CHAPTER 1

Introduction
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Photosynthesis

Across evolution, living organisms have developed different strategies to cope with their energy needs, among which is photosynthesis. Photosynthesis is the ensemble of processes producing chemical energy using sunlight. Different types of photosynthesis exist in Nature, with the most widespread being the “chlorophyll-based form” (1), in which sunlight is harvested by chlorophylls (Chl) and carotenoids (Cars) (and bilins in some organisms). Oxygen is a by-product of photosynthesis in plants, algae and cyanobacteria, but is not produced by most of the prokaryotes, which are therefore called anoxygenic bacteria.

Oxygenic photosynthesis consists of four successive phases: (i) Light harvesting and excitation energy transfer by the antenna systems (ii) Charge separation in the reaction center (photochemistry) (iii) Secondary electron transfers, resulting in the synthesis of NADPH (reductive agent) and ATP (energy) (iv) Carbon fixation, for which NADPH and ATP are used in the Calvin-Benson cycle to synthesize stable organic products from CO₂. The three first phases are called ‘light reactions’ and the last phase ‘dark reactions’. However, only the first phase depends directly on the light (photon absorption) whereas the others could be considered light-driven reactions.

Light harvesting in oxygenic organisms relies on pigments coordinated to multi-protein complexes. These complexes are embedded in (or associated with) membranes called thylakoids. In photosynthetic eukaryotes, thylakoid membranes form the inner network of an organelle specialized in photosynthesis called the chloroplast. Four major trans-membrane proteins are involved during the first three phases: Photosystems (PS) I and II, cytochrome b₆f and ATP synthase. Electrons travel linearly through these complexes (Figure 1), from water, the first electron donor, to NADP⁺, the final electron acceptor of the electron transport chain (ETC).

The electron transfer from water to NADP⁺ is not spontaneous as shown by the redox potential of their related couples \( E'_0 \) (\( \text{O}_2/\text{H}_2\text{O} \)) = +0.82V > \( E'_0 \) (\( \text{NADP}^+/	ext{NADPH} \)) = -0.32V). After excitation by sunlight, Chls become highly reductive. In the reaction centers (RCs) of the PSs, these highly reductive excited Chls can react with nearby oxidative species. Photochemistry consists in the formation of successive radical pairs by charge separation (CS). Primary electron transfer steps end with final radical pairs involving \( P_{700}^+ \) in PSI and \( P_{680}^+ \) in PSII (each named after its absorption wavelength, (2) and details for PSI below). \( P_{700}^+ \) and \( P_{680}^+ \) have strong oxidative power and extract electrons from plastocyanine (PC) and water. These RC Chls, back in their ground states, are then ready for new light excitation. Secondary chemical reactions involve the successive acceptors of the ETC starting from ferredoxin (Fd) reduction on the donor side of PSI and from plastoquinone (PQ) reduction on the donor side of PSII (Figure 1). In conclusion, sun light provides the energy necessary to fill
the free energy gap between water and NADP\(^+\) and to initiate a cascade of spontaneous oxido-reduction reactions (also called the “Z-scheme”).

Figure 1: Thylakoid membrane of higher plants with the four main photosynthetic multiprotein complexes: the two photosystems (PS) and their Light Harvesting Complexes (LHC), PSI-LHCI and PSII-LHClI, the cytochrome b6f (cyt_b6f) and the ATP synthase. Under light, electrons travel linearly from H\(_2\)O to NADPH (solid arrows) and at the same time protons translocate from the stroma to the lumen (long dashed arrow). The proton gradient is used by the ATP synthase for the production of ATP. Cyclic electron transfer (short dashed arrow) only produces ATP which adjusts the NADPH:ATP stoichiometry. Fd: ferredoxin; FNR: ferredoxin-NADP-reductase; PQH\(_2\): plastoquinol; PC: plastocyanin. Picture adapted from (3).

After CS in the RC, a high electric field of 10\(^7\) V.m\(^{-1}\) is created across the membrane (considering a voltage of 100 mV across a membrane of 10 nm (4)). Due to water oxidation and the PQ cycle, protons (H\(^+\)) accumulate in the lumen (the inner space of the thylakoid) resulting in a proton gradient across the membrane. This proton gradient is used by the ATP synthase to produce ATP.

**Antenna principle**

As just mentioned, the photosynthetic ETC is activated thanks to the energy harvested from the sun. In principle, the more light is harvested, the more electrons are transported from water to NADP\(^+\). Considering that a Chl has a cross-sectional area of about 15 Å\(^2\) and that the photon flux is 100 μmoles photons/m\(^2\)/s on average, the average probability for a photon to be absorbed on this Chl is equivalent to one photon per second. In the case of a Chl in an RC, this would lead to the generation of one electron per second (considering a quantum yield of 1). Within the PSs, pigments are densely packed around the RC Chls and can transfer their excitation energy to it. In this way, the RC benefits from energy harvested by the entire network, which increases the probability of generating an
electron in the RC. For instance, 155 Chls (a and b) and 35 carotenoids (26 β-carotene, five lutein and four violaxanthin molecules) compose the PSI pigment network in higher plants ((5), Figure 2).

Chls are substituted porphyrins whose nitrogen atoms coordinate a central magnesium atom. The conjugated doubled bonds of the porphyrin ring permit π-electrons delocalization and the absorption of visible light. The various substituents to the ring change its molecule symmetry tuning the absorption properties. A long phytol chain makes the molecule hydrophobic. Cars are linear polyene chains potentially terminated by rings at one or both ends. Xanthophylls contain oxygen in their molecular structure while carotenes are unsaturated hydrocarbons. Depending on the length of the conjugated system different wavelengths can be absorbed.

