## Natural strategies for light harvesting in oxygenic photosynthesis: from excess light to shade

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# Summarizing discussion

### A glance into the methods used in the thesis

This thesis explores the flexibility of photosynthetic light harvesting using ultrafast spectroscopic techniques, such as transient absorption (TA) and time-resolved fluorescence (TRF). These techniques take advantage of the optical properties of photosynthetic pigments in the visible and near-infrared spectral regions. Their time resolution (100 fs for TA and 5 to 50 ps for TRF) allows us to access the timescales of the energy transfer, trapping and relaxation processes following light absorption, whereas their spectral resolution allows the identification of the pigment excited states involved in such processes. Besides the differences in time resolution that prevalently stem from the different detection systems, these two techniques display two other important differences. One is that TRF can only detect fluorescent pigments (such as chlorophylls, Chls, and bilins), whereas with TA is potentially more complete, since also dark excited states (those of carotenoids, for instance) can be observed. On the other hand, TRF setups are generally more robust and have a much higher sensitivity, which allows using significantly lower laser powers (and measuring times) as compared to TA. This feature is essential in the study of photosynthetic complexes, not only because these are particularly prone to photodamage, but also because multi-chromophoric systems are typically subject to power-dependent artifacts (singlet-singlet and singlet-triplet annihilation). The latter ones occur when multiple excitations are simultaneously produced in the same complex and their probability increases with the cross-section (i.e. the number of energetically connected pigments). These are among the main reasons why TRF is still the technique of choice for most measurements on photosynthetic samples.

While spectroscopy is the common thread of the entire thesis, other experimental and theoretical methodologies are used to obtain more insights on the systems of interest. In chapters 2, 3, and 4, which focus on the minor antenna of plants CP29, the data related to the wild-type (WT) protein are consistently compared to those of two Chl knock-out (KO) mutants to disentangle the contribution of specific pigments to the observed physical processes. Global analysis of time-resolved spectroscopic data is also used in all chapters to gain a basic knowledge of the timescales and spectral changes associated with the excited-state dynamics. Some specific cases required also more sophisticated data analysis techniques. In chapters 3 and 6, for instance, target analysis is used to relate the experimental data to the dynamics of certain pigment excited states by testing specific kinetic models, which is required to conclude about the underlying molecular

mechanisms. Both the global and target analyses described so far, despite the different levels of complexity, implicitly assume a first-order kinetics, i.e. the time evolution of the excited states can be described by rising and decaying exponentials. This assumption might be too strong in very large systems, such as antenna aggregates, where the presence of hundreds to thousand nearly iso-energetic pigments translates into a diffusive regime of energy transfer and in non-exponential kinetics[219]. This is why in chapter 4, which addresses the quenching mechanism of CP29 aggregates, we use a different data analysis technique - multivariate curve resolution - to extract the signatures of the various fluorescent states participating in the excited-state dynamics from the TRF data. With this method, the spectrum and kinetic profile of each component can be resolved without postulating an exponential model, which would be inappropriate for describing the data. Besides spectroscopy and data analysis, chapters 2 and 4 also employ simulations based on different levels of theory (see introduction).

**Part I. The heterogeneous landscape of the antenna complexes of plants.**

Excluding the introductory chapter, the first half of the thesis (chapters 2, 3, and 4) explores the heterogeneous energy landscape of plant light-harvesting complexes (LHCs). While most *in vitro* studies on LHCs have focused on trimeric LHCII, which is by far the most abundant LHC in the membrane, we deliberately chose to address the monomeric antenna CP29 for a number of reasons. The first one is the central role played by Chl KO mutants in the first chapters for highlighting the importance of specific Chls in light harvesting and quenching. Currently, it is only possible to perform *in vivo* site-directed mutagenesis for the monomeric antennae[288,394,395], but not for trimeric LHCII, which is the product of multiple genes. This condition is necessary to design and purify mutant complexes with a native pigment content. A second reason is that the exciton structure of LHCII has been intensively studied for two decades[275,283,286,287], assisted by an over 15-years old high resolution crystal structure[116]. Conversely, the lack of structural data has made the energy landscape of the minor antennae much less accessible. More recently, however, various cryo-electron microscopy (EM) structures of the photosystem II (PSII) supercomplex have revealed the detailed pigment organization of all LHCs, including the minor antennae[38,118,278]. These structures have confirmed that, as a consequence of the high structural homology, most chlorophyll and carotenoid binding sites are highly conserved in all LHCs. At the same time, a question arises whether some differences in the pigment energy landscape exist that justify the specific position and organization of each LHC in the supercomplex and facilitate energy transfer towards the reaction centers (RCs).

