelengths of the pigments and influences the width of their absorption bands. This effect can be direct, due to the protein environment surrounding the pigment (for example, presence of charged residues in close contact with the pigments[42] or formation of H-bonds between amino acid side chains and pigments[49,50]), or indirect, via the stabilization of a particular pigment conformation (in the case of phycobilins) or the modulation of pigment-pigment interactions[37] (**Fig. 6e**). In **Figure 6h** the absorption spectrum of Lhca4, a family member of LHCII, is described in terms of the contribution of the individual Chls. In particular, the Chl *a* molecules associated with this complex absorb between 660 and 705 nm. The large spread in absorption in comparison with the spectrum of Chl *a* in solution (**Fig. 6g**) is due mainly to the different environment of each Chl *a* in the protein. It has been shown that the lowest-energy absorption (represented by a broad Gaussian curve peaking at 705 nm) can be shifted to shorter and longer wavelengths by changing individual amino acids near the three interconnected Chls[49,51] (**Fig. 6i**).

The protein also determines the binding affinity for specific types of pigments. In the case of LHCs of plants and green algae, which

coordinate Chl *a* and *b*, the selectivity for Chl *b* is linked to the possibility of H-bond formation involving the Chl *b* CHO group[46,47], whereas the occupancy of the Chl *a* sites seems to be determined mainly by the availability of the pigments[52].

Acclimation or survival in a changing environment

Whereas adaptation to different environmental conditions has led to the development of antenna complexes with very different characteristics, acclimation requires fine tuning of the light-harvesting properties with different short- and long-term strategies[53,54]. Long-term changes in light quantity lead to modulation of the antenna size in practically all organisms, through regulation of expression and degradation of the antenna system[55–57]: in low light the number of excitations per RC can be increased by increasing the size of the antenna, and in high light wasteful saturation can be avoided by again reducing the size. In some cyanobacteria, long-term acclimation to different colors induces the synthesis of different pigments for an optimal match to growth light, a phenomenon called chromatic acclimation. The molecular mechanisms of its regulation are starting to be revealed[58] and might also provide interesting applications for optimizing light harvesting in other organisms.

Very often, changes in light quality and quantity occur on a timescale much faster than the one on which protein and pigment synthesis and degradation take place. In high light, surviving becomes imperative, and the organisms switch on protective mechanisms, dissipating a large part of the harvested light energy as heat to avoid ROS formation in a collection of processes called nonphotochemical quenching (NPQ)[59]. In general, this quenching relies on changes in pigment-pigment interactions, achieved by conformational changes of the proteins[60] or rapid chemical or structural modification of pigments[59]. In many eukaryotic organisms, members of the LHC family (PsbS[61] and LhcSR[62–64]) act as stress sensors, becoming protonated as a result of the low lumenal pH occurring during high light. They then undergo and/or induce in other LHCs a conformational change that leads to effective quenching of the excited-state energy[65–67] owing to Chl-carotenoid and/or Chl-Chl interactions[68–72]. At the same time, the low pH induces the conversion of the xanthophyll violaxanthin into zeaxanthin, which contributes to the quenching[73]. Cyanobacteria use a different mechanism: quenching is not regulated by the pH but through blue-green light absorption by a carotenoid in the orange carotenoid protein (OCP)[74,75]. Every time OCP absorbs a photon there is a finite possibility that it changes color and conformation and switches into an active red form that binds to the phycobilisome core, transforming 80% of the phycobilisome excitations into heat before they can reach the RCs[76]. This is a statistical process that happens with low probability, but in high-light conditions it occurs more frequently. In solution the protein can stay in the active form for more than 10 min[74], but *in vivo* the fluorescence recovery protein (FRP) is responsible for switching off the quenching[75].

Responses to changes in light quality are also necessary for optimal light harvesting. Because PSI and PSII work in series, their excitation should be balanced to optimize linear electron transport from water to NADPH while minimizing photodamage. The difference in absorption properties of the two photosystems (with PSI dominating above 690 nm and PSII around 475 nm and 650 nm; **Fig. 1**) makes it challenging to maintain this balance. A process known as state transitions, in which LHCII is thought to move from one photosystem to the other upon phosphorylation or dephosphorylation, furnishes an elegant solution to the problem[77]. It was recently shown that LHCII functions as an antenna for both photosystems, allowing the simultaneous regulation of their antenna size by changing the amount of two gene products upon long-term acclimation[56], and that phosphorylation is responsible for fine tuning, thereby evenly distributing the excitations between the two photosystems or inducing antenna detachment and concomitant quenching of part of it[78,79]. Although in the past, state transitions and NPQ were regarded as independent processes activated in different light regimes, recent results indicate that they may be strongly interconnected[79–81] and may even exploit the same mechanisms. The different mechanisms of light-harvesting regulation should then be considered as a set of highly integrated processes that have been optimized in concert.

The design of an antenna

Although the basic requirements for an efficient antenna may differ for natural, biohybrid and entirely artificial systems[82,83], in general, good coverage of the available light spectrum is needed in combination with fast EET that leads to efficient charge separation.

The coverage of a wide region of the solar spectrum can be achieved with many different pigments, but for efficient light harvesting the presence of uphill intermediates should be avoided, and EET pathways should be optimized. For this purpose, nature uses a limited number of pigments and a polymeric scaffold that acts as a smart matrix, allowing the control of the assembly in terms of affinity of the binding sites for different pigments, geometrical arrangement (correct orientation and high density without self-quenching) and tuning of the pigments' spectroscopic properties. In addition, the scaffold permits the switching on and off of light harvesting to respond to light fluctuations. For the design of optimal antennae, it is thus essential to achieve complete control of the smart matrix. Efforts in this direction are ongoing[48,49,84], but a full understanding is still required.