According to the Franck-Condon principle, the most probable transition from the ground state will occur with the vibrational level of the excited electronic state whose wavefunction overlaps the most with the one of the ground state. In the excited state, the electrons reorganize which modifies the energy landscape compare to the ground state. In Figure 3, electronic transitions are represented for Chl b: the most probable transitions are \((S_0, v_0)\) to \((S_n, v_2)\) in the blue region and \((S_0, v_0)\) to \((S_1, v_2)\) in the red region (\(v_2\) is an arbitrary
choice to visualize the principle). These most probable transitions are named the Soret bands in the blue and the Q bands in the red. If there is no overlap of the wavefunctions, the transition is not possible (also said “forbidden”). An $S_0$-$S_1$ transition is forbidden for Cars leading to the absence of red absorption.

Figure 3: (A) Absorption (dark green) and fluorescence (brown) spectra of Chls $b$ (in acetone) schematized by (B) a simplified Jablonski diagram. Different excited levels are populated after absorption (solid red or blue arrows) and depopulated after fluorescence (solid brown arrow) or internal conversion (IC, blue and red dashed arrow). Other de-excitation pathways are possible but not represented (see main text).

The absorption intensity is also determined by the polarization properties of the transition. For the Chls, $Q_y$ (first excited state, $S_1$ in the Figure 3) and $Q_x$ (second excited state, not represented in Figure 3) transitions correspond to transition dipole moments $\vec{\mu}$ of the molecule that are oriented (more or less) along the two axes of the porphyrin ring (Figure 2). The transition is enhanced when the electric field is polarized parallel to the
transition dipole moments. By comparing the transition strength upon different electric field polarizations, we can estimate the molecule orientation.

The intensity of a transition to one excited state (all vibrational levels together) is quantified by the oscillator strength $f$. It can be seen as a proportion of electrons in the molecule able to oscillate with the frequencies $\nu$ of the absorption band of the excited state. The oscillator strength $f$ is proportional to the area under the absorption spectrum, $A = \int_\Delta \nu \varepsilon(\nu) \, d\nu$ with $\varepsilon(\nu)$ the extinction coefficient (in $\text{M}^{-1} \cdot \text{cm}^{-1}$). $\varepsilon(\nu)$ will vary depending on the refractive index (6).

The oscillator strength of the $Q_y$ transition of Chl $b$ is 0.7 times the one of Chl $a$ (7). The dipole strength $d = (\mu \cdot) ^2$ is another quantification of the transition strength and is related to the extinction coefficient as follow $d = 9.186 \times 10^{-3} = n \int_\Delta \varepsilon(\nu) \, d\nu$ (in Debye$^2$, (6, 8, 9)). The dipole strength enables to obtain the radiative rate $k_{\text{rad}}$ (10). The intrinsic fluorescence lifetimes are consistent with experiment ($\tau_i = \frac{1}{k_{\text{rad}}} = \frac{\tau_{\text{obs}}}{\phi_F}$ with $\tau_{\text{obs}}$ the measured value in situ and $\phi_F$ the fluorescence yield) when considering carefully the influence of the refractive index (8). To summarize, energy levels are populated in different proportion depending on the excitation wavelengths, on the selection rules of the Franck-Condon principle and on the orientation of the transition dipole moments of the molecules with respect to the electric field polarization.

After absorption, the excitation energy decays rapidly by successive heat dissipation steps: (i) dissipation to the lowest vibrational level ($S_n, v_n$) of the excited state; then (ii) dissipation from $S_n$ state to one of the vibrational levels of $S_1$ (internal conversion, IC in Figure 3) and finally (iii) dissipation to the lowest vibrational state of $S_1$. Since internal conversion is very fast (rate constant $\sim$(200 fs)$^{-1}$ (11-13)), very little fluorescence is observed from $S_2$ of Cars, and $S_n$ in Chls exhibits none. Fluorescence is a radiative process during which the molecule returns from ($S_n, v_0$) to one of the vibrational levels of the ground state by emission of a photon (Figure 3). From $S_1$, both IC and fluorescence are possible with predominant IC in Cars (rate constant $\sim$(10 ps)$^{-1}$ in Cars (14) against $\sim$(50 ns)$^{-1}$ in Chls) and predominant fluorescence in Chls (rate constant $\sim$(2 μs)$^{-1}$ in Cars and $\sim$(13.3 ns)$^{-1}$ in Chls (15)). Even though $S_1$-$S_0$ transition is optically forbidden in Cars, this transition can receive some oscillator strength from the strongly allowed $S_0$-$S_2$ transition after perturbation of the Car structure, explaining the observed fluorescence from $S_1$ (13, 15). In addition to fluorescence and IC, excitation energy can decay from $S_1$ via inter-system crossing (ISC). ICS consists in a spin-flip of $S_1$ leading to the formation of a triplet excited state $T_1$ from which phosphorescence will occur. For a Chl, when considering the IC, fluorescence and ISC (rate constant $\sim$(8 ns)$^{-1}$ for Chl ISC (16)) competing pathways, the chance to create a Chl triplet is $\sim60\%$. This long living Chl triplet (phosphorescence with a lifetime of ms (17))
quenched, can react with molecular $^3\text{O}_2$ (triplet in its ground state) and form reactive oxygen species (ROS). Singlet oxygen ($^1\text{O}_2$) and hydroxyl radicals HO$^-$ are the most reactive ROS leading to dramatic photodamage, such as oxidation of lipids, proteins and nucleic acids (18). Several photoprotective mechanisms exist (19-21) to avoid formation of ROS (Chl triplet interaction with Cars to form Car triplet which are lower in energy than singlet oxygen) or to quench them (direct scavenging of the ROS by Cars). The generalized coordinates (or the bond length in the case of a diatomic molecule) differ between $S_1$ and $S_0$ states and the energy landscapes of the two states do not overlap (Figure 3). During emission, different vibrational levels of the ground state will be populated depending on their wavefunction overlaps with $(S_1, v_0)$. The most probable transition energy is not expected to be with $(S_0, v_0)$ but rather with higher vibrational levels of $S_0$ instead. This will result in the shift of the fluorescence maximum toward lower energy as compared with the absorption maximum in the Q$_y$ (Figure 2). The difference in energy is called the reorganization energy (Figure 3) which is approximated as half of the Stokes Shift (22).