**Chapter 2.** In chapter 2 we address these points by reconstructing the excitonic structure of the Chls of CP29 and comparing it to that of the well-characterized LHCII. CP29 occupies a strategic position in PSII, bridging the peripheral antennae to the core. We found that this antenna has an overall highly conserved exciton structure very similar to that of LHCII. However, besides the Chl 610-611-612 cluster, which is believed to carry

the lowest energy states in all LHCs of PSII (LHCbs), CP29 also possesses an additional cluster of low-energy Chls, Chl 602-603-609. The organization of CP29 in PSII shows that, while the Chl 610-611-612 cluster faces the terminal emitters of peripheral LHCII, the Chl 602-603-609 cluster is connected to the core antenna CP47. Such a configuration allows CP29 to efficiently work as a conduit in the energy flux from the periphery to the core of PSII. The differences in the low-energy Chls of CP29 and LHCII possibly originate from the slightly different binding pocket associated with the Chl 602–603–609 cluster in these two complexes. The results of this chapter clarify how the LHC architecture can count on a range of common motifs that ensure robust and efficient light harvesting but is also flexible enough to allow the fine tuning of the energy landscape and, consequently, the carrying out of specific functions. This combination of robustness and tunability can be advantageous for designing artificial photosynthetic units that concentrate solar energies for other purposes. A recommendation for future studies is to extend the same approach to the other minor antennae, CP26 and CP24, whose structure is also available, in order to get a full energetic picture of the PSII supercomplex.

**Chapter 3.** It is widely accepted that the LHCs regulate the level of excitations in the membrane by switching from a long-lived state functional for light-harvesting to a short-lived (quenched) state that dissipates the absorbed energy as heat. However, the molecular mechanism of quenching and the nature of the photoprotective switch are still highly debated. One of the main reasons for this controversy is the challenging nature of the samples under study. The high complexity of the thylakoid membrane restricts the applicability of most spectroscopic studies to *in vitro* systems, where the number of variables into play is drastically reduced. However, isolated LHCs are prevalently in their light-harvesting state[224], which hinders the identification of their photoprotective mechanism. In contrast, other *in vitro* conditions such as LHC clustering show an increased level of quenching but introduce additional experimental problems. One of them is the higher chance of incurring in power-dependent artifacts, particularly in the case of TA measurements, as they typically require high excitation powers[235,321]. We face this challenge in chapter 3 by investigating the quenching mechanism in monomeric detergent-solubilized CP29. This was done by taking advantage of a substantial subpopulation of quenched complexes (about 16%, with a lifetime of less than 100 ps) that is in equilibrium with the majority of long-lived complexes. This property of CP29 makes it particularly suited for this study, whereas a similar approach would be more difficult to apply to detergent-solubilized trimeric LHCII, whose light-harvesting state is overwhelming. Using a target kinetic model, we were able to extract the spectroscopic signatures associated with the quenching mechanism and assign it to excitation energy transfer from the Chls to a dark state of a nearby xanthophyll. Furthermore, a comparison between the quenching behavior of the WT complex and of some Chl deficient mutants reveals that one of the low-energy Chls, Chl 612, is involved in the mechanism of energy dissipation. This implies that the carotenoid responsible for the observed quenching is the L1 lutein.