What is the best overall organization for efficient EET? Nature uses two basic designs to deliver excitations efficiently to the RCs. The first one makes use of the overall funnel principle: many, more or less isoenergetic, pigments exchange excitations in a high-energy pool, from which they cascade down in energy via one or more lower-energy pools to the RC. A concomitant decrease in the number of pigments per pool focuses the excitations near the RC. Without such focusing capacity, chlorosomes, for instance, would become very inefficient. This design works because the pigments involved have relatively long excited-state lifetimes, and the dipole strengths of the low-energy transitions are large, which is needed for fast EET.

The second strategy makes use of small local funnels, where excitations of high-energy pigments are rapidly transferred to nearby low-energy pigments (Chls *a* in plants and green algae), which subsequently transfer excitations to the RC with little energetic and spatial directionality. With this design, use can be made of pigments, such as carotenoids, that cover an important part of the solar spectrum but have short lifetimes and/or small dipole strengths for the absorption to the S_1 state. This design requires very short distances between the pigments involved in the local funnel to assure fast EET. The local-funnel concept has recently been applied for constructing biohybrid antennae[85]. Chromophores with largely varying absorption characteristics were covalently linked to newly introduced cysteine residues in a truncated polypeptide from a purple bacterium antenna. EET from these chromophores to BChl *a* bound to a histidine residue of the same polypeptide is followed by EET between the BChl *a* molecules in larger assemblies.

The implementation of both the overall and local funnel design in nature correspond to high quantum efficiencies, but a lot of energy is lost, as absorption occurs over a broad spectral region but only a (relatively small) part of the energy of the absorbed photons is ultimately used for charge separation. In artificial designs it would therefore be far more efficient to collect different spectral regions separately in different layers and use as much of the energy as possible (as in multi-junction solar cells), using the principles of efficient EET and focusing the excitations if needed.

How large should the antenna be? Although an increase in antenna size leads to more excitations, it decreases the quantum efficiency. If excitations migrate infinitely fast, the rate of photosynthesis k_p

is equal to the intrinsic rate of charge separation (k_{CS}) in the RC multiplied by the probability that the excitation is on the primary donor and not in the antenna. This probability decreases when the antenna size increases, although to a lesser extent for a deep funnel than a shallow one. The optimal antenna size thus is determined by k_{CS}. When EET is relatively slow, the optimal size is smaller (as for PSII in plants)[15].

How can the light-harvesting system respond to environmental fluctuations? Natural light harvesting is finely regulated at the membrane level. The capacity of switching light-harvesting complexes on and off requires the presence of a 'stress sensor' (severable protonable residues in plants and green algae and a 'photon counter' in cyanobacteria), integrated in a smart matrix. The stress leads to conformational changes that alter the organization of the light-harvesting system, enhancing particular pigment-pigment interactions that lead to quenching. A similar mechanism has been implemented in an artificial system, in which a photochromic control moiety is able, after photoisomerization, to quench porphyrin excited states in an artificial photosynthetic unit[86]. As in cyanobacteria, a certain fraction of the excitations induce NPQ. The level of NPQ depends also on the rate for back-switching to the unquenched state.

Improving light harvesting in living cells

It was recently discussed[87] that extending the PAR region of Chl *a*–containing organisms to 750 nm using Chl *d* and Chl *f* could increase the quantum yield of oxygenic photosynthesis by ~20%. This requires reduction of the number of Chls per photosystem to avoid saturation[87]. Antenna size reduction has been proposed for green algae, to allow a better light distribution in the mass culture[88], through reduction of the amount of wasted energy owing to NPQ in the top layers and increasing light penetration. Recently, antenna size regulation and/or reduction has also been proposed for plants, where it can be advantageous in the canopy[89]. Attempts to validate this concept are in progress, and the results are at present partially contradictory[90,91]. However, the effects of antenna truncation on EET and photoprotection still need to be evaluated because these factors can also strongly influence photosynthetic efficiency.

How could the spectral composition of organisms be changed to catch more photons? The addition of Chl *d* to organisms that use Chl *a* might require only one additional, but yet unidentified, enzyme[87]. However, the full replacement of Chl *a* by Chl *d* would increase the green gap, and additional pigments might be needed to refill the gap. The absorption spectrum can be further broadened by Chl *f*, but regulation of its amount, and probably also specific binding in the right sites, is needed, otherwise the efficiency, especially of PSII, might become too low. Regulation of Chl *f* synthesis can be inspired by the known regulation of Chl *b* synthesis[52,92]. All these changes require Chl *d* or *f* to replace Chl *a* in the photosynthetic complexes, which seems feasible, as the binding sites of LHCs are not completely selective for one type of Chl[52,93]. An interesting alternative is to broaden the spectrum by tuning the protein environment of Chls that are naturally present in the organisms[49,50], an approach that only requires small modifications of the photosynthetic apparatus. A combination of these methods might lead to a substantial increase of light-harvesting capacity in natural systems.

The future of light harvesting

Harvesting sunlight is vital for life on Earth and needs to be fully exploited to assure future food and energy supplies. To achieve this goal, full light-harvesting power must be used, meaning that both living organisms and artificial systems need to be improved. The study of photosynthetic organisms teaches us that the perfect light-harvesting system does not exist because light-harvesting performance is highly dependent on the environment, and the system is often balancing between maximizing photon capture and minimizing photodamage. Evolution has led to many different solutions in very diverse environments but have done so by combining a relative small number of building principles. These principles are summarized here, and although there is still a long way to go to fully understand all the molecular details, the way to improve light-harvesting system is definitively open.

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Competing financial interests

The authors declare no competing financial interests.

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