The proteic environment influences the electron distribution and therefore the energy levels of the pigments. The influence of the environment on a single pigment is described by the homogeneous and inhomogeneous broadenings of the absorption (or fluorescence) band (Figure 4). One pigment in a specific protein binding-site will experience (even slight) conformational changes of the protein. The $(S_0, v_0)\leftrightarrow(S_1, v_0)$ transition (also called the zero-phonon line) can take as many values as protein conformations whose distribution determines the inhomogeneous broadening. The inhomogeneous broadening is well described by a gaussian distribution (23) whose full width at half maximum (FWHM) $\Gamma_{\text{inhom}}$ is represented in Figure 4.
The homogeneous broadening corresponds to the broadening of the zero-phonon line by the phonon side-wing. Phonons are low-frequency vibrations of the protein (the bath) that can couple with the electronic transition. The difference in energy between the zero-phonon line and the phonon side-wing maximum corresponds to the reorganization energy which equals the product of $S$, the strength of the electron-phonon coupling (or Huang-Rhys factor), and $\nu$ the mean frequency of the protein matrix phonons (22, 25). The total broadening of the electronic transition is characterized by a FWHM $\Gamma_{\text{tot}}$ whose square $\Gamma_{\text{tot}}^2 = \Gamma_{\text{inhom}}^2 + \Gamma_{\text{hom}}^2$.

We can consider now the absorption of a protein that binds more than one pigment. Each of them will have different maximum corresponding to different binding sites in the protein. Nevertheless, each absorbing form will have a contribution which can be decomposed in homogeneous and inhomogeneous broadenings as represented in Figure 5A for two different Chls $a$. Summing all the absorbing forms’ contributions will result in the pigment-protein complex absorption spectrum (black in Figure 5B).
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Figure 5: (A) Absorption spectra of two different Chls a decomposed in homogeneous (pink) and inhomogeneous (black) broadenings. (B) Absorption spectrum of a pigment-protein complex (black) containing several Chls a (dark cyan) and Chls b (dark orange).

**PSs: Core & Lhcs. Differences between organisms**

The harvesting capacities of the PSs are achieved thanks to a very large number of pigments, either Chls or Cars, bound to different subunits of the PSs. Even though different, the subunits of PSI and PSII antenna systems can be grouped in two moieties: the core (or inner) and the peripheral (or outer) antennae. The core antenna only binds Chls a while the peripheral antenna can bind other types of Chls, like Chls a and b in plants and algae.

**Well conserved core complexes**

In cyanobacteria, PSII core is made of 20 subunits binding 35 Chls a and 11 β-carotenes (26) and PSI core is made of 12 subunits binding 98 Chls a and 22 β-carotenes (24). The core of both PSs in eukaryotic organisms is almost identical to that of cyanobacteria (27). In particular, in higher plants (5, 28, 29), 12 subunits of the PSI core (PsaA-L) have been resolved among which 10 are homologous to cyanobacterial PSI (24, 30). PsaG, PsaH, PsaN and PsaO are four subunits unique to PSI core of higher plants (31, 32) but the two latter have not been resolved in the crystal structures yet. In higher plants, PsaG interacts with PsaB and the peripheral antenna (5, 29). This contributes to stabilize the whole supercomplex which was still observed in the absence of PsaG but to a smaller extent (33, 34). PsaH is located in higher plants where monomers interact in cyanobacterial PSI trimer (28, 30) and is important for the overall stability of higher plant PSI (5, 29, 35). Furthermore, PsaH was observed to interact during state transition with Lhcbs (36, 37). PsaO was also reported to play a role during state transition in binding Lhcbs (38). (Lhcbs and state transition are described in the followings). No crystal structure is available for the PSI of the green alga *Chlamydomonas reinhardtii*. However, because of the well conserved core subunits compared with cyanobacteria and higher plant (39), we expect the same structural
features for PSI core of *C. reinhardtii*. The pigments composition is also very similar between cyanobacterial PSI and eukaryotic PSI cores (5, 24, 29).

**Peripheral antenna complexes**

On the contrary, the peripheral antennae of eukaryotic PSI vary a lot, not only as compared with cyanobacteria but also between different eukaryotic organisms (40). In cyanobacteria, the soluble proteins, which are called phycobilisomes, serve as peripheral antenna of both PSs, whereas, in plants and green algae, the peripheral antenna is made of trans-membrane proteins called Light Harvesting Complexes (LHC), either LHCIs (or Lhcas) for PSI or LHCIIIs (or Lhcbis) for PSII. Concerning PSI-LHCI of eukaryotic organisms, large differences have been observed: PSI outer antenna of *C. reinhardtii* is more than twice larger than in higher plants (Figure 6). More precisely, among the six Lhca genes reported in *Arabidopsis thaliana* (41), Lhca1-4 encode for PSI peripheral antenna (28, 42, 43) while the Lhca5 and Lhca6 proteins are present in sub-stoichiometric amount with the PSI core (44). In *C. reinhardtii*, nine genes were related to PSI outer antenna (45, 46) with all of them being expressed. The products of all the genes assembled in the PSI supercomplex (47, 48) in the form of two concentric half rings on one side of the core (48, Figure 6). The pigments number of the PSI core is increased by 58% with the presence of LHCIs in higher plants and by 128% in *C. reinhardtii* (Table 1) if we consider that each LHCI binds 14.25 Chls (on average) and three Cars (5).