One of the most intriguing aspects of this mechanism is the structural basis of the photoprotective switch, which we can only speculate about based on the current data. The question is how can the rate of energy transfer between a Chl and a Car change so drastically when the two pigments are tightly bound to the same rather rigid binding pocket? And what is the physical origin of the Car excited state responsible for the quenching? There are several elements that can influence the energetic connectivity between the Chl $Q_y$ energy donor and the Car dark state acceptor and, consequently, the extent of quenching[239,396]. First of all, a change in the relative transition energies of the two states, which is the determining factor according to the 'gear-shift' model[42], can alter the directionality of energy transfer, either from Car to Chl or from Chl to Car. In the former case, the Car dark state would be functional for light-harvesting, in the latter for quenching. Since the two-photon excitation spectrum of the lutein $S_1$ state in organic solvents is very broad[397], a very weak dependence of its spectral overlap with the Chl $Q_y$ can be expected over a large range of $S_1$ average excitation energies. For such a reason, the actual relevance of this parameter for the photoprotective switch has been ruled out by a number of theoretical studies[239,396], which assigned instead a predominant role to the tuning of the Chl-Car electronic coupling. Due to the constraints imposed by the binding pocket, it is very unlikely that the distance between the two chromophores can be relevant for this regulation. This leaves two main factors: (i) a fine-tuning of the relative orientation between pigments, as supported by a work on LHCs that combines molecular dynamics (MD) simulations and semi-empirical quantum mechanical (QM) calculations[239] and (ii) the transition dipole strength of the Car dark state. A recent study has shown that while the Car lowest transition remains essentially dark even after symmetry breaking, structural distortions that lead to a change in bond length alternation of the polyene chain can possibly increase its dipole strength[398]. Potentially effective distortions include rotations of the partially conjugated end-rings, which are also the most flexible elements of the centrally bound xanthophylls according to MD simulations[19,135]. Interestingly, our target analysis of CP29 TA data reveals that the quencher has a lifetime typical of a carotenoid singlet excited state. However, its spectrum is blue-shifted with respect to $S_1$ and resembles that of the triplet state, supporting its assignment to a Car $S^*$ state[20]. Based on analogue spectroscopic signatures, a similar quenching mechanism has also been proposed for a variant LHCII that binds astaxanthin as the only xanthophyll and is constitutively quenched[19]. In this view, $S^*$ represents a distinct excited state of lutein 1 and the spectral difference with respect to $S_1$ argues for a different electronic structure, which we tentatively assign to a subtle difference in the ground state geometry of the Car (possibly localized at the end-rings)[18,53]. This distortion might be responsible for an increase in the transition dipole of $S^*$ relative to $S_1$ and, possibly, for a different relative orientation with respect to Chl 612, reflecting into a substantially larger coupling between these two pigments[239]. Interestingly, the blue-shift of the $S^*$-$S_N$ transition with respect to $S_1$-$S_N$ also supports the idea that $S^*$ is lower in energy than $S_1$. This might increase the directionality of energy transfer from the Chls to the Car, in agreement with a recent theoretical study which re-evaluated the importance of the Car excited-state energy in the

regulation of quenching[399]. A full understanding of the structural basis of the photoprotective switch can only result from an improvement in the level of theory (both QM and MD methods). One of the main difficulties is that the QM calculations involve the excited-state properties of Car dark states, which so far have been shown to be strongly model-dependent. Two other missing pieces are the exact energy and lineshape of the Car dark states in the protein, which so far have been only extrapolated from experimental data in solution. Furthermore, the sampling of the conformational space of the LHCs via MD simulations needs to be enhanced to search for other possible pigment configurations.

Another important outcome of this work, which is further investigated in the following chapter, is that the extent of quenching in monomeric CP29 is drastically reduced but not fully suppressed when Chl 612 is lost. This implies that, although the Chl 612 – lutein 1 pair is the main quenching site, other pigment clusters can also function as quenchers. An interesting question is whether in the KO612 mutant lutein 1 remains the quencher (accepting excitations from a different Chl) or the quenching is taken over by a different carotenoid, violaxanthin in L2, for instance. The vanishingly small amount of quenched complexes prevents further investigation in the KO612 mutant of CP29, but it would be interesting to test these hypotheses in other LHCs.

