

## Summary

Oxygenic photosynthesis is not only fundamental because it enables some living organisms to meet their energy needs, but also because it plays a major role in oxygenizing the atmosphere which supports much of life on earth. The photosynthetic process uses photons from sunlight to produce intermediate chemicals involved in oxidation-reduction (redox) reactions. In higher plants and algae, light is harvested by two photosystems (PS) PSI and PSII, located in the thylakoid membrane of the chloroplast. These multi-protein complexes, together with the cytochrome  $b_6f$  complex, form an electron transport chain that also creates a proton motive force across the photosynthetic membrane which drives the ATP synthase. In the end, water and  $CO_2$  are consumed, and oxygen and sugars are produced.

The light harvesting function of PSs is assured by two types of antenna: the core antenna, which only coordinates chlorophylls (Chls) *a* and carotenoids (Cars), and the peripheral antenna, which also coordinates Chls *b*. The core antenna contains the reaction center (RC) where charge separation (CS) occurs. The peripheral antennae are also called Light-harvesting Complexes (LHC). Structure and pigment organization of the core antenna is highly conserved among species, while the peripheral antenna varies in size and composition. This thesis describes the capacity of PSI to harvest light energy and promote charge separation in two algae *Chlamydomonas reinhardtii* and *Nannochloropsis gaditana*.

PSI in *C. reinhardtii* contains nine LHCl in its peripheral antenna. This is five additional LHCl compared to higher plants. They form an outer half ring on the same side of the PSI core as the four LHCl also present in higher plants. A characteristic of PSI is the presence of low-energy chlorophylls called red forms. Red forms are Chls *a* that absorb (and emit) at lower energy than other Chls, and have a broad absorption bandwidth and a large stoke shift. Concurrent with its comparatively larger size, PSI-LHCl in *C. reinhardtii* has red forms with higher energy (less red-shifted) compared to PSI of higher plants. The lower in energy the red forms are, the more they act as local traps and slow the excitation energy transfer (EET). The larger the antenna size, the more photons are harvested, but the migration time of the excitation energy in the antennae is longer. From time-resolved fluorescence decay measured on isolated PSI particles, we related PSI EET and trapping kinetics with the antenna size and red forms content. In the **second chapter** we detail how the significant antenna enlargement in *C. reinhardtii* PSI-LHCl compared to plants is compensated by less red-shifted red forms, resulting in similar average decay time of  $\sim 50$  ps in both organisms. We show that most of the Chls are in very fast equilibrium ( $\sim 150$  Chls *a* over 183 Chls *a*) including Chls in the LHCl most distant from the core antenna. *C.*

*reinhardtii* PSI-LHCI efficiency is as high as 97% despite slower EET migration due to the larger number of Chls in its antennae (estimated at 225 Chls *a* and *b* vs 155 Chls higher plants). Another PSI particle was isolated from *C. reinhardtii* without Lhca2 and Lhca9, two LHCI that were previously characterized as containing red forms. By comparing both five- and nine-LHCI PSI particles of *C. reinhardtii*, we show that the presence of Lhca2 and Lhca9 slows down the overall kinetics mainly because of their red forms (details in **Annex of Chapter 3**). However, it is shown that the red-most forms were in, or functionally close to, other LHCI.

In response to changes in light intensity and quality, cells can modify peripheral antenna's size and composition in a process known as state transition. For example, in response to changes in light quality, some LHCI can detach from PSII and associate with PSI (state 2) in order to balance the energy distribution. From cells of *C. reinhardtii* induced in state 2, PSI-LHCI was isolated with seven LHCI (one monomer and two trimers) in addition to the nine LHCI. The LHCI remain associated to PSI on the PsaF/J side while the LHCI attach opposite on the PsaH side. The **third chapter** determines the energy transfer efficiency of LHCI to PSI core and their influence on the trapping yield of the photosystem. Energy transfer between LHCI and PSI core (~60 ps lifetime) is slower than between LHCI and PSI core (~7ps) mainly because of a looser connectivity (details in **Annex of Chapter 3**). From a reconstructed 3D model of PSI-LHCI-LHCI structure scaled from EM images, we observed that the distance of the closest Chls (nearest edge-to-edge distance) between LHCI Chls and PSI Chls is 18 Å. Despite the increase in Chls number by ~43% (322 Chls) and the relatively slow EET step between LHCI and PSI core, the overall kinetics of PSI-LHCI-LHCI has an average decay time of 78 ps (or less depending on the excitation wavelength) which is fast enough to maintain a very high trapping efficiency (above 96%).

In parallel with characterizing the EET and trapping kinetics of PSI-LHCI in *C. reinhardtii*, we studied the LHCI and the core antenna separately. LHCI in higher plants show highly conserved proteins structure and pigment organization but there are a few notable biochemical and spectroscopic properties that vary between the monomers. For example, some LHCI are enriched in red forms of Chl *a*, increasing their absorption in the far red. *C. reinhardtii* LHCI also contain varying red form content. The **fourth chapter** characterizes the decay kinetics of excitation energy in the nine LHCI of *C. reinhardtii*. We observe a decay time of 1.9 ns for all the monomers. LHCI have shorter lifetime than LHCI indicating that they are in a more quenched state. Since no correlation was observed between LHCI lifetimes and their red form contents, we conclude that red forms do not act as quenchers. This is in agreement with previous observation made in plant LHCI.

After the peripheral antenna, the second moiety analyzed in PSI-LHCI was the core antenna. In the **fifth chapter**, we have purified *C. reinhardtii* PSI core to homogeneity to characterize its spectral and excitation energy trapping properties. In PSI core, red forms absorb at 701.2 nm and can be related to an average decay time of ~18 ps. This is slower than cyanobacterial PSI devoid of red forms (~14 ps) but faster than PSI core of higher plants (21.3 ps) or other cyanobacterial PSIs (up to 40 ps) whose red forms are lower in energy compared to *C. reinhardtii*. By analyzing PSI core kinetics in parallel with PSI-LHCI kinetics, we show that red forms in the core are functionally distinct from the bulk Chls, in contrast to red forms of the peripheral antenna which are always found in fast equilibrium with bulk Chls. Despite highly conserved structure between species, red form content in PSI core varies tremendously and small conformational changes of PSI core subunits could considerably affect the red form properties. The best candidate for the reddest red form in cyanobacteria is a Chl trimer located at the periphery of the core, on the luminal side of PsaG. In higher plants and *C. reinhardtii*, the presence of LHCI at this position was proposed to change the environment and thus the Chl trimer organization.

The **sixth chapter** characterizes PSI-LHC in the heterokont alga *Nannochloropsis gaditana*. Differently from higher plants and *C. reinhardtii*, all LHCs were not located on the same side of the core. Two LHCs are located at the same position as Lhca2 and Lhca3 of higher plants while three others are located on the Psa/L side of the core. PSI-LHC of *N. gaditana* has an average decay time of ~30 ps which is much faster than in higher plants. Despite the antenna enlargement by one additional LHC in *N. gaditana*, the quicker trapping kinetics can be explained by higher energy (bluer) red forms than in plants, acting less as local traps. From an evolutionary point of view, *N. gaditana* gives another example of supramolecular organization of PSI-LHC that results in a very high trapping yield.

In this thesis, we have explored PSI in *C. reinhardtii* and *N. gaditana* and compared its composition and energy kinetics to higher plants. Despite evolutionary conserved components, remarkable differences in antennae size and composition, as well as tremendous variety in the location, number and spectral properties of red forms of Chl *a*, were observed. Nevertheless, PSI maintains an almost perfect trapping efficiency, even in a context of loose connectivity.