Figure 6: EM picture of PSI-LHCI in *C. reinhardtii* (48) superimposed with the crystal structure of PSI-LHCI in higher plants (49), PDB 2WSC, stromal view) with the core antenna (green) and the peripheral antenna (brown). The core subunits PsaH and PsaL are represented in yellow and pink respectively. The asterisks locate the additional LHCIs present in *C. reinhardtii*. Blue and yellow arrows correspond to either Lhca2 or Lhca9 (of interest in the followings). Scale bar 10 nm. Figure modified from (48).

Concerning PSII peripheral antenna, many Lhcb genes produce six proteins in *A. thaliana* (41) and 11 in *C. reinhardtii* (50, 51). LHCII trimers, the major LHCIIIs, are constituted by three Lhcb proteins in *A. thaliana* (Lhcb1-3) and by nine in *C. reinhardtii* (LhcbM1-9). Two trimers per monomeric PSII core were found in *A. thaliana* PSII supercomplex (52-55) versus three in *C. reinhardtii* (56, 57). Monomeric antennae in PSII-LHCII, which also called minor
antennae, are located between the core and the trimers. Three per monomeric PSII core were found in *A. thaliana* PSII supercomplex, namely CP24 (Lhcb6), CP26 (Lhcb5) and CP29 (Lhcb4) while only two are present in *C. reinhardtii*. CP24 is missing in *C. reinhardtii* PSII-LHCI supercomplex and its position is occupied by one LHCI trimer instead (57). Considering the number of Chls and Cars in each LHC (14 Chls and four Cars per monomer in the LHCI trimer (58), 10 Chls and two Cars estimated in CP24 (59, 60), 14 Chls and four Cars in CP26 that we assume to have the same pigment composition as a monomer of the LHCI trimer, 13 Chls and three Cars in CP29 (61)), the pigments number of the PSII core antenna is increased by 333% with the presence of Lhcbs in higher plants and by 424% in *C. reinhardtii* (Table 1).

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<th>Core antenna</th>
<th>Peripheral antenna</th>
<th>Total for each type of pigments (and increase of core antenna %)</th>
<th>Total of Chls+Cars (and increase of core antenna %)</th>
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Table 1: Estimation of the Chls and Cars number in PSI and PSII of *C. reinhardtii* (C.r.) and *A. thaliana* (A.t.).

Not only the PS peripheral antenna size differs between organisms but also their affinity for Chl b: the Chl a/b ratio of LHCs in *C. reinhardtii* is lower than in higher plants; but in both organisms, the Chls a/b ratio of Lhcbs is lower than in Lhcas (5, 58, 62-64). Furthermore, *C. reinhardtii* has a particular Car composition with the presence of loriocxanthin (48, 65), in addition to β-carotene, lutein, violaxanthin and neoxanthin present in higher plants.

**Light harvesting capacities versus PS efficiency?**

The question is whether ETC and PS performances always increase with the increase of the light harvesting capacities. This thesis focuses on light harvesting, excitation energy transfer (EET) and trapping capacities of PSI which determine the overall trapping efficiency of this photosystem.

The trapping efficiency $\Phi_{CS}$ is the quantum yield of CS and can be measured by comparing the excitation lifetime in the two following cases: when the excitation energy promotes CS or not. When CS is possible, this channel competes efficiently with ISC so that
the possibility to form triplets is strongly reduced (see above). Aside CS and ISC, fluorescence is another pathway for the energy to decay. As it can be measured, fluorescence gives indirectly access to CS yield, if all the other decay rates are unchanged. $\Phi_{CS}$ can then be written as $\Phi_{CS} = 1 - \tau_{CS}/\tau_{no\ CS}$ (15) where $\tau_{CS}$ is the fluorescence lifetime (called average decay time, in the followings) when the RC is able of CS, and $\tau_{no\ CS}$ the one when CS does not occur.

The average decay time $\tau_{CS}$ can be interpreted in terms of migration and trapping times such as $\tau_{CS} = \tau_{mig} + \tau_{trap}$ (15, 66, 67). The migration time $\tau_{mig}$ is the time required to reach thermal equilibrium and is the time for the excitation to arrive at the RC for the first time. If this component dominates, the process is \textit{diffusion-limited}. The migration time can contain a term representing the time of the last energy transfer step to the RC Chls therefore called delivery time $\tau_{del}$. If this term dominates, the diffusion process is called \textit{transfer-to-the-trap} limited (68). The trapping time $\tau_{trap}$ is the ratio between the intrinsic time of the CS $\tau_{CS}$, and the probability that the excitation is located on the RC after thermal equilibrium. This probability decreases with the increasing number of pigments, i.e. the antenna size. In a simple situation of the antenna composed of only isoenergetic pigments, the trapping time would be $\tau_{trap} = N^*\tau_{iCS}$ with N the number of isoenergetic pigments in the antenna system. $\tau_{trap}$ does not depend on the initial excitation location. If this term dominates, the process is \textit{trap-limited}. If the antenna system is modelled as an ensemble of very well coupled (infinitely fast migration time) isoenergetic pigments, $\tau_{CS} = \tau_{trap} = N^*\tau_{iCS}$. The reality is more complex and the three contributions (diffusion, transfer-to-the-trap and trap) in $\tau_{CS}$ of PSI-LHCI are under debate (69).