**Chapter 4.** In chapter 3, we found that monomeric CP29 can access different fluorescent states by virtue of its vast conformational landscape and we identified the mechanism responsible for energy dissipation in the quenched state. The next step is to understand how this heterogeneous landscape is regulated in different environments, which is particularly relevant if we wish to transfer these results to the *in vivo* situation. In order to address these points and study LHCs in more physiologically relevant conditions, chapter 4 focuses on LHC clusters *in vitro*. This condition is of particular interest because while isolated LHCs are prevalently in a light-harvesting conformation, LHC clusters are a highly quenched sample, which has led to the suggestion that aggregation is responsible for quenching *in vivo*[244]. However, the origin of the quenching and its regulation in these LHC clusters is not yet understood. Our TRF experiments at room and cryogenic temperature show that the oligomers represent a very heterogeneous ensemble where the CP29 units can switch between various fluorescent states with different lifetimes and spectra, similar to what previously observed in single protein experiments[163,229]. A detailed kinetic model also extracts other properties of these states, such as their lifetime, occurrence, and trapping strength. We found that most complexes have a spectrum identical to that of monomeric CP29. A fraction of the latter complexes is strongly quenched, with a lifetime and trapping strength compatible with the quenching mechanism described in chapter 3 for CP29 monomers. This suggests that the quenching mechanism adopted by CP29 in the monomeric and oligomeric state is the same (Chl to Car energy transfer). The oligomers also contain a sub-population of long-lived red-shifted complexes that are not involved int quenching, but compete with the quenched complexes in the energy trapping, particularly at low temperatures. This explains the temperature-dependent far-red enhancement typically observed in the emission of LHC

clusters. Our quantification indicates that, while clustering can stabilize the quenched conformation to a certain degree, quenching in the aggregates is largely achieved by energetically connecting complexes that span the same light-harvesting and dissipative states adopted when isolated. Finally, by using Chl KO mutants of CP29, we show that LHCs have multiple pigment clusters responsible for quenching and for red-shifted states, which is another proof of the robustness of their architecture.

For the reasons discussed above, the findings of this and the previous chapters are based on measurements on CP29. This minor antenna has been suggested to play a role in NPQ in the membrane[152,208,395], although other studies have assigned the most prominent role in NPQ to LHCII[201,203,206]. Anyway, whether or not CP29 has a direct role in NPQ *in vivo*, the high degree of structural homology with LHCII and other antennae suggests that our discoveries can be extended to the other members of the LHC family. Furthermore, a similar model used to describe the quenching of CP29 oligomers *in vitro* (chapter 4) has been successfully applied to LHCII-enriched thylakoid membranes[245]. This strongly suggests that the same type of quenching mechanism observed in CP29 monomers and oligomers should be active *in vivo*. To summarize, chapters 3 and 4 provide new insights on the heterogeneous landscape of plant LHCs and on the effect of protein clustering on such landscape. What is missing at the moment is a full understanding of how the photoprotective switch is regulated in the membrane. First of all, chapter 4 shows that the lifetime of CP29 drops from 3 ns to 100-200 ps upon clustering. This is very different from what is typically found in the membrane, where the protein density is also very high but the average fluorescence lifetime in the absence of NPQ is substantially longer (1-2 ns)[56–58,122]. Since we have proved that even a small amount of short-lived complexes can efficiently quench an entire LHC cluster, it follows that the PSII antenna needs to avoid the formation of any strong quencher to allow for efficient light harvesting. At the same time, switching a few complexes to a dissipative state upon NPQ should be enough to protect the entire membrane from photodamage. But what makes this switch possible? Based on the results of chapters 3-4, it is plausible that the interaction of the outer antenna with PsbS – activated by PsbS protonation at the lumen – increases the probability of the LHCs to switch to a quenched conformation, while in light-harvesting conditions this probability is kept as low as possible. The photoprotective effect of the newly formed quenchers might be enhanced by protein-protein interactions, which increase the energetic connectivity with the other complexes and further stabilize the quenched conformation, as it is the case for LHC oligomers *in vitro*. To test this hypothesis, the structural and spectroscopic effects of the interaction between PsbS and the LHCs need to be investigated in detail, possibly in a native environment. Locating the docking site of PsbS in the PSII supercomplex (if any) can also help to identify the interaction partners of this protein.

**Part II. How to perform photosynthesis in the shade**

Oxygenic photosynthesis relies on Chl *a* as photochemically active pigment and needs visible light (wavelength up to 680 nm for PSII and 700 nm for PSI, representing the so called 'red limit') to power charge separation. Some recently discovered cyanobacteria, however, can acclimate to use far-red light (FRL, 700-800 nm) for photosynthesis by remodeling their photosynthetic apparatus and synthesizing the far-red absorbing Chl *f* (and, to a minor extent, Chl *d*, while Chl *a* remains the dominant Chl)[2]. This acclimation process is named FaRLiP (far-red light induced photoacclimation) and has become a very hot topic in plant research also because of its potential to increase crop productivity. Indeed, the spectrum of the available light under a dense plant canopy is highly enriched in far-red photons[4], which cannot be absorbed efficiently by the pigments present in plants. As a result, the insertion of Chl *f* in plant photosynthetic units could result in higher crop yields due to the increased light utilization. The implementation of the latter approach requires, however, a clear understanding of the mechanisms of light-harvesting and photochemistry in the presence of red-shifted Chls. Due to its different energetics, Chl *f* could in principle alter the excited-state dynamics in the photosystems and thus undermine their performances. Chapters 5 and 6 are therefore devoted to the investigation of the photosystems expressed in FRL (FRL-photosystems) and containing Chl *f*. These are compared to the "canonical" photosystems expressed in white light (WL) and containing Chl *a* only. FaRLiP also involves the expression of red-shifted phycobiliproteins (PBPs), which are the subject of chapter 7.

**Chapter 5.** In chapter 5, we performed TRF measurements on intact cells and isolated photosystems of two cyanobacterial strains able to perform FaRLiP. In both their FRL-PSI and FRL-PSII, the few red-shifted pigments are shown to harvest excitations from the nearby Chls *a* very rapidly (10-20 ps). However, energy transfer between the Chls *f* and the overall photochemistry are significantly slower than in the WL-units. The fluorescence lifetime rises from 30-40 ps to 130-140 ps for PSI and from less than 100 ps to over 500 ps for PSII upon Chl *f* insertion. While this has only a minor impact on the photochemical quantum yield of PSI, it decreases that of PSII substantially (from about 70-80% to 40% *in vivo*). In the case of FRL-PSII, we also investigated the reasons for the reduced rate of photochemistry (we remind to chapter 6 for a more insightful discussion about PSI). One reason for the slower trapping is the reduced driving force of charge separation caused by the lowering of the energy of the primary electron donor (from 680 nm in WL-PSII to above 725 nm in FRL-PSII[253]). This hypothesis is confirmed by the fact that the PSII of *Acaryochloris marina* (AM), where the primary donor is a Chl *d* that is red-shifted to a similar extent[270], also displays slower photochemistry than canonical PSII. These findings represent an elegant confirmation of the validity of the so-called red limit, which stems from the energetic requirement of the photosynthetic electron transfer reactions[35]. This limit can be approached to be able to harvest FRL, but at the expense of the photochemical rate and efficiency (not to mention the higher chance to incur into harmful charge recombination)[253]. Despite the similar energy of the primary donor, however, charge separation by FRL-PSII is substantially slower than in the PSII of AM. The most

likely interpretation for this difference is the weak energetic connectivity between the Chls *f* in the antenna and the RC pigments of FRL-PSII, which results in a migration-limited trapping (this point is further discussed in chapter 7).