\textbf{Excitation energy transfer}

The migration of the excitation energy in the antenna systems depends on the types of interaction between pigments. When bound to proteins, pigments are oriented and separated in a specific way that will influence their interaction (3, 15). To picture interaction of several pigments (isoenergetic if $S_1$ levels have the same energy $\varepsilon_e$), we usually represent them by superimposing their energy diagrams (Figure 7A). We can also think of them as an ensemble by describing the state space (Figure 7B): the ground state of the ensemble is when all the pigments are in their ground state (|\textit{g}\rangle), n states correspond to the first exciton states when one of them is in $S_1$ (|\textit{e}\rangle), n*(n-1)/2 states correspond to the second exciton states when two of them are in $S_1$...etc. If the interaction of each pigment with the related bath (independent bath for each pigment) dominates above the interaction between pigments, the first exciton states will all have the same energy $\varepsilon_e$ (degenerated energy
levels) and the EET will be described in the frame of the Förster theory (red arrows in Figure 7B).

If the interaction between pigments is larger than the one with the baths, the first exciton states will have energy around $\varepsilon_e$ within an energy band ($\Delta \varepsilon_e$). In this case, interaction between pigments can be described in the frame of the Redfield theory (red arrow in Figure 7C) and the excitation delocalized over the ensemble. Since the exciton states of the first band are not eigenstates of the individual pigments, the excitation of the individual pigments will oscillate (Figure 7C). The population of each state will also depend on the orientation of the transition dipole moments of the related pigments as well as on their spatial arrangement (15). Energy exchange with the bath, even though weak, triggers transition to other energy states and finally result in a spatial migration of the excitation. A lot of debate exists in the scientific community whether different exciton states lead to coherent oscillations. Whether this excitonic coherence exists long enough to be of relevancy for biological processes is beyond this thesis scope but a review is available in (70).

To come back to the Förster theory, the interaction energy $V$ between weakly coupled pigments D and A can be approximated by a dipole-dipole interaction:

$$V = \frac{1}{4\pi\varepsilon} \frac{\mu_D \cdot \mu_A^*}{R_{DA}^3} 3(\mu_D \cdot \vec{r}_{DA})(\mu_A \cdot \vec{r}_{DA})$$

where $\mu_D$, $\mu_A$ are the transition dipole moments of D and A respectively (in Debye), $\vec{r}_{DA}$ is the normalized vector between the centers of D and A distant of $R_{DA}$, and $\varepsilon$ is the permittivity of the medium. The relative orientation of the transition dipole moments as well as their arrangement in the pair, together with the distance between the two pigments will influence the strength of the interaction. The EET is described between a donor (D) that transfers its energy to an acceptor (A) with a rate...
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described by the Förster equation (71-73): 

\[ k_{DA} = \frac{1}{\tau_R^D} \cdot \frac{R_0^6}{R_{DA}^6} \]

where \( \tau_R^D \) is the intrinsic radiative lifetime of the donor D and \( R_0 \) the Förster radius such as 

\[ R_0^6 = \frac{9 \ln 10}{128 \pi^3} K^2 \frac{c^4}{N_n^4} J(v) \]

where \( c \) is the speed of light in vacuum, \( N \) the Avogadro number, \( n \) the refractive index of the surrounding, \( \kappa \) the dimensionless orientation factor and \( J(v) \) the spectral overlap integral 

\[ J(v) = \int_0^\infty \frac{\varepsilon_A(v) F_D(v)}{v^4} \, dv \]

with \( \varepsilon_A(v) \) the molar extinction coefficient of the acceptor and \( F_D(v) \) the fluorescence emission of the donor (normalized to 1 on the frequency scale \( v \)). If the spectral overlap integral \( J(v) \) increases, the rate of energy transfer from D to A increases. When pigments are isoenergetic, they have similar absorption and emission spectra (represented in Figure 8 with the Stokes shift) which overlap over some frequency (case 1). For non isoenergetic pigments, energy transfer to a pigment lower in energy will be faster (larger integral in case 2 than in case 1, Figure 8) while the transfer from this pigment lower in energy will be slower (case 3).

![Figure 8](image-url)

Figure 8: Overlap integral (green hatch) between the fluorescence emission (dash) of a donor (D) and the molar extinction coefficient (solid) of an acceptor (A). The two pigments 1 and 2 are either isoenergetic (Case 1) or non isoenergetic, with the more energetic pigment 1 (black) being the donor (Case 2) or the acceptor (Case 3) of energy to (or from, respectively) a less energetic pigment (red).