Despite their reduced performances, in environments enriched in FRL (where Chl *a* barely absorbs), the FRL- photosystems still display a higher energy output than those containing only Chl *a*. This proves that the use of Chl *f* represents a promising strategy for extending the photosynthetic active spectrum in other species (e.g. algae and crops). However, our results show that the positioning of the Chls *f* in the antenna can play a crucial role in determining the efficiency of light harvesting and must be carefully taken into account in the design of new photosynthetic units. Another crucial element affecting the photochemical yields is the energetics of the RC pigments. It is indeed clear that lowering the energy of the primary donor has a negative impact on the charge separation rate. However, a red-shifted RC might be indispensable for harvesting the excitations from the Chls *f* in the antenna, especially in the case of PSII, whose trapping strength is typically lower than PSI.

It is important to mention that, in this chapter, the fitness of the cyanobacterial cells is estimated exclusively from the fluorescence measurements. Since the photosynthetic performances also include other factors, it would be insightful to extend the comparison between WL- and FRL-acclimated cells to other parameters, such as the rate of $O_2$ production and that of $CO_2$ assimilation. Furthermore, while the efficiency of PSII photochemistry can be effectively calculated by comparing the fluorescence lifetime with open and closed RCs, for PSI photochemistry this possibility is precluded. The photochemical quantum yield of PSI is typically estimated by assuming a long lifetime (4 ns, for instance) in the absence of photochemistry. This assumption, however, might be too strong, especially when comparing WL- and FRL-PSI, which embed different pigments and might follow different energy relaxation pathways. An alternative way to accurately determine the efficiencies of the PSI complexes would be performing electrochemical measurements that directly detect the conversion of photons into electrons.

**Chapter 6.** In chapter 5 we show that Chl *f* insertion has the effect of slowing down the photochemistry and reducing its efficiency in both photosystems, which is at least partially a result of approaching the red-limit of oxygenic photosynthesis. In the case of FRL-PSI, however, a 3 to 4-fold increase in the excited-state lifetime seems a relatively small price to pay when considering that its Chls *f* can harvest photons up to 800 nm, 100 nm above the red limit. This corresponds to an uphill energy gap of about 200 meV, i.e. about 8 times the thermal energy at room temperature, which appears as an insurmountable barrier even for the most efficient reducing enzyme in nature. The most intuitive way to explain why 800-nm excitations can still drive photochemistry is that a Chl *f* is present in the RC, reducing the energy required for charge separation similarly to what happens in FRL-PSII. This has been proposed based on recent spectroscopic data[253,342], which have also reported that charge separation can be powered by 750 nm

photons even at cryogenic temperatures, when uphill energy transfer is blocked. Even more recently, however, structural data of FRL-PSI from two different FaRLiP strains have shown no evidence for Chls *f* in the RC[256,257], giving rise to a yet unresolved controversy about the identity of the RC pigments and the mechanism of charge separation.

Here we address this challenge with a comprehensive spectroscopic study of both WL- and FRL-PSI from four different cyanobacterial species. FRL-PSI contains two distinct Chl *f* pools emitting around 750 and 800 nm. A target analysis of the TRF data shows that both pools transfer energy to the RC pigments and suggests that the slower photochemistry observed in FRL-PSI is mainly a consequence of the reduced energetic connectivity between the Chls *f* in the antenna and the RC. The rate of photochemistry remains, however, surprisingly high, and so does the efficiency of light conversion. We also provide further evidence for the photochemical quenching of 750-nm excitations at cryogenic temperatures, in line with previous spectroscopic measurements[253]. Careful quantitative analysis indicates that all these observations can only be explained by lowering the energy required for photochemistry in FRL-PSI, i.e. by 'breaking the red limit'. The reported structures of FRL-PSI, however, exclude the possibility that a low-energy Chl *f* is directly involved in photochemistry. We therefore propose that charge separation is initiated by a low-energy charge transfer state involving only Chls *a*, a mechanism that has been proposed to play a minor role also in canonical PSI[381]. This charge-transfer state provides an alternative trapping pathway for the low-energy excitations and circumvents the otherwise unfavorable uphill energy transfer of 100 nm to the canonical primary donor, $P_{700}$. This mechanism reconciles the controversial spectroscopic and structural data reported so far for FRL-PSI.

Assisted by the structural data, we also provide insights into the location of some Chl *f* spectral forms in the complex. Both spectroscopic and structural data indicate that the most red-shifted Chls are very close to the RC pigments. This is most likely the only configuration that allows the RC pigments to harvest 800-nm excitations at such a formidable rate.