The forward and backward rate constants ratio between pigment 1 and pigment 2 is expressed at equilibrium using the (Gibbs) free energy difference \( \Delta G \) function of the equilibrium constant \( K \): 

\[ \Delta G_{12} = G_2 - G_1 = -k_B T \ln(K_{1 \rightarrow 2}) \]

with \( K_{1 \rightarrow 2} = k_{1 \rightarrow 2}/k_{2 \rightarrow 1} \), where \( k_{1 \rightarrow 2} \) is the rate constant of EET from pigment 1 to pigment 2 (and reverse for \( k_{2 \rightarrow 1} \)), \( k_B \) the Boltzmann constant and \( T \) the absolute temperature. Under constant pressure and temperature, a negative free energy difference corresponds to a spontaneous process. The free energy difference is defined as 

\[ \Delta G_{12} = \Delta H_{12} - T \Delta S_{12} \]

with \( H \) the enthalpy and \( S \) the entropy. When we consider only two pigments, the entropy difference is zero and the rate constants are
directly related to the energy levels of the pigments (when $S_1$ energy levels are taken to represent the enthalpy). A transfer from high energy pigment to low energy pigment will be “spontaneous” ($K_{1\to2}>1$, down-hill) while the reverse transfer not ($K_{2\to1}<1$, up-hill). The population of the highest level occurs thanks to thermal disorder. The populations of the two levels are related by the Boltzmann distribution $\frac{P_2}{P_1} = \exp\left(-\frac{(E_2-E_1)}{k_B T}\right)$ with $E$ the $S_1$ energy levels. When the temperature decreases, the pigment with the lowest $S_1$ will be populated the most. It follows that the 77K fluorescence is dominated by the “red” forms emission. When there is more than one pigment of each type, the pool size of pigments 1 ($N_1$) and pigments 2 ($N_2$) will lead to an entropy difference $\Delta S_{12} = -k_B \ln\left(\frac{N_1}{N_2}\right)$. The entropy difference will possibly contribute to the free energy difference so that the latter become negative and the transfer spontaneous even though up-hill at first. The detailed balance $\frac{k_{1\to2}}{k_{2\to1}} = \frac{N_2}{N_1} \exp\left(-\frac{(H_2-H_1)}{k_B T}\right)$ characterizes equilibrium between pools of pigments.

To summarize, the type of interactions will define the EET between pigments described either in the frame of Redfield theory or Förster theory. Both types of interaction take place in the antenna systems. The Förster theory applies for transfer times above 1 ps. The transfer between two isoenergetic Chls $a$ takes $\sim$1.3 ps on average when they are distant by 1.5 nm and randomly oriented (15). We will use the Förster theory to study the EET between weakly coupled non isoenergetic Chls $a$ in the antenna systems in PSI-LHCI. In particular, we will focus our interest on special Chls which absorb at lower energy than the RC or, more generally, than the bulk Chls $a$, the so called “red forms” (63, 74-77).

This thesis aims at characterizing the EET and trapping kinetics of PSI-LHCI in order to determine PSI efficiency relative to the size and spectral properties of the antenna. We will thus focus on describing PSI-LHCI in the followings.

**PSI-LHCI enriched in red forms**

The “red forms” are excitonically coupled Chls $a$ (43, 78-81) whose lowest exciton state mix with a charge transfer (CT) state (80, 82-85). Since the red form excited state has a CT character, its electron distribution will be very different from the ground state leading to significantly different dipole moments between ground and excited states. This explains the large Stokes shift and the large homogeneous broadening observed for the red forms (63, 76, 80, 82, 86). Furthermore, from the CT character, the dipole moment of the red form excited state will easily feel polar changes in the proteic environment cause by (even small) conformational changes of the protein. Both homogeneous and inhomogeneous broadenings of red-form containing antennae range between 170-360 cm$^{-1}$, each depending
on species, (23, 80, 82, 87) and are larger than broadenings reported for red-forms devoid antennae, like LHCII (88, 89).

The red forms can be located in both core and/or peripheral antennae (Figure 9). In the core antenna, candidates were proposed based on potentially strong excitonic interactions calculated from the crystal structure of cyanobacterial PSI ((24), purple in Figure 9).

Figure 9: Stromal and transmembrane views of PSI-LHCI of higher plants (PDB 4XK8) showing the red forms candidates in the core antenna (purple, PDB 1JB0) and the red forms characterized in the LHCIs of higher plants (red, PDB 4XK8).

Despite very similar PSI core subunits’ structure and pigment organization (see above), the red forms composition varies tremendously between organisms (77, 90, 91) possibility because the CT character gives to the red forms the capacity to sense even small
conformational changes of the protein. Gaussian deconvolutions of 6K absorption spectra permitted to identify several pools of red forms in cyanobacteria absorbing at 708, 719 or 740 nm (75, 90). One additional pool absorbing at 714 nm was identified by hole-burning studies (85, 92-94). Trimeric PSI is enriched in red forms compared to the monomeric PSI (75) with the red-most forms found at 740 nm in *Arthrospira platensis* PSI trimers (90, 95-97). Only one pool at 705 nm was reported in higher plant PSI core (76). Time-resolved measurements do not confirm the presence of this pool in the core of higher plants (98). Similarly in *C. reinhardtii*, identification of red forms in the core is still debated (87, 99-105). At 77K, PSI-LHCI of *C. reinhardtii* emits at 712-717 nm (48, 106) then at higher energy than *A. thaliana* PSI-LHCI (maximum at 735 nm, (107)).

Even though well conserved between species, a noticeable difference in the PSI core structure is the size of the PsaB loop on the luminal side, whose extension was proposed to stabilize a Chl trimer B31-B32-B33 possibly the most red forms in *Synechococcus elongatus* (24). This PsaB extension is missing in other cyanobacteria and eukaryotes, which could destabilize the Chl trimer stacking and explain the differences in the energy levels of the red forms between these species (30, 49). A Chl bound to PsaG and Lhca1 has been recently found in higher plants (5, 29) and results in the formation of another stacked Chl trimer at the same place as in *S. elongatus*, even in the absence of the extended PsaB loop. This luminal trimer was therefore suggested to be responsible for the reddest forms also in higher plant PSI core, which somehow are not as low in energy as in *S. elongatus*.