**Chapter 7.** We complete the study of the photosynthetic units expressed upon FaRLiP by investigating the organization and energetics of the phycobiliproteins (PBPs). Similar to most cyanobacteria, the FaRLiP strains grown in WL assemble their phycocyanins (PCs) and allophycocyanins (APCs) into a large phycobilisome (WL-PBS), which is organized to funnel excitations to the attached photosystems. Upon acclimation to FRL, however, new APC subunits are expressed that assemble into bicylindrical cores (FRL-BCs) and contain far-red absorbing bilin pigments[259,260]. The goal of this chapter is to assess the connectivity of the FRL-BCs to the FRL-photosystems and its effect on the efficiency of light harvesting and photochemistry.

As already observed in chapter 5, the FRL-acclimated cells display a very heterogeneous photosynthetic apparatus. Besides FRL-photosystems binding Chl *f* and Chl *d*, and FRL-BCs containing red-shifted APC, they maintain units expressed in WL, especially WL-

PSII and WL-PBS, although their amount is species-dependent. These WL-units might be preserved to harvest visible photons in the case of rapid fluctuations in light spectrum (since the full acclimation requires at least several days)[258]. We also show that while the FRL-BCs are mostly coupled to FRL-PSII, the remaining WL-PBS are mostly connected to WL-photosystems. From a spectroscopic point of view, the association between WL-PBS and WL-PSII and that between FRL-BCs and FRL-PSII exhibit some important differences. It is well known that the excitations formed in the WL-PBS are processed by the WL-PSII RCs more slowly than those formed in the Chls *a* of the WL-PSII antenna. This happens because the excitations formed on the peripheral PBPs need some time to travel across the PBS (about 100 ps or more)[179] and, before reaching the RCs of WL-PSII, they also need to pass through the Chls *a* in the antenna[178,187]. On the other hand, the equilibration between the antenna of WL-PSII and the RC is relatively fast (50 ps or less)[87,390], which means that the excitations in the PBS and in PSII can equilibrate in few hundred ps. In the case of the FRL-BCs however, the excitations on the APC pigments do not equilibrate completely with the Chls *f* in the antenna of FRL-PSII even after 1-2 ns. In addition, the RCs seem to trap the excitations from the FRL-BCs more rapidly and efficiently than from at least a subgroup of Chls *f* in the FRL-PSII antenna. This supports the hypothesis that one red-shifted Chl of FRL-PSII (which we tentatively locate in CP43 based on the structural model) bridges the FRL-BCs to the RC forming a shortcut for the bilin excitations. At the same time, it is clear that the antenna of FRL-PSII contains other Chls *f* that are less well connected to each other and also to the RC. This picture is consistent with the findings of chapter 5, where we hypothesized that the slow trapping achieved in FRL-PSII is partly a consequence of a limitation in the rate of exciton migration within the complex.

In other words, while the coupling between WL-PBS and WL-PSII greatly extends the antenna size and the active spectrum for charge separation at the expense of some efficiency, the association of the FRL-BCs to FRL-PSII kills two birds with one stone. Not only is the absorption cross-section of the RCs larger in FRL, but their photochemistry is also more efficient. These findings also indicate that the positioning of the Chls *f* in FRL-PSII might be optimal to work in association with the FRL-BCs but not on its own, which is consistent with the fact that the FRL-BCs carry the largest amount of far-red absorbing pigments. This evidence could be very enlightening in the quest for inserting Chl *f* in the photosystems of plants, which cannot harbor phycobilisomes due to a very different thylakoid organization. As a result, there might be more efficient ways of organizing the Chls *f* in the antenna of plant PSII and improve their connectivity to the RC. This type of research would obviously benefit from more detailed structural data about FRL-PSII, which are still lacking at the moment. The design of new photosynthetic units might also involve modifying the selectivity of specific binding sites towards Chl *f* and Chl *a*. This task, however, also requires a better understanding of the molecular factors underlying this kind of selectivity.