More is known about the red forms associated with the peripheral antennae. Lhca3 and Lhca4 in higher plants have red forms that absorb at 704 nm and 708 nm respectively (80, 108). Lhca2, Lhca4 and Lhca9 in *C. reinhardtii* present characteristic features of red forms containing antenna, but given that their red absorption spectra at 77K do not show obvious structures, the lowest energy state could be determine only for Lhca9 and Lhca2 for which a contribution in the second derivative is detected at 692 nm and 693-697 nm, respectively (63). The 77K emission spectrum reveals that red forms in *C reinhardtii* Lhcas are higher in energy than *A. thaliana* Lhcas: the red emission with a maximum at 717 nm, was observed for Lhca2 in *C reinhardtii* (63), while Lhca3 and Lhca4 of higher plants emit at 725 nm and 733 nm respectively (80). The less red-shifted 77K emission peak correlates linearly with a decrease of Stokes shift in *C reinhardtii* (63).

Different roles were attributed to the red forms: (i) they increase the absorption cross section of PSI-LHCI in the red/far-red region (109) and (ii) in a context of non-equilibrium, they could help in concentrating excitation close to the RC (102, 110). Because of a CT character, the red forms were proposed to act as quenchers (82, 111) although no
correlation between the presence of the red forms and the lifetime of the complexes was observed (62, 112, 113).

Last but not least, the red forms’ influence on the EET and trapping kinetics of PSI has been observed in different organisms. The average decay time is ~22 ps for the PSI core of higher plants (114) and ranges from ~20 ps to ~40 ps in different cyanobacteria species depending on the red form content (90): the more red forms, the slower the trapping kinetics of the PSI core. Cyanobacterial PSI devoid of red forms has a lifetime of ~14ps (115). The red forms present in the peripheral antenna of eukaryotic organisms were also shown to significantly slow down the EET and trapping kinetics of PSI-LHCI (76, 114, 116, 117). In A. thaliana, the “blue” antenna complexes (low content of red forms, Lhca1 and Lhca2) transfer excitation energy to the core four time faster than the “red” antenna (high content of red forms, Lhca3 and Lhca4)(114). The overall lifetime of PSI-LHCI is ~50 ps in higher plants (114).

**Trapping on the RC of PSI**

The cofactors of the ETC of PSI form two symmetrical branches (one branch bound to PsaA and the other one to PsaB) arranged in three pairs of Chls a (P700, A, A0) and one pair of phylloquinone (A1) (Figure 10). Both branches transfer electrons to the sulfur-iron clusters (Fx, Fa, Fb) on the stromal side. In C. reinhardtii, the PSI RC was reported to absorb at ~697 nm (100, 103, 118, 119). Since C. reinhardtii PSI core particles have very little absorption above 700 nm (100, 101), electron transfer steps of the RC could be well characterized in this alga: the accessory Chl A was revealed to be the primary electron donor, whereas P700 is oxidized in a second electron transfer step (2, 103, 105, 120)(Figure 10).
The first radical pair $A^+A_0^-$ was shown to form after $\sim6$ ps. Within $\sim2$ ps, the antenna excited-state equilibrium seems to be completed and the red emission observed in PSI core was attributed to repopulation of the exciton state of the six RC Chls after recombination of the first radical pair (98, 104). In this model, which is called the “charge recombination model”, PSI is described as a shallow trap, limiting the decay kinetics of the supercomplex. Nevertheless, cyanobacterial PSI devoid of red forms has its fluorescence quantum yield drastically decreased at 77K (115) suggesting that the RC Chls are very good quenchers. As shown in higher plants (121), we have also observed that P700 and P700$^+$ in *C. reinhardtii* have similar quenching efficiency (data not shown). Other works proposed to model PSI kinetics differently: the first radical pair was only formed after $\sim20$ ps because of a limiting migration time (76, 122), or because of a shallow-trap not able to recombine (100, 118, 123). Because PSI kinetics can be modeled in multiple ways, conclusions on purely trap-limited or purely diffusion-limited kinetics are not possible (124).

**Acclimation**

As mentioned above, the quantum yield of the PSs depends on the absorption spectra of the PSs, their antenna size, the loss of excitation in the antennae before CS
(excitation energy transfer efficiency and photoprotection) and the trapping. These factors are adjusted to enable the highest performances of the ETC and the least photodamage (125) by remodeling the photosynthetic membrane either on a short time scale after sudden change of light intensity/quality (short term acclimation) or on a long time scale when change of light is maintained (long term acclimation). Both types of acclimation can lead LHCII to be part of PSI peripheral antenna. Different PSI core subunits were reported to interact with Lhcbs (see above).

**Long term acclimation.** In higher plants, in addition to the adjustment of the RC stoichiometry (126-128), the long term acclimation involves regulation of Lhcb genes expression (129, 130). Indeed, Lhcbs were shown to function as antenna for both PSs: under continuous growing light, 40% to 65% of the PSI contains Lhcbs in its peripheral antenna (131). Less is known in *C. reinhardtii*.

**Short term acclimation.** After sudden change of light intensity/quality, the excitation energy is redistributed between PSs via migration of Lhcbs from one PS to another. This process is known as state transitions (132-134). State 1 occurs when all Lhcbs transfer their excitation energy to PSII and State 2 when part of the Lhcbs transfer their energy to PSI (135, 136). After low light growing conditions, we can calculate an equivalent of 1-2 “extra” LHCII trimers per monomeric PSII in higher plants (130) and 3-4 in *C. reinhardtii* (65) when considering the Chls a/b ratio in the cells (2.7 in *A. thaliana* (137) and 2.3 in *C. reinhardtii* (65) respectively), the PSI/PSII ratio (0.71 after growth under 20 uE.m\(^{-2}\).s\(^{-1}\) in *A. thaliana* (130) and 0.97 in *C. reinhardtii* in 20 uE.m\(^{-2}\).s\(^{-1}\) (65)) and the total number of Chls estimated in each PSs (Table 1). The pool size of the “extra” LHCII in the membrane can vary with the light intensity (130, 137). These “extra” Lhcbs were shown to be involved in state transitions in higher plants (138, 139). In *C. reinhardtii*, how the “extra” LHCII trimers are involved in state transitions is under debate. Several recent studies report very few Lhcbs migrating to PSI under State 2 in *C. reinhardtii* (140, 141) instead of 80% of the Lhcbs reported before (142). Ünlü et al. (141) found that the absorption cross section of PSI-LHCI in *C. reinhardtii* cells increases by less than one LHCII trimer in state 2.

**PSI-LHCl-LHCII.** The size of isolated PSI-LHCl-LHCII depends on the protocol of purification, which if too strong can dissociate possibly weakly connected Lhcbs. PSI-LHCl-LHCII from plants has been purified with only one LHCII trimer (138, 143). In partially solubilized membranes (using styrene-maleic acid copolymers), supercomplex with three trimers could be obtained in higher plants (144). In *C. reinhardtii*, PSI-LHCl-LHCII was isolated with different antenna size (65, 145-147). The largest PSI-LHCl-LHCII isolated so far ((57), Figure 11) contains seven Lhcbs (two LHCII trimers and one monomer located on the PsaH/L side of the core), in addition to the nine Lhcas located on the other side of the core (48).
The PSI-LHCI-LHCII supercomplex of *C. reinhardtii* PSI binds 322 Chls and 76 Cars (see SI of Chapter 3 for details on this estimation) and thus 45% more pigments than PSI-LHCI resulting in a significant increase of light harvesting capacities.

**Time resolved fluorescence with the streak camera**

To study the EET and trapping kinetics of PSI complexes as a function of the antenna composition size and organization, we used time-resolved fluorescence measurements at a picosecond time-scale. Because of the pigment composition of the core antenna (with Chl *a* only) is different from the peripheral antenna (Chl *a* and *b*), it was possible to excite differently the two parts of the supercomplex by setting excitation wavelengths corresponding to preferential absorption of one pigment or another (117).

In this thesis, picosecond-time-resolved fluorescence measurements were performed mainly with a streak camera setup described previously in (90, 148) with some adaptations as in Figure 12.
Figure 12: Streak camera set-up of the LaserLab in 2015. See main text for explanations.

A Coherent Vitesse Duo contains an integrated 10W Verdi CW laser (output wavelength 532 nm) that seeds the Vitesse solid-state ultrafast Ti:S oscillator (output wavelength 800nm, average power ≈100 mW, pulse width ≈100 fs, repetition rate 80 MHz) and pumps the regenerative amplifier Coherent RegA 9000 (output wavelength 800 nm, average power ≈1W, pulse width 180-200 fs, tunable repetition rate between 10 kHz and 300 kHz).

The output of the RegA feeds the optical parametric amplifier Coherent OPA 9400 (output wavelength from 470 nm to 770 nm, average power up to a few mW). The frequency-doubled light (400 nm) in the OPA could also be used as an output. The repetition rate was set to 250 kHz and the OPA was set to generate either the 400 nm or the 475 nm excitation wavelength in all experiments reported in this thesis.

The light intensity was modulated and residual 800 nm light and white light from the OPA was removed. The excitation polarization was set vertical with a Berek polarization compensator (New Focus, model 5540). The light was focused in the sample with a 15 cm focal length lens resulting in a spot diameter of 50 μm in the sample. Fluorescence emission was collected at right angle by two identical achromatic lenses (B. Halle UV-Achromat f=100 mm) to collimate the light and then focus it on the input slit (100 μm) of a spectrograph (Chromex 250IS, 50 grooves/mm ruling, blaze wavelength 600 nm, spectral resolution of 2nm). Scattered excitation light was removed with an optical long-pass filter. A polarizing filter (Spindler & Hoyer, Type 10K) was placed in between the two achromatic lenses to
collect light polarized at magic angle (54.7°) with respect to the excitation polarization. After the spectrograph, the light was focused on the input slit (40 μm) then on the photo-cathode of the streak camera Hamamatsu C5680 mounted with the M5675 Synchroscan unit (triggered by the Vitesse oscillator) and the Digital CCD Camera Hamamatsu Orca R2 (read out speed 8.5 frame/s). From the photo-cathode of the Synchroscan unit, photo-electrons will go through an electric field whose amplitude increases linearly with time: early electron will be less deviated than late electrons. Electrons will impact the phosphor screen (and then the CDD) at different spots depending on their time of generation at the photo-cathode. In other words, the delay of emission from the sample, i.e. the fluorescence decay, is mapped along the vertical dimension. An example of a streak camera image obtained through measurement is given in Figure 12.

Spectral calibration was done with an Argon lamp (Oriel Instruments Argon lamp model 6030) and spectrottemporal sensitivity (shading) correction (148) with a homogeneous white light source (Xenon lamp, Osram HLX 64642 24V 150W GER i 028). The time resolution depends on optics, filters used after the sample but predominately on the grating ruling, the spectrograph input slit (100 μm), the streak camera input slit (40 μm) and on the streak camera detector. The streak camera set-up used in this thesis has a final instrument response function (IRF) of 3-4 ps corresponding to a time resolution of ~2 